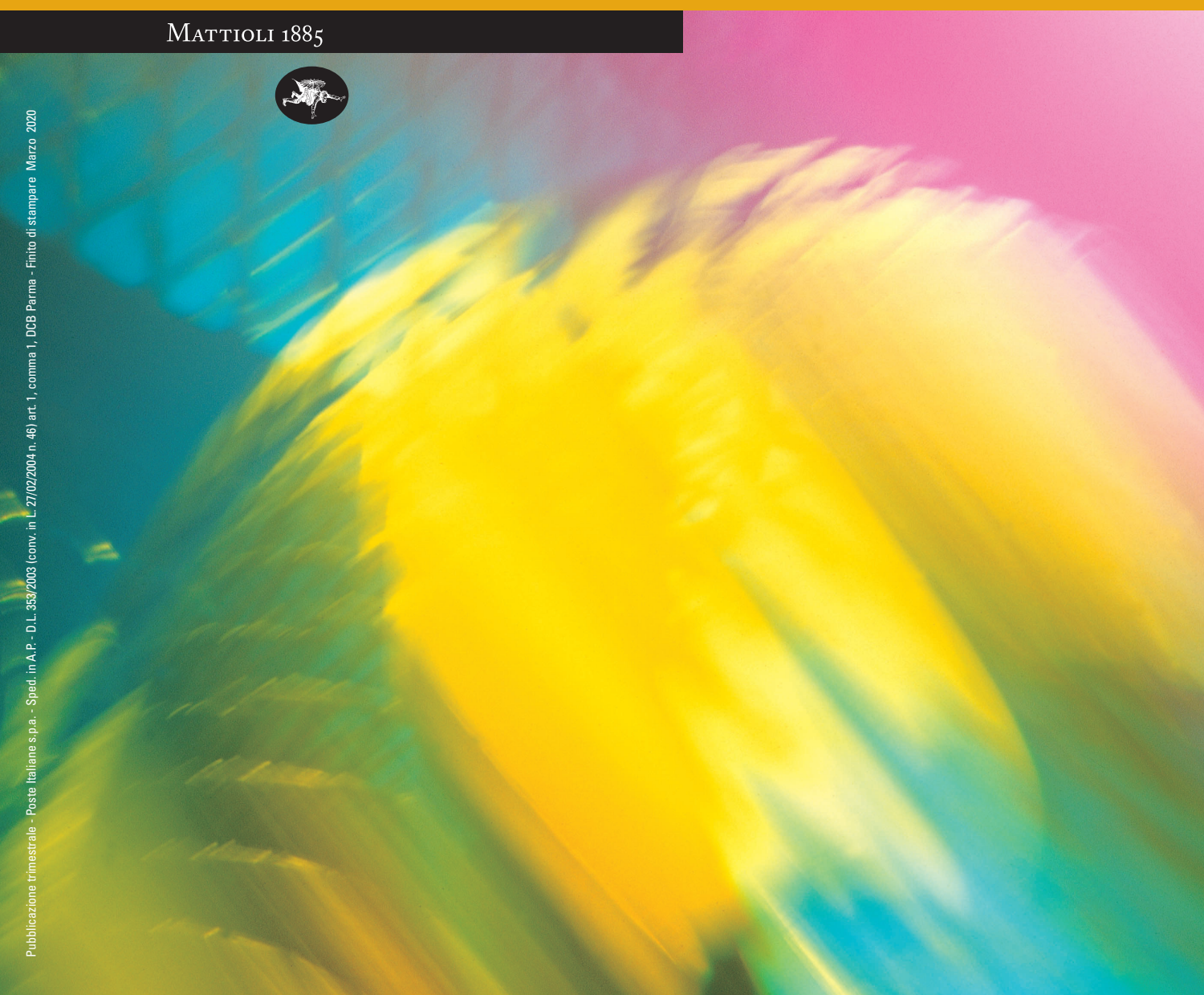
Progr. in Nutr. - Vol. 22 - N. 1 - March 2020 | ISSN 1129-8723

PROGRESS IN NUTRITION

Journal of Nutrition and Internal Medicine

Indexed in Science Citation Index Expanded (SciSearch®); Journal Citation Reports/Science Edition; Excerpta Medica/Embase, ISI Web of Science, Scopus (Elsevier) and Bibliovigilance. Impact Factor: 0,265

Mattioli 1885



Pubblicazione trimestrale - Poste Italiane s.p.a. - Sped. in A.P. - D.L. 353/2003 (conv. in L. 27/02/2004 n. 46) art. 1, comma 1, DCB Parma - Finito di stampare Marzo 2020

PROGRESS IN NUTRITION

JOURNAL OF NUTRITIONAL AND INTERNAL MEDICINE

Official Journal of the Italian Society of Nutritional Science (SISA)
With the support of Nutrition and Food Research Association (ARNA)

MATTIOLI 1885

srl- Strada di Lodesana 649/sx
Loc. Vaio - 43036 Fidenza (Parma)
tel +39 0524 530383
fax +39 0524 82537
www.mattiolihealth.com
E-mail: redazione@mattioli1885.com

EDITORIAL OFFICE
Valeria Ceci
E-mail: valeriaceci@mattioli1885.com

Mattioli 1885
srl- Strada di Lodesana 649/sx
Loc. Vaio - 43036 Fidenza (Parma)
tel 0524/530383
fax 0524/82537
www.mattioli1885.com

Direzione Generale
Direttore Generale
Paolo Cioni
Vice Presidente e Direttore Scientifico
Federico Cioni

Direzione Editoriale
Editing Manager
Anna Scotti
Editing
Valeria Ceci
Eugenio Nadotti
Foreign Rights
Nausicaa Cerioli

Marketing e Pubblicità
Responsabile Area ECM
Simone Agnello
Project Manager
Natalie Cerioli
Massimo Radaelli
Responsabile Distribuzione
Massimiliano Franzoni

PROGRESS IN NUTRITION
Registrazione Tribunale di Parma
N. 4 del 21/1/1999

Spedizione in abbonamento postale
Abbonamento annuale euro 57

Progress in Nutrition is indexed by: Science Citation Index Expanded (SciSearch®); Journal Citation Reports/ Science Edition; Excerpta Medica/Embase, ISI Web of Science. Scopus (Elsevier), Bibliovigilance

Impact Factor (released in June 2018): 0.265

Index

Volume 22 / n. 1 March 2020

Reviews

Original Articles

Progress in Nutrition 2020; Vol. 22, N. 1: 7-11 DOI: 10.23751/pn.v22i1.9203

Review

Linoleic acid: have we understood how it works in Psychopathologies and Ischemic Cardiovascular Diseases?

Massimo Cocchi[1,2], Elisabetta Mondo[1], Chiara Minuto[1], Giovanni Lercker[3]

[1]Department of Veterinary Medical Sciences, University of Bologna-E-mail: massimo.cocchi@unibo.it; [2]Research Institute for Quantitative and Quantum Dynamics of Living Organisms, Center for Medicine, Mathematics & Philosophy Studies; [3]Department of Agri-Food Sciences and Technologies, University of Bologna

Summary. The question about Linoleic Acid, which remains arguable, is whether the Linoleic Acid is inversely correlated to the Cardiovascular Disease or not and whether increasing the intake over the Recommended Daily Allowance (RDA) is positive or negative. The scientific literature, past and recent, is still controversial about the matter. To try to answer this controversy, it is important to remember the role of the Linoleic Acid in the membrane and its relationship with cholesterol. Furthermore, it is important to examine the effect of the oxidation of Linoleic Acid, its impact on the gut microbiota and the consequences on the gut epithelial integrity.

Highlights. Nutritional considerations about Linoleic Acid > Characteristic of oxidizability of Linoleic Acid with respect to the cell membrane function > Linoleic Acid in platelets > The position of Linoleic Acid with respect to Psychopathology and Ischemic Cardiovascular Disease > Linoleic Acid and its effect on gut bacteria adhesion to gut epithelial barrier.

Key words: linoleic acid, psychopatology, cardiovascular disease, membrane mobility

Nutrionional Characteristic of Linoleic Acid

The essentiality of Linoleic Acid (n-6), and alpha Linolenic Acid (n-3), was discovered by Burr & Burr in 1929 (1). It mainly consists in preventing tissue degeneration until death (1) and it is considered that the 1- 2% of total calories is the right amount to prevent deficiency (2). The assumption of 3-6 grams of Linoleic Acid is considered safe for average adults.

If we consider an average of 2000 Kcal per day, the amount of Linoleic Acid should be provided by about 4 grams of the fatty acid.

The increased consumption of vegetable oils has increased the intake of Linoleic Acid to about the 6% of total calories (3), which means that in diets with an intake of 2000 calories, the amount of Linoleic Acid has increased of about 3 times increasing the risk of oxidation.

Linoleic Acid: oxidizability and cell membrane mobility

There is a relationship between the molecular structure and the environmental behavior of all chemical species, both in chemical and biological systems, although it is not always the same for the two systems.

The interpretation of an event in complex systems such as biological ones is often also due to the knowledge of the molecular structures involved.

The fatty acids, that can be found bound or in their free form in different types of substances, have a

carboxyl group able to bind different chemical species, while the hydrocarbon chain can give chemicalphysical interactions of energy that is lower than in a bond, but still very important for biology (4).

By the existence of van der Waals forces, the hydrocarbon chains of different molecules can approach

each other to produce an electrostatic attraction for the coupling of those linearly similar molecular parts due to the deformations of the electronic clouds, with the genesis of electrostatic effects. The presence of double bonds in the hydrocarbon chain keeps part of the juxtaposed chains and decreases the total energy due to van der Waals forces, appearing among the molecules involved as critical points of minor forces and greater spatial bulk. For molecular structures rich in hydrocarbon, linear or not, the melting point, the viscosity and the respective structural distances between molecules will be different and indicative of the "interaction" intensity. In a biological membrane, dominated by the presence of phospholipids and cholesterol, its fluidity and functionality will be conditioned by these molecular interactions, with variations due to the presence of all the minor components of the membrane.

In addition to these effects, the thermostatic control will be a necessity in order to facilitate the homeostasis of many interactions, maintained by a series of self-regulations, triggered by any changes that have occurred.

The diet influences the composition and behavior of the membranes, which the living being will rebalance in relation to their respective needs. For membranes, saturated fatty acids and cholesterol will be used to increase the stiffness of the membrane folds, while polyunsaturated fatty acids will have the task of fluidizing it. The oxidation of fatty acids or their derivatives is an inevitable event for the needs of living organisms to produce energy (beta-oxidation) and for chemical transformations, i.e., the synthesis of oxygenated derivatives (prostaglandins, thromboxanes, leukotrienes, etc.).

Unfortunately, alongside the needs of the desired oxidative process, other undesired ones may occur.

Reactive Oxygen Species (ROS), considered as the cause of undesired biological oxidation, are "buffered" by numerous antioxidant systems present in our body.

The problem of oxidation arises when its quantity is very high, and the antioxidant means are not enough.

The connection between oxidation and inflammation, with the possible consequences, increases the importance of the oxidation argument.

Lipid oxidation mechanisms have been studied extensively through research conducted on foods to protect them, but little in biological systems. Oxidation in the aqueous-lipidic system has a very different behavior from that of lipids in the homogeneous phase. In fact, in the latter, it follows the perfect correspondence of speed with the level of unsaturation of the fatty acids (5, 6), while in the aqueous-lipidic system the Linoleic Acid (LA) is the most oxidizable and the Docosahexaenoic Acid (DHA) the least oxidizable (7).

The explanation of this behavior is attributable to the ability of the DHA to twist itself, in a watery environment, based on the presence of six double bonds reaching a structure with minimal surface exposed to water, the exact opposite behavior with respect to the lipidic system (8).

These phenomena explain the central role of Linoleic Acid in the regulation of cell membrane mobility and explain how this fatty acid, whose molecular size is greater than any other fatty acid, conditions both, cholesterol levels and exposure of membrane receptors.

Linoleic Acid, membrane viscosity, Ischemic Heart Disease and Psychopathology

Fatty acids and membrane viscosity of platelets (cells primarily involved in cardiovascular pathologies), have been extensively studied in patients with Ischemic Cardiovascular Disease (ICVD) and Major Depression-Bipolar Disorder (9-14).

In these studies, Linoleic Acid in platelets is, by far, lower than in normal subjects and this finding could confirm that lower levels are characteristic of the ICVD and Depression-Bipolar Disorder.

The question is whether a higher intake of Linoleic Acid can reduce cardiovascular risks.

Linoleic Acid facts

To better understand the position of Linoleic Acid in ICVD and Depression-Bipolar Disorder risk, some arguments should be addressed:

Linoleic Acid concentration in platelets of ICVD and Depression-Bipolar Disorder subjects.

The platelet concentration of Linoleic Acid in normal subjects with respect to the pathologic subjects is higher at such extent that is practically impossible to reach normal conditions (19% vs 10% in ICVD subjects) (9,11,14).

The platelet concentration of Linoleic Acid in in suicide risk and severe heart ischemia reaches a very low concentration, about 5% (11, 15).

Linoleic Acid and Cholesterol have the same behavior in cell membrane

Linoleic Acid and Cholesterol act the same way in the membrane. The larger molecular dimension of the first increases the fluidity of the membrane, but an increase of cholesterol contrasts this to maintain the balance of the membrane mobility (16, 17).

Cholesterol protects Linoleic Acid from oxidation being the latter the trigger of endothelial activation which leads to pro inflammatory effects (18).

Linoleic Acid is, among all polyunsaturated fatty acids, the most oxidizable

It is demonstrated that Linoleic Acid, because of the above characteristic, is more oxidizable with respect to the other polyunsaturated fatty acids (19) and that the cell membrane has an increased risk of oxidation (20-22).

Despite the large number of scientific papers claiming a positive effect of Linoleic Acid in ICVD risk reduction, other papers claim the responsibility of Linoleic Acid in ICVD because of its peroxidative role in LDL, VLDL and HDL and the production of oxidized metabolites such as 9-HODE (9-hydroxy-10,12- octadecadienoic acid) and 4HNE (4-Hydroxynonenal) (23, 24).

Linoleic Acid influences the gut bacterial adhesion to the epithelial gut opening the door to the silent inflammation which is considered one of the major events in ICVD and Psychopathology

Complex cellular and inflammatory interactions are involved in the progress of vascular diseases, such as, for example, the association between pro-inflammatory cytokines with endothelial dysfunction and atherosclerosis (25, 26).

Several studies, including the ones conducted by Arif et al. (27) have demonstrated that the increased membrane fluidity of bacteria reduces the adhesion to the epithelial wall influencing the opening of the tight junctions and giving access to the pro-inflammatory cytokines.

The excessive presence of PUFAs in general, in particular Linoleic Acid, both in the culture media of the various bacterial strains and in the daily diet, such as seed oils, lecithin, etc. (28) should be avoided in order to prevent the silent inflammation involved in ICVD and in Psychopathologies. Further, it is of interest the activity of *B. longum* and *L. acidophilus* in their antioxidant capacity on the Linoleic Acid and in their scavenging activity on malondialdehyde (29).

Conclusion

The evidence of a reduced plasma and cellular concentration of Linoleic Acid in cardiovascular risk and in the course of ischemic cardiovascular disease has certainly led many researchers to believe that it is advisable to take a greater amount of Linoleic Acid than recommended.

This work is not a criticism against the opinion of researchers (29) who recommend increasing the intake of Linoleic Acid over the recommended amount; however, it wants to draw attention to the biochemical aspects of Linoleic Acid. Based on the tissue distribution of Linoleic Acid, ranging from about 1% of the brain (30) to about 20% of the cardiac cells (31) it is recognized the role that Linoleic Acid plays in the regulation of the ion channels flux (32, 33). The authors suggest respecting the quantities recommended to not compromise the delicate biochemical role of this fatty acid with the risk of an increased oxidizability with respect to the heart and brain functions (15).

Authors' contributions

Conceptualization, investigation and writing-original draft preparation, Massimo Cocchi and Giovanni Lercker; review and editing, Elisabetta Mondo and Chiara Minuto.

References

1. Burr GO, Burr, MM. A New Deficiency Disease Produced by the Rigid Exclusion of Fat from the Diet. Journal of Biological Chemistry. 1929; 82: 345-367.
2. Holman RT. Biological activities of and requirements for polyunsaturated acids. In: Progress in the Chemistry of Fats and Other Lipids, Vol. 9. Pergamon Press. New York: 1971.p.607-682.
3. Ronald JJ. Linoleic Acid: A Nutritional Quandary. Healthcare. 2017; 5:25.
4. Lercker G, Cocchi M. Il grasso del latte: membrane, composizione e struttura. Progress in Nutrition. 2010; 12 (2): 183-194.
5. Gunstone FD, Harwood JL, Padley FB. The lipid handbook. London; New York; 1986. p. 453-457.
6. Belitz HD, Grosch W, Schieberle P. Food Chemistry. Springer Verlag ed. Berlin; Heidelberg; New York; London; Paris; Tokyo; 1987. p. 175.
7. Cosgrove JP, Church DF, Pryor WA. The Kinetics of the Autoxidation of Polyunsaturated Fatty Acids. Lipids. 1987; 22(5): 299-304.
8. Gawrisch K, Eldho NV, Holte LL. The Structure of DHA in Phospholipid Membranes. Lipids. 2003; 38: 445-452.
9. Cocchi M, Gabrielli F, Tonello L. Platelet's Fatty Acids Secrets in Coronary Artery Disease (CAD). BMJ. 2013;347.
10. Cocchi M, Tonello L. Bio molecular considerations in Major Depression and Ischemic Cardiovascular Disease. Central Nervous System Agents in Medicinal Chemistry. 2010; 10: 97-107.
11. Cocchi M, Tonello L, Lercker G. Fatty acids, membrane viscosity, serotonin and ischemic heart disease. Lipids in Health and Disease. 2010: 9:97.
12. Cocchi M, Tonello L. Platelets, Fatty Acids, Depression and Cardiovascular Ischemic Pathology. Progress in Nutrition. 2007; 9: 94-104.
13. Cocchi M, Tonello L, Tsaluchidu S, Puri BK. The use of self-organizing maps to study fatty acids in neuropsychiatric disorders. BMC Psychiatry. 2008; 8: S3.
14. Cocchi M, Tonello L, Bosi S, Cremonesi A, Castriota F, Puri B, Tsaluchidu S. Platelet oleic acid as Ischemic Cardiovascular disease marker. BMJ. 2007.
15. Cocchi M, Minuto C, Tonello L, Gabrielli F, Bernroider G, Tuszynski JA, Cappello F, Rasenick M. Linoleic acid: Is this the key that unlocks the quantum brain? Insights linking broken symmetries in molecular biology, mood disorders and personalistic emergentism. BMC Neuroscience. 2017; 18: 38.
16. McMurchie EJ. Dietary lipids and the regulation of membrane fluidity and function. In: Aloia RC, Curtain CC, Gordon LM editors. Physiological Regulation of Membrane Fluidity. New York: Liss AR; 1988. p.189–237.
17. Cocchi M, Minuto C, Tonello L, Tuszynski JA. Connection between the Linoleic Acid and Psychopathology: A Symmetry-Breaking Phenomenon in the Brain? Open Journal of Depression. 2015; 4: 41-52.
18. Lee YW, Park HJ, Hennig B, Toborek M. Linoleic Acid Induces MCP-1 Gene Expression in Human Microvascular Endothelial Cells through an Oxidative Mechanism. The Journal of Nutritional Biochemistry. 2001; 12: 648-654.
19. Araseki M, Kobayashi H, Hosokawa M, Miyashita K. Lipid Peroxidation of a Human Hepatoma Cell Line (HepG2) after Incorporation of Linoleic Acid, Arachidonic Acid, and Docosahexaenoic Acid. Bioscience, Biotechnology e Biochemistry. 2005; 69(3): 483–490.
20. Silaste M, Rantala M, Alfthan G, Aro A, Witztum JL, Kesaniemi YA, Horkko S. Changes in dietary fat intake alter plasma levels of oxidized low-density lipoprotein and lipoprotein (a). Arteriosclerosis, Thrombosis, and Vascular Biology. 2004; 24: 498–503.
21. Jira W, Spiteller G, Carson W, Schramm A. Strong increase in hydroxy fatty acids derived from Linoleic Acid in human low-density lipoproteins of atherosclerotic patients. Chemistry and Physics of Lipids. 1998; 91: 1–11.
22. Reaven P, Parthasarathy S, Grasse BJ, Miller E, Steinberg D, Witztum JL. Effects of oleate rich and linoleate-rich diets on the susceptibility of low-density lipoprotein to oxidative modification in mildly hypercholesterolemic subjects. Journal of Clinical Investigation. 1993; 91: 668–76.
23. DiNicolantonio JJ, O'Keefe JH. Omega-6 vegetable oils as a driver of coronary heart disease: the oxidized Linoleic Acid hypothesis. Open Heart. 2018; 5: e000898.
24. Asselin C, Ducharme A, Ntimbane T, Ruiz M, Fortier A, Guertin MC, Lavoie J, Diaz A, Levy E, Tardif JC, Des Rosiers C. Circulating Levels of Linoleic Acid and HDL-Cholesterol are Major Determinants of 4-Hydroxynonenal Protein Adducts in Patients with Heart Failure. Redox Biology. 2014; 2: 148-155.
25. Kofler S, Nickel T, Weis M. Role of cytokines in cardiovascular diseases: a focus on endothelial responses to inflammation. Clinical Science. 2005; 108: 205–213.
26. Ross, R. Atherosclerosis: an inflammatory disease. The New England Journal of Medicine. 1999; 340: 115–126.
27. Arif I, Meddings JB, Ratnam S, Sherman PM. Modulation of host cell membrane fluidity: a novel mechanism for the prevention of bacterial adhesion. The American Physiological Society. 1999; 277(1): G201-8.
28. Kankaanpää PE, Salminen SJ, Isolauri E, Lee YK. The influence of polyunsaturated fatty acids on probiotic growth and adhesion. FEMS Microbiology Letters. 2001; 194 (2): 149-153.
29. Meei-Yn L, and Chyuan-Liang Y. Inhibition of Lipid Peroxidation by Lactobacillus acidophilus and Bifidobacterium longum. J. Agric. Food Chem. 1999; 47, 3661–3664.
30. Marklund M, et al. Biomarkers of Dietary Omega-6 Fatty Acids and Incident Cardiovascular Disease and Mortality: An Individual-Level Pooled Analysis of 30 Cohort Studies. Circulation. 2019; 21;139(21): 2422-2436.
31. Cocchi M, Minuto C. Linoleic Acid: A Milestone in Brain Evolution? Human evolution. 2015; 245-257.
32. Rocquelin G, Guenot L, Justrabo IE, Grynberg A, David M. Fatty Acid Composition of Human Heart Phospholip-

ids: Data from 53 Biopsy Specimens. Journal of Molecular and Cellular Cardiology. 1985; 17: 76-773.
33. Giroud S, Frare C, Strijkstra A, Boerema A, Arnold W, Ruf T. Membrane Phospholipid Fatty Acid Composition Regulates Cardiac SERCA Activity in a Hibernator, the Syrian Hamster (Mesocricetus auratus). PLoS One. 2013; 8: e63111.
34. Harrell MD, Stimers JR. Differential Effects of Linoleic Acid Metabolites on Cardiac Sodium. The Journal of Pharmacology and Experimental Therapeutics. 2002; 303: 347-55.

Correspondence:
Massimo Cocchi
Department of Veterinary Medical Sciences,
University of Bologna
E-mail: massimo.cocchi@unibo.it

Progress in Nutrition 2020; Vol. 22, N. 1: 12-19 DOI: 10.23751/pn.v22i1.9341

Review

Potential therapeutic effects of alpha lipoic acid in memory disorders

Leonardo Triggiani

Neurology Department, Ospedale Santo Spirito, Rome, Italy - E-mail: ltriggiani@gmail.com

Summary. With ageing, biological processes promote a gradual loss of the ability to maintain homeostasis, leading to a progressive deterioration in the body's biochemical and physiological functions, thereby increasing susceptibility to disease. ALA, a low-molecular-weight dithiol with a chiral centre, is a dietary supplement thought to have potential therapeutic effects for the prevention or treatment of neurodegenerative diseases. In addition, treatment with ALA is able to regulate inflammatory cell infiltration into the central nervous system and to down-regulate VCAM-1 and human monocyte adhesion to epithelial cells. In neurodegenerative disease models, treatment with ALA is able to improve the function of the dopamine, serotonin and norepinephrine neurotransmitters. Scientific evidence shows that ALA possesses the ability to improve memory capacity in a number of experimental neurodegenerative disease models and in age-related cognitive decline in rodents. Studies have shown that this substance is able to reduce memory loss in various behavioural paradigms of Alzheimer's disease and in age-related cognitive dysfunctions.

Key words: alpha lipoic acid, ALA, memory disorders

Cognitive decline

Ageing is a multi-factorial process that includes genetic, social and environmental factors. With ageing, biological processes promote a gradual loss of the individual ability to maintain homeostasis, followed by a progressive deterioration in the body's biochemical and physiological functions, thereby increasing susceptibility to age-related diseases. The cognitive functions also undergo a decline with age. Alzheimer's dementia and mild cognitive impairment (MCI) are common amongst the elderly and are characterised by a progressive loss of the cognitive functions, memory, speech and reasoning ability. However, even those who do not suffer from these conditions can present minor cognitive changes that affect the activities of daily living and quality of life.

Alpha lipoic acid

Over the years, increasing attention has been dedicated to alpha lipoic acid (ALA) as a dietary supplement with potential therapeutic effects for the prevention and treatment of neurodegenerative diseases and age-related cognitive dysfunctions.

ALA (1,2-dithiolane-3-pentonoic acid, or thioctic acid) was discovered in 1937 by Snell (1) and characterised by Reed in 1951 (2); however many of its properties are yet to be clarified.

ALA is a low-molecular-weight dithiol with a chiral centre and its structure is formed of eight carbon atoms, two oxygen atoms in the carboxyl group and two sulphur atoms (Figure 1). A substantial part of ALA is reduced to dihydrolipoic acid (DHLA) by lipoamide dehydrogenase with the involvement of the NADH and NADPH system (3,4). ALA contains an asymmetrical carbon atom that determines two optical isomers: the

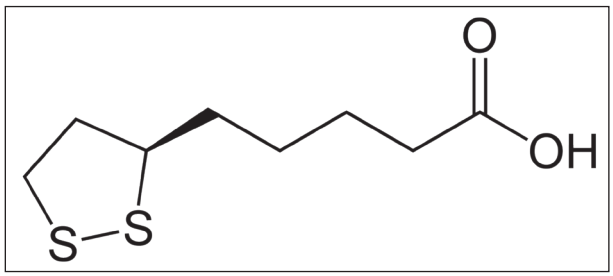


Figure 1. Structural formula of alpha lipoic acid

S form and the R form, the former being synthesised endogenously (5). ALA can be taken in through the diet and, due to its endogenous synthesis capacity, it is not considered a vitamin, rather it is structurally considered a member of the B vitamin family (6). When taken as a dietary supplement in the racemic mixture form, it contains two isomers (1:1 ratio of R-ALA and S-ALA) of which S-ALA can prevent the polymerisation of R-ALA and therefore increases its bioavailability (5). ALA is found in foods of both vegetable and animal origin. R-ALA is most abundantly present in vegetables like spinach, broccoli and tomatoes; amongst the foods of animal origin it is found most copiously in bovine kidney, heart and liver (7). Even when taken in through food and supplements, ALA is absorbed, metabolised and excreted rapidly. Up to 93% of an oral dose is absorbed in the digestive tract and undergoes considerable pre-systemic elimination. Between 27 and 34% of the oral intake is available for tissue absorption; the liver is one of the main clearance organs on account of its high absorption and storage capacity (8). Gastrointestinal absorption varies greatly and would appear to be reduced with dietary intake, suggesting that the absorption of ALA competes with that of other nutrients. ALA is rapidly absorbed in the digestive tract and its plasma presence is followed by rapid clearance. The plasma half-life of ALA is approximately 30 minutes. Peak urinary excretion occurs 3-6 hours after intake. Approximately 45% is excreted in urine within 24 hours and just 3% is excreted in stools. A small amount of ALA is excreted in an unmodified form (9). There are no guidelines regarding the recommended daily dose and the dose that could have adverse effects on human health is not known.

At cell level, ALA is an essential substrate for energy metabolism and the formation of amino acids (9). It is a fundamental cofactor for mitochondrial dehydrogenase complex enzymes including pyruvate-dehydrogenase (PDH), α-ketoglutarate-dehydrogenase (α-KGDH) and branched-chain keto acid dehydrogenase (10).

The presence of a dithiol ring in both the oxidised form (ALA) and the reduced form (DHLA), makes both forms potent natural antioxidants (11). They react with both reactive oxygen species (ROS), such as the hydroxyl, peroxyl and superoxide radicals (12), and with reactive nitrogen species (RNS), and therefore their functions are considered part of the cell protection mechanisms against those conditions in which oxidative stress plays the main aetiological role (13).

A high level of oxidative stress contributes to making the inflammatory processes chronic. A number of studies show that ALA alters nuclear factor (NF-κβ) signal transduction at cell level. NF-κβ is a redox-sensitive transcription factor that plays an important role in inflammation by regulating the expression of cytokines, internal tissue factors and adhesion factors in the endothelial cells. The expression of adhesion molecules causes an interaction between white blood cells and endothelial cells through the bloodstream. In this situation, other inflammatory mediators, such as monocyte chemo-attractant protein-1 (MCP-1), metalloprotease 9 and various cytokines, are involved by NF-κβ (14,15). As an inhibitor of NF-κβ, ALA has been studied in cytokine-mediated inflammation (16). In addition, treatment with ALA is able to regulate inflammatory cell infiltration into the central nervous system and to down-regulate vascular cell adhesion molecule-1 (VCAM-1) and the human monocyte adhesion to epithelial cells, and to inhibit the expression of NF-κβ-dependent metalloprotease 9 (17,18).

Metals can mediate the generation of free radicals, induce oxidative damage and exert a potential toxic and carcinogenic action. In addition to the direct antioxidant properties of ALA, some studies have shown that both ALA and DHLA and a great capacity to chelate redox-active metals, such as copper, free iron, zinc and magnesium, albeit in different ways (19, 20). The evidence from research seems to suggest that ALA modulates the free radical actions induced by metals in transition metal accumulation sites. More specifically, it has been shown that iron and copper chelation with DHLA may explain the low level of free radical dam-

age in the brain and the improvement in the pathobiology of Alzheimer's Disease (21). It has also been demonstrated that treatment with ALA reduces both iron absorption and the size of iron accumulations in the cerebral cortex (22).

Experimental models

Alzheimer's Disease (Figure 2) in a progressive, chronic neurodegenerative disease. It is characterised by a progressive loss of cognitive and memory capacity, as well as self-sufficiency in daily life and communication and interpersonal skills. In the past, it was postulated that the aggregation of Beta-Amyloid peptides (Aβ) to form amyloid plaques constituted the first event in the pathological cascade of Alzheimer's disease; this mechanism was known as the "amyloid cascade hypothesis". The so-called "oligomer hypothesis" now suggests that Alzheimer's disease is initiated by a synaptic dysfunction caused by soluble oligomers of Aβ (23). Furthermore, scientific evidence has shown that multiple neurotransmitters and the cholinergic system undergo a change under this condition (24,25). These changes can be defined as a deterioration of the basal cholinergic neurons of the frontal cortex and a reduction in acetylcholine production (26). As acetylcholine metabolism is associated with

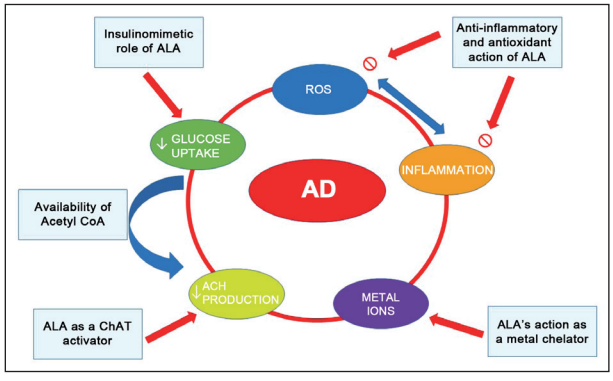


Figure 2. ALA can improve cognitive performance and could be a potential treatment for the pathogenesis of Alzheimer's disease through a number of mechanisms: (1) increased acetylcholine production; (2) increased glucose uptake and therefore greater availability of acetyl-CoA for the production of ACh; (3) inhibition of hydroxyl radical formation, removal of ROS, inflammatory process down-regulation; (4) potent metal chelator.

the glycolytic pathway and the formation of pyruvate, acetylcholine synthesis will be altered by the depletion of glucose in the brain of patients with Alzheimer's disease (27). Energy depletion and oxidative stress are therefore fundamental biochemical characteristics of the disease. Oxidative stress is triggered by the astroglia and microglia activated through an inflammatory process (28) that leads to the formation of free radicals and to extensive oxidative stress in the progression of Alzheimer's disease (29). Superoxide species and free radicals therefore cause neuronal damage in this condition (30,31). Observational studies suggest that antioxidant supplementation can modulate oxidative stress and reduce the risk of Alzheimer's disease (32). Many mechanisms have been postulated to explain the effect of ALA in Alzheimer's disease: one recent pilot study showed that combination therapy with omega-3 and ALA may reduce functional and cognitive decline in patients with mild and moderate Alzheimer's disease (33). ALA down-regulates the pro-inflammatory redox-sensitive transduction processes, including NF-ϰβ translocation, with a reduction in the release of further free radicals and cytotoxic cytokines. ALA is able to down-regulate inflammatory processes by modulating the pro-inflammatory cytokines (29), and it can influence oxidative stress by increasing intracellular glutathione levels (34,35). One study has shown that ALA improves mitochondrial enzyme activity and reduces the levels of lipid peroxidation in the hepatic and renal mitochondria of rats (36). Another study has shown that ALA may increase the mitochondrial synthesis of ATP in the brain of elderly rats, thereby increasing the activity of the mitochondrial enzymes (37). In an age-related reaction, the excess of metal ions (copper, iron and zinc) in the brain causes peptide precipitation and the formation of plaques. The abnormal combination of Aβ with copper and iron ions induces the production of hydrogen peroxide from molecular oxygen (38) with the consequent production of neurotoxic hydroxyl radicals. ALA is able to chelate transition metal ions and, therefore, modulate the iron- and copper-mediated oxidative stress in Alzheimer's plaques (39). This results in an increase in the extraction of Aβ peptide from the cortical areas (40). In an experimental mouse model of Alzheimer's, ALA was seen to increase the extraction of Aβ from the frontal cortex

that, like other metal chelators, could reduce the amyloid load in affected patients (40). ALA may also play a role in the activation of the choline acetyltransferase enzyme (ChAT), which is essential in the anabolism of acetylcholine (41,42). One experimental study has shown that in rats that had been administered ALA there was an inversion in the cognitive dysfunction with an increase in ChAT activity in the hippocampus (43). Another study showed the complete disappearance of enzymatic activity of purified ChAT after the dialytic removal of DHLA, whereas activity was restored by adding DHLA. The authors concluded that DHLA plays an essential role in the activity of this enzyme and that the relationship between reduced and oxidised ALA is important for the synthesis of acetylcholine (42). DHLA can act as a coenzyme in the ChAT reaction or it can reduce an essential functional cysteine residue of ChAT that no other physiological antioxidant, including reduced glutathione, is able to reduce.

Clinical studies

A number of clinical studies have been conducted to evaluate the effects of ALA in Alzheimer's disease. Unfortunately, as they all have methodological limitations, the conclusions that can be drawn from them have a merely indicative value.

In 2001, Hager et al. studied 9 subjects with Alzheimer's aged over 45 years. These patients received a standard treatment with acetylcholinesterase inhibitors - donepezil or rivastigmine - for three months before starting a dose of 600 mg of ALA for 12 months. The neuropsychological scales used were the Mini-Mental State Examination (MMSE) and the Alzheimer's disease assessment score cognitive subscale (ADAScog). After starting treatment with ALA, the results of these tests remained constant throughout the follow-up period of almost a year (44). A few years later, the same group published the results of a 48-month follow-up study including 43 patients with mild, moderate-early and moderate-advanced dementia. The results show a lower disease progression rate in the subjects taking ALA (600 mg/day) (45). However, the small sample size of the study and the absence of a control group constitute important limits.

Galasko et al. in 2012 conducted a double-blind, placebo-controlled trial on 78 Alzheimer's patients to evaluate biomarkers in the cerebrospinal fluid (CSF). In this trial, patients were randomised to three groups that received: (a) a combination of 800 IU/day of vitamin E (E), 500 mg/day of vitamin C (C) and 900 mg/day of ALA; (b) 1200 mg/day on co-enzyme Q; (c) placebo. Although the C/E and ALA combination reduced the CSF biomarker F2-isoprostane, showing a reduction in cerebral oxidative stress, none of the antioxidants altered the levels of CSF biomarkers associated with amyloid disease or tau protein. Furthermore, the E/C/ALA group presented a faster cognitive decline, based on MMSE scores, than the placebo group. These results suggest that further clinical studies are required to evaluate the benefits of ALA in cognitive decline (46).

In 2014, Shinto et al. studied 39 subjects with Alzheimer's Disease in a randomised, double-blind, parallel-group, 3-arm trial evaluating the efficacy of omega-3 fatty acids alone or in combination with ALA (600 mg/day) versus placebo. The purpose of the trial was to evaluate changes in oxidative stress biomarkers. The following cognitive and functional tests were also performed: MMSE, ADAScog and Activities of Daily Living / Instrumental Activities of Daily Living (ADL/IADL). After 12 months, none of the 3 groups presented significant differences in F2-isoprostane levels. The group treated with omega-3 presented a halt in decline on the IADL but no change in the MMSE and ADL scores. It is interesting to note that the omega-3 + ALA combination caused a slow-down in cognitive decline (considering both the MMSE and the IADL scores but not the ADAScog scores) (47).

In 2005, Bragin et al. suggested ALA as an approach for integrated treatment in patients with moderate dementia (MMSE < 15) and depression. In this trial, 35 subjects with an average age of 71 years were followed for 24 months. The treatment was composed of antidepressants, cholinesterase inhibitors, supplements and vitamins. Patients were also given dietary, exercise and stress control technique instructions. Neuropsychological assessment scales were used. The results showed that this treatment, administered for 2 years, slowed cognitive decline and improved the per-

formance of these patients. This effect can be partly explained by an improvement in the patients' overall mental health, due primarily to the depression treatment. The main limitations of this study were the lack of information on patient compliance and the concomitant use of other medicinal products. It is also difficult to establish which of the interventions produced a positive effect (48).

Possible mechanisms of the neuroprotective effect of alpha lipoic acid

In vivo and *in vitro* studies have been conducted to define the cellular and molecular effects of ALA underlying its activity on memory processes. The effects of ALA on oxidative markers in various areas of the brain have been discussed in a number of studies on animal models of ageing and degenerative diseases. The administration of ALA reduces lipid peroxidation in different areas of the brain and increases the activity of antioxidants such as ascorbate (vitamin C), α-tocopherol (vitamin E), glutathione, and also the activity of superoxide dismutase, catalase, glutathione-peroxidase, glutathione-reductase, glucose-6-P-dehydrogenase. Furthermore, administration of ALA inverts the increase in carbonyl protein levels in a radiation-induced cognitive dysfunction model and causes a decrease in carbonyl protein levels in elderly SAMP8 rats (49-51).

Liu et al. examined the effects of ALA on the mitochondrial structure, hippocampal neurodegeneration, and the nucleic acid oxidative damage in the hippocampus and cortex of elderly rats. ALA supplementation significantly reduced the levels of oxidised RNA and inverted age-induced structural mitochondrial damage in the hippocampus (52). In another study, conducted to evaluate the protective effect of ALA against damaged caused by arsenic-dichlorvos in rats, showed that cerebral oxidative stress and cholinergic dysfunction were significantly reduced by the administration of ALA (53).

The SAMP8 mouse is an experimental model that shows increased oxidative stress and memory decline associated with a rapid ageing process. The mechanisms underlying the inversion in the cognitive decline of SAMP 8 mice caused by ALA were determined by studying the expression and specific carbonylation of protein in the brain of 12-month-old SAMP8 mice after the administration of ALA or carrier. The levels of 3 proteins (neurofilament L triplet protein, a-enolase and ubiquitous mitochondrial creatine kinase) were significantly elevated, whereas protein carbonylation was reduced in lactate dehydrogenase B, in dihydropyraminidase-like protein 2 and in the a-enolase of elderly mice receiving ALA, suggesting that, in addition to improving learning and memory, ALA is able to restore certain proteins in the brains of elderly SAMP 8 mice (54).

In neurodegenerative disease models, treatment with ALA can improve the function of the dopamine, serotonin and norepinephrine neurotransmitters (49). In an Aβ vaccine-induced Alzheimer's disease model, mice treated with ALA presented increased levels of serotonin, dopamine and norepinephrine, whereas the concentration of the metabolites 5-hydroxyindole, acetic acid and homovalinic acid gradually normalised (55). Another study demonstrated that ALA can improve the neurological damaged induced by excess Aβ and aluminium, thereby restoring AChE activity (56). In a $AlCl_3$-induced neurodegeneration model, ALA showed the ability to improve cognitive functions and increase cholinergic system functions. Treatment with ALA increased the expression of genes encoding for muscarine receptors M1 and M2 and for choline acetyltransferase in the group treated with $AlCl_3$- (57).

Both ALA and DHLA have been seen to inhibit the formation of Aβ fibrils and their expansion, and they also weaken pre-formed fibrils in a dose-dependent manner (58). Furthermore, DHLA alone has been seen to have a significant neuroprotective action against the neurotoxicity induced by Aβ and by iron/hydrogen peroxide (59).

Conclusions

Although the mechanisms of action of ALA are not yet completely clear, it is evident that a multitude of pathways underlie its neuroprotective capacity.

Scientific evidence shows that ALA possesses the ability to improve memory capacity in a number of ex-

perimental neurodegenerative disease models and in age-related cognitive decline in rodents. Studies have shown that this substance is able to reduce memory loss in various behavioural paradigms of Alzheimer's disease and in age-related cognitive dysfunctions.

In vivo and *in vitro* studies have shown that ALA has a positive intervention in the neurodegenerative processes of the hippocampus, by reducing neuronal apoptosis and supporting a neuroprotective role mediated by the mitochondrial cell death process. ALA is also able to inhibit the formation of Aβ fibrils, thereby improving the consequent neurological damage, and it significantly restores AChE activity. This latter property suggests that ALA has a potential role in improving cholinergic and cognitive function. These neuroprotective effects can be associated with ALA's ability to improve the memory loss associated with neurodegenerative diseases.

Although they have yielded promising results, the clinical studies conducted in humans to date present a number of limitations - small sample size, open-label design, concomitant use of other antioxidants - that make it impossible to draw appropriate conclusions regarding the use of ALA in neurodegenerative diseases and memory disorders.

The improvements in memory and cognitive capacity and the neuroprotective activity shown by ALA support the hypothesis that it could be used as a supplementary treatment in neurodegenerative diseases and provide the rationale for clinical trials with an *ad hoc* design.

References

1. Snell EE, Strong FM, Peterson WH. Growth factors for bacteria: Fractionation and properties of an accessory factor for lactic acid bacteria. Biochem J. 1937 Oct;31(10):1789-99
2. Reed LJ, DeBusk BG, Gunsalus IC, Hornberger CS Jr. Crystalline alpha-lipoic acid; a catalytic agent associated with pyruvate dehydrogenase. Science. 1951 Jul 27;114(2952):93-4
3. Arivazhagan P, Ramanathan K, Panneerselvam C. Effect of DL-alpha-lipoic acid on glutathione metabolic enzymes in aged rats. Exp Gerontol. 2001 Dec;37(1):81-7.
4. Bilska A, Dubiel M, Sokołowska-Jezewicz M, Lorenc-Koci E, Włodek L. Alpha-lipoic acid differently affects the reserpine-induced oxidative stress in the striatum and prefrontal cortex of rat brain. Neuroscience. 2007 Jun 8;146(4):1758-71
5. De Araújo DP, Lobato Rde F, Cavalcanti JR, Sampaio LR, Araújo PV, Silva MC, Neves KR, Fonteles MM, Sousa FC, Vasconcelos SM. The contributions of antioxidant activity of lipoic acid in reducing neurogenerative progression of Parkinson's disease: a review. Int J Neurosci. 2011 Feb;121(2):51-7
6. Xing ZG, Yu GD, Qin L, Jiang F, Zhao WH. Effects and mechanism of lipoic acid on beta-amyloid-intoxicated C6 glioma cells. Genet Mol Res. 2015 Oct 30;14(4):13880-8
7. Lodge JK, Packer L. Natural sources of lipoic acid in plant and animal tissues. In: Antioxidant food supplements in human health. Eds. Packer L, Hiramatsu M, Yoshikawa T. San Diego, CA: Academic Press. 1999:121-134
8. Hagen TM, Ingersoll RT, Lykkesfeldt J, Liu J, Wehr CM, Vinarsky V, Bartholomew JC, Ames AB. (R)-alpha-lipoic acid-supplemented old rats have improved mitochondrial function, decreased oxidative damage, and increased metabolic rate. FASEB J. 1999 Feb;13(2):411-8
9. Bustamante J, Lodge JK, Marcocci L, Tritschler HJ, Packer L, Rihn BH. Alpha-lipoic acid in liver metabolism and disease. Free Radic Biol Med. 1998 Apr;24(6):1023-39
10. Ambrus A, Tretter L, Adam-Vizi V. Inhibition of the alpha-ketoglutarate dehydrogenase-mediated reactive oxygen species generation by lipoic acid. J Neurochem. 2009 May;109 Suppl 1:222-9
11. Bilska A, Włodek L. Lipoic acid - the drug of the future? Pharmacol Rep. 2005 Sep-Oct;57(5):570-7
12. Jones W, Li X, Qu ZC, Perriott L, Whitesell RR, May JM. Uptake, recycling, and antioxidant actions of alpha-lipoic acid in endothelial cells. Free Radic Biol Med. 2002 Jul 1;33(1):83-93
13. Petersen Shay K, Moreau RF, Smith EJ, Hagen TM. Is alpha-lipoic acid a scavenger of reactive oxygen species in vivo? Evidence for its initiation of stress signaling pathways that promote endogenous antioxidant capacity. IUBMB Life. 2008 Jun;60(6):362-7
14. Zhang WJ, Frei B. Alpha-lipoic acid inhibits TNF-alpha-induced NF-kappaB activation and adhesion molecule expression in human aortic endothelial cells. FASEB J. 2001 Nov;15(13):2423-32
15. Kim HS, Kim HJ, Park KG, Kim YN, Kwon TK, Park JY, Lee KU, Kim JG, Lee IK. Alpha-lipoic acid inhibits matrix metalloproteinase-9 expression by inhibiting NF-kappaB transcriptional activity. Exp Mol Med. 2007 Feb 28;39(1):106-13.
16. Abdelkarem HM, Fadda LH, Hassan AAG. Potential Intervention of α- Lipoic Acid and Carnitine on Insulin Sensitivity and Anti-Inflammatory Cytokines Levels in Fructose-Fed Rats, a Model of Metabolic Syndrome. J Diet Suppl. 2017 Jan 2;14(1):54-64
17. Kunt T, Forst T, Wilhelm A, Tritschler H, Pfuetzner A, Harzer O, Engelbach M, Zschaebitz A, Stofft E, Beyer J. Alpha-lipoic acid reduces expression of vascular cell adhesion molecule-1 and endothelial adhesion of human monocytes after stimulation with advanced glycation end prod-

ucts. Clin Sci (Lond). 1999 Jan;96(1):75-82
18. Guo J, Gao S, Liu Z, Zhao R, Yang X. Alpha-Lipoic Acid Alleviates Acute Inflammation and Promotes Lipid Mobilization During the Inflammatory Response in White Adipose Tissue of Mice. Lipids. 2016 Oct;51(10):1145-1152
19. Shay KP, Moreau RF, Smith EJ, Smith AR, Hagen TM. Alpha-lipoic acid as a dietary supplement: molecular mechanisms and therapeutic potential. Biochim Biophys Acta. 2009 Oct;1790(10):1149-60
20. Ou P, Tritschler HJ, Wolff SP. Thioctic (lipoic) acid: a therapeutic metal-chelating antioxidant? Biochem Pharmacol. 1995 Jun 29;50(1):123-6
21. Bush AI. Metal complexing agents as therapies for Alzheimer's disease. Neurobiol Aging. 2002 Nov-Dec;23(6):1031-8
22. Suh JH, Moreau R, Heath SH, Hagen TM. Dietary supplementation with (R)-alpha-lipoic acid reverses the age-related accumulation of iron and depletion of antioxidants in the rat cerebral cortex. Redox Rep. 2005;10(1):52-60.
23. Tamaoka A. The pathophysiology of Alzheimer's disease with special reference to "amyloid cascade hypothesis". Rinsho Byori 2013;61(11):1060–9
24. Nordberg A. In vivo detection of neurotransmitter changes in Alzheimer's disease. Ann NY Acad Sci 1993;695(1):27–33
25. Whitehouse PJ, Au KS. Cholinergic receptors in aging and Alzheimer's disease. Prog Neuropsychopharmacol Biol Psychiatry 1986;10(3):665–76
26. Tomaszewicz M, Roßner S, Schliebs R, Cwikowska J, Szutowicz A. Changes in cortical acetyl-CoA metabolism after selective basal forebrain cholinergic degeneration by 192IgG-saporin. J Neurochem 2003;87(2):318–24
27. Fu W, Jhamandas JH. Role of astrocytic glycolytic metabolism in Alzheimer's disease pathogenesis. Biogerontology 2014;15(6):579–86
28. Avila-Muñoz E, Arias C. When astrocytes become harmful: functional and inflammatory responses that contribute to Alzheimer's disease. Ageing Res Rev. 2014;18:29–40
29. Manczak M, Anekonda TS, Henson E, Park BS, Quinn J, Reddy PH. Mitochondria are a direct site of Aβ accumulation in Alzheimer's disease neurons: implications for free radical generation and oxidative damage in disease progression. Hum Mol Gen 2006;15(9):1437–49
30. Butterfield DA, Boyd-Kimball D, Castegna A. Proteomics in Alzheimer's disease: insights into potential mechanisms of neurodegeneration. J Neurochem 2003;86(6):1313–27
31. Lovell MA, Markesbery WR. Oxidative damage in mild cognitive impairment and early Alzheimer's disease. J Neurosci Res 2007;85(14):3036–40
32. Morris M. The role of nutrition in Alzheimer's disease: epidemiological evidence. Eur J Neurol 2009;16(s1):1–7
33. Galasko DR, Peskind E, Clark CM, Quinn JF, Ringman JM, Jicha GA, et al. Antioxidants for Alzheimer disease: a randomized clinical trial with cerebrospinal fluid biomarker measures. Arch Neurol 2012;69(7):836–41
34. Farr SA, Price TO, Banks WA, Ercal N, Morley JE. Effect of alpha-lipoic acid on memory, oxidation, and lifespan in SAMP8 mice. J Alzheimers Dis 2012;32(2):447–55
35. Moraes TB, Dalazen GR, Jacques CE, de Freitas RS, Rosa AP, Dutra-Filho CS. Glutathione metabolism enzymes in brain and liver of hyperphenylalaninemic rats and the effect of lipoic acid treatment. Metab Brain Dis 2014;29(3):609–15
36. Arivazhagan P, Ramanathan K, Panneerselvam C. Effect of DL-α-lipoic acid on mitochondrial enzymes in aged rats. Chem Biol Interact 2001;138(2):189–98
37. Ajith TA, Nima N, Veena RK, Janardhanan KK, Antonawich F. Effect of palladium α-lipoic acid complex on energy in the brain mitochondria of aged rats. Altern Ther Health Med 2013;20(3):27–35
38. Huang X, Atwood CS, Hartshorn MA, Multhaup G, Goldstein LE, Scarpa RC, et al. The Aβ peptide of Alzheimer's disease directly produces hydrogen peroxide through metal ion reduction. Biochemistry 1999;38(24):7609–16
39. Suh JH, Zhu B-Z, deSzoeke E, Frei B, Hagen TM. Dihydrolipoic acid lowers the redox activity of transition metal ions but does not remove them from the active site of enzymes. Redox Rep 2004;9(1):57–61
40. Fonte J,Miklossy J, AtwoodC, Martins R. The severity of cortical Alzheimer's type changes is positively correlated with increased amyloid-β levels: resolubilization of amyloid-β with transition metal ion chelators. J Alzheimers Dis 2001;3(2):209–19
41. Sancheti H, Akopian G, Yin F, Brinton RD,Walsh JP, Cadenas E. Age-dependent modulation of synaptic plasticity and insulin mimetic effect of lipoic acid on a mouse model of Alzheimer's disease. PloS one 2013;8(7):e69830
42. Haugaard N, Levin RM. Activation of choline acetyl transferase by dihydrolipoic acid. Mol Cell Biochem 2002;229(1–2):103–6
43. de Freitas RM. Lipoic acid increases hippocampal choline acetyltransferase and acetylcholinesterase activities and improvement memory in epileptic rats. Neurochem Res 2010;35(1):162–70
44. Hager K, Marahrens A, KenkliesM, Riederer P, Münch G (2001) Alphalipoic acid as a new treatment option for Alzheimer [corrected] type dementia. Arch Gerontol Geriatr 32:275–282
45. Hager K, Kenklies M, McAfoose J et al (2007) Alpha-lipoic acid as a new treatment option for Alzheimer's disease–a 48 months follow-up analysis. J Neural Transm Suppl:189–193
46. Galasko DR, Peskind E, Clark CM, Quinn JF, Ringman JM, Jicha GA, Cotman C, Cottrell B, Montine TJ, Thomas RG, Aisen P, Alzheimer's Disease Cooperative Study (2012) Antioxidants for Alzheimer disease. Arch Neurol 69:836–841
47. Shinto L, Quinn J, Montine T, Dodge HH, Woodward W, Baldauf-Wagner S, Waichunas D, Bumgarner L, Bourdette D, Silbert L, Kaye J (2014) A randomized placebo-controlled pilot trial of omega-3 fatty acids and alpha lipoic acid in Alzheimer's disease. J Alzheimers Dis 38:111–120
48. Bragin V, Chemodanova M, Dzhafarova N et al (2005) In-

tegrated treatment approach improves cognitive function in demented and clinically depressed patients. Am J Alzheimer's Dis Other Dementias 20(21–26):6p
49. Arivazhagan P, Panneerselvam C. Neurochemical changes related to ageing in the rat brain and the effect of DL-alpha-lipoic acid. Exp. Gerontol. 2002;37,1489–1494
50. Arivazhagan, P, Panneerselvam C. Effect of DL-alpha-lipoic acid on neural antioxidants in aged rats. Pharmacol. Res. 2000;42, 219–222
51. Farr SA, Poon HF, Dogrukol-Ak D, Drake J, Banks WA, Eyerman E. The antioxidants alpha-lipoic acid and Nacetylcysteine reverse memory impairment and brain oxidative stress in aged SAMP8 mice. J. Neurochem 2003;84, 1173–1183
52. Liu J, Head E, Gharib AM, Yuan W, Ingersoll RT, Hagen TM. Memory loss in old rats is associated with brain mitochondrial decay and RNA/DNA oxidation: partial reversal by feeding acetyl-L-carnitine and/or R-alpha -lipoic acid. Proc. Natl. Acad. Sci. U.S.A. 2002;99, 2356–2361
53. Dwivedi N, Flora G, Kushwaha P, Flora SJ. Alphalipoic acid protects oxidative stress, changes in cholinergic system and tissue histopathology during co-exposure to arsenic-dichlorvos in rats. Environ. Toxicol. Pharmacol. 2014;37, 7–23
54. Poon HF, Farr SA, Thongboonkerd V, Lynn BC, Banks WA, Morley JE. Proteomic analysis of specific brain proteins in aged SAMP8 mice treated with alpha-lipoic acid: implications for aging and age-related neurodegenerative disorders. Neurochem. Int. 2005;46, 159–168
55. Jesudason EP, Masilamoni JG, Kirubagaran R, Davis GD, Jayakumar R. The protective role of DL-alpha-lipoic acid in biogenic amines catabolism triggered by Abeta amyloid vaccination in mice. Brain Res. Bull. 2005;65,361–367
56. Ahmed HH. Modulatory effects of vitamin E, acetyl-L-carnitine and alpha-lipoic acid on new potential biomarkers for Alzheimer's disease in rat model. Exp. Toxicol. Pathol. 2012;64, 549–556
57. Mahboob A, Farhat SM, Iqbal G, Babar MM, Zaidi NU, Nabavi SM. Alpha-lipoic acid-mediated activation of muscarinic receptors improves hippocampus- and amygdala-dependent memory. Brain Res. Bull. 2016;122, 19–28
58. Ono K, Hirohata M, Yamada M. Alpha-lipoic acid exhibits antiamyloidogenicity for beta-amyloid fibrils in vitro. Biochem. Biophys. Res. Commun. 2006;341, 1046–1052
59. Lovell MA, Xie C, Xiong S, Markesbery WR. Protection against amyloid beta peptide and iron/hydrogen peroxide toxicity by alpha lipoic acid. J. Alzheimers Dis. 2003;5, 229–239

Correspondence:
Leonardo Triggiani
Neurologist. UOC Neurologia ASL Roma 1,
Ospedale Santo Spirito, Rome
E-mail: ltriggiani@gmail.com

Progress in Nutrition 2020; Vol. 22, N. 1: 20-24 DOI: 10.23751/pn.v22i1.7098

Review

The Mediterranean diet could be an exceptional support for patients with chronic renal disease

Francesco Cacciabaudo[1], Roberta Altomare[2], Vincenzo Davide Palumbo[2,3], Giuseppe Damiano[2], Salvatore Fazzotta[2], Leonardo Gulotta[2], Giulia Lo Monte[4], Francesco Maltese[3], Attilio Ignazio Lo Monte[2,3]

[1] "P. Giaccone" University Hospital, School of Clinical Nutrition, University of Palermo, Italy - E-mail: f.cacciabaudo@yahoo.it; [2] "P. Giaccone" University Hospital, School of Medicine, University of Palermo, Italy; [3] Department of Surgical, Oncological and Stomatolgical Disciplines, University of Palermo, Italy; [4] "P. Giaccone" University Hospital, School of Biotechnology, University of Palermo, Italy

Summary. Chronic renal disease (CKD) is a social problem affecting millions of patients characterized by loss of renal function and related to metabolic diseases. The approach from the dietary point of view to this problem could be a perfect strategy to slow down the progression of the disease and at the same time the problems of malnutrition typical of CKD. Several studies show that the Mediterranean diet (MD) may play a key role in the control of the early stages of the disease. Even if the MD showed to be the best diet for the control of metabolic diseases and for the general well-being, in the case of the patient with CKD, should be adapted in what is called the DASH diet, characterized by the typical roles of the MD but with a controlled intake of minerals and proteins.

Key words: Mediterranean diet, chronic renal disease, obesity, cardiovasvascular disease, DASH diet.

Introduction

Chronic Kidney Disease (CKD) is characterized by a physiological alteration of the kidneys, manifested by abnormal albumin excretion or decreased kidney function, quantified by measuring glomerular filtration rate (GFR), that is detectable for more than three months (1).

CKD occurs from many reasons that alter the function and structure of the kidney irreversibly. CKD is one of the biggest health problem in our society; the number of CKD patients rises every year, reflecting the growing elderly population and the increasing number of metabolic diseases (2).

The causes of CKD may be different: diabetes, hypertension and obesity are the main reasons of this disease (3).

The insulin resistance typical of the metabolic syndrome is present in mild kidney disease, and gets worse as kidney function diminishes (4).

For this reason, the first approach for patients affected by CKD is designing an optimal diet because there is an interrelationships between diet and CKD, but it is not easy to find a perfect balance. The diet plan should be balanced in all nutrients, depending on the stage of the individual patient's illness to maintain the requirements for calories (5). This is not the only problem, since the requirement for calories is also influenced by CKD; in fact, CKD results in the development of insulin resistance and eventual impairment of carbohydrates and lipids utilization. This is relevant because a high intake of protein is generally accompanied by an increase in dietary salts (6).

Up to now, many studies in CKD have focused on protein intake, in particular the guidelines have sug-

gested a protein restriction to get a modest benefit.

The Mediterranean Diet (MD) has received attention for decreasing cardiovascular risk and all the metabolic and inflammatory diseases, including the CKD. In particular, it brings improvements in blood pressure (BP), lipid profile, endothelial function, and systemic inflammation (7).

Clinical practice guidelines for adult patients with CKD have recommended dietary protein intake of 0.6-0.8 g/kg body weight per day and energy intake of 30-35 kcal/kg ideal body weight per day. However, protein restriction is only a part, though a very relevant part, of a more complex dietary management of CKD patients. Phosphate intake should be reduced (700-400 mg/day), as well as sodium intake (2-3 g/day). Dietary energy intake must cover energy requirements up to 35 kcal/kg/day for 65 years old patients (8).

Could Chronic kidney disease induce malnutrition?

Malnutrition is defined as an alteration of nutritional status resulting from imbalanced nutrients intake; this phenomenon is very common in patients with CKD. The patients affected by CKD show reduced body weight, due to the loss of fat and muscle mass and the low levels of some plasmatic and visceral proteins (9). Various studies show high incidence of malnutrition in 23-76% of haemodialytic (HD) patients and in 18-50% of peritoneal dialysis (PD) patients. The reason of this malnutrition may be related to factors such as age, diseases and quality of dialysis therapy. In many CKD patients the reason of this phenomenon is not really clear; it may be related to a poor food intake, nausea and vomiting due to uremic toxicity, hormonal derangements, acidosis and increased resting energy expenditure and depression of the patient (10).

In CKD, malnutrition is strongly related to many inflammatory metabolic diseases such as atherosclerosis. For this reason, a rapid atherosclerosis that occurs in advanced CKD has been reported by many authors and this is probably due to different mechanisms such as inflammation, malnutrition, oxidative stress and genetic components.

Early evaluation of pre-dialysis CKD patients for malnutrition, paying more attention to their diet and correcting metabolic disorders like acidosis, may help to mitigate development of cardiovascular disease (CVD) (11).

The role of the Mediterranean diet in several diseases

The discovery of the health benefits of the MD is attributed to the American scientist Ancel Keys who suggested a relationship among lifestyles, nutrition and CVD in different populations (12). From this study comes out, as the populations that follow a diet based on the MD present a very low incidence of metabolic and chronic diseases. The reason of this data are mainly due to the plentiful use of olive oil, bread, pasta, fish, vegetables, herbs, garlic, red onions, and other foods of vegetable origin compared to a rather moderate use of meat (13).

Many studies and clinical trials have shown that the MD reduces the risk of CVD and metabolic syndrome that are related to the CKD. In particular, it has been observed in many trials performed on patients, the decrease of abdominal circumference, an increase in high density lipoprotein (HDL), a decrease in triglycerides, a lowering of blood pressure and a decrease in the concentration of glucose in the blood and all these values are related to the reduction of many chronic diseases (14).

The typical balance of the MD maintains intestinal eubiosys whose imbalance is closely related to systemic inflammatory and metabolic diseases that, as reported, increase the incidence of CKD (15).

The MD provides the high consumption of olive oil that is very rich in monounsaturated fatty acids (MUFA); furthermore, in the traditional MD pattern, the intake of dairy products and meats is lower than in the Western diet and animal fats are mainly from goats and sheep, which provide a higher content in medium-chain fatty acids that are less atherogenic than long-chain fatty acids (16).

Results of the PREDIMED trial pointed out that a MD with relatively high fat intake (35-40%), mostly from Extra virgin olive oil (EVOO), was associated with primary prevention of CVD. Oleic acid is the main MUFA representative component and it is present in EVOO, which is also rich in polyphenols and vitamin E; these compuonds have high anti-inflammatory, antioxidant and vasculoprotective properties. Increased olive oil consumption has been consistently associated with a lower risk of all-cause mortality, car-

diovascular mortality, cardiovascular events and stroke in the general population and in individuals with manifest CVD (17).

From these observations, it is possible to say that a low-fat diet, coupled with the widespread availability of zero- or low-fat foods (e.g., milk, yogurt), is not essentially healthy; in fact, it may induce the people to have higher consumption of refined starch-and sugar-rich foods, thus contributing to excessive energy intake, overweight, obesity, and related complications (i.e., CVD). In support of this concern, there is some evidence that, compared to carbohydrates, both MUFA and polyunsatered fatty acids (PUFA) in the MD eating pattern, tend to reduce low-level density lipoprotein cholesterol (LDL-C) and triglycerides, while increasing HDL-C (18).

Worth of notice is that in the MD the most intake of fats comes from olive oil and fish that are rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and for this reason many evidences support the health benefits of a MD eating pattern against metabolic diseases (19).

Epidemiological data and various clinical trials support a correlation between moderate red wine consumption and lower risk of CVD, but also with lower risk of CKD and end-stage renal disease (ESRD) in individuals with normal kidney function (20, 21).

Mediterranean Diet for the Chronic Kidney Disease

Traditional dietary management of CKD focuses predominantly on the quantity of calories and proteins, and the restriction of single micronutrients, with little mention of dietary quality. Emphasis on restriction of sodium, potassium and phosphorus in CKD may possibly compromise overall diet quality (22). Fruit and vegetable-rich diets such as the MD are recommended for primary and secondary disease prevention. Many evidences in patients with CKD suggests that the MD may be helpful to delay progression and prevent complications, but in the same time the scientific community has reluctance to recommend a normal MD to the CKD patient; in fact, some of the typical components of the MD pyramid could conflict with the traditional dietary restrictions of CKD (23).

The importance of the MD comes from the balance among all the nutrients but even if a lot of evidence recommend this diet for special needs, in CKD, some values in nutrients should be readapted (for example protein intake in the MD aligns with a controlled protein diet for CKD – 0.8 g/kg/day). An interesting aspect is the source of protein, which in the MD comes predominantly from vegetables, fish and white meat. Red meat and processed meats are less often consumed, which may convey a lower amount of dietary sodium, phosphate and potassium. Such habits have been associated with lower risk of CKD and ESRD in individuals with a normal kidney function (22). In some studies have been showed the benefits of plant-based versus animal-based protein in patients with CKD, slowing or even blocking the progression of the disease (24, 25).

The Mediterranean diet for CDK treatment may become DASH

The Dietary Approaches to Stop Hypertension (DASH) provides high intakes of fruit, vegetables, legumes, and nuts, moderate amounts of low-fat dairy products, low amounts of animal proteins and sweets; sodium reduction is part of this diet (26). Although the DASH diet was originally designed for blood pressure (BP) reduction, several characteristics, such as higher intakes of whole grain and lower intakes of red and processed meat, are similar to the MD; this may suggest that the DASH diet may be the adapted version of the MD for CKD patients. The DASH diet also improves low-density lipoprotein cholesterol control, insulin sensitivity and reduces the risk for coronary heart disease, heart failure and stroke, all highly important treatment targets for patients with CKD.

For all these reasons, the DASH diet is strongly considered as a MD modified for electrolyte and protein intake suggesting that it may be safe for these specific patients (27).

BP control is a mainstay of treatment for patients with CKD to prevent both the progression of CKD and its associated CVD-related complications.

The National Kidney Foundation - Kidney Disease Outcome Quality Initiative (KDOQI) guidelines do not recommend the DASH eating plan for individuals with "advanced" CKD (defined as eGFR < 60 ml/min/1.73m^2). KDOQI guidelines suggest that non-dialysis patients with advanced CKD should limit

protein intake to 0.6 – 0.75 g/kg/day. Reasons cited for this recommendation include observational studies that show an associated reduction in the generation of nitrogenous wastes products and inorganic ions with protein restriction (28).

It is well known that a reduced intake of protein is recommended for CKD patients since it diminishes uremic symptoms, improves hyperkalemia, hyperphosphatemia, and calcium or sodium balance control, protects against oxidative stress which may aggravate progression of CKD, and delays the initiation of dialysis (29).

The DASH diet may be safe in any case for preventing Hypertension and in the population at risk for kidney diseases.

In general there are many evidences that the patients following the DASH diet experience a lower prevalence of metabolic complications and may be most likely to experience slower progression of CKD with adequate BP control (30).

On the other hand, there are many instances when the DASH eating pattern may not be appropriate for patients with CKD. This occurs for individuals who have already experienced metabolic complications or are at high risk for their development. That is, if a patient has high-normal or elevated serum potassium and phosphorus values, the DASH eating pattern should not be initiated (28). A larger study of patients with lower kidney function is clearly needed to definitively establish the efficacy and safety of the DASH diet in this population, considering the risk of hyperkalemia (30).

Conclusions

A proper nutritional regimen is important for patients with CKD, and the MD which is rich in fruits, vegetables, fish, cereals, whole grains, fibers and polyunsaturated fatty acids but low in saturated fatty acids, could be beneficial for this category of patients.

Malnutrition often occurs in CDK patients, causing metabolic inflammatory diseases, such as atherosclerosis, and the development of complications that may induce patient early death.

For this reason, choosing accurately the dietary plan is really important, because it could improve the patient's quality of life.

The re-adaptation of the MD in the DASH diet makes this regimen even more appropriate and safe for the treatment of those patients in an initial or intermediate CKD. In fact, the adjustment of nutrients such as proteins, sodium and potassium, make this diet an ideal therapeutic approach for these patients.

Finally, the DASH diet has a positive effect on blood pressure and those markers of mineral metabolism and kidney function. Further studies are necessary to confirm the therapeutic and safe effects to be adopted worldwide as treatment for the patient in CKD.

References

1. Thomas R, Kanso A, Sedor JR. Chronic kidney disease and its complications. Prim Care 2008; 35(2):329-44.
2. Webster AC, Nagler EV, Morton RL, Masson P. Chronic Kidney Disease. Lancet. 2017; 389(10116):1238-52.
3. Spinelli G, Tomasello G, Damiani F, Damiani P, Lo Monte AI. Endoscopic findings in chronic renal failure: review of literature. Acta Med Mediterr 2012; 28(3):261.
4. Liao MT, Sung CC, Hung, KC, Wu CC, Lo L, Lu K C. Insulin Resistance in Patients with Chronic Kidney Disease. J Biomed Biotechnol. 2012: 691369.
5. Nashar K, Egan BME. Relationship between chronic kidney disease and metabolic syndrome. Diabetes. Metab Syndr Obes. 2014; 7: 421–435.
6. Mitch WE, Remuzzi G . Diets for patients with chronic kidney disease, should we reconsider? BMC Nephrology. 2016; 17:80.
7. Khatri M, Moon YP, Scarmeas N, Gu Y, Gardener H, Cheung K, Wright CB, Sacco RL, Nickolas TL, Mitchell SV. The Association between a Mediterranean-Style Diet and Kidney Function in the Northern Manhattan Study Cohort. Clin J Am Soc Nephrol. 2014; 7; 9(11): 1868–1875.
8. Aki K, Yasushi O, Reibin T, Toshiyuki A, Sonoo M, Toyoko O, Atsushi A, Ken S. Association between Low Dietary Protein Intake and Geriatric Nutrition Risk Index in Patients with Chronic Kidney Disease: A Retrospective Single-Center Cohort Study. Nutrients. 2016; 8, 662.
9. Chung S, Koh ES, Shin SJ, Park CW. Malnutrition in patients with chronic kidney disease, Open J Int Med 2012; 2:89-99.
10. Stenvinkel PO, Heimbürgerb O, Lindholm B, Kaysen GA, Bergströmm J. Are there two types of malnutrition in chronic renal failure? Evidence for relationships between malnutrition, inflammation and atherosclerosis (MIA syndrome). Nephrol Dial Transplant. 2000; 15(7):953-60.
11. Adejumo, O, Okaka, E. Malnutrition in pre-dialysis chronic kidney disease patients in a teaching hospital in Southern

Nigeria. Afr Health Sci. 2016; 16(1):234-241.
12. Feinleib M. Seven countries :a multivariate analysis of death and coronary heart disease JAMA. 1981; 245(5):511-512.
13. Menotti, A, Keys, A, Blackburn, H, Kromhout, D, Karvonen, M, Nissinen, A, Pekkanen, J, Punsar S, Fidanza F, Giampaoli S, Seccareccia F, Buzina R, Mohacek I, Nedeljkovic S, Aravanis C, Dontas A, Toshima H, Lanti M. Comparison of multivariate predictive power of major risk factors for coronary heart diseases in different countries: results from eight nations of the seven countries study, 25-year follow-up. J Cardiov. Risk, 1996; 3; 69-75.
14. Altomare R, Cacciabaudo F, Damiano G, Palumbo VD, Gioviale MC, Bellavia M, Tomasello G, Lo Monte AI. The Mediterranean Diet: A History Of Health. Iranian J Publ Health, 2013; 42(5): 449-457.
15. Sinagra E, Tomasello G, Cappello F, Leone A, Cottone M, Bellavia M, Rossi F, Facella T, Damiani P, Zeenny MN, Damiani F, Abruzzo A, Damiano G, Palumbo VD, Cocchi M, Jurjus A, Spinelli G, Lo Monte AI, Raimondo D. Probiotics, prebiotics and symbiotics in inflammatory bowel diseases: state-of-the-art and new insights. J Biol Regul Homeost Agents. 2013; 27(4):919-33.
16. Widmer RJ, Flammer AJ, Lilach OL, Lerman A. The Mediterranean Diet, its Components, and Cardiovascular Disease, Am J Med. 2015;128(3):229-38.
17. Violi F, Loffredo L, Pignatelli P, Angelico F, Bartimoccia S, Nocella C, Cangemi R, Petruccioli A, Monticolo R, Pastori D, Carnevale R. Extra virgin olive oil use is associated with improved post-prandial blood glucose and LDL cholesterol in healthy subjects. Nutr Diabetes. 2015. 5(7): e172.
18. Russo GL. Dietary *n* − 6 and *n* − 3 polyunsaturated fatty acids: From biochemistry to clinical implications in cardiovascular prevention. Biochem Pharmacol 2009;15:937-946.
19. Mekki K, Bouzidi-Bekada N, Kaddousb A, Bouchenaka M. Mediterranean diet improves dyslipidemia and biomarkers in chronic renal failure patients. Food Funct 2010;1:110-115.
20. Ix JH, Sharma K. Mechanisms Linking Obesity, Chronic Kidney Disease, and Fatty Liver Disease: The Roles of Fetuin-A, Adiponectin, and AMPK. J Am Soc Nephrol 2010;21:406-412.
21. Moreno-Luna R, Muñoz-Hernandez R, Miranda ML, Costa AF, Jimenez-Jimenez L, Vallejo-Vaz AJ, Muriana FJ, Villar J, Stiefel P. Olive Oil Polyphenols Decrease Blood Pressure and Improve Endothelial Function in Young Women with Mild Hypertension. Am J Hypertens 2012;25:1299-1304.
22. Bellizzi V, Cupisti A, Locatelli F, Bolasco P, Brunori G, Cancarini G, Caria S, De Nicola L, Di Iorio BR, Di Micco L, Fiaccadori E, Garibotto G, Mandreoli M, Minutolo M, Oldrizzi L, Piccoli GB, Quintaliani G, Santoro D, Torraca S, Battista FV. Low-protein diets for chronic kidney disease patients: the Italian experience. BMC Nephrol. 2016;17(1):77.
23. Huang X, Jiménez-Moleón JJ, Lindholm B, Cederholm T, Ärnlöv J, Risérus U, Sjögren P, Carrero JJ. Mediterranean Diet, Kidney Function, and Mortality in Men with CKD. Clin J Am Soc Nephrol. 2013; 8(9):1548-1555.
24. Chauveau P, Aparicio M, Bellizzi V, Campbell K, Hong X, Johansson L, Kolko A, Molina, P Sezer S, Wanner C, Wee PM, Teta D, Fouque D, Carrero JJ. Mediterranean diet as the diet of choice for patients with chronic kidney disease. Nephrol Dial Transplant. 2017; 1–11.
25. Gorayaa N, Wessona DE. Dietary management of chronic kidney disease: protein restriction and beyond. Curr Opin Nephrol Hypertens. 2012; 21(6):635-40.
26. Fung TT, Hu, FB, Wu K, Chiuve SE, Fuchs CS, Giovannucci E. The Mediterranean and Dietary Approaches to Stop Hypertension (DASH) diets and colorectal cancer. Am J Clin Nutr; 2010; 92(6):1429–35.
27. Tyson CC, Lin PH, Corsino L, Batch BC, Allen J, Sapp S, Barnhart H, Nwankwo C, Burroughs J, Svetkey LP. Short-term effects of the DASH diet in adults with moderate chronic kidney disease: a pilot feeding study. Clin Kidney J. 2016; 9(4): 592–598.
28. Tyson CC, Nwankwo C, Lin PH, Svetkey LP. The Dietary Approaches to Stop Hypertension (DASH) Eating Pattern in Special Populations. Curr Hypertens Rep; 2012.14(5):388-389.
29. Rysz J, Franczyk B, Ciałkowska-Rysz A, Gluba-Brzózka A. The Effect of Diet on the Survival of Patients with Chronic Kidney Disease. Nutrients. 2017; 9(5): 495.
30. Tyson CC, Kuchibhatla M, Patel UD, Pun PH, Chang A, Nwankwo C, Joseph M, Svetkey L, P. Impact of Kidney Function on Effects of the Dietary Approches to Stop Hypertension (Dash) Diet. J Hypertens 2014;3.pii:1000168.

Correspondence:
Francesco Cacciabaudo
"P. Giaccone" University Hospital, School of Clinical Nutrition, University of Palermo, Italy
E-mail: f.cacciabaudo@yahoo.it

Progress in Nutrition 2020; Vol. 22, N. 1: 25-29 DOI: 10.23751/pn.v22i1.8029

Original article

Impact of food behavior on children's health. A case study

Laurentiu Gabriel Talaghir[1], Octavian Barna[2], Cristian Serea[2], Iuliana Petronela Barna[1], Elena Ariela Banu[3]

[1]Faculty of Physical Education and Sport, University Dunarea de Jos Galati Romania; [2]Department of Food Science and Biotechnology, Faculty of Food Science, University Dunarea de Jos, Galati - Email: octavian.barna@yahoo.com; [3]Faculty of Medicine and Pharmacy, University Dunarea de Jos

Summary. The current study evaluated the impact of food behavior on the incidence of gastrointestinal disorders, food allergies, and overweight, on children. For this purpose, a series of questionnaires regarding diet, eating habits, and the incidence of childhood affections, were completed by the parents of 212 children aged between 0 and 14 years, patients in the St. John Children's Hospital of Galati, Romania. Eating patterns were investigated, including the frequency of eating breakfast, fruits and vegetable intake, fast food, and carbonated drinks consumption. The results showed an increase in the incidence of digestive disorders, by 6.13% for boys and by 4.72% for girls, especially for those who eat daily fast food compared to children who consumed fast food only two to three times a week and respectfully, an increase in the incidence of food allergies by 8.96% for boys and 6.60% for girls in case of daily consumption of carbonated drinks compared to children who consumed this type of beverages only once a week or less. In the case of overweight children, the results also displayed a low-frequency consumption of fresh fruits and vegetables, by 5.19% for boys and 4.71% for girls, less than two times a week, compared with the daily consumption of these products.

Key words: children diet, food allergies, food-behavior, fast-food, gastrointestinal disorders, overweight children.

Introduction

Health is one of our most important values, both for us as individuals and also for our society, and is also a very important condition for the sustainable development of a nation (1). With the recent globalization processes, besides various economic and political challenges, we also need to be aware of the challenges for our health.

The globalization that characterized and influenced our society seems to include even the food that we eat (2). Many nutritional factors may influence disease, including changes to the production and availability of food, changing food preferences, increased supply of processed foods for consumption (3).

In this context, some of the most important challenges to our health at this stage are the chronic diseases: heart disease, stroke, cancer, diabetes, and particularly obesity and especially the role of diet in the development of obesity that was poorly understood (4). Among other challenges of globalization, there is an urgent need to identify dietary factors that contribute to obesity among children and young adults so that prevention efforts can be effectively made from an early age (5).

Most components of metabolic syndrome are related separately to lifestyle factors such as weight control, diet, and physical activity (6). It was found that all these chronic diseases are governed by both genetic and lifestyle factors (7).

One of the major negative effects of an unhealthy lifestyle is obesity, the genesis of which begins in childhood. Obesity has reached epidemic proportions in the world (8). Obesity *per se* is an important risk factor for atherosclerotic cardiovascular disease, type 2 diabetes,

dyslipidemia, hypertension, and other chronic diseases (5). A recent study conducted in 79 countries revealed that there were 250 million obese worldwide, including an estimated 22 million children under the age of 5 years, emphasizing the idea that 50% of obese children will become obese adults (9).

According to WHO statistics, the prevalence of obesity has tripled since 1980, and in 2010, there were over 40 million overweight children under the age of 5 worldwide (10). Specialty literature and studies have shown a doubling of the prevalence of childhood obesity in the world over the past 30 years in both industrialized and developing countries (11).

Therefore, the question is: can we prevent obesity somehow (12). It is very important to know what we eat, so we can make the best choices (13). Individual recommendations included the decreasing of sugar and fat intake, increasing the consumption of fruit and vegetables as well as whole grains and cereals and exercising regularly (60 minutes a day for children) (14).

Materials and Methods

Cross-sectional survey

A cross-sectional survey was used in St John Child Hospital in Galati, Romania, to quantify the impact of food behavior on a group of children in Romania. For analysis, a child eating behavior questionnaire has been used and also anthropometric measurements, like the body weight, and height was used to calculate the body mass index. For this specific case study, the graphics from the World Health Organization website were used for calculating percentile and *Z* score.

The study information was provided by the parents on behalf of their children. A total of 212 parents gave their consent, and from the information obtained, 110 were boys and 102 were girls.

From this starting point, three groups of children were created, first one with the age between 0 and 3 years, the second one with the age between 3 and 7 years and the third group, with the age between 7 and 14 years.

Eating patterns of these groups of children were investigated, that included frequency of eating breakfast, fruits and vegetable intake, fast food consumption, and carbonated drinks consumption. More specific, it was assessed the impact of food behavior on the incidence of gastrointestinal disorders, food allergies, and obesity on the 212 children.

Statistical analysis

Statistically significant results were obtained with the CrossTabs in IBM SPSS Statistics 23 software. The threshold of significance we considered is $\alpha = 0.05$. Database associated variables were of a nominal or ordinal type.

Results and Discussion

First of all, it was studied the correspondence between eating habits represented by the frequency of taking breakfast, considered by most specialists the most important meal of the day, and weight calculation for a healthy life represented by *Z* score.

Centralized data can be seen in Table 1.

From the data in Table 1, it can be seen that the children with *Z* score within the normal range have had the habit of having breakfast daily in the proportion of 14.15% boys and 13.21% girls. Since the calculated value of the Pearson chi-square test, χ^2, is 38,168 and the probability associated with this value $p < 0.001 < \alpha = 0.05$, it was accepted that there was a link between *Z* scores and the frequency of breakfast.

Usually, the absence of breakfast is offset by the consumption of fast foods during breaks between school hours, which also lead to excessive weight gain. Similar results have also been observed by Janssen et al., 2005 (15) and Barlow 2007, (16) who appreciated that the global increase in the prevalence of obesity and overweight is due, on the one hand, to an increase in energy intake, especially high-calorie foods rich in fat and sugars, by so-called fast food or junk food, and, on the other hand, the decrease in physical activity due to the increase in sedentary prevalence.

The incidence of digestive disorders depending on fast food consumption was also investigated. The centralization of results can be seen in the chart in Figure 1.

There was an increase in the incidence of digestive disorders especially in children who ate fast food daily by about 6.13% for boys and by 4.72% for girls who eat fast food only two to three times a week. Children,

Table 1. *Z* scores according to the frequency of breakfast.

Z scores	Less than –2		Between –2 and 1		Between 1 and 2		Over 2	
Gender	**Boys (%)**	**Girls (%)**	**Boys (%)**	**Girls (%)**	**Boys (%)**	**Girls (%)**	**Boys (%)**	**Girls (%)**
Daily	0.94	0.94	10.85	10.38	3.30	2.83	0.94	0.47
3–6 days a week	1.42	1.89	5.66	5.66	6.60	6.13	1.89	1.42
0–2 days a week	2.83	3.30	4.25	3.30	9.91	8.96	3.30	2.83

who ate fast food once a week or less, have had digestive disorders more than three times a year only in the proportion of 3.77% boys and 2.83% girls.

Other authors have found as well, the undesirable effect of fast food consumption. It has been underlined by Shau et al. in 2016 (17) that the fast food consumption may contribute to a positive association with the development of functional gastrointestinal disorders. For instance, junk food is unhealthy for the digestive system as it slows down the digestion process making the stomach bloated. In Shau's study, it has been reported that 26.8% had a history of at least one gastrointestinal disorder and 88.1% of the subjects reported fast food consumption. Another inconvenience is that fast food can replace other, more nutritious and healthier products (18).

It was investigated also if there was a link between the incidence of food allergies in children and the consumption of carbonated beverages. The data centralization results can be seen in the graph in Figure 2.

There was an increase in the incidence of food allergies by 8.96% among boys and 6.60% among girls in case of daily consumption of carbonated drinks compared to children who have this type of beverages only once a week or less.

However, increasing the incidence of food allergies to less than three per year was likely to be influenced by other factors such as genetic ones because the same dependence on the frequency of carbonated beverages was no longer observed.

The literature provides reports of carbonated drinks that can cause a change in the child's immune system that can cause allergic reactions (19). Another study showed that carbonated beverages not only favored weight gain but also affected the body's ability to process sugar and thus, the increased risk of developing diabetes (20).

Finally, the link between eating habits in terms of fresh fruit and vegetables and obesity in children was analyzed. The results of the centralized data can be tracked in the graph of Figure 3.

From the chart in Figure 3, it is noticeable that in the case of overweight children (percentile 85–97), the consumption of fresh fruit and vegetables with a frequency of fewer than two times a week is with 5.19% more common for boys and with 4.71% for girls than

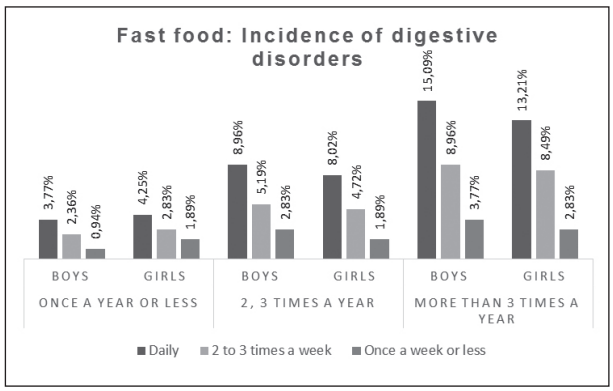


Figure 1. The incidence of digestive disorders and fast food consumption.

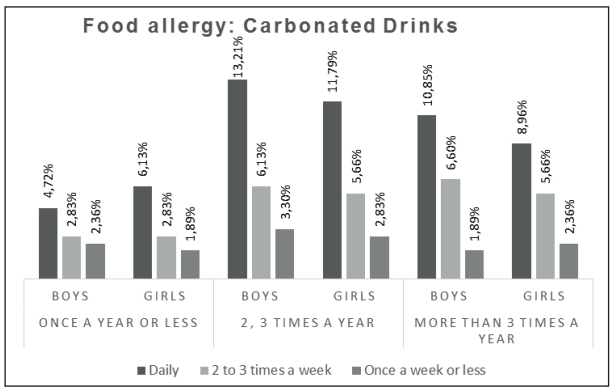


Figure 2. The incidence of food allergy and carbonated beverages consumption.

the daily consumption of these products. It is also observable in the case of obese children (over 97 percentile), that the consumption of fresh fruit and vegetables less than two times a week is 3.01 times more common for boys and 5.02 times for girls than the daily consumption of these products.

Regarding prevention, European Union dietary guidelines recommended that every child should consume vegetables and fruits daily, along with cereals (including bread, rice, pasta, and noodles) preferably whole, lean meats, fish or poultry, milk, yoghurt, and fresh cheese; to choose the water as a drink; to choose low-salt foods, sugar, and additives in the menu, and also very important, to do daily outdoor exercise (21).

Thus, a healthy diet and physical activity reduce the risk of obesity in adult life, also ensuring a harmonious development in the child (22). Increased consumption of fruit and vegetables has been postulated to be associated with a decrease in the prevalence of asthma and rhinitis through their antioxidant properties, which may protect against inflammation (23).

Conclusions

The results obtained showed that it was an increase in the incidence of digestive disorders especially in children who eat daily fast food by about 6.13% for boys and by 4.72% for girls who eat fast food only two to three times a week. It was observed also an increase in the incidence of food allergies by 8.96% in the case of boys and 6.60% in the case of girls in case of daily consumption of carbonated drinks compared to children who consume this type of beverages only two to three times a week. In the case of overweight children (percentile 85–97), the consumption of fresh fruit and vegetables with a frequency less than two times a week was 5.19% more common for boys and 4.71% for girls than the daily consumption of these products.

This data has been confirmed by other researchers in the literature. In terms of prevention and the continuous education, children who will be educated early on to eat breakfast daily, to eat fruits and vegetables, but also to do physical exercise, to treasure their health, they will practice these skills in their adult life and they will educate their own children based on these principles (24).

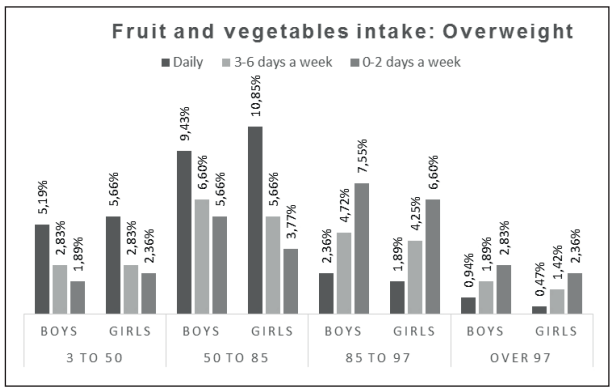


Figure 3. Fruit and vegetable intake and overweight at children.

References

1. Holden E, Linnerud K, Banister D. Sustainable development: our common future revisited. Global Environ Change 2014; (26):130–9; doi:10.1016/j.gloenvcha.2014.04.006.
2. Friedman J. Being in the world: globalization and localization. Theory Cult Soc 1990; 7(2–3):311–28.
3. Popkin BM, Adair LS, Ng SW. Global nutrition transition and the pandemic of obesity in developing countries. Nutr Rev 2012; 70(1):3–21.
4. Hruby A, Hu FB. The epidemiology of obesity: a big picture. Pharm Econ 2015; 33(7):673–89; doi:10.1007/s40273-014-0243-x.
5. Nicklas TA, Baranowski T, Cullen KW, Berenson G. Eating patterns, dietary quality and obesity, Gerald Berenson. J Am Coll Nutr 2001; 20(6):599–608.
6. Yoo S, Nicklas T, Baranowski T, Zakeri IF, Yang SJ, Srinivasan SR, et al. Comparison of dietary intakes associated with metabolic syndrome risk factors in young adults: the Bogalusa Heart Study. Am J Clin Nutr 2004; 80:841–8.
7. Kujala UM. Physical activity, genes, and lifetime predisposition to chronic disease. Eur Rev Aging Phys Act 2011; 8(1):31–6; doi:10.1007/s11556-010-0077-2.
8. World Health Organization (WHO). Obesity: preventing and managing the global epidemic (No. 894). WHO, Geneva, Switzerland, 2000.
9. Wang Y, Lobstein T. Worldwide trends in childhood overweight and obesity. Int J Paediatr Obes 2006; 1:11–25.
10. Nguyen DM, El-Serag HB. The epidemiology of obesity. Gastroenterol Clin 2010; 39(1):1–7.
11. Flynn MA, McNeil DA, Maloff B, Mutasingwa D, Wu M, Ford C, et al. Reducing obesity and related chronic disease risk in children and youth: a synthesis of evidence with 'best practice' recommendations. Obese Rev 2006; Suppl 1:7–66.
12. Friedman JM. A war on obesity, not the obese. Science

2003; 856–8.
13. Worsley A. Nutrition knowledge and food consumption: can nutrition knowledge change food behavior? Asia Pac J Clin Nutr 2002; 11(s3):579-85.
14. World Health Organization. Essential nutrition actions: improving maternal, newborn, infant and young child health and nutrition. Geneva, Switzerland, WHO, 2013.
15. Janssen I, Katzmarzyk PT, Boyce WF, Vereecken C, Mulvihill C, Roberts C, et al. Comparison of overweight and obesity prevalence in school-aged youth from 34 countries and their relationships with physical activity and dietary patterns. Obes Rev 2005; 6:123–32.
16. Barlow SE. The expert committee recommendations regarding the prevention, assessment, and treatment of child and adolescent overweight and obesity: summary report. Paediatrics 2007; 120:164–92.
17. Shau JP, Chen PH, Chan CF, Hsu YC, Wu TC, James FE, et al. Fast foods: are they a risk factor for functional gastrointestinal disorders. Asia Pac J Clin Nutr 216; 25(2):393–401.
18. Harris JL, Bargh JA, Brownell KD. Priming effects of television food advertising on eating behavior. Health Psychol 2009; 28(4):404.
19. Fellows PJ. Food processing technology: principles and practice. ISBN: 9780081019078, UK, Elsevier, 2009.
20. Malik VS, Popkin BM, Bray GA, Després JP, Willett WC, Hu FB. Sugar-sweetened beverages and risk of metabolic syndrome and type 2 diabetes: a meta-analysis. Diabetes Care 2010; 33(11):2477–83.
21. WHO. 2003. Available via http://www.cdc.gov/growthcharts
22. Fussenegger D, Pietrobelli A, Widhalm K. Childhood obesity: political developments in Europe and related perspectives for future action on prevention. Obes Rev 2008; 9(1):76–82.
23. Ronald L, Prior I, Guohua C. Antioxidant Phytochemicals in Fruits and Vegetables: Diet and Health Implications. HortScience 2000; 35(4):588–92.
24. Benton D. Role of parents in the determination of the food preferences of children and the development of obesity. Int J Obes 2004; 28(7):858.

Correspondence:
Octavian Barna
University Dunarea de Jos Galati Romania
E-mail: octavian.barna@yahoo.com

Progress in Nutrition 2020; Vol. 22, N. 1: 30-35 DOI: 10.23751/pn.v22i1.7951

Original article

Strong association between malnutrition, inflammation, and depression in elderly patients. A new novel geriatric complex based on malnutrition; MID complex?

İbrahim Halil Türkbeyler[1], Zeynel Abidin Öztürk[2], Zeynel Abidin Sayiner[3], Mehmet Gol[4], Eyüp Murat Efendioglu[1], Seyma Sahinbeyoglu[1]

[1]Ersin Arslan Education and Research Hospital, Department of Internal Medicine, Division of Geriatric Medicine, 27100 Sahinbey, Gaziantep, Turkey - E-mail: turkbeyler@mynet.com; [2]Gaziantep University, Faculty of Medicine, Department of Internal Medicine, Division of Geriatric Medicine, 27100 Sahinbey, Gaziantep, Turkey; [3]Gaziantep University Faculty of Medicine, Department of Internal Medicine Division of Endocrinology, 27100 Sahinbey, Gaziantep, Turkey; [4]Faculty of Medicine, Department of Physiology, Gaziantep University, 27100, Sahinbey, Gaziantep, Turkey

Summary. *Background/Aim:* The proportion of elderly individuals is increasing worldwide and geriatric syndromes (GS) are associated with a decreased life span. Malnutrition and depression are highly prevalent among the elderly and associated with poor clinical prognosis. This study investigated the frequency of inflammation and depressive symptom comorbidity in the context of a cause-and-effect relationship among elderly patients with malnutrition and/or malnutrition risk. *Material and Methods:* Participants in this cross-sectional study included 217 individuals aged 65 years and over. Comprehensive geriatric assessment was performed to participants. Malnutrition and depression were diagnosed according to the Mini-Nutritional Assessment (MNA-SF) Tool and Yesavage Geriatric Depression Scale (YGDS), respectively. Inflammation status were diagnosed according to the C-reactive protein (CRP) levels. *Results:* According to MNA-SF, 41 (18.9%) patients were malnourished, 82 (37.8%) at a risk of malnutrition, and 94 (43.3%) possessed normal nutrition. Among the malnourished patients, 51.2% experienced CRP elevation and 70.7% displayed symptoms of depression. Patients at risk of malnutrition experienced 39.0% CRP elevation, and 46.3% displayed depression symptoms. There was a statistically significant negative correlation between MNA-SF scores and YGDS and CRP (r: -0,201, p: 0,003, r: -0,495, p: 0,000, respectively). The incidence of inflammation-depression association in malnourished patients was 36.6%, 12.2% in those at risk for malnutrition, and 10.6% in those with normal nutrition. *Conclusion:* Physicians should be informed regarding the association among malnutrition, inflammation, and depression in geriatric patients.

Key words: malnutrition, inflammation, depression

Introduction

In comparison to the general population, the proportion of elderly individuals is increasing worldwide. According to data reported by the Turkey Statistical Institute, the geriatric population constitutes 8.2% of the total population in Turkey (1). In developed countries, the geriatric population has risen to 15%, and it is estimated that 22% of the world's population will be elderly by 2020 (2).

In elderly patients, geriatric syndrome (GS) refers to clinical conditions comprised of atypical symptoms and cannot be fully explained by a specific disease definition. In addition, Kane et al. have proven that GS is associated with a decreased life span (3).

Geriatric malnutrition is a major problem worldwide. Malnutrition is highly prevalent among the elderly and is associated with poor clinical prognosis, decreased functional status, and increased morbidity and mortality (4). Among patients diagnosed with

GS, the prevalence of geriatric malnutrition has been found to be 22.8% (5). Moreover, inflammation plays an important role in the development of GS and is thought to be related to malnutrition (6). Depression is a common health problem among elderly individuals which impacts several functions, leads to public health expenditure, and increases mortality rates (7). According to a recent meta-analysis, the prevalence of major depression ranged from 4.6% to 9.3%, and the prevalence of all depressive symptoms ranged from 4.5% to 37.4% among individuals aged 75 years and older (8). In several other studies, the role of inflammation in the etiopathogenesis of depressive disorders has been supported in different ways. In recent years, findings supporting the elevation of proinflammatory cytokine levels in patients diagnosed with depressive disorders has increased (9).

This study investigated the frequency of inflammation and depressive symptom comorbidity in the context of a cause-and-effect relationship among elderly patients with malnutrition and/or malnutrition risk.

Material and Methods

Subjects

Participants in this cross-sectional study included 217 individuals aged 65 years and over who had been admitted for routine medical care to the outpatient clinic of the Department of Internal Medicine, Division of Geriatric Medicine at Gaziantep University Hospital. All patients were administered comprehensive geriatric assessment tests via a one-on-one interview method. Patient demographics were also recorded during this interview, including age, sex, height, weight, marital status, household size (including spouse, children, and relatives), educational status, exercise habits, comorbidities (diabetes mellitus, cardiovascular diseases, neuropsychiatric diseases, musculoskeletal diseases, and respiratory system diseases) and polypharmacy status (currently using ≥4 drugs). Patients excluded from this study were those under 65 years of age; those with active malignancy or a gastrointestinal pathology directly causing malnutrition; those residing in nursing homes; those with visual or hearing problems potentially complicating the interview; those with schizophrenia, mental retardation, and/or bipolar disorder; and those with a Mini-Mental State Examination (MMSE) score less than 17. All participants provided informed consent, and the procedures followed throughout the course of this study were in accordance with the institutional ethical standards of the responsible committee on human experimentation. The study protocol was approved by the Gaziantep University Local Research Ethics Committee.

Comprehensive Geriatric Assessment Tests

Patients were administered a standardized comprehensive geriatric assessment, and detailed patient histories were recorded using several clinical testing modalities including the 15-question Yesavage Geriatric Depression Scale (YGDS) (10), MMSE (11), Barthel Index of Daily Living Activities (12), Lawton-Brody Instrumental Activities of Daily Living Scale (13), and an abbreviated form of the Mini-Nutritional Assessment (MNA-SF) Tool (14). MMSE assesses five different areas in cognitive functions such as orientation, registration, attention and calculation, recalll and language. Moreover, GDS scores of 5 and over indicate depression. The nutritional status of participants was determined by utilizing the MNA-SF, which is a simple and validated screening tool for nutritional risk. Scores ≤7 indicate malnutrition, those 7-11 indicate malnutrition risk, and those >12 designate normal nutritional status.

Blood Samples

Blood samples were obtained between 8:30 and 10:00 a.m. from the antecubital veins of all subjects, who had fasted for at least eight hours prior. The results of laboratory tests consisting of complete blood count analysis (CBC), erythrocyte sedimentation rates (ESR), and C-reactive protein (CRP) were analyzed via a hospital auto-analyzer. Moreover, CBC analysis was performed via a Beckman Coulter (High Wycombe, UK) Gen-S automated analyzer within 2 hours.

Statistical Analysis

SPSS 17.0 software for Windows was utilized for statistical analysis. All data were entered into a database and verified by a second independent person. The variables were examined using visual (histograms and

probability plots) and analytical methods to determine whether or they were normally distributed. Data are presented as mean ± SD for normally distributed variables and as median (minimum-maximum) ± IQR for skew-distributed continuous variables. Categorical variables are displayed as frequencies. The Pearson's Chi-square method for categorical parameters and the Mann–Whitney U Test for skew-distributed parameters were conducted for univariate analysis. Moreover, correlation analyses were performed via the Spearman's Rank Correlation Analysis for Non-Normal Data, and two-sided values of p< 0.05 were considered as statistically significant. A One-way ANOVA was used to compare normally distributed variables, and the Levene Test was employed to assess the homogeneity of variances. Post-hoc Tukey or Tamhane T2 tests were performed according to the homogeneity of variances.

Results

Patient ages ranged from 65 to 90 years, and the median age was 72.48 ± 5.98 years. A total of 128 patients (59%) were female and 89 (41%) were male. According to MNA-SF, 41 (18.9%) patients were malnourished, 82 (37.8%) at a risk of malnutrition, and 94 (43.3%) possessed normal nutrition. The current study observed that malnutrition and/or malnutrition risk increased in elderly patients who were living alone and unmarried (p> 0.005). In terms of laboratory values, Hb decreased while PLT, CRP, and ESR increased in elderly patients with malnutrition/malnutrition risk compared to elderly patients with normal nutrition (p <0,05). The amount of medication used regularly by patients ranged from 0 to 20 units, and the mean amount was 4.67 ± 2.98. The most common diseases were diabetes mellitus, cardiovascular disease, neuropsychiatric disease, musculoskeletal disease, and respiratory system disease, respectively. Patients' social and demographic information is displayed in Table 1. There was a statistically significant difference between the groups according to the MNA-SF score in terms of YGDS, Bartel ADL, Lawton-Brody IADL, loneliness frequency, marital status, walking speed, hand strength, CRP, ESR, and PLT (p<0005) (Table 1). Among the malnourished patients, 51.2% experienced CRP elevation and 70.7% displayed symptoms of depression. Patients at risk of malnutrition experienced 39.0% CRP elevation, and 46.3% displayed depression symptoms. There was a statistically significant negative correlation between MNA-SF scores and YGDS and CRP (r: -0,201, p: 0,003, r: -0,495, p: 0,000, respectively) (Table 2). The incidence of inflammation-depression association in malnourished patients was 36.6%, 12.2% in those at risk for malnutrition, and 10.6% in those with normal nutrition.

Discussion

Over the past century, the improvement of living conditions, technology, and science as well an increase in the elderly population has continued. With this increase, the frequency of GS in malnutrition-depression patients has increased, as well. This study is the first study that demonstrates the relationship among malnutrition, depression, and inflammation in elderly patients. Besides this is the first usage of the term of MID (Malnutrition, Inflammation, Depression) complex.

Malnutrition among the elderly is a public health problem that is important and often neglected. Moreover, it has been shown that more than one-third of the elderly (37-40%) are unable to meet their daily energy needs from food on a daily basis and that two out of three elderly individuals skip at least one meal a day (16). Studies have demonstrated that deteriorating nutritional health among the elderly decreases their quality of life, increases their hospital visits and lengths of stay, increases their frequency of infection, delays wound healing, disturbs their gait, increases their fall-fracture risk, and increses the likelihood of sudden death (17). The present study observed that the Bartel GYA, Lawton-Brody EGYA, YSGD, and hand - muscle strength values of elderly patients malnourished or at risk of malnutrition were negatively affected (p <0,05) compared to elderly patients with normal nutrition.

The second factor examined in this study, inflammation, plays a protective role in the body. Acute phase response is a major pathophysiologic event that accompanies inflammation and is associated with the increased activity of proinflammatory cytokines. If increased inflammation becomes permanent, a decrease

Table 1. The social and demographic distribution of the patients. Various letters describe statistically significant difference.

	Malnutrition	With Malnutrition Risk	Normal Nutrition	P
N:217	41	82	94	
Age	74.1±7.5	72,8±6,1	71,5±4,9	0,054
Gender				
· Male	31.7%	43.9%	42.6%	0,398
· Femal	68.3%	56.1%	57.4%	
Smoking (+)	%24.4(10)	24,4%(20)	20,2%(19)	0,765
Alcohol (+)	2,4%(1)	9,8%(8)	5,3%(5)	0,232
Exercise (+)	14,6%(6)	30,5%(25)	19,1%(18)	0,080
Living Alone	31,7%(13)[a]	20,7%(17)[b]	12,8%(12)[c]	0,003
Education Status				
· Unaeducated	51,2%(21)	50%(41)	59,6%(56)	0,103
· Elementary	46,3%(19)	34,1%(28)	24,5%(23)	
· High School	2,4%(1)	7,3%(6)	9,6%(9)	
· College	0%	8,5%(7)	6,4%(6)	
Diagnosis				
· Diabetes mellitus	46.3%(19)	39,0%(32)	29,8%(28)	
· CVD	29,3%(12)	39,0%(32)	35,1%(33)	0,187
· SVD	14,6%(6)	9,8%(8)	9,6%(9)	
· Respiratory Diseases	4.9%(2)	3,7%(3)	12,8%(12)	
· Musculoskeletal Diseases	4.9%(2)	8,5%(7)	12,8%(12)	
Marital Status				
· Married	53,7%(22)a	75,6%(62)[b]	76,6%(76)[b]	0,015
Barthel ADL	3,68±7,02/±1,10[a]	2,42±6,19/±0,68[b]	0,87±3,58/±0,37[c]	0,015
Number of daily drugs	5,39±2,75	4,70±3,45	4,32±2,63	0,188
Lawton Brody IADL	9,44±6,20[a]	12,03±5,42[b]	13,11±4,01[b]	0,001
MMSE	25,95±4,09	25,81±4,18	26,00±3,18	0,971
MNA-SF	6.78±1,84[a]	9,46±1,60[b]	12,44±1,77[c]	0,000
YGDS	6.42±3.96[a]	5.87±3.98[a]	4.41±3.39[b]	0,011
CRP	10.7±8.7[a]	7.2±6.6[b]	3.6±3.4[c]	0,001
ESR	42,14±25,80[a]	23,62±17,90[b]	17,79±12,80[c]	0,000
Hemoglobin	11,79±1,54[a]	13,38±1,60[b]	13,66±1,49[b]	0,000
White blood cell	8243±2463	8074±2460	7736±2368	0,466
Platelets	324.550±122.362[a]	262.797±65.383[b]	253.963±69.171[b]	0,000
CRP>5	51.2%(21)[a]	39,0%(32)[b]	14.6%(20)[c]	0,039
YGDS>5	70,7%(29)[a]	46,3%(38)[b]	21,3%(20)[c]	0,012
CRP>5+YGDS>5	36.6%(15)[a]	12.2%(10)[b]	10.6%(10)[b]	0,002

CVD: cardiovascular diseases, SVD: Serebrobascular diseases, ADL: activities of daily living, IADL: Instrumental activities of daily living, MMSE: mini-mental state examination, MNA-SF: mini-nutritional assessment, YGDS: Yesavage Geriatric Depression Scale, ESR: erythrocyte sedimentation rates, CRP: C-reactive protein

Table 2. The correlation between MNA(SF), YGDS, and CRP

	MNA(SF)		YGDS		CRP	
	r	p	r	p	r	p
MNA(SF)	1		-0.201	0.003	- 0.495	0.000
YGDS	-0.201	0.003	1		0.264	0.000
CRP	-0.495	0.000	0.264	0.000	1	

in appetite can lead to negative consequences such as skeletal muscle loss and protein loss in tissues. It is also believed that inflammation plays an important role in the prevalence of aging-related cardiovascular disease and mortality (18-20). In older people, increased IL-6 and CRP have been shown to increase mortality, cause functional regression, and reduce physical activity (21-23). Yukiko et al. have observed that higher levels of plasma tumor necrosis factor alpha (TNF-α) are associated with various parameters related to the nutritional status of the elderly (24). In the present study, the ESR and CRP values of patients with malnutrition and/or malnutrition risk were significantly higher than those with normal nutrition ($p < 0.005$). Moreover, there was a statistically significant negative correlation (r: -0.495, p: 0.000) between MNA-SF scores and CRP in all patients with CRP elevation in more than half of malnourished patients (51.2%).

In addition to inflammation and malnutrition, depression also significantly impacts much of the elderly population. Studies have demonstrated that inflammatory responses play an important role in the pathophysiology of depression. In patients with depression, higher proinflammatory cytokines, acute phase proteins, chemokines, and cellular adhesion molecules are higher (25,26). In a population study conducted among 2,861 individuals, a positive correlation was observed between depressive symptoms and IL-6, TNF-α and CRP (27). Moreover, a relationship between the somatic symptoms of both depression and anxiety and IL-6, TNF-α and CRP levels was observed. Thus, it has been suggested that the somatic symptoms of depression and anxiety may be related to inflammation (27). Psychological stress can also increase inflammatory response via sympathetic and parasympathetic pathways (28). The results of the present study have demonstrated a positive correlation between CRP elevation and YGDS as well as CRP levels in 34% of patients with depressive symptoms. Patients who were either malnourished or at risk for malnutrition also exhibited increased depressive symptom frequency (r: 0,264, p: 0,000). While 14.6% of patients with depressive symptoms also experienced malnutrition and inflammation, 24.3% experienced malnutrition risk and inflammation comorbidity.

Regarding the association among malnutrition, inflammation, and depression, Choi et al.'s recent study among chronic hemodialysis patients described these, along with atherosclerosis, as a MIDA complex proven to be an independent risk factor for cardiovascular disease as well as chronic hemodialysis for all causes of death (29).

Limitations

There are some limitations of the present study that should be considered when interpreting the findings. First, CRP in this study were only measured once, so it was not possible to determine whether an acute and brief episode of infection or chronic inflammation was responsible for the observed correlation. Moreover, as this study was cross-sectional in design, it was not possible to establish a causal relationship among malnutrition, inflammation, and depression. Further studies conducted among a larger number of participants over a longer period of time are warranted to confirm the cause-and-effect relationship among MID complex components.

Conclusion

Depression and inflammation may occur frequently as well as simultaneously in elderly malnutrition patients. Thus, physicians in outpatient clinics should be informed regarding the association among malnutrition, inflammation, and depression in these individuals. Finally, in the presence of one or two MID components, physicians should seek to identify the second or/and third components. But further comprehensive studies are needed to gain general acceptance of MID complex in the clinics.

References

1. TUIK, 2016, TC. Turkish Statistical Institute The Results of Address Based Population Available from: http://www.tuik.gov.tr/ doid=246382016. Number: 21507, 26 January 2017.
2. Situation of Elderly People in Turkey and National Action Plan on Ageing. General Directorate of Social Sectors and Coordination; The Repuclic of Turkey: 2007.
3. Kane RL, Shamliyan T, Talley K, Pacala J. The association between geriatric syndromes and survival. J Am Geriatr Soc 2012; 60(5): 896-904.
4. Harith S, Shahar S, Yusoff M, Kamaruzzaman B, Hua P. The magnitude of malnutrition among hospitalized elderly patients in university Malaya medical centre. Health Environ J. 2010;1:64-72.
5. Kaiser MJ, Bauer JM, Ramsch C, Uter W, Guigoz Y, Cederholm T, et al. Frequency of malnutrition in older adults: a multinational perspective using the mini nutritional assessment. Journal of the American Geriatrics Society. 2010;58(9):1734-8.
6. Shingh T, Newman AB. Inflammatory markers in population studies of aging. Aging Res Rev 2011;10: 319-329.
7. Taylor WD. Clinical practice. depression in the elderly. N Engl J Med 2014;371:1228.
8. Luppa M, Luck T, König HH, Angermeyer MC, Riedel-Heller SG. Natural course of depressive symptoms in late life. An 8-year population based prospective study. Journal of Affective Disorders 2012;142 (1-3):166-71.
9. Krishnadas R, Cavanagh J. Depression: an inflammatory illness? J Neurol Neurosurg Psychiatry 2012; 83:495-502
10. Yesavage JA, Brink TL, Rose TL, Lum O, Huang V, Adey M, Leirer VO. Development and validation of a geriatric depression screening scale: a preliminary report. J Psychiatr Res 1982–1983; 17: 37–49.
11. Folstein MF, Folstein SE, McHugh PR Mini-Mental State: A practical method for grading the cognitive state of patients for clinician. J Psychiatr Res, 1975;12: 189-98.
12. Mahoney FI, Barthel DW. Functional evaluation: The Barthel Index. Md State Med J. 1965;14:61-65.
13. Lawton MP, Brody EM. Assessment of older people: Self-maintaining and instrumental activities of daily living. Gerontologist 1969;9(3):179-86.
14. Kaiser MJ, Bauer JM, Ramsch C, Uter W, Guigoz Y, Cederholm T, et al. Validation of the Mini Nutritional Assessment short-form (MNA-SF): a practical tool for identification of nutritional status. The journal of nutrition, health & aging. 2009;13(9):782-8.
15. Bahat G, Tufan A, Tufan F, Kilic C, Akpinar TS, Kose M, Erten N, Karan MA, Cruz-Jentoft AJ. Cut-off points to identify sarcopenia according to European Working Group on Sarcopenia in Older People (EWGSOP) definition. Clin Nutr. 2016 Dec;35(6):1557-1563. doi: 10.1016/j.clnu.2016.02.002.
16. Morley JE. Anorexia of aging: physiologic and pathologic. Am J Clin Nutr 1997;66:760-73.
17. Agathe Raymond-Simon. Virtual Clinical Nutrition University: Malnutrition in The Elderly, Epidemiology and Consequences. E-SPEN. 2009;4(2):86-89.
18. Newman Dorland W, Anderson D. Dorland's Illustrated Medical Dictionary. Philadelphia, PA: Saunders; 2000.
19. Kushner I. Acute-phase proteins and other systemic responses to inflammation. N Engl J Med. 1999;340:448-54.
20. Suffredini AF, Fantuzzi G, Badolato R, Oppenheim JJ, O Grady NP. New insights into the biology of the acute phase response. J Clin Immunol. 1999;19:203–14.
21. Maggio M, Guralnik JM, Longo DL, Ferrucci L. Interleukin 6 in aging and chronic diseases: a magnificient pathwaty. J Gerontol A Biol Sci Med Sci 2006;61:575-584.
22. Trayhurn P, Wood IS. Signalling role of adipose tissue. Adipokines and inflammation in obesity. Biochem Soc Trans 2005;33:1078-1081.
23. Reuben DB, Cheh AI, Harris TB, Ferruci L, Rowe JW, Tracy RP et al. Peripheral blood markers of inflammation predict mortality and functional decline in high-functioning community-dwelling older persons. J Am Geriatr Soc. 2002 Apr;50(4):638-44.
24. Oe Y, Mochizuki K, Miyauchi R, Misaki Y, Kasezawa N, Tohyama K et al. Plasma TNF-α Is Associated with Inflammation and Nutrition Status in Community-Dwelling Japanese Elderly. J Nutr Sci Vitaminol (Tokyo). 2015;61(3):263-9. doi: 10.3177/jnsv.61.263.
25. Howren MB, Lamkin DM, Suls J. Associations of depression with C-reactive protein, IL-1, and IL-6: a meta-analysis. Psychosom Med 2009; 71:171-186.
26. Dowlati Y, Herrmann N, Swardfager W, Liu H, Sham L, Reim EK et al. A meta-analysis of cytokines in major depression. Biol Psychiatry 2010; 67:446-457.
27. Duivis HE, Vogelzangs N, Kupper N, de Jonge P, Penninx BW. Differential association of somatic and cognitive symptoms of depression and anxiety with inflammation: findings from the Netherlands Study of Depression and Anxiety (NESDA). Psychoneuroendocrinology 2013; 38:1573-1585.
28. Raison CL, Capuron L, Miller AH.Cytokines sing the blues: inflammation and the pathogenesis of depression. Trends Immunol. 2006 Jan;27(1):24-31.
29. Choi MJ, Seo JW, Yoon JW, Lee SK, Kim SJ, Lee YK, et al. The malnutrition-inflammation-depression-arteriosclerosis complex is associated with an increased risk of cardiovascular disease and all-cause death in chronic hemodialysis patients. Nephron Clin Pract. 2012;122(1-2):44-52.

Correspondence:
Asoc. Prof.Ibrahim Halil Turkbeyler,
Dr. Ersin Arslan Education and Research Hospital, Department of Internal Medicine, Division of Geriatric Medicine, 27100 Sahinbey, Gaziantep, Turkey.
E-mail: turkbeyler@mynet.com

Progress in Nutrition 2020; Vol. 22, N. 1: 36-47 DOI: 10.23751/pn.v22i1.7441

Original article

Evaluation of orientation and efficiency of schoolchildren nutrition in recreational period

Leonid Podrigalo[1], Sergii Iermakov[2], Olga Rovnaya[1], Tetiana Yermakova[3], Mirosława Cieślicka[4], Radosław Muszkieta[5]

[1] Department of Human Hygiene and Physiology, Kharkiv State Academy of Physical Culture, Kharkiv, Ukraine; [2]Department of Tourism and Recreation, Gdansk University of Physical Education and Sport, Gdańsk, Poland, E-mail: sportart@gmail.com; [3]Kharkiv State Design and Art Academy, Kharkiv, Ukraine; [4]Department of Human Physiology Nicolaus Copernicus University, Collegium Medicum, Bydgoszcz, Poland; [5]Nicolaus Copernicus University in Torun, Torun, Poland.

Summary. *Background:* determination of antioxidant orientation schoolchildren of nutrition in recreational period and evaluation of its efficiency. *Methods*: it is studied nutrition of 221 schoolchildren aged 7-15 years old at children's recreational institution. It is used the method of analysis of menu production records with the subsequent calculation of average daily set of foods, specifying its chemical composition and energy value. The efficiency of nutrition was estimated according to indicators of vitamin and antioxidant statuses. The dynamics of excretion of vitamins C, B2 and B6 was studied in hour-long portion of urine. The features of urine bio-chemiluminescence in 9 indicators are estimated. Studies were conducted on 2-3 and 22-23 days of recreation. *Result*: it is confirmed the existence of antioxidant direction of nutrition by means of sufficient content of total protein, aromatic amino acids, phospholipids, tocopherols, B vitamins, S, Zn. Content of Se, ascorbic acid in food ration was insufficient. It is determined 16 indicators which are most objectively characterized antioxidant direction of food ration. *Conclusion*: The nutritional factor influenced positively on vitamin and antioxidant status of organism. The determined correlation dependences between indicators of biochemiluminescence and level of vitamins egestion confirmed the efficiency of nutritional factor for influence on schoolchildren's health.

Key words: nutrition, schoolchildren, recreational period, health, indicators

Introduction

Nowadays the exacerbation of social and economic situation is led to adverse health changes of children and youth, increase in probability of formation the pre nosological states (1, 2). The analysis of eating behavior of schoolchildren revealed prevalence of pre nosology of nutritional genesis (3). These states displayed themselves by overweight, deficiency of essential nutrients (vitamins, minerals, dietary fibers), functional digestive disorder. It demands development of the target actions directed to correction of nutrition and nutritional status. Such approach allows increasing the potential of adaptative opportunities of children, providing organism with necessary biologically active agents (4, 5). It is advisable to carry out such programs in period of rest and recreation.

Nutrition is important preventive and remedy of modern medicine. Correctly composed food ration allows preventing development of illnesses, providing optimization of eating behavior (6). The import value is attached to nutrition of preventive direction. Such food ration allows limiting influence of risk factors influenced on the person. It is reached by enrichment of food ra-

tion with natural antioxidants and adaptogens (7). Such food ration is characteristic of Korean ethnic cuisine as one of the healthiest in the world. It is determined that keeping of national cuisine traditions promoted higher antioxidant status. It is emphasized that in the conditions of oxidative stress just healthy nutrition couldn't provide the high antioxidant status. It is also necessary the additional enrichment of food ration (8).

It is necessary to enrich food ration with antioxidants for prophylaxis of health problems. In this case the essential value has the level of such microelements as Se, Cu and Zn (9). Important risk factors of digestive disorder system are oxidative stress and inflammation. Therefore giving of antioxidant direction to food ration by means of enrichment with vitamins and amino acids allows decreasing the level of oxidative stress (10).

It is important to study balance of oxidants – antioxidants for the prognosis for illness development in children's and adult age. Study of oxidative stress is offered for early diagnostics. It allows revealing intermediate states between health and illness (11). In turn, enrichment of food ration with antioxidants is recommended as a factor of protection from oxidative stress and complications caused by it (12). It is distinguished vitamin C, vitamin E, Se and vitamin A among significant antioxidant factors of nutrition. Such elements give corresponding direction to food ration. This is achieved by prophylaxis of many illnesses (13).

There are quite certain connections between level of carotenoids consumption and children body structure in 2-18 year old. It is confirmed that decrease of carotenes level in food ration is risk factor of chronic illnesses development and also causes overweight (14). The negative correlation between antioxidant activity and markers of overweight is also determined. It proves that the level of oxidative stress is closely connected with overweight development (15). In other research groups with excess weight / overweight had much lower average doses of critical microelements necessary for body growth in comparison with normal children (16). Authors specify that low average consumption of critical microelements among all age groups from 2 up to 18 year old indicates the need of adequate interferences for solution the problem of food insecurity and undernutrition. Other authors determined that undernutrition among children is the main problem of public health care in developing countries (17). These problems are especially characteristic of schoolchildren living in regions with intense ecological situation (18).

Mode of life and behavior in social environment are distinguished among many factors of overweight development in youth. The mode of life of children with excess weight / overweight and teenagers influences on efficiency of treatment (19). Authors determined that family multidisciplinary approach is effective in the short term in health condition improvement, eating habit and physical indicators in children and teenagers. In other researches was revealed that decrease of general physical activity and increase in consumed food is closely connected with mode of life (20, 21). It is necessary to recommend to teenagers to practice sport actively to avoid consequences of abdominal overweight (22, 23). At the same time the physical activity of schoolchildren has to have pedagogical control (24). It is also necessary to use various approaches to assessment of risks of excess weight and overweight (25).

Saturation of food ration with antioxidants allows decreasing negative impact of many adverse factors: influence of toxic metals, ionizing radiation, stress, digestive disorder. Researches (26) confirmed the increased tension of antioxidant protective systems in conditions of influence of toxic metals, in particular, of Cd. Authors developed and proved the diet of protected direction which is characterized by the high level of antioxidants. Its application has allowed decreasing toxic influence of Cd on organism. In other research (27) the oxidative and antioxidative statuses were analyzed in children with various levels of protein and energy insufficiency. Undernutrition led to decrease in level of antioxidant enzymes and increase of concentration of lipids' peroxidation. Special methodical recommendations were developed for improvement the children from Chernobyl disaster zone (1986, Ukraine) (28). They are based on the principle of increase in adaptation potential of organism. It is reached by use of the developed complex of improving actions. The nutrition with antioxidant direction is a part of this complex.

The purpose of the research was determination of antioxidant direction of nutrition of schoolchildren in the recreational period and assessment of its efficiency.

Materials and Methods

Participants

It was studied nutrition features of 221 schoolchildren in 7-15 year old in children's recreational institution.

Study design

The design of research presupposes study of nutrition features of schoolchildren in recreational period and assessment of dynamics of indicators characterizing vitamin and antioxidant status as criteria of efficiency of food ration.

The analysis of nutrition was carried out by means of the calculation method providing study of the menu production records during the recreation period (not less than 7-10 days). The analysis of daily consumed food allows calculating their daily average set (7). The chemical composition and energy value of food ration is calculated on the basis of this set and with the help of chemical composition tables of food (29, 30). Recommendations of food set for children from Chernobyl disaster zone for summer recreational period was used for comparison (28).

The efficiency of nutrition was estimated on indicators of vitamin and antioxidant status in 2-3 and 22-23 days of recreation. Vitamin saturation was estimated by ascorbic acid, riboflavin and pyridoxine egestion in hour portion of urine. Level of ascorbic acid was determined by titration with Tillman's indicator. Level of B vitamins was determined by fluorometric method (31). Ascorbic acid level lower than 0,4 mg hour – was considered low; 0,4-0,69 mg hour – was considered average; 0,7 mg hour and more – was considered normal. Egestion level of riboflavin not less than 10 mkg hour is taken for norm. Egestion level of pyridoxine not less than 40 mkg hour is taken for norm. The quantity of studied participants was: for vitamin C – 42 persons; for B vitamins – 15 children in 10-14 year old.

The antioxidant status was estimated according 9 parameters of biochemiluminescence of urine on HLM1C–01 device. The spontaneous luminescence (SL) (impulse/second) was defined in urinalysis samples of 2 ml. Stimulation of luminescence were carried out with the help of fresh reagent 6% of hydrogen peroxide. It was determined 8 parameters of stimulated luminescence: intensity (IL), (impulse/second), initial amplitude of "flash" (IA) and final amplitude (FA) of research (relative units), indicator of activation (IA = IL – SL), (impulse/second). It was defined average intensities of luminescence of the first (I1) and the last (I2) 10 seconds of research (impulse). It was estimated the correlation of these values (CI) and also relative value of slope ratio of chemiluminogram (TK). The research involved 39 children at the age of 10-14 year.

Statistical analysis

The statistical analysis of results is carried out with use of the licensed tables of Excel. Indicators of descriptive statistics were defined: arithmetic means, standard deviations and mean values. Considering sample value, the reliability of differences in groups was estimated by means of parametrical Student's t-test. In making an assessment of levels of vitamins egestion was used nonparametric sign test. It was determined correlation by Pearson between indicators of antioxidant and vitamin statuses. Study of dependence of B vitamins level in urine and indicators of biochemiluminescence included: determination of correlation coefficient; assessment of F-test. It was estimated the reliability of the revealed dependence on the basis of F-test. Use of F-test allows to level to some extent the distortion appeared at the small sample value.

Ethical consideration

The study protocol was approved by the Ethical Committee of Academy of Physical Culture. In addition, children and their parents or legal guardians were fully informed about all the features of the study, and a signed informed-consent document was obtained from all the parents. A written informed consent was obtained from all participants prior to data collection. Schoolchildren were approached during mandatory course class and invited to participate in the study. In addition, children and their parents or legal guardians were fully informed about all the features of the study, and a signed informed-consent document was obtained from all the parents. They had a right to withdraw from the study at any time. The study was conducted in accordance with the Declaration of Helsinki.

Results

Data on average daily consumption of the main food products are provided in table 1. Taking into consideration need of realization of set within 7-10 days the deviation of recommended within 10% was considered reliable. The reliability of differences of main food products consumption was estimated by Student's t-test.

It is determined that in June levels of milk and dairy products consumption, meat and meat products consumption, juice and fats consumption were within reliable deviations. The content in food ration of grain and noodle products, bread and bakery, sugar and confectionery was reliably overestimated. Total consumption of vegetables and fruit was underestimated.

The consumption of milk and dairy products, vegetables and fruit, fats generally corresponded to recommendations in July. Specific weight of meat and meat products in nutrition increased. This indicator reliably exceeded the recommended level. The consumption of grain, noodle and bakery products, sugar and confectionery decreased. This indicator exceeded recommended level. Content of juice in food ration decreased. There were absence of fish, fish products and sea products in children nutrition. Also there was no additional vitaminization.

Data of chemical composition and energy value of nutrition also confirm ambiguity of effect of alimentary factor (tab. 2). According one indicators (content of total protein, total of aromatic and sulfur-containing amino acids, tocopherols etc.) food ration coincides with recommendations. The energy value, content of sugars, Na, chlorides, B vitamins exceed level of recommendations. According to the content of proteins of animal origin, Se, ascorbic acid, I food ration is significantly in arrearages of recommendations.

16 most characteristic indicators were chosen for specification of antioxidant direction of food ration. Data are submitted in the figure 1. Recommended consumption of nutrients according to the developed food ration is represented in the form of circle (28). Their values in percentage corresponding to content in nutrition in recreation period are represented in the form of the dispersing beams.

In our opinion, the graphic representation reflects existence of essential antioxidant direction of food ration. The majority of indicators don't significantly differ from the recommended levels. However there are significant deviations as upwards (protein, B vitamins), and downwards (Se, ascorbic acid).

The food ration was characterized by sufficient content of proteins (in June and July in 3,9% and 9,9% higher than recommended). Specific weight of proteins of animal origin decreased (in June in 20,9%, in July – in 9,6%).

Assessment of amino acid content was carried out using amino-acid score method. It allows to consider amount of amino acids of nutrition and to compare it to ideal protein. It is the most optimum way of assessment of protein full value of nutrition. The obtained data confirm the sufficient and optimum balanced content of amino acids in food ration. Thus, part of lysine is: in June – 5,4%; in July – 7,5% at recom-

Table 1. The average daily consumption of the main food products by schoolchildren in recreation period

Food	*Content in food ration, g*		
	recommended	*June*	*July*
Cereals and noodle products	85	128,07±7,7[1]	119,4±11,3[1]
Bread and bakery	260	423,8±13,2[1]	415,0±9,3[1]
Vegetables and fruit	880	537,2±0,9[1]	897,0±83,8
Butter and fats	50	54,9±2,3	48,8±2,0
Milk and dairy products	510	505,3±24,3	444,4±28,2
Vegetable and fruit juices	250	197,5±14,7	182,5±10,6[1]
Meat and meat products	215	191,8±11,4	282,5±25,5[1]
Sugar and confectionery	70	102,5±6,6[1]	96,9±9,5[1]

[1] – *Differences of recommended consumption are reliable (p<0,05).*

Table 2. The chemical composition and energy value of food ration of schoolchildren in period of recreation

Nutrients	*Content in food ration*		
	June	*July*	*recommended*
Proteins, g	117,6	124,4	113,2
among them animal proteins, g	56,6[1]	64,7	71,6
Lipids, g	113,5[1]	113,9[1]	101,3
among them vegetable fats, g	28,2	24,5[1]	27,8
Carbohydrates, g	491,9[1]	494,7[1]	341,1
of them sugar, g	139,0[1]	141,3[1]	121,7
Amylum, g	352,9[1]	353,4[1]	219,4
Amino acid, g			
Lysin	6,318[1]	7,096	7,56
Tryptophan	1,331	1,503[1]	1,39
Methionine + cystine	3,920	4,384	4,00
Phenylalanine + tyrosine	8,870	10,130	9,53
Histidine	2,694[1]	3,167	3,19
Phospholipids, g	5,44	6,75[1]	5,83
Polyunsaturated fatty acid (PUFAs), g	16,53	13,11[1]	18,34
Saturated fatty acid (SAFA), g	48,18	47,07	44,17
Dietary fiber, g	24,19[1]	27,77[1]	32,86
among them pectin, g	1,95[1]	3,19[1]	4,25
Minerals, mg			
Na	3616,5[1]	36,47,1[1]	2575,1
K	4363,28	5608,7	5534,6
Ca	975,9[1]	899,9[1]	1210,5
Mg	485,9[1]	541,8	547,2
P	1848,7[1]	1983,5	2067,2
Fe	29,92	29,06	30,58
S	815,0	1090,7[1]	903,4
Chlorides	4484,5[1]	5418,9[1]	3383,4
I, mcg	40,5[1]	91,11[1]	136,58
Se, mcg	17,88[1]	15,23[1]	29,55
Zn, mcg	13156,5[1]	15825,0[1]	15197,3
Vitamins, mg			
Carotenes	2,37[1]	3,54[1]	5,19
Retinol	0,49[1]	1,42[1]	4,24
Thiamine	1,97	2,08	1,82
Riboflavin	1,95[1]	2,26[1]	3,12
Niacin	22,47	29,53[1]	21,42
Ascorbic acid	62,31[1]	108,57[1]	186,80
Pyridoxine	3,22	4,17[1]	3,46
Cyancobalamin, mcg	4,63[1]	12,70[1]	32,25
Tocopherol	22,02	20,46	22,23
Folate, mcg	317,31[1]	291,48[1]	379,01
Choline	499,78[1]	586,22[1]	672,4
Energy value, kcal	3459,5[1]	3501,5[1]	2729,6

[1] – *difference of recommended consumption more than 10%.*

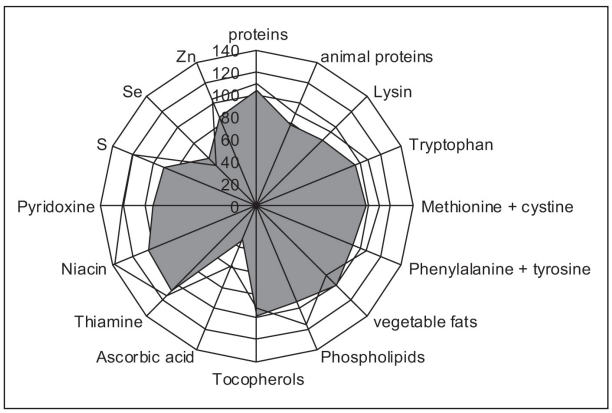


Figure 1. Assessment of antioxidant direction of food ration of schoolchildren in period of recreation.

mended level 5,5%. The part of tryptophan is 1,1-1,2% (recommended level – 1%). The part of phenylalanine and tyrosine is 7,5-8,1% (recommended level – 6%). The part of methionine and cystine is 3,3-3,5% (recommended level – 3,5%).

The lipid component of nutrition exceeded the recommended level in 10-12%. Specific weight of vegetable fats in June increased, and in July –decreased in comparison with recommended level. The food ration was adequately provided with phospholipids. Their contents in June wasn't differ, and in July exceeded the recommended level in 15,8%. The balance of proteins, lipids, carbohydrates on mass was: in June – 1:0,97:4,18 g; in July – 1:0,92:3,98 g (recommended level was 1:0,89:3,01 g).

Maintenance of balance of saturated fats (SAFA) and unsaturated fats (PUFAs), lipids and tocopherols is relevant for assessment of antioxidant protection of nutrition. The studied food rations were characterized by balance of SAFA/PUFAs – 0,34-0,28, (recommended level was 0,42). The content of PUFAs was below the recommended level (in June – 4,3% and in July – 3,4% of daily food energy, recommended level was 6%). The quantity of SAFA in nutrition exceeded the recommended level. The quantity of vitamin E to the level of PUFAs' consumption was rather high: 1,33-1,53 (recommended level was 1,21).

The content of tocopherol generally corresponded to recommend. Content of thiamine, niacin and pyridoxine deviated in June, and even exceeded in July. The level of ascorbic acid consumption was above the physiological standard for children of this age, but below the recommended for this category.

The disproportion of food ration caused the insufficient content of vitamin A and its precursors – carotenes. The retinol equivalent was 17,4% (June) and 39,3% (July) of the recommended quantity.

The mineral component of food ration couldn't be estimated definitely. On the one hand, the content of S, Zn, Fe, consumption of K, Mg and P was rather high (in July it generally corresponded to recommended level). The balance of Ca, P and Mg was 1:1,89:0,5 (June) and 1:2, 2:0,6 (July), the recommended level was 1:1,71:0,45. On the other hand, the absence of seafood in nutrition (as main source of I) caused its insufficient level during the observation period. Deviations from recommended daily set caused decrease of Ca level in 19-24%. The amount of Se didn't exceed a quarter of recommended level.

Results of studied status of ascorbic acid of its egestion with urine in dynamics of recreation are presented in fig. 2, 3. The third part of participants had the low level of egestion of this vitamin. Besides, all children were characterized by unsatisfactory indicators of egestion of riboflavin and pyridoxine. They was, respectively, (4,25±0,51) and (5,06±0,48) mkg/hour. It is confirmed the vitamins deficiency states among schoolchildren. Moderate level of vitamin C egestion was found in 45, 2% of examined children, normal level – in 21,7%.

Use of antioxidant nutrition influenced positively on vitamin status of children. The part of children with a low ascorbic acid egestion decreased almost by 3 times (11,9%). The number of children with

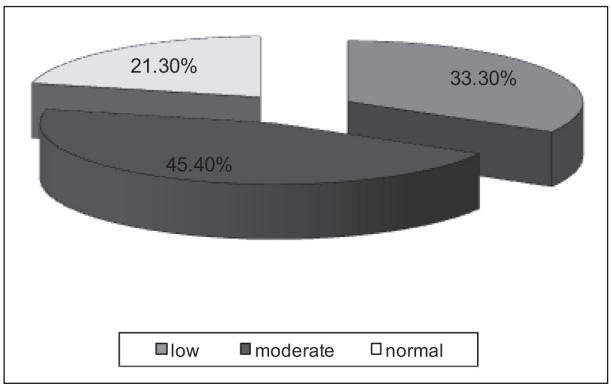


Figure 2. Level of ascorbic acid egestion at the beginning of recreation period

Table 3. Dynamics of indicators of antioxidant status of children during the recreation period

Indicator	At the beginning	At the end	Expression (%)
Spontaneous luminescence, impulse/second	9,84±0,98	4,96±0,89[2]	64,1
Stimulated luminescence, impulse/second	411,88±59,53	280,73±35,47	30,8
Index of activation, impulse/second	402,63±59,25	271,93±36,17	30,8
Initial amplitude, relative units	92,00±10,76	54,90±9,40[1]	25,6
Final amplitude, relative units	45,75±4,17	34,06±2,41[1]	41,0
Slope of chemilumigramm	0,48±0,09	0,22±0,08[1]	33,3
Initial intensity, impulse	818,50±80,17	550,18±68,29[1]	28,2
Final intensity, impulse	455,07±37,62	356,42±23,55[1]	43,6
Intensity correlation	2,00±0,27	1,35±0,18[1]	28,2

[1] – differences are reliable (p<0,05), [2] – differences are reliable (p<0,001).

normal egestion of this vitamin increased up to 26,2%. To the end of recreation period the main part was children with moderate level of this vitamin egestion.

The similar increase in levels of egestion is revealed for B vitamins. The content of riboflavin and pyridoxine in hour portion of urine increased, respectively in 1,3 and 1,7 times. At the end of the recreational period the average level of pyridoxine egestion was (9,00±0,95) mkg/hour. This indicator was reliably higher (p<0,01), than at the beginning. The egestion of riboflavin was (5,56±0,49) mkg/hour. The comparison of data revealed tendency to reliability of differences (t=1,85, p<0,1). Use of non-parametric test of signs allowed to confirm the importance of differences in both cases: z=1 for pyridoxine; z=3 for riboflavin, (p<0,05).

The assessment of dynamics of antioxidant status was carried out by individual comparison of the studied indicators at the beginning and the end of recreation period: the part of children with expressed effect was defined. In addition, the frequency of effect expression by quantity of the changed indicators was estimated.

The received results confirmed favorable dynamics of the majority of parameters (tab. 3). It was expressed in decrease of super weak luminescence of urine in 25,6-64,1% of children.

Study of frequency of expression changes demonstrates the favorable dynamics: one indicator – in 41,1% of children; 2-3 indicators – in 23%; 4-5 – in 17,9%; 7-9 – in 18%. Comparison of indicators of BCL and hour egestion of vitamin C allowed to reveal existence of the inverse correlation dependence (p<0,05) with 4 indicators: TK (g=-0,36), CI (g=-0,31), I1 (g=-0,35), IA (g=-0,35).

It is revealed the existence of the inverse correlation dependence between pyridoxine egestion level with urine and indicators of BCL (g<0,3). However results weren't reliable (p>0,05) that could be connected with small value of sample. Feedback of weak and average power is revealed between the riboflavin level and indicators of IL (g=-0,52), IA (g=-0, 515), IA (g=-0,517) и I1 (g=-0,513). Comparison of riboflavin and pyridoxine egestion allowed to reveal direct correlation of average power between them (g=-0,533).

At the end of recreational period the interrelation of riboflavin egestion and indicators of BCL was characterized by weak inverse correlation, and with IL and IA even of average power. But the small number of observations didn't allow considering it reliable. Assessment of interrelation of BCL and pyridoxine egestion levels revealed reliable strong inverse correlation

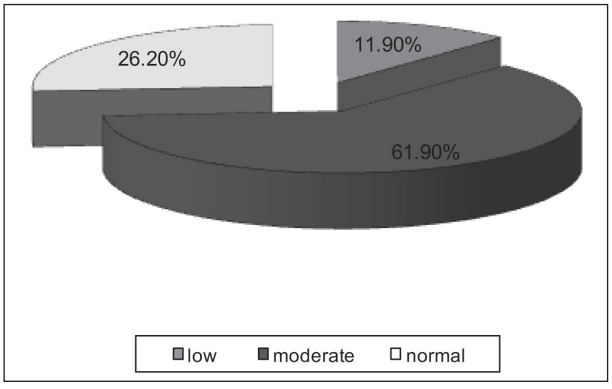


Figure 3. Level of ascorbic acid egestion at the end of recreation period

dependence in IL indicators (t =-0,713, p<0,05) and IA (=-0,711, p<0,05). The inter vitamin dependence at the end of research was also characterized by strong direct correlation (g=0.799, p<0,01).

Discussion

The used study of research is standard in hygiene of food and nutritional biology. Assessment of "food – is health of the person" system is based on the study of nutrition features and analysis of its efficiency of influence on health.

Nowadays the large number of techniques is applied to estimate the food ration. Some authors (12) recommend using the questionnaire of consumption frequency of certain food products for assessment of nutrition features. Other authors recommend using 24-hour dietary recalls method (32). Results of researches confirmed its efficiency in the analysis of nutrition features in middle school age children. Other authors (33) by means of 24-hour dietary recalls method studied communications between consumption of fruit and vegetables and vitamins level in blood serum of European teenagers. It is defined that normal consumption of fruit and vegetables is characterized by reliable higher levels of B_6 vitamins, folic acid, C, E and -carotene. The method of nutrition analysis was used in our research according to the menu production records. It is most convenient for application at the collective level. This technique allows to level fluctuations in consumption of the main food products by means of nutrition assessment during rather long period (not less than 7-10 days). Besides, it is defined the deviations in consumption of food products allow to predict deviations of the main nutrients from standards and influence of food on organism.

Our results (tab. 1) allow determining some disproportions in consumption of food. It is revealed the expressed increase in consumption of grain products (grain, noodle products and bread). It allows assuming the increased consumption of starchy complex carbohydrates, B vitamins. The lack in food ration of fresh vegetables and fruit causes deficiency of dietary fiber and water-soluble vitamins, especially ascorbic acid. This deficiency in a certain measure is leveled by sufficient consumption of vegetable and fruit juices. At the same time the sufficient consumption of dairy, meat products allows to predict optimum level in food ration of essential amino acids, Ca, P, Mg, S and other minerals. Excess of recommendations in sugar and confectionery could lead to increase in consumption of monosaccharide.

The similar methodological research is conducted by other authors (34). They defined adequacy of consumption of Fe and vitamin C in children's food rations. It was studied levels of food consumption and their chemical composition. It is indicated the need of group and individual consumption levels with direction to average physiological norms of consumption. In other research (32) was defined that diets of children contained enough polyphenols in relation to the recommended values. The consumption of carotene usually exceeded the recommended retinol equivalent. Conversely, vitamin C didn't meet the requirements. The conclusion is drawn about need of increase in consumption of fresh vegetables and fruit.

Definition of average daily set of food allows calculating average chemical composition and energy value of food ration according to special reference tables. It gives the grounds for the decision of nutrition direction. The carried-out analysis confirmed competency of the made prognosis.

Thus, the level of consumption of essential amino acids is associated with processes of growth and children development. The defined balance of tryptophan and total of aromatic amino acids in the studied food rations coincides with found in nutrition of long-living persons from Abkhazia (35). Researches of authors confirmed direct dependence of this value and lifetime by means of increase in capacity of protective systems of organism.

Decrease of PUFAs' content caused some reduction of pro-oxidant features of food ration. It also has to be estimated as increase of antioxidant direction of nutrition. At the same time, the insufficient consumption of ascorbic acid has to be estimated as illustration of decrease of antioxidant potential of nutrition. Its value is confirmed by several researches (36). Authors emphasize the importance of ascorbic acid (as water-soluble antioxidant), necessary for ensuring many functions of organism. The food ration with

high level of this vitamin by means of vegetables and fruit is prophylaxis factor of many illnesses. It is connected with decrease in level of oxidative stress. Some authors (37) determined that optimum level of vitamin C consumption is 200 mg/day. It allows to maximize potential advantage of this vitamin for health and to minimize risk of adverse effects for health.

The general antioxidant protection of organism is formed by means of complex of factors. It requires complex assessment of food ration direction; this was used by us (fig. 1). In our opinion, closeness of square of figures (formed by raying) and circle confirms sufficient value of antioxidant direction of nutrition. The similar technique of assessment of direction of nutrition was developed and patented with our help (38). It was based on the planimetric analysis of square of the specified figures.

The conclusion about complex assessment of antioxidant direction of nutrition is confirmed by several researches. Authors (39) emphasize that general antioxidant activity of nutrition and blood serum are considered adequate tools of study of influence of antioxidants on health. The questionnaire of frequency of consumption of certain products was used for assessment of direction of nutrition. It was applied the correlations of indicators of oxidative stress to antioxidant activity and to the level of antioxidants in food ration As criteria of efficiency.

The competency of application of nutrients correlations for assessment of nutrition direction is confirmed by other researches (40). Authors give a row of the indicators and indexes used in the analysis of nutrition features. At the population level it is proved that food ration with the high content of antioxidants is associated with low risk of development of illnesses.

Similar correlations were applied in our research. Our results confirmed the sufficient level of antioxidant protection of food. The optimum quantity of tocopherols, their high correlation with the PUFAs level, and the optimum correlation of PUFAs/SAFA testifies to the expressed antioxidative direction of food ration.

It is repeatedly proved the efficiency of nutrition enriched with antioxidants for neutralization of action of unfavorable ecological factors. Experiments on animals (41) were confirmed the efficiency of the diet enriched with antioxidants. It allowed decreasing effect of radiation. Tocopherols and native protein promoted decreasing of damage diaphragms and DNA by X-rays.

In other research (42) is noted that addition of antioxidants within 4 months improved antioxidant and oxidative balance and moderately improved functions of liver. It is determined reliable correlations between oxidative stress and level of inflammatory conditions of liver.

It is confirmed the sufficient antioxidant direction of nutrition of schoolchildren in recreational period by means of the content of certain nutrients and their optimum ratios. Use of this food ration allows assuming its preventive effect, positive influence on the vitamin and antioxidant status of children.

Definition of vitamin egestion with urine is considered one of the most adequate tests of assessment of nutrition efficiency (31). The preventive character of nutrition caused positive changes of vitamin status of schoolchildren. The majority of schoolchildren were characterized by moderate and normal level of ascorbic acid egestion, significant increase of B vitamins egestion. On the other hand, at the end of the recreation period were found out children with low egestion of vitamin C. The normal level of B vitamins egestion wasn't reached. It gives grounds for recommendations to increase in vitamin component of food ration by means of additional intake of multivitamin preparations.

The preventive character of nutrition assumes limitation of risk factors and neutralization of damaging mechanisms. And the most optimal of them is the intensification of free radical oxidation (43). That's why pathogenetically substantiated method of research was use biochemiluminescence for assessment of antioxidant status.

Other researches (44) confirmed efficiency of this method for assessment of free radical oxidation. Authors proved its availability and high informational content to the analysis of adaptative potential. Other authors (45) used blood chemiluminescence assessment method for the analysis of antioxidant potential of organism. The increase in consumption of strawberry decreased chemiluminescence rest level in blood and urine increased the antioxidant potential of organism. Indicators of chemiluminescence correlated with

the level of leucocytes. Decrease of chemiluminescence at rest is estimated as result of depression of oxidizers' formation and decrease of risk of systemic imbalance between oxidizers and antioxidants. Such dynamics is determined in our research. Our results testify (tab. 3), that decrease of indicator of spontaneous luminescence is the most essential. Improvement of this indicator most often met in the examined collective, and also had the most expressed dynamics. Decrease of indicators of TK and CI was observed in 33,3% and 28,2% of schoolchildren. These criteria reflect change of character of chemiluminogram and serve as illustration of improvement of balance of work of protective anti-oxidative systems of organism.

Similar processes are confirmed by dynamics of indicators of amplitudes and intensity of luminescence. Excess of initial indicators over final observed up to the end of recreation, however its expression decreases. It can be interpreted as tendency to improvement of antioxidant status.

In 30,8% of examined schoolchildren had decrease of indicators of intensity of the initiated luminescence and indicator of activation. However in this case was revealed the tendency to reliability ($p<0,1$).

Determination of correlation dependence revealed existence of reliable inverse average and strong connection between indicators of vitamin status and BCL. It confirms a potential possibility of influence on intensity of free radical oxidation in organism by means of a nutritional factor. The most interesting dependence is between TK and CI. The found interrelation confirms a possibility of influence on the anti-oxidative status by means of modification level of vitamin C in organism.

Assessment of interrelation of BCL and levels of pyridoxine egestion at the end of recreation period reflects decrease of potential ability of biological substrates to oxidation, restoration of functionality of anti-oxidative systems. It is caused by normalization of the vitamin status.

The most expressed correlation communication at the end of recreation is found for pyridoxine. Its level in urine at the end of recreational period was authentically increased. It proves that normalization of vitamin status, increase of indicators of egestion will contribute to normalization of intensity of free radical oxidation in organism of children and teenagers.

The competency of the drawn conclusions is confirmed by the available results. Other researches (12) confirmed existence of positive association of antioxidant direction of nutrition with consumption of dietary fiber, folic acid, Mg and A, C and E vitamins. The body weight index, indicator of standard deviation of body weight index and the general body fat were connected with antioxidant direction of food ration only at patients with overweight. These data show that the antioxidant activity of nutrition could be potential indicator of risk of development of the signs connected with overweight and can be considered as a useful method at assessment of consumption of antioxidants.

Conclusion

The received results confirm efficiency of preventive nutrition of schoolchildren in the recreational period. Use of our recommendations allowed increasing significantly antioxidant direction of nutrition of schoolchildren. The nutritional factor positively influenced the vitamin and antioxidant status of organism. Dynamics of vitamin status is characterized by increase in levels of B vitamins egestion with urine, decrease in number of children with the low level of ascorbic acid egestion. It occurs due to transition to moderate and normal levels of egestion. Indicators of antioxidant status illustrate depression of level of free radical oxidation in organism. About a half of the schoolchildren examined by us were characterized by improvement more than on one indicator of biochemicoluminescence. The determined correlation dependences between indicators of biochemiluminescence and level of egestion of vitamins are confirmed by efficiency of use of nutritional factor for influence on health of schoolchildren.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

References

1. Podrigalo LV, Danylenko GN. Prenosological condition in children, adolescents and young adults: diagnosis, prognosis and hygienic correction. Kiev: Geneza; 2014 (in Russian)
2. Podrigalo L, Iermakov S, Rovnaya O, Zukow W, Nosko M. Peculiar features between the studied indicators of the dynamic and interconnections of mental workability of students. J Phys Educ Sport 2016; 16(4):1211-1216.
3. Podrigalo LV, Iermakov SS, Avdiievska OG, Rovnaya OA, Demochko HL. Special aspects of Ukrainian schoolchildren's eating behavior. Pedagogics, Psychology, Medical-Biological Problems of Physical Training and Sports 2017; 21(3):120-125.
4. Bodnar IR, Kindzera AB. Leisure of Ukrainian schoolchildren and place of motor functioning in it. Pedagogics, Psychology, Medical-Biological Problems of Physical Training and Sports 2016; 20(5): 11-18.
5. Radziminska A, Weber-Rajek M, Lulinska-Kuklik E, Kazmierczak U, Moska W. Academic youth's health behavior. Physical Education of Students 2016; 20(6):55-62.
6. Hart M. The importance and elements of healthy nutrition. Advances in Eating Disorders 2016; 4:14–30.
7. Martinchik AN, Maev IV, Ianushevich OO. General Nutrition. Moscow: MEDpress-Inform; 2005. (in Russian)
8. Jung S, Darvin ME, Chung H-S, Jung B, Lee S-H, Lenz K. Antioxidants in Asian-Korean and caucasian skin: the influence of nutrition and stress. Skin Pharmacol Physiol 2014; 27:293–302.
9. Bhattacharya PT, Misra SR, Hussain M. Nutritional Aspects of Essential Trace Elements in Oral Health and Disease: An Extensive Review. Scientifica 2016; 1:1–12.
10. Vargas Robles H, Citalán Madrid AF, García Ponce A, Silva Olivares A, Shibayama M, Betanzos A. Experimental Colitis Is Attenuated by Cardioprotective Diet Supplementation That Reduces Oxidative Stress, Inflammation, and Mucosal Damage. Oxid Med Cell Longev 2016; 1:1–9.
11. Chiavaroli V, Giannini C, De Marco S, Chiarelli F, Mohn A. Unbalanced oxidant–antioxidant status and its effects in pediatric diseases. Redox Report 2011; 16:101–7.
12. Puchau B, Ochoa MC, Zulet MÁ, Marti A, Martínez JA, Members G. Dietary total antioxidant capacity and obesity in children and adolescents. Int J Food Sci Nutr 2010; 61:713–21.
13. Wu X, Cheng J, Wang X. Dietary Antioxidants: Potential Anticancer Agents. Nutrition and Cancer 2017; 69:521–33.
14. Gust JL, Logomarsino JV. The Association Between Cartenoid Status and Body Composition in Children 2 – 18 Years of Age – A Systematic Review. Int J Vitam Nutr Res 2017; 1–24.
15. Rowicka G, Dyląg H, Ambroszkiewicz J, Riahi A, Weker H, Chełchowska M. Total Oxidant and Antioxidant Status in Prepubertal Children with Obesity. Oxid Med Cell Longev 2017; 1–6.
16. Alshammari E, Suneetha E, Adnan M, Khan S, Alazzeh A. Growth Profile and Its Association with Nutrient Intake and Dietary Patterns among Children and Adolescents in Hail Region of Saudi Arabia. Biomed Research International 2017.
17. Endris N, Asefa H, Dube L. Prevalence of Malnutrition and Associated Factors among Children in Rural Ethiopia. Biomed Research International 2017;
18. Lebedinskiy VY, Koipysheva EA, Rybina LD, Kudryavtsev MD, Iermakov SS, Osipov AY, et al. Age dynamic of physical condition changes in pre-school age girls, schoolgirls and students, living in conditions of Eastern Siberia. Physical Education of Students 2017; 21(6):280-286.
19. Ranucci C, Pippi R, Buratta L, Aiello C, Gianfredi V, Piana N, et al. Effects of an Intensive Lifestyle Intervention to Treat Overweight/Obese Children and Adolescents. Biomed Research International 2017.
20. Bodnarchyk ON, Stefanyshyn VM, Malanchuk GG. Dynamic of primary school age pupils' physical fitness. Pedagogics, Psychology, Medical-Biological Problems of Physical Training and Sports 2017; 21(4):152-156.
21. Pryshva OB. Influence of mature men way of life on highly intensive physical activity. Pedagogics, Psychology, Medical-Biological Problems of Physical Training and Sports 2017; 21(3):126-130.
22. Mohammed Z, Noureddine A, Abdullah B. Abdominal obesity and their association with total body: fat distribution and composition. Case of Algerian teenager male high school students. Physical Education of Students 2017; 21(3):146-151.
23. Mohammed Z. Body composition versus body fat percentage as predictors of posture/balance control mobility and stability among football players under 21 years. Physical Education of Students 2017; 21(2):96-102.
24. Ivashchenko OV. Special aspects of motor abilities development in 6-10 years' age girls. Pedagogics, Psychology, Medical-Biological Problems of Physical Training and Sports 2017;21(3):105-110.
25. Pop CL. Overweight and obesity risk assessment - two methods, different results. Physical Education of Students 2016; 20(3):53-57.
26. Colacino JA, Arthur AE, Ferguson KK, Rozek LS. Dietary antioxidant and anti-inflammatory intake modifies the effect of cadmium exposure on markers of systemic inflammation and oxidative stress. Environ Res 2014; 131:6–12.
27. Khare M, Mohanty C, Das BK, Jyoti A, Mukhopadhyay B, Mishra SP. Free radicals and antioxidant status in protein energy malnutrition. Int J Pediatr, 2014;254396.
28. Besedina AA, Krivonosov MV, Podrigalo LV. The organization of improvement for schoolchildren who were influenced by radiative effects in summer camps of sanatorium type. Kharkov; 1993. (in Russian)
29. Skurikhin IM, Volgarev MN. Chemical composition of food. vol. 1. Moscow: Agropromizdat; 1987. (in Russian)
30. Skurikhin IM, Volgarev MN. Chemical composition of food. vol. 2. Moscow: Agropromizdat; 1987. (in Russian)
31. Spirichev VB. Methods of assessment and control of vitamin provision of population. Moscow: Science; 1984. (in

Russian)
32. Kopeć A, Cieślik E, Leszczyńska T, Filipiak-Florkiewicz A, Wielgos B, Piątkowska E, et al. Assessment of Polyphenols, Beta-Carotene, and Vitamin C Intake with Daily Diets by Primary School Children. Ecol Food Nutr 2013; 52:21–33.
33. Mielgo-Ayuso J, Valtueña J, Huybrechts I, Breidenassel C, Cuenca-García M, De Henauw S, et al. Fruit and vegetables consumption is associated with higher vitamin intake and blood vitamin status among European adolescents. Eur J Clin Nutr 2017; 71:458–67.
34. Barbosa RMS, Peixoto NG de A, Pereira A da S, Vieira CBL, Soares EA, Lanzillotti HS. A study on the prevalence of adequacy of iron and vitamin C in children's diets. Rev Bras Epidemiol 2014; 17:543–56.
35. Grigorov IuG, Kozlovskaia SG. Nutrition and phenomenon of longevity. Kyiv: Knowledge; 1988. (in Russian)
36. Padayatty SJ, Katz A, Wang Y, Eck P, Kwon O, Lee J-H, et al. Vitamin C as an Antioxidant: Evaluation of Its Role in Disease Prevention. J Am Coll Nutr 2003; 22:18–35.
37. Frei B, Birlouez-Aragon I, Lykkesfeldt J. Authors' Perspective: What is the Optimum Intake of Vitamin C in Humans? Crc Cr Rev Food Sci 2012; 52:815–29.
38. Krivonosov MV, Nazarian RS, Podrigalo LV. Way of diagnostics of treatment-and-prophylactic orientation of nutrition for persons with parodont diseases. Patent Ukraine No. 2003031958 Feb. 2004. (in Ukrainian)
39. Rendo-Urteaga T, Puchau B, Chueca M, Oyarzabal M, Azcona-Sanjulián MC, Martínez JA, et al. Total antioxidant capacity and oxidative stress after a 10-week dietary intervention program in obese children. Eur J Pediatr 2014; 173:609–16.
40. Bawaked RA, Schröder H, Ribas-Barba L, Izquierdo-Pulido M, Pérez-Rodrigo C, Fíto M, et al. Association of diet quality with dietary inflammatory potential in youth. Food & Nutrition Research 2017; 61:1328961.
41. Shin SJ, Yamada K. Adequate Intakes of Vitamin E and Protein Prevent Increases of Oxidative Damage to DNA, Lipids, and Protein Induced by Total Body Irradiation in Mice. Nutrition and Cancer 2002; 44:170–4.
42. Murer SB, Aeberli I, Braegger CP, Gittermann M, Hersberger M, Leonard SW, et al. Antioxidant Supplements Reduced Oxidative Stress and Stabilized Liver Function Tests but Did Not Reduce Inflammation in a Randomized Controlled Trial in Obese Children and Adolescents. The Journal of Nutrition 2014; 144:193–201.
43. Koekkoek WAC (Kristine), van Zanten ARH. Antioxidant Vitamins and Trace Elements in Critical Illness. Nutr Clin Pract 2016; 31:457–74.
44. Kontorshchikova KN, Tikhomirova YR, Ovchinnikov AN, Kolegova TI, Churkina NN, Kuznetsova SY, et al. Indices of Free Radical Oxidation in the Oral Fluid as Markers of Athletes' Functional State. Sovremennye Tehnologii v Medicine, 2017; 9:82.
45. Bialasiewicz P, Prymont-Przyminska A, Zwolinska A, Sarniak A, Wlodarczyk A, Krol M, et al. Addition of Strawberries to the Usual Diet Decreases Resting Chemiluminescence of Fasting Blood in Healthy Subjects—Possible Health-Promoting Effect of These Fruits Consumption. J Am Coll Nutr 2014; 33:274–87.

Correspondence:
Sergii Iermakov
Department of Tourism and Recreation, Gdansk University of Physical Education and Sport, Gdańsk, Poland
E-mail: sportart@gmail.com

Progress in Nutrition 2020; Vol. 22, N. 1: 48-57 DOI: 10.23751/pn.v22i1.8110

Original article

Standardizing the recipes mainly used in the menus of commercially operating institutional food services: their nutritional values and cost analysis

Ayhan Dağ, Türkan Kutluay Merdol

Department of Nutrition and Dietetics, Faculty of Health Science, Cyprus International University, Lefkoşa, Northern Cyprus, E-mail:adag@ciu.edu.tr

Summary. *Objective:* This research was carried out at Bilkent University cafeterias to standardize the recipes that are not available for specific dishes which are mainly used by commercially operating mass feeding institutions. Throughout the study, 75 food recipes classified under 9 categories (soups, meat, chicken, fish, vegetables cooked with meat, cold vegetable dishes cooked with olive oil, pastries, salads and desserts) were standardized for 100 portions and written to the forms redeveloped by the researchers. All of the dishes were prepared, cooked and served by the cooks working at Bilkent University main kitchen. Recipe base line information was created by combining the data collected both from the well experienced cooks and famous cook books. The organoleptic evaluation of the recipes to be standardized were made by using a 5 points scale evaluation form which was based on 5 criteria (colour-shape, general appearance, flavour-taste, texture-consistency, portion size) and graded by the panellists composed of dietitians, university students, university staff and cooks. Fifty nine of these recipes were standardized following their initial, 9 after their second, and 7 after their third trial of production. The recipes which were perceived to be average and/or below by the panellists were produced again considering their shortcomings until the desired points were achieved. Energy and nutrient content of the recipes were calculated using BEB S (computerized program giving the energy and nutrient values of given food and recipes that are specific for Turkish dishes) program. The cost of the recipes was calculated as food cost and the total cost. The food cost was calculated by the ingredients' cost indexed to the value of American Dollar is due to its consistent rate compared to Turkish Liras. Total cost was achieved by the factors affecting the cost of the dish such as the cost of employee and other technical costs. Total cost was calculated to determine the sale price of the dishes. Energy and nutrient content and the total cost of the dishes were shown on the standardized recipe forms. It was found that the percentages of the food, labour and the operational cost of the total cost were 33.3 %, 29.9 % and 26.4% of the total cost respectively.

Key words: standardized recipes, cost evaluation, food cost, nutritional values.

Introduction

Standard recipes are one of the factors influencing the quality, effectiveness and the cost control at food service establishments together with purchasing methods, well trained staff, layout and equipments and quality control procedures. By using standardized recipes, it is possible to serve the food with the same cost, quality, consistency, and taste. They also allow the operators to control the portion size and the total yield to be produced (1-12).

The first advantage of using standardized recipes is consistency. Standard recipes are one of the four factors that help achieve the quality, consistency and control-

ling costs at Institutional Food Services. By using standardized recipes, prepared foods will have the same cost, quality, portion control, consistency, and taste, regardless of whom they are prepared for, who prepared the food and the time of preparation. The other factors that help achieve quality, consistency and controlling costs are standardized purchasing methods, well trained staff and quality control procedures (3,10,13-15).

Standardized recipes and standard portions are the main pillars of cost control program, and give constant and valid information for the program. By using the information gathered from standardized recipes, exact cost of items and services could be calculated and analyzed. This is very critical for the strategic planning and control of the business (5,12).

Today most of the commercially operating institutions in Turkey do not use standardized recipes, thus nutritional value of foods served are not known and the cost analysis of the foods is not easy to substantiate (16). This study was planned and carried out to standardize the recipes that are not available for the dishes mostly served in the commercially operating institutions and to define their nutritional values and total cost.

Materials and Methods

The recipes chosen for standardization

In this study seventy-five different dishes were standardized for one hundred portions. The criteria for the selection of the dishes for their recipe standardization were

1) to be seen in the menus of commercially operating institutional food services.

2) not having standardized recipes.

The dishes were chosen from 9 different dish groups i.e. soups, meat, chicken, fish, vegetables cooked with meat, cold vegetables dishes cooked with olive oil, börek-pasta, salads and desserts. All recipes were tried and produced at the Bilkent University kitchens by well trained cooks under the supervision of the researchers. The dishes that are chosen for standardization are shown in Table 1.

Bebis 8 (nutrition information system) program is used in the calculation of energy and nutrient content of meals.

Methods Used in WritingThe Recipes to the Forms

Recipes were documented on a form redeveloped by the researchers. This form contains information about the name of the dish, group number of the dish, portion size,

Table 1. The Dishes Chosen for Standardization

Dish Group	Number	Name*
Soups	10	Carrot, broccoli, minestrone, spinach, vegetable, bezir, mushroom, chicken, ezogelin, corn soups.
Meat	18	Kebabs (kağıt, orman, yörük, islim, with puree), lamb tendaur, shepherds sautee, roasted lamb, hünkar beğendi, elbasan tava and meatballs (roast, hasanpaşa, grilled, dalyan).
Chicken	13	Chicken with soybean sauce, chicken stuffed with spinach, sauteed chicken with mushroom, roasted chicken roti, chicken sautee with vegetables, chicken topkapı, köylüm chicken,
Fish	1	Trout sautee.
Vegetables Cooked With Meat	3	Vegetables augratin, cauliflower augratin, zucchini mousakka.
Cold Vegetable Dishes Cooked With Olive Oil**	4	Artichoke, stuffed aubergine, stuffed cabbage, şakşuka.
Böreks, Pastas	5	Spagetti napoletana, su böreği, milföy börek with cheese, yufka böreği with spinach, vermicelli with cheese and walnut.
Salads and Appetizers	7	Salads (Mediterranean, garden, shepherds, aubergine and potatoes), carrot tarator and fava.
Desserts	14	Tulumba tatlısı, kalburabastı, keşkül, şekerpare, revani, irmik tatlısı, lokma tatlısı, fırın sütlaç, kazandibi, sakızlı muhallebi.

**Orginal names of some dishes are given and explained in Table 4, as they don't have direct translation into English.*

*** In Turkish Cuisine, there is a group of dishes titled "Olive Oiled Dishes". These dishes are cooked with olive oil and served cold. Recently others oils (corn, sunflower etc) were started to be used instead of olive oil, but the dishes are still called oliveoiled dishes. Vegetables, legumes, rice are main ingredients of this group. The dish is mostly named after the main ingredients such as olive oiled green beans, olive oiled artchoke, olive oiled stuffed green pepper, etc.*

utensils used to control portion size, equipments used in preparation and cooking, preparation and cooking time, total yield, ingredients; their net, gross weights and average measurements, the steps to be followed for preparation and cooking, the cost and energy and nutrient content of one serving size.

While calculating the energy and nutritional value of the dishes, the net quantity of the foods in the dishes was used. Gross quantities of the foods were shown on a separate column at the form to determine the purchasing amount and transferring amount of the foods from the dry and cold stores to the kitchen on a given day. Net values of the food were calculated by subtracting the waste from the gross values. All net and gross values of the foods were given in kilograms. For simplifying the procedure for the users, third column is allocated for the ingredients average amounts such as pieces, bunches, glass etc. Some foods that were not purchased as kilograms but in pieces, such as lemon, parsley etc, were stated in kilograms to be used in calculating their nutritional values. The order of the ingredients were written as the order of their process in the preparation and the cooking of the dish. Each new step to be processed were separated by a horizontal line to make the recipe easy to follow.

Organoleptic Evaluation of the Dishes

Each dish was evaluated by ten panelists consisting of two dietitians, two staff members, two cooks and four university students from Bilkent University. A form, created by Kurtcan and Gönül (17) based on grading the criteria determined for the evaluation, was given to the panelist to be filled after they tasted the given dish.

The criteria stated on this form were colour-shape, general appearance, flavour-taste, texture-consistency, and portion size of the dish. As the appearance of quality criteria on the forms is important, they were written as above mentioned order. These five criteria have been graded on a 1 to 5 points scale (18) which are: Unacceptable: 1 point, Acceptable: 2 points, Average: 3 points, Good: 4 points, Excellent: 5 points. Each dish would get a minimum of ten and a maximum of fifty points on this grading method with a panel of ten evaluators. The range of points in grading and their explanation are shown in Table 2.

At the end of the evaluation, the dishes that were graded as an average of 34 points and above were considered acceptable and standardized consequently. The dishes that were graded below 34 points were reproduced until they get the acceptable grade.

Each panelist were trained on the purpose and the grading criteria of the study prior to the evaluation. The dishes to be tested were served on the plates standardized for each panelist and the survey. As one of the evaluation criteria is portion adequacy, the dishes were served at lunch time (12.00-13.00) in the cafeteria. Much effort was given to make sure each panelist were served the dishes at the same inner temperature (19).

Points Considered During the Trials of the Recipes

Dishes were prepared by the cooks under the supervision of the researchers and some notes such as preparation and cooking time and measurements results (such as wastes and absorbed oil etc) were taken. The amount of waste during vegetable preparation can be seen in Table 3.

Table 2. The Points of Grading and Their Explanation Used in the Evaluation of the Standardized Recipes

Points of Grading	Explanation
10 -17	Unacceptable
18 - 25	Acceptable
26 - 33	Average
34 - 41	Good
42 +	Excellent

Table 3. Percentage of the Vegetable Waste

Vegetable	Waste (%)	Vegetable	Waste (%)
Potato		Tomato	
Peeling By Hand	25	Pitting only the top	1
Peeling By Machine	10	Peeling	20
Peeling After Boiling	10	Scooping	30
Zucchini	20	Onion	12
Scooping	45	Spring onion	30
Celery Root	35	Aubergine	20
Carrot	20	Cabbage	30
Cauliflower	45	Radish	25
Garlic	5	Green Pepper	10
Dill	35	Parsley	40
Broccoli	25	Spinach	25+
Brussel Sprout	10	Iceberg	25
Garden Cress	30	Lemon (80g) juice	25 g

Preparation Time

The time spent for preparation was categorized into 3 groups to show the time spent by the cooks (during washing, peeling, chopping etc.), the time that passes to hold the food for specific reason (soaking the beans in water, the rising of dough etc.) and time spent by the cooks after cooking the food (slicing the roasted meat etc.).

Cooking Time

The time spent for cooking was also categorized into 3 to show the time spent by cooks (frying, sautéing, stirring the food etc.), the time not needed staff interference (in the oven, boiling in the pots etc.) and the time needed to make the dish ready to serve (holding rice to become fluffy, cooling deserts and olive oil dishes that are served cold etc.) Preparation and cooking times that are seen on the recipe forms are the averages of the staff performance for one person.

Cost Analysis of the Recipes

Standardized recipes' portion food costs were calculated with the help of an MRP (Material Requirement Program) system and the unit prices that were used on food cost analysis were taken from purchasing lists of the production kitchen. Food Cost was calculated by taking into consideration the gross weights of the ingredients and the prices were indexed to the American Dollar due to its consistent rate. While calculating energy and nutritional value of the fried foods, oil absorption were taken into consideration and noted on the recipe charts. In addition to the above mentioned analysis, labor cost and operational cost were also calculated to find the total cost of the dishes. In determining these costs the following procedure was used. Food costs were calculated with the help of an MRP (Material Requirement Program) system, labor and running costs were calculated by dividing the number of meals produced annually by the number of cafeterias producing meals.

Results and Discussion

From the seventy nine foods produced, fifty nine (79%) were standardized during the first, nine (12%) were standardized during the second, and seven (9%) were standardized during the third trial of the production. All recipes were written into a specific standardization form redeveloped by the researchers. Table 4 shows an example of a standardized recipe for "Chicken Stuffed with Spinach". The nutritional value, food cost and the total cost of the standardized recipes are given in Table 4. The components which are the basis for cost analysis and their percentages are shown in Table 5. Food cost were found to be as 33.3% of the total cost. Labor cost and the operational cost were 29.9% and 26.4% respectively Table 6.

The first step in the preparation process is washing of food.Thus, food, stone, mud, dust, pesticides harmful to health are largely purified (20). The extraction of food from all kinds of foreign matter and bruises affects not only health and economy but also the taste of food (21). In this study, the purchased food items were first extracted and washed. After the extraction process, the shear rates between the gross and net quantities were calculated.

Time, temperature and humidity control are the most important factors in giving proper shape and consistency to the food. The better these three factors are set, the better the quality of the food (1). In this study, these three factors which have a direct effect on the quality of the food were meticulously followed, the preparation and cooking times, the cooking temperatures were checked.

Standardization of the recipes was achieved mostly after the first trial. Dissatisfaction reasons stated by the panelists for the dishes that were needed to be tried for second and third time concentrated on two evaluation criteria, consistency and taste. Taste stands much higher between other sensory quality factors for acceptance of food by the consumer and differs widely from individual to individual. When dissatisfaction reasons were analyzed; surface dryness, undercooking, too much fat content, mushy, unsatisfactory taste, improper cooking time were found to be the mostly stated points. No inadequacy was found on color-shape and portion size criteria of the dishes. There were no low grading for the portion size showing the quantity of foods that form the standardized portion size of the recipes were normal. As the energy value of the lunch meal is suggested to be one third of the daily energy

Table 4. An Example of the Standardized Recipes.

Total Amount: 100 PORTION					
Name	**:**	**Chıcken Stuffed Wıth Spınach**	**Preparation Time**	**:**	1 s. 3' - 0 - 25'
Group	**:**	1	**Cooking Time**	**:**	12' - 1 h. 25' – 0
Portion Size	**:**	250 g	**Total Weight (Kg)**	**:**	25
Portion Measurement	**:**	1 piece	**Food Cost**	**:**	0.60.- USD
Cooking Pots	**:**	Caserol, Oven Tray, Oven	**Total Cost**	**:**	1.17.-USD

Ingredıents	Weıght		Measure	Procedure	Period	Notes
	GROSS (kg)	NET (kg)				
Chicken Steak	15.000	15.000	100 Piece	Wash the chicken steaks and pound, prepare to wrap.	(10 ')	As they will wait till the filling is ready keep cold or make ready while the filling is prepared by someone else.
Spinach	8.000	6.000		Clean, wash carefully and chop the spinach.	(15 ')	
				Braise the spinach.	10 '	
Onion	1.500	1.320	10 MS	Peel, wash and finely chop the onion.	(9 ')	
Mushroom	2.000	2.000		Clean mushroom and slice to a case which is filled with water.**	(8 ')	
				Strain when to be used.	(3 ')	
Margarine	1.000	1.000	4 Pack	Melt the margarine in a pan, add onion, mushroom and spinach and sautee.	10 '	
Black Pepper	0.050	0.050	3 Spoon	Add black pepper, red pepper and salt to the sautéed mixture.	2 '	
Red Pepper	0.050	0.050	3 Spoon			
Salt	0.100	0.100	3 Spoon			
				Spread mixture on the beefs which were prepared before and wrap up like roll.	(20 ')	
Tomato	1.500	1.485	10 MS	Clean and wash tomatoes. Slice tomato's middle size.	(5 ')	
Green Pepper	1.500	1.350		Clean and wash the green pepper and cut into two pieces.	(10 ')	
				Place the rolls to the oven tray and decorate surface of the rolled beefs with a slice of tomatoes and pepper.	(3 ')	
Boiling Water	1.000	1.000	5 Glass	Add the boiling water to the oven tray and put it into the oven (175 ^{0}C).	75 '	

Energy and Nutritent Content of One Serving

Energy	Protein	Fat	CHO	Calcium	Iron	Vitamin C	Thiamin	Riboflavin	Niacin	β. Karoten	Cholesterol
308.4 kal	46.4 g	12.2 g	2.3 mg	294.8 mg	3.6 mg	36.5 mg	0.2 mg	0.4 mg	13.4 mg	3.6 mg	93.2 mg

***Vegetables loose much of their nutritional value while holding in water. Darkening of vegetables is also important during mass production, thus holding time of vegetables in water must be kept as short as possible.*

value, the energy content of the dishes were also consequently indicating the adequacy as most of the meals consist of three course and bread.

Vegetable waste percentages found in this study were in accordance with another study (1) carried out for standardization of the recipes mostly used in public institutions. In another study, the differences between the wastage rates in the comparison with the wastage ratios were determined. In this study, since the dishes are produced according to 6-8 portions and in the laboratory environment, the difference between the controlled production in this environment and the number of serv-

Table 5. Energy and Nutrient Content, Food Cost and Total Cost of the Standardized Recipes

Name of the Dish	Energy (kcal)	Protein (g)	Fat (g)	CHO (g)	Vitamin A (µg.)	Vitamin C (mg.)	Ca (mg.)	Vitamin B1 (mg.)	Vitamin B2 (mg.)	Niacin (mg.)	Fe (mg.)	Food Cost (USD)	Total Cost (USD)
Chicken sautee with soy sauce	315.1	39.9	13.9	7.3	88.0	0.1	27.7	0.1	0.1	12.4	1.1	0.51.-	1.07.-
Chicken stuffed with spinach	308.4	46.4	12.2	2.3	708.6	36.5	294.8	0.2	0.4	13.4	3.6	0.60.-	1.17.-
Roasted chicken	405.3	43.0	23.0	6.7	85.8	0.8	35.5	0.2	0.4	10.6	3.0	0.50.-	1.07.-
Chicken sautee with mushroom	229.7	34.1	9.0	2.6	138.3	15.8	42.8	0.1	0.3	12.5	1.5	0.48.-	1.05.-
Chinese chicken	302.9	34.0	12.6	13.3	1041.8	6.1	75.6	0.2	0.2	10.6	2.3	0.45.-	1.01.-
Roasted chicken thighs	359.2	43.3	18.7	4.2	112.0	12.8	42.3	0.2	0.4	10.7	3.3	0.49.-	1.06.-
Chicken sautee with vegetable	299.0	35.2	10.0	16.3	490.9	17.7	46.5	0.2	0.2	11.2	1.5	0.46.-	1.02.-
Chicken ball	491.8	34.5	17.0	49.1	95.9	12.9	105.0	0.2	0.2	9.1	1.7	0.43.-	1.00.-
Piliç Topkapı[1]	306.1	40.7	11.6	9.4	88.3	0.8	42.2	0.1	0.1	12.4	2.3	0.51.-	1.07.-
Broiled chicken	337.6	47.8	13.2	6.5	675.5	18.4	81.3	0.2	0.2	14.4	1.9	0.62.-	1.19.-
Köylüm Chicken[2]	444.4	40.5	23.5	17.4	461.2	14.0	212.1	0.2	0.3	11.4	1.5	0.52.-	1.09.-
Chicken schinitzel	534.2	54.7	16.8	40.2	740.9	18.1	95.8	0.3	0.3	14.7	2.8	0.76.-	1.33.-
Chicken sautee	360.1	34.5	17.7	15.3	146.6	24.4	49.7	0.2	0.2	11.2	1.6	0.46.-	1.02.-
Rainbow trout Sautee	489.6	52.3	16.4	31.8	233.0	33.0	94.1	0.3	0.2	6.9	3.0	0.82.-	1.43.-
Kağıt Kebap[3]	367.8	36.2	19.2	12.4	657.1	13.9	80.9	0.2	0.3	5.9	4.9	1.01.-	1.58.-
Yörük Kebap[4]	457.1	42.2	23.9	18.1	138.1	17.4	169.0	0.2	0.5	6.5	5.2	0.97.-	1.54.-
İslim Kebap[5]	452.4	38.5	25.7	16.2	237.7	48.6	95.5	0.2	0.4	6.5	4.7	1.16.-	1.73.-
Orman Kebap[6]	401.4	35.6	22.4	14.3	856.3	9.3	59.6	0.1	0.3	5.2	5.8	0.85.-	1.43.-
Kebab with puree	409.7	38.5	20.4	17.3	139.8	32.0	70.6	0.2	0.4	5.9	4.9	0.88.-	1.44.-
Lamb tendour	363.1	27.9	27.5	1.6	6.4	1.9	50.4	0.1	0.3	4.6	2.6	1.52.-	2.09.-
Roasted lamb	416.6	43.0	20.9	13.9	5626.1	10.0	84.8	0.2	1.5	10.2	8.9	1.56.-	2.13.-
Ankara Tava[7]	385.2	42.0	20.2	8.6	141.1	21.4	54.7	0.1	0.3	6.4	4.0	1.75.-	232.-
Çoban Kavurma[8]	349.4	25.1	25.9	4.1	107.0	35.7	35.4	0.2	0.3	6.2	3.4	0.86.-	1.97.-
Boiled veal	369.4	36.6	17.9	15.5	1048.6	8.8	32.4	0.1	0.3	5.4	4.6	0.87.-	1.43.-
Beef with mashroom sauce	453.3	38.9	22.5	23.2	48.2	14.7	68.3	0.2	0.4	5.8	5.0	1.00.-	1.56.-
Hünkar Beğendi[9]	491.1	44.2	21.4	29.5	139.2	239.7	11.2	4.3	0.2	0.5	6.1	1.17.-	1.73.-
Roasted Köfte	339.2	30.9	18.3	12.6	114.6	56.1	18.8	3.9	0.2	0.3	5.4	0.63.-	1.19.-
Hasanpaşa Köfte[10]	468.6	36.0	21.8	31.5	117.0	248.5	18.2	3.9	0.2	0.4	5.8	0.66.-	1.22.-
Grilled meatballs	370.9	32.1	18.4	18.8	372.2	66.6	19.1	4.3	0.2	0.3	5.6	0.69.-	1.26.-
Dalyan Köfte[11]	439.3	36.4	21.4	25.2	780.2	86.4	19.6	4.9	0.2	0.4	5.8	0.67.-	1.23.-
Elbasan Tava[12]	456.4	35.7	26.2	19.1	108.3	182.3	14.7	3.9	0.2	0.4	5.0	0.80.-	1.36.-
Vegetables au gratin	409.7	24.5	25.5	20.4	561.1	253.6	16.0	2.8	0.2	0.4	3.7	0.49.-	1.05.-
Cauliflower au gratin	355.3	27.7	15.4	25.8	113.3	276.3	52.7	2.8	0.2	0.4	3.3	0.70.-	1.26.-
Zuccini mousakka	257.4	17.8	17.0	8.2	159.4	77.5	22.8	4.4	0.2	0.3	3.3	0.32.-	0.88.-
Veal sautee with mushrooms	346.1	36.1	19.5	6.5	84.2	34.7	24.0	4.9	0.2	0.5	6.8	0.95.-	1.51.-

Table 5. Energy and Nutrient Content, Food Cost and Total Cost of the Standardized Recipes

Name of the Dish	Energy (kcal)	Protein (g)	Fat (g)	CHO (g)	Vitamin A (µg.)	Vitamin C (mg.)	Ca (mg.)	Vitamin B1 (mg.)	Vitamin B2 (mg.)	Niacin (mg.)	Fe (mg.)	Food Cost (USD)	Total Cost (USD)
Artichokes cooked with olive oil	231.8	4.8	17.3	14.0	660.7	98.3	11.6	2.1	0.1	0.1	1.2	0.75.-	1.31.-
Stuffed aubergines cooked with oilve oil	278.7	4.2	22.5	15.0	96.7	51.5	14.3	2.2	0.1	0.1	1.1	0.23.-	0.79.-
Fava[13]	141.7	4.9	10.3	7.5	223.7	30.3	2.7	1.1	0.1	0.1	0.5	0.07.-	0.64.-
Stuffed cabbage cooked with olive oil	238.2	4.4	17.3	16.2	82.7	103.6	49.4	2.5	0.1	0.1	1.8	0.16.-	0.72.-
Şakşuka[14]	171.6	2.1	14.3	8.5	87.2	28.5	26.4	0.8	0.1	0.1	0.7	0.11.-	0.67.-
Spaghetti Napoliten	391.1	8.5	19.8	44.6	141.4	34.6	28.7	1.4	0.1	0.1	1.7	0.07.-	0.66.-
Layered börek	293.1	9.4	15.5	29.0	182.2	138.0	6.7	1.0	0.1	0.2	0.4	0.11.-	0.68.-
Vermicelli with walnuts and cheese	399.9	12.9	19.3	43.3	29.0	154.7	0.1	1.2	0.1	0.1	1.3	0.16.-	0.73.-
Phyllo pastry stuffed with cheese	559.7	15.2	38.2	39.4	330.9	261.9	3.6	1.1	0.1	0.3	0.8	0.18.-	0.73.-
Börek with spinach	368.5	11.1	14.3	48.0	726.9	177.7	22.4	4.1	0.1	0.2	0.8	0.17.-	0.75.-
Carrot soup	131.5	1.4	10.5	8.3	709.0	36.1	4.1	0.5	-	-	0.2	0.02.-	0.59.-
Broccoli soup	168.2	2.0	15.8	5.0	40.8	58.3	15.5	0.4	-	0.1	0.2	0.06.-	0.62.-
Minestrone Çorba	122.9	2.5	6.0	14.5	437.0	28.9	11.8	0.8	0.1	0.1	0.7	0.04.-	0.61.-
Spinach soup with cream	156.4	2.0	14.7	4.2	401.6	73.9	8.7	1.5	-	0.1	0.2	0.11.-	0.68.-
Vegetables soup with cream	149.8	1.5	12.6	7.8	255.7	27.9	3.6	0.4	-	-	0.3	0.08.-	0.65.-
Mushrooms soup with cream	178.1	1.9	15.6	7.9	54.9	21.0	1.1	0.4	-	0.1	1.0	0.10.-	0.67.-
Kremalı Bezir çorba[15]	241.3	9.7	17.7	10.9	207.4	99.6	5.9	1.1	0.1	0.2	2.4	0.18.-	0.75.-
Chicken soup with cream	181.5	6.3	14.7	6.4	58.0	21.6	0.2	0.3	-	-	1.7	0.13.-	0.69.-
Ezogelin çorba[16]	114.7	2.7	6.6	11.1	60.5	20.3	2.4	1.5	-	-	0.5	0.03.-	0.60.-
Corn soup	142.7	2.0	10.7	9.8	32.3	27.3	6.1	0.4	-	0.1	0.4	0.05.-	0.61.-
Carrot sautee with yoghurt	269.8	6.0	21.8	13.4	1997.2	128.5	6.1	1.2	0.1	0.2	0.6	0.25.-	0.82.-
Meditarranean salad	130.1	3.7	9.6	7.0	1071	92.2	14.9	0.8	0.1	0.1	0.7	0.14.-	0.70.-
Garden salad	106.1	1.4	8.3	6.2	1089.4	37.1	18.7	0.9	0.1	0.1	0.6	0.11.-	0.68.-
Shepherds salad	113.0	1.8	8.4	7.0	184.2	41.9	52.2	1.2	0.1	0.1	0.8	0.11.-	0.68.-
Aubergine salad	207.0	2.6	18.4	7.7	178.9	44.5	55.2	1.2	0.1	0.1	1.0	0.20.-	0.76.-
Potatoes salad	184.9	3.8	7.1	25.2	116.9	32.0	63.5	1.2	0.2	0.1	2.0	0.07.-	0.64.-
Cheesecake	610.2	12.3	41.2	47.6	458.5	145.1	0.8	0.7	0.1	0.3	0.2	0.92.-	1.48.-
Triamisu	549.0	14.2	28.5	57.4	207.6	75.0	0.3	3.2	0.4	0.6	3.7	0.65.-	1.21.-
Tulumba Tatlısı[17]	512.3	4.3	11.9	95.8	42.7	15.6	0.2	0.8	-	0.1	0.1	0.17.-	0.73.-
Kalburabastı[18]	411.4	4.5	12.2	70.1	102.1	23.1	0.2	1.1	0.1	0.1	0.9	0.10.-	0.67.-
Keşkül[19]	455.0	8.4	13.5	74.0	69.5	260.5	1.8	0.7	0.1	0.3	0.4	0.20.-	0.77.-
Şekerpare[20]	482.6	5.0	13.6	84.2	122.2	25.3	0.2	0.9	-	0.1	0.2	0.15.-	0.71.-
Revani[21]	367.6	4.8	11.4	60.9	145.4	19.6	0.2	0.8	-	0.1	0.1	0.11.-	0.68.-

Table 5. Energy and Nutrient Content, Food Cost and Total Cost of the Standardized Recipes

Name of the Dish	Energy (kcal)	Protein (g)	Fat (g)	CHO (g)	Vitamin A (µg.)	Vitamin C (mg.)	Ca (mg.)	Vitamin B1 (mg.)	Vitamin B2 (mg.)	Niacin (mg.)	Fe (mg.)	Food Cost (USD)	Total Cost (USD)
İrmik Tatlısı[22]	270.4	5.2	5.4	49.4	45.0	183.8	1.3	0.2	-	0.2	0.1	0.12.-	0.69.-
Supangle[23]	408.5	9.2	11.1	66.7	66.1	270.8	1.7	1.0	0.1	0.4	0.3	0.19.-	0.75.-
Lokma Tatlısı[24]	383.3	3.9	10.0	66.7	14.5	9.0	0.3	0.9	0.1	0.1	0.5	0.13.-	0.70.-
Fırın Sütlaç[25]	355.0	7.0	7.2	64.4	60.0	244.7	1.7	0.2	-	0.3	0.2	0.16.-	0.72.-
Krem Şokola[26]	447.6	7.4	13.9	72.3	54.0	216.2	1.1	1.2	0.1	0.3	0.3	0.23.-	0.80.-
Kazandibi[27]	306.8	5.1	6.2	56.8	51.1	183.5	1.3	0.2	-	0.2	0.1	0.12.-	0.69.-
Sakızlı Muhallebi[28]	431.6	9.1	11.7	71.3	56.5	235.0	2.3	1.0	0.1	0.3	0.6	0.38.-	0.95.-

[1]Grilled chicken stuffed with rice.
[2]Oven grilled chicken breast with vegetables.
[3] Sautee lamb pieces wrapped in grease-proof paper with vegetables and cooked in the oven.
[4]Poached and sautéed veal finished in the oven with vegetables and served over vermicelli.
[5]Roasted pieces of lamb wrapped with aubergine and cooked in the oven.
[6]Sauteed lamb with potatoes, carrots and onions.
[7]Poached lamb, cooked in the oven over rice.
[8]Sauteed lamb with tomatoes, onions and green peppers.
[9]Sauteed lamb served over aubergine puree with roux.
[10]Stuffed meatballs with potatoes puree.
[11]Roasted meatball stuffed with cucumbers, eggs and beans.
[12]Sauteed veal and vegetables with béchamel au gratin
[13]Broad bean puree.
[14]Fried vegetable cubes (aubergine, green pepper and potatoes) garnished with tomatoes sauce.
[15]Chicken and bean soup with creamy roux.
[16]Lentil, whole wheat and rice soup.
[17]Syrup soaked pastry (flat and round shaped).
[18]Syrup soaked pastry (big flat round shaped).
[19]Milk pudding with almonds.
[20]Syrup soaked pastry (ball shaped, fried).
[21]A sweet made of semolina, flour and eggs.
[22]Cooked semolina with milk and vanilla.
[23]Chocolate pudding.
[24]Syrupy fried pastry.
[25]Baked rice pudding.
[26]Cream chocolate.
[27]A kind of pudding, bottom part is burnt.
[28]Milk pudding with mastica.

ings and the rate of wastage in our study is considered normal (22). It can be concluded that these vegetable wastage values can be used as a guidance for institutional food services to calculate the amount to purchase and to calculate the nutritional value of foods served.

Standardized recipes are the main component of the food services to maintain the quality and cost control in a desired level. With the help of this study, food cost, total cost, energy and nutritional value of the dishes mostly used in commercially operating establishments were standardized. This may help the institutions where quality and cost control is the primary objective.

Increased competitiveness in the field of mass catering industry has decreased the flexibility for errors. For this reason, companies could achieve customer satisfaction through using standardization recipes which

Table 6. Total Cost Criteria and their Percentages.

Cost component	(%)
• Food Cost	33,3
• Labor cost	29,9
• Operational	26,4
Transportation	*7,6*
Cleaning	*2,8*
Energy-Fuel	*2,9*
Investment Expenses	*2,8*
Washing	*2,2*
Uniform	*1,4*
Care-Repair	*1,5*
Amortizaton	*1,2*
Consumables	*0,8*
Health	*0,4*
Stationery-Documents	*0,3*
Telephone-Fax	*0,3*
Disinfectation	*0,2*
Laboratory	*0,2*
Insurance	*0,2*
Other	*1,6*
• Profit	10,4

would improve their productivity and decrease costs in every aspect of their work, from procurement to cooking and service.

Standardized recipes are not just lists of cooking procedures. At the same time they are preparation and service directions for the people who are responsible for these. In addition, these are used by managers when deciding on the equipment and amount to buy for the company as well as personnel needs and qualities.

By using standardized recipes the production stages of food can be tracked. Besides, food cost control, the quality, taste and portion standards and nutrient ingredients can be achieved. For this reason, recipes should be standardized in all mass feeding institutions and these should be made available in bulletin boards, a feeding list, and calculation folders and the control mechanism should be established accordingly.

When standardizing recipes it is essential that HACCP regulations should be considered when producing meals with the risk of hygienic concerns, especially those which are prepared without cooking.

Moreover, price determination strategies can be created by using standardized recipes. Price determination strategies and cost controls are important not only for a lot of catering services but also for institutional food services as well. All companies should check their costs. Commercial institutions should do this in order to have appropriate profit. In addition, institutional food services should also do this within their budget.

Food cost was found to be as 33,3% of the total cost. This result was between 30-35 % in the studies carried out in other countries (23-28). Running costs were calculated as 26,4% and this is a much higher ratio than other studies' 20% ratio (23-26). Energy prices (electricity, natural gas, gasoline etc), corporate tax ratio, VAT ratio and income tax ratios, transportation, and sanitation costs are all affecting factors of this cost which are much higher in Turkey than other countries. Labor costs were found to be 29,9% of total cost. This result is consistent with other studies. One would expect a lower cost with the industries' wage rate, however lack of technology in kitchens and unqualified personnel increases the labor cost. This affects the profit. As it is seen in Table 6, the profit is 10.4 % for our study which is lower than other studies of 15-20 % (23-26).

References

1. Kutluay, Merdol. T. Standart Yemek Tarifeleri. 7. Edition, Hatipoğlu Yayınları, 2018, p. 77.
2. Kutulay Merdol, T., Dağ, A., Baş, M. et al. Genel Beslenme. T.C. Anadolu Üniversitesi Yayını No:2768. 3. Edition. 2018, p.45-60.
3. Ninemeier, J. D. Planning and Control for Food and Beverage Operations. Fourth Edition, Educational Instıtute American Hotel and Motel Association,1998, p. 38-41.
4. Gisslen, W. The Recipe: Its Structure and its Use. Professional Cooking. 6th Edition, 2006, p. 64-73.
5. Mizer, A. D., Porter, M., Sonnier, B. Recipes and Measurement. Food Preparation for The Professional. Third Edition, John Willey and Sons, 1999, p. 93-95.
6. Magris, M., Mc Creery, C., Brıghton, R. Menu Planning. Introduction to Catering. Oxford, 1996, p. 242-249.
7. Türksoy, A. Yiyecek ve İçecek Hizmetleri Yönetimi.Turhan Kitapevi, 2nd Edition, Ankara, 2002.
8. Kinton, R., Ceserani, V., Fosket, D. The Theory of Catering. 11th Edition, Hodder and Stonghton Publishing. London, 2007.
9. Tolve, P, A. Standardizing Foodservice For Quality and Efficiency. Restaurant and Institutional Foodservice Management Department of Home Economics, Bowling Green State University, Avi Publishing Company, Inc. Westport Connecticut, 1984.

10. Cousins, J., Foskett, D., Shortt, D. Food and Beverage Management. Thames Valley University, Longman, Fourth Edition, 2016.
11. Coltman, M, M. Cost Control For the Hospitality Industry. Van Nostrand Reinhold, Newyork, Second Edition, 1998
12. Measuring Success With Standardized Recipes U. S. Department of Agriculture, Food and Nutrition Service and The National Food Service Management Institute, 2002.
13. Scanlon, N. L. Criteria for menu pricing. Catering Menu Management. Newyork, Third Edition, 2006, p. 88-93.
14. Ninemeier, D. J. Standart Product Costs and Pricing Strategies. Food and Beverage Management. Second Edition, Educational Institute American Hotel and Motel Association, 1995, p.131-138.
15. Kivela, J. Menü Planning For the Hospitality Industry. Hospitality Press, Melbourne, 1994, p.138-148.
16. Başoğlu, S. Yöresel Yemek Tarifelerinin Standartlaştırılması ve Besin Değerleri Üzerine Bir Araştırma. Hacettepe Üniversitesi Sağlık Bilimleri Enstitüsü Bilim Uzmanlığı Tezi, Ankara, 1985.
17. Kurtcan, Ü., Gönül, M. Gıdaların Duyusal Değerlendirmesinde Puanlama Metodu. Ege Üniversitesi Mühendislik Fakültesi Dergisi, Seri B:5,1. 1987, p.137-145
18. Sümbüloğlu, K., Sümbüloğlu, V. Biyoistatistik, Özdemir Yayıncılık, 17th Edition, 2017.
19. Dağ, A. Yiyecek İçecek İşletmelerinde Standart Tarifeler Maliyet ve Hijyen Kontrolü, 1. Edition Baskı, Ankara, 2006.
20. Baysal, A. Beslenme. Hatipoğlu Yayınevi, 18. Baskı, Ankara, 2018.
21. Arlı, M., Şanlıer, N., ve arkadaşları, Yiyecek Üretimi 1, Gazi Üniversitesi Meslek Eğitim Fakültesi Aile Ekonomisi ve Beslenme Eğitimi ABD, Turan Ofset, İstanbul, 2002.
22. Baysal, A., Merdol, T. K,. Ciğerim, N. Başoğlu, S. Sacır, H. Türk Mutfağından Örnekler. T.C. Kültür Bakanlığı Tanıtma Eserleri/56, 4. Baskı Türk Tarih Kurumu Basımevi, Ankara, 2005.
23. Miller, J. E., Pavesic, D. V. Menu Pricing Strategies. Menu Princing and Strategy. Fourth Edition, Newyork,1996, p. 133-145.
24. Seaberg, A. G. Producing A Menu. Menu Desing Merchandising and Marketing, Fourth Edition, Newyork, 1991, p.1-4.
25. Davis, B., Sally, S. Food and Beverage Management. Heinemann- London, 3rd Edition, 1998, s. 45-46.
26. Iverson, M. K. Food and Beverage Opeations. Introduction to Hospitlity Management, Newyork, 1989, p. 60-78.
27. Kotschevar, L. H. Management by Menu. Fourth Edition, The Educational Foundation National Restaurant Association, 2007, p. 100-114.
28. Wright-Thompson, A. Piche, L. Nutritional analysis of along term care menu before and after an increase in the raw food cost allowance, Can J Diet Pract Res. 2011 Fall; (72):141-5.

Correspondence:
Ayhan Dağ
Department of Nutrition and Dietetics, Faculty of Health Science, Cyprus International University
Lefkoşa, Northern Cyprus
E-mail:adag@ciu.edu.tr

Progress in Nutrition 2020; Vol. 22, N. 1: 58-67 DOI: 10.23751/pn.v22i1.8033

Original article

The effects of media tools on food consumption and obesity in adolescents

Fatma Kartal[1], *Neslişah Rakıcıoğlu*[2]

[1] Gümüşhane University, Faculty of Health Sciences, Department of Nutrition and Dietetics, 29100 Gümüşhane, Turkey; [2]Hacettepe University, Faculty of Health Sciences, Department of Nutrition and Dietetics, 06100-Sıhhiye Ankara, Turkey - Email: neslisah@hacettepe.edu.tr

Summary. *Purpose:* The present study was aimed to define the quantities and types of foods consumed by adolescents during use of media tools to evaluate the contribution of energy and nutrient intake during the use of media tools to daily energy and nutrient intake. *Methods:* A total of 73 adolescents who visits an internet cafe, were included in the study. Participants were evaluated when they were using a computer in an internet cafe, food consumptions during the use of media tools and daily consumption and physical activity level were recorded. *Results:* It has been found that consumption of cakes, pastries and cookies during the use of media tools constitute more than half of daily consumption. At the same time, consumption of oily seeds, consumption of sweet foods and saturated fat consumption during the use of media tools were contributed to the daily consumption significantly. It was found that, adolescents consumed less egg, legumes, milk and dairy products, and vegetables in front of the media tools ($p<0.05$). A significant correlation was observed between the energy and fat content of foods consumed in front of media tools, and obesity. Also, time spend in the internet cafe are increasing as the energy intake during the use of the media tools increases ($p<0.05$). *Conclusion:* Consequently it is necessary to reduce the time spend in front of media tools and regulate the consumed foods during the use of media tools to live a healthier life and reduce the risks of development chronic disease in the future lives.

Key words: adolescent, media tools, nutritional habits, obesity, physical activity

Introduction

The adolescent period is an important period in the gaining of healthy dietary habits in which more physiological and behavioral changes occur than in other stages of life, apart from infancy, and that encompasses the years extending from the start of puberty until the start of adulthood (1). Updated findings obtained from the general youth population demonstrate that there is an inverse relationship between sedentary behaviors and healthy dietary habits and also that there is a positive relationship between the consumption of snacks and sedentary behaviors (2). Sedentary behaviors like watching television or playing video games independently influence dietary consumption (3). The nutritious value of the diets of children and adolescents who regularly use media tools for a longer period of time is found to be lower than their peers who whose media tools less (4). Tools of mass communication, radio, television, and advertisements found in print are powerful factors that influence the dietary habits of children and adolescents (5). In the past 30 years, the lifestyles of children and adolescents have changed drastically with the increase in their use of television, video games, and computers. The daily time spent watching television for children and adolescents in the United States (US) increased from 3 hours and 45 minutes in 1999 to 4 hours and 30 minutes in 2009. Rates for computers and video games increased even greater (6). As a result of sedentary activity taking the

place of physical activity and at the same time being exposed to stimulants that encourage unhealthy foods alongside the increase in the use of media tools, the increase in the consumption of high-energy content, low-nutritional content foods like high-fat foods and sugary snacks or beverages has also increased the risk of obesity in children and adolescents (7). The global prevalence of childhood obesity has increased significantly. Childhood obesity rates were 4.2% in 1990 and increased to 6.7% in 2010 (8). Turkey Nutrition and Health Survey 2010: According to the Final Report on the Assessment of the Status and Habits of Nutrition, of children between the ages of 6-18 throughout Turkey, 14.3% and 8.2% are overweight or obese, respectively. This study conducted in Turkey reported that obesity is seen most frequently in the 12-14 age group (9.8%) and least frequently in the 9-11 age group (6.0%) (9). Childhood obesity can have harmful effects in different ways over body composition and health. Obese children are at risk in terms of cardiovascular diseases, impaired glucose tolerance, Type 2 Diabetes, insulin resistance, respiratory problems like sleep apnea and asthma, musculoskeletal system disorders, fatty liver disease, gallbladder stones, acid reflux, psychological stress, low self-esteem, and various health problems like impaired physical, social, and emotional functionality (10). Childhood and adolescent obesity, which is a growing problem in our society, may be slowed down if the causes are addressed and measures are taken against this. The co-implementation of diet and physical activity interventions is very important for the prevention of obesity and being overweight. Focusing on these causes reduces the risk of childhood and adolescent obesity with time and helps to create a healthier society (11). One of the factors that lead to the increase of the prevalence in childhood and adolescent obesity is spending long periods of time in front of media tools. The aim of this study was to determine that the amount and variety of the nutrition that adolescents consume within the period of time they spend each day using computers and other media tools at Internet cafes. Also, to evaluate the contribution this makes in their daily intake of nutrients by determining the amount of energy and other nutrients provided by the consumed foods.

Methods

A total of 73 adolescent individuals (36 females-37 males) between the ages of 11 and 18, who visit the Internet cafe found in the center of the Gümüşhane province were included in this study. The ethics committee permission required for the study was obtained from the Hacettepe University Non-Interventional Clinical Research Ethics Board (GO 16/94). The "Parental Approval Form and Child Approval Form Clarified for Research-Purpose Study" was read to and signed by the adolescents and who agreed to participate in the study and their parents. Information regarding the general characteristics and the status of their use of television and computers were obtained with a face-to-face interview technique in the first section of the survey form. Adolescents were asked about the status of their physical activities.

Anthropometric measurements of body weight, height, and waist circumference measurements were taken in accordance with the method by the researcher (12). 24-hour recall was used to determination of food consumption (13). The consumption amounts of foods were determined using a photographic food catalog (14) and the intake of energy and nutrients were calculated with the Nutritional Information System 7.2 computer program (15). The percentages of meeting the daily energy and nutrients requirements were evaluated using the Nutritional Guide Specific to Turkey (16). The meet of daily requirements of the nutrient intake of >67% was accepted as sufficient intake (12).

Statistical Analysis

The SPSS 11.5 packet program was used in the evaluation of the data. The Single Sampling Kolmogorov Smirnov Test was utilized to determine whether the data were normally distributed. The homogeneity test was conducted with the One-Way Anova. The Mann-Whitney U Test was used to analyze the significant of the difference between the two independent groups. The Spearman Correlation Analysis was used to test whether there was a relationship in the proportional data, and the Pearson Chi-square Test was used in the categorical data (17).

Results

A total of 73 adolescents, which 37 were males and 36 were females, who visit the Internet cafe were included in the study. The average age of the adolescents was 14.7±1.9 years, and it was seen that 53.4% of the individuals attended middle school and 46.6% attended high school. Of the adolescents, 15.1 were overweight (≥85th-<95th percentile) and 9.6% were obese (>95th percentile), and the rate of obesity was found to be 16.2% and 2.8% for the males and females, respectively. The rate of overweight is 10.8% in male adolescents and 19.4% in females. Of the adolescents, 69.9% do not regularly play sports. The average waist circumference was found to be 77.0±13.7cm for the male adolescents and 76.4±9.1cm for the females. The body mass ındex (BMI)average of the adolescents was found to be 21.1±4.5 kg/m^2 and 21.4±3.3 kg/m^2 in the males and females, respectively. While the total energy expenditure was 2961.8±821.1 kcal/day in males, it was 2425.5±396.0 kcal/day in females. The average numbers of main meals and snacks for all adolescents was found to be 2.71±0.5 and 2.35±0.9, respectively (Table 1).

It was found that 75.3% of the adolescents exceeded the recommended duration of the use of computers (<2 hours) on weekdays, and that 83.6% exceeded this period on the weekends. The percentages of adolescents who have a personal computer were found to be 35.6%. It was seen that 84.9% of the adolescents enjoy watching television. The adolescents overall have an average of one (1.3±0.5) in their homes. While the rate of male adolescents who have a television in their bedrooms is 5.4%, it is lower than the rate for female adolescents (22.2%) (Table 2).

Male adolescents (75.7%) and 86.1% of the female adolescents watch television for more than the recommended time (<2 hours). The rates of female and male adolescents who watch television on the weekends more than the recommended <2 hours were 91.6% and 89.2%, respectively. While the adolescents use the computer for an average of 3.35±2.0 hours on weekdays, they use the computer for an average of 4.30±2.8 on the weekend. The amount of time passed in to use a computer on the weekends is greater than weekdays, significantly ($p<0.05$). Similarly, the amount of time

Table 1: General charecteristics of the adolescents

	Male (n=37)	Female (n=36)	Total (n=73)
	($\bar{x}$±SD)	($\bar{x}$±SD)	($\bar{x}$±SD)
Age (year)	14.5±1.7	14.8±2.1	14.7±1.9
Educational status*			
Middle School	21 (56.8)	18 (50.0)	39 (53.4)
High School	16 (43.2)	18 (50.0)	34 (46.6)
Body weight (kg)	57.0±15.0	53.8±9.0	55.4±12.5
Length (cm	163.5±11.0	158.3±7.1	160.9±9.6
Body mass index (kg/m^2)	21.1±4.5	21.4±3.3	21.3±3.9
BMI percentile*			
<5	1 (2.7)	1 (2.8)	2 (2.7)
5-15	6 (16.2)	3 (8.3)	9 (12.3)
15-85	20 (54.1)	24 (66.7)	44 (60.3)
85-95	4 (10.8)	7 (19.4)	11 (15.1)
≥95	6 (16.2)	1 (2.8)	7 (9.6)
Waist circumference (cm)	77.0±13.7	76.4±9.1	76.7±11.6
Doing regular sports*	16 (43.2)	6 (16.7)	22 (30.1)
Total energy expenditure (kcal/day)	2961.8±821.1	2425.5±396.0	2697.3±697.3
Number of main meals	2.73±0.5	2.69±0.5	2.71±0.5
Number of snacs	2.25±1.0	2.44±0.8	2.35±0.9

*n (%)

Table 2. The use of media tools in adolescents

	Male n= 37		Female n=36		Total* n=73		
	n	**%**	**n**	%	**n**	**%**	**p***
Use of computer (hour)							
Weekdays §	3.6±2.1		3.06±2.04		3.35±2.0		
0-2	8	21.6	10	27.8	18	24.7	
≥ 2	29	78.4	26	72.2	55	75.3	
Weekend §	4.7±3.1		3.8±2.3		4.30±2.8		0.000**
0-2	4	10.8	8	22.2	12	16.4	
≥ 2	33	89.2	28	77.8	61	83.6	
Do you have a personel computer?							
Yes	14	37.8	12	33.3	26	35.6	
No	23	62.2	24	66.7	47	64.4	
Do you like watching television?							
Yes	33	89.2	29	80.6	62	84.9	
No	4	10.8	7	19.4	11	15.1	
Number of televisions at home §	1.3±0.4		1.3±0.5		1.3±0.5		
Do you have a televisions in your bedroom?							
Yes	2	5.4	8	22.2	10	13.7	
Time of watching television (hour)							
Weekdays §	3.0±1.8		3.2±1.6		3.14±1.7		
0-2	9	24.3	5	13.9	14	19.1	
≥ 2	28	75.7	31	86.1	59	80.8	
Weekend §	3.8±2.1		3.6±1.8		3.77±2.0		0.02*
0-2	4	10.8	3	8.4	7	9.6	
≥ 2	33	89.2	33	91.6	66	90.4	
Time of stay at internet cafe (hour)							
<1	9	24.3	10	27.7	19	26.0	
1-4	22	59.5	20	55.6	42	57.5	
4-6	6	16.2	4	11.1	10	13.7	
≥6	-	-	2	5.6	2	2.7	

*§($\bar{x}$±SD); *p<0.05, **p<0.01*

the adolescents spent watching television on average on the weekends (3.77±2.0) was significantly greater than the amount they spent watching television on weekdays (3.14±1.7) (p<0.05). While 57.5% of the adolescents spend 1-4 hours at the Internet cafe, 13.7% spend 4-6 hours, and 2.7% spend ≥6 hours (Table 2).

The daily vitamin B_1 intake in male adolescents aged 14-18 years was found to be below the daily requirement. The total folic acid intake was below the daily requirement in male adolescents age 14-18 and in all the female adolescents. Calcium intake was similarly found to be below the requirement in all age groups. When considering the status of the adolescents meeting the requirement for iron intake, it was determined that all of the male adolescents and the 11-13 age group of female adolescents were within the normal limits for the requirement of iron intake but that female adolescents aged 14-18 were under the requirement for iron intake. The percentages of the meet requirement of vitamins B_2, vitamin C and zinc intakes were found to be sufficient in all adolescents. The amount of dietary sodium, except for salt, was above the requirement in all age groups (Table 3).

Tablo 3: Daily energy and nutrient intake and percentage of daily requirements to meet in adolescents (%)

	Male				Female			
	11-13 year (n=11)		14-18 year (n=26)		11-13 year (n=9)		14-18 year (n=27)	
Nutrients	**Daily intake x±SD**	**Percentage of requirements to meet**	**Daily intake x±SD**	**Percentage of requirements to meet**	**Daily intake x±SD**	**Percentage of requirements to meet**	**Daily intake x±SD**	**Percentage of requirements to meet**
Energy (kcal/day)	2282.8±875.3	93	2351.4±837	82	2017.9±659.8	93	2037.3±635.9	90
Protein (g/day)	54.8±24	100	71.3±24.6	99	53.8±20.7	118	66.2±23.3	100
Protein (%)	9.8±1.7		12.8±3.1		10.6±2.5		13.4±3.9	
Fat (g)	85.5±33.7		93.3±39.8		77.1±35.4		81.3±34.9	
Fat (%)	34.0±7.0		35.4±6.5		33.4±9.0		34.9±9.8	
Carbohydrate (g)	305.4±123.2		291.3±105.9		273.4±88.5		250.6±96.5	
Carbohydrate (%)	55.9±7.2	101	51.5±6.0	93.6	55.7±9.8	101.2	51.4±11.2	93,4
Saturated fat (g)	27.5±9.0		32.2±14.0		26.2±7.3		28.0±14.0	
Fiber (g)	21.4±9.1	73	20.4±10.3	70	24.8±15.7	95	21.2±10.8	81
Cholesterol	194.7±152.4		305.9±153.7		122.8±103.1		245.6±205.4	
Vitamine A(mcg)	897±325.2	149	1048±589	116	737.3±331.4	122	913.9±468.4	130
Vitamine E (mg)	18.4±9.6	167	16.4±11.6	109	14.1±10.5	128	16.8±10.5	112
Vitamine B_1 (mg)	0.87±0.35	96	0.76±0.37	63*	0.77±0.27	85	0.75±0.24	75
Vitamine B_2 (mg)	1.0±0.25	111	1.28±0.54	98	1.04±0.33	115	1.21±0.53	134
Vitamine B_6 (mg)	1.52±0.6	152	1.79±2.23	137	1.43±0.59	143	1.24±0.37	103
Vitamine B_{12} (mcg)	2.02±1.37	112	4.0±2.3	166	2.72±1.53	151	3.48±3.15	145
Vitamine C (mg)	85.1±39.5	113	69.1±51.4	92	83.9±73.2	111	82.9±50.8	110
Total folic acid (mcg)	258.4±94.4	86	268.3±114.6	66*	210.5±83.2	52*	236.8	59*
Calcium (mg)	553.6±152.5	42*	738.2±340.6	56*	580.0±294.3	44*	642.1±303.5	49*
Iron (mg)	11.3±4.9	113	11.9±5.1	119	11.9±4.8	119	11.5±3.8	63*
Sodium (mg)§	3830.8±1864.3	166	4673.9±1980.2	203	3102.0±1672.0	135	4061.3±2020.9	177

**Values are below the requirement (<67 %). § Does not contain sodium from the salt*

The percentages of contribution total consumption of milk and dairy foods across from the media tools is were 11.9±27.3% and 33.9±41.1% of daily consumption in male and female adolescents, respectively. Female adolescents consumed more milk and dairy products than males while using media tools (51.6±81.9 and 26.3±71.2, respectively, p<0.05). Additionally, the contribution to daily consumption of the milk and dairy products that female adolescents consumed while using media tools was found to be significantly greater than males (p<0.05). The total consumption of meat and similar foods during the use of media tools was determined as 21.3±32.6 g in male adolescents and 29.2±46.7 g in females (p>0.05). However, the consumption of red meat during the use of media tools in females (8.1±20.2 g) was found to be significantly more than in males (3.4±18.7 g) (p<0.05). The contribution to bread consumption during the use of media tools to daily consumption was found to be significantly higher in females (38.7±43.8%) than in males (11.7±27.5%) (p<0.05). Similarly, the contribution of saturated fat consumption during the use of media tools to daily consumption was significantly higher in female adolescents than in males (46.2±47.7% and 18.3±37.0%, respectively) (p<0.05). The total amount of sweets consumed during the use of media tools was found to be significantly higher in females than in males (17.6±22.2 g and 7.2±14.2 g, respectively, p<0.05) (Table 4).

Table 4: The average consumption of food groups by adolescents during the use of media tools and contribution of daily consumption (%) (n=73)

	Consumption of during the use of media tools ($\bar{x}$±SD)			Percentage of contribution to daily consumption ($\bar{x}$±SD)		
Food groups (g)	**Male**	**Female**	**p**	**Male**	**Famele**	**p**
Milk and dairy products						
Total	26.3±71.2	51.6±81.9	0.038*	11.9±27.3	33.9±41.1	0.009**
Milk, yogurt	19.8±48.7	44.6±79.9	0.192	15.1±30.8	34.9±45.1	0.157
Cheese	6.5±34.5	6.9±13.6	0.093	9.0±26.8	31.2±43.9	0.039*
Meat and egg group						
Total	21.3±32.6	29.2±46.7	0.656	16.2±23.7	35.5±47.3	0.178
Red meat	3.4±18.7	8.1±20.2	0.023*	7.9±29.8	20.4±36.1	0.123
Egg	1.5±3.5	2.1±6.7	0.657	6.5±19.8	19.4±37.9	0.539
Legumes	0.7±4.2	6.5±31.2	0.521	11.1±33.3	20±42.1	0.606
Nuts and oily seeds	15.6±24.0	12.4±22.6	0.592	48.0±51.8	57.4±47.5	0.315
Vegetables and fruits						
Vegetables	19.1±60.9	12.5±44.2	0.522	21.8±29.2	28.6±39.0	0.223
Gren leafy vegetables	2.0±8.7	0.5±1.7	0.954	13.9±34.2	13.5±32.2	0.945
Fruits	91.1±140.9	132.8±264.3	0.595	46.2±46.5	39.7±44.3	0.609
Citrus	4.0±24.6	-	0.324	16.6±40.8	-	0.317
Bread and cereal						
Bread	22.0±57.2	41.4±55.3	0.019*	11.7±27.5	38.7±43.8	0.003**
Rice, pasta, flour	25.8±50.2	44.5±84.7	0.283	16.2±28.2	36.0±44.2	0.131
Cake and biscuits	38.9±54.8	28.1±49.9	0.141	73.5±40.5	50.3±47.2	0.087
Fats and oils	2.5±6.1	5.0±12.3	0.286	11.6±25.7	28.0±40.5	0.093
Fats	1.0±3.3	2.1±3.8	0.2	18.3±37.0	46.2±47.7	0.028*
Oils	1.4±4.9	2.9±10.8	0.935	10.8±28.9	14.0±31.9	0.576
Sweets	7.2±14.2	17.6±22.2	0.040*	36.4±43.1	53.7±44.4	0.143

**p<0.05, **p<0.01*
 # The Mann-Whitney U test

Table 5: The status of correlation with regard to some anthropometric measurements regarding adolescents, dietary habits, and the intake of energy and other nutrients

Variable	BMI	Waist circumference	Duration of stay at internet cafe
Waist circumference (cm)	0.677**		0.206
	p=0.000		p=0.080
Energy intake (kcal/day)	0.368*	0.259*	0.114
	p=0.001	p=0.027	p=0.336
Fat (g/day)	0.398**	0.224	0.049
	p=0.000	p=0.057	p=0.683
Energy intake during the use of media tools (g/day)	0.294*	0.050	0.278
	p=0.011	p=0.673	p=0.024*
Fat intake during the use of media tools (g/day)	0.399**	0.053	0.195
	p=0.000	p=0.654	p=0.099
Number of main meals	-0.066	-0.052	0.219
	p=0.581	p=0.661	p=0.063
Number of snacks	0.032	0.053	0.329*
	p=0.809	p=0.686	p=0.010
Total energy expenditure (kcal/day)	0.194	0.137	0.029
	p=0.099	p=0.248	p=0.810

**p<0.05, **p<0.01 (BMI: body mass index)*

Table 5 is shown that the status of correlation with regard to some anthropometric measurements regarding adolescents, dietary habits, and the intake of energy and other nutrients. There is a positive relationship between the waist circumference and BMI ($p<0.05$). Also, as BMI and waist circumference increase, the daily consumed energy increases, too ($p<0.05$). A positive relationship was determined between energy consumed during the use of media tools and the BMI values of the adolescents ($p<0.05$). A significant, positive correlation was also determined between the amount of fat consumption provided by foods during the use of media tools and the BMI values of adolescents ($p<0.05$). There was also a significant, positive relationship found between the energy intake during the use of media tools and the duration of stay at the Internet cafe ($p<0.05$). Although there was no significant relationship between the number of main meals consumed each day and the duration of stay at the Internet cafe, there was a significant, positive relationship between the number of snacks and the duration of stay at the Internet cafe. As the duration of stay at the Internet cafe increased, the number of snacks also increased ($p<0.05$) (Table 5).

Discussion

The increase in the rates of obesity in adolescence originates from a significant increase in the sedentary behaviors especially including the use of technological tools in the past twenty years (18). With the use of media tools, it's not just the issue of the replacement of sedentary activity with physical activity but the issue of the exposure of youth to stimulants that at the same time encourage unhealthy foods that has been confronted (7). It is known that each additional hour to the total amount of time spent watching television in a day increases the status of being overweight by 20-30% (19). Stroebele et al. (20) demonstrated that watching television is correlated with an increase in the frequency of meals and with the consumption of more energy. They also found that consumption of foods while watching television reduces the time spent on physical activity, that participants who watched more television had greater body weights and a larger BMI compared with those who watched less television and had a greater daily consumption of fat. This study found similarly that as the amount of time spent at the Internet cafe increased, the number of snacks and the energy intake during the use of media tools increased. There is also a positive, significant relationship between

the energy intake during the use of media tools and the BMI values of the adolescents ($p<0.05$). For this reason, the use of media tools should be considered in the prevention of obesity in children, just like in other sedentary activities. The increase of the number of snacks consumed during the use of media tools and the constitution of foods consumed in snacks with high-energy content, low-nutritional value snacks leads to a significant increase in the intake of energy. For this reason, long-term use of media tools is causally connected with a gradually increasing prevalence of obesity in adolescents because it leads to a greater intake of energy and to the rate of energy from fat being greater and because it affects their nutritional preferences.

The media environment changes as the days pass, and within the past five years, the rate of children and adolescents between the ages of 8-18 who have a computer in their homes rose from 73% to 86%, and the rate of homes with an Internet connection rose from 47% to 74%. Video games have been improved and begun to be made more realistically in order to influence children and adolescents (21). As a result, adolescents spend more than what they spend in school or sleeping using various media tools (22). In this study, it was found that 75.3% and 83.6% of adolescents use the computer more than the recommended time (<2 hours) on weekdays and weekends, respectively, and that 80.8% and 90.4% watch more than the recommended amount of television on weekdays and on the weekends, respectively. The increase today of the number and accessibility of media tools like television, computers and computer games, and the internet also increase the inactive amount of time that children and adolescents spend in front of these tools. Borraccino et al. (23) showed that more than 80% of adolescents aged 15 years have more than 2 hours of sedentary, screen-related activities each day. Watching television for long periods of time contributes to the increasing rates of childhood and adolescent obesity by leading to the exposure of children and adolescents to commercials for unhealthier foods and to their being physically inactive. The American Academy of Pediatrics (24), recommends for this reason that children and adolescents limit their time spent watching television to 2 hours.

The global prevalence of childhood obesity increases with each passing day. While the prevalence of childhood obesity was 4.2% in 1990, it reached 6.7% in 2010. It is estimated that this increase will continue and that 9.1% of children around the world will be obese in the year 2020. It has also been shown that the prevalence of overweight and obesity in developed countries is twice as great as that in developing countries and that children are largely affected (11.7% overweight, 6.1% obese) (8). The prevalence of overweight and obesity were determined 15.1% and 9.6% in this study, respectively. While the prevalence of obesity has been determined as 16.2% in male adolescents and 2.8% in females, the condition of overweight has been determined as 10.8% in male adolescents and 19.4% in females. Similarly, in the scope of the WHO European Childhood Obesity Surveillance Initiative (COSI) study, the prevalence of overweight and obesity in 7-8year old, school-aged children in Turkey (Childhood (ages 7-8) Obesity Research (COSI-TUR) of Turkey 2013) were found to be 14.2% and 8.3%, respectively (25). Good dietary habits are an important part of a healthy lifestyle (26) and a protective factor in the prevention of obesity (27). Healthy nutrition in the early stages of life like childhood and adolescence is very important and should be encouraged (12). When the daily food consumption of adolescents was asked in this study, it was determined that the consumption of vitamin B_1 (thiamin) was lower than the normal limits (67%-133%) of the percentage to meet the requirement. Problems like nervous and digestive system disorders, reduced appetite, and fatigue may emerge with a deficiency of vitamin B_1. Moreover, the percentage that met the requirement of total folic acid consumption in the female adolescents in the 11-13 and 14-18 age groups was found to be below normal limits. Insufficient folic acid intake and folic acid deficiency can result in an increase of sensitivity to leucopenia, intestinal malabsorption, impaired blood clotting, and infection and in macrocytic anemia, which is the second most common type of nutritional anemia after iron deficiency (28). The percentage that meet the requirements in all age groups for the consumption of calcium by the adolescents in the study was found to be low. Similarly, Assumpçao et al. (29) determined that they conducted over adolescents between the ages of 10 and 19 that 88.6% of the adolescents were unable to meet the recommended intake of calcium in a study. Calcium is a nutrient necessary in the provision of bone mineralization and in the protection of bone health. Bone mass reaches forty times

its size from birth to adulthood and peaks at the end of puberty. For this reason, the sufficient intake of calcium in adolescence is an important factor in the creation of adequate bone mass and in the protection of bone health (29). Together with growth during adolescence, some nutrient requirements also increase. One of these nutrients is iron (12). In this study found that the female adolescents in the 14-18 age group were unable to meet the recommended intake of iron. The risk of anemia is quite high especially in the girls in this age group because of low iron intake and at the same time due to the loss of blood that occurs during the menstrual cycle. Iron deficiency may originate from an insufficient dietary intake of iron or from absorption disorders (30). Foods right in iron should be added to the diets of adolescents for this reason. Micronutrients are quite important for growth and development, and insufficient consumption is associated with various diseases and developmental risks. Therefore, their deficiencies may lead to growth developmental retardation and bone disorders in adolescents (12). Additionally, this study found the consumption of dietary sodium - except for salt - to be greater than necessary in all age groups. This situation is very important in terms of adolescent health. It creates risk for many chronic diseases like sodium hypertension and cardiovascular diseases (31). The sodium content of the diets of adolescents should be carefully examined and initiatives introduced to decrease sodium content.

In this study, the percentage that contribution daily consumption of the total consumption of dairy products during the use of media tools by adolescents was found to be much greater in females than males ($p<0.05$). The percentage of the contribution daily consumption of the consumption of meat foods during the use of media tools was determined as 35.5±47.3% in females and 16.2±23.7% in males ($p>0.05$). Additionally, the consumption of fatty seeds during the use of media tools constitutes more than half of daily consumption in females (57.4±47.5%) and almost half in males (48.0±51.8%). Along with this, adolescents do not consume any chicken or fish during the use of media tools. The consumption of eggs and legumes were also found to be quite low during the use of media tools. The percentage of the contribution to the total daily consumption of vegetables by the consumption of vegetables during the use of media tools was found to be 21.8±29.2% in males and 28.6±39.0% in females. The total consumption of fruit during the use of media tools was greater in contrast to conducted studies, and the consumption of citrus was found to be less. The most consumed foods from the wheat group during the use of media tools were varieties of cakes, pastries, and cookies. The consumption of bread during the use of media tools was found to be greater in females than in males ($p<0.05$). The contribution to daily consumption of the fat consumption was also found to be greater in females than in males ($p<0.05$). The contribution to the daily consumption of sweets was again found to be 36.4±43.1% in males and 53.7±44.4% in females. In light of this information, it can say that the consumption of fatty seeds, fruits, and varieties of cakes, pastries, and cookies during the use of media tools by adolescents constitutes a significant portion of daily consumption, that they consume more fat and sugar relating to this, that they consume less eggs, legumes, and less vegetables and citrus, and that they do not consume any chicken and fish. Falbe et al. (32) reported that each increase of an hour in the total use of electronic media is associated with the increase in the consumption of unhealthy foods like sugary drinks, fast-food, and sweet and salty snacks and with a decrease in the consumption of vegetables and fruits. Based on the results of this study, the use of media tools is associated with a greater consumption of snacks and unhealthy foods. The use of media tools may lay the groundwork for the development of obesity in adolescents by leading to a greater consumption of energy, a greater intake of fatty foods, and spending less energy, due to it being associated with either unconscious eating or replacing physical activity and due to it affecting the variety of foods consumed. For this reason, the effect of media tools on childhood and adolescent obesity should be carefully examined, the amount of time spent in front of media tools should be limited, and low-energy content, high-nutrient content foods like fresh fruits and vegetables should be preferred in place of unhealthy foods that are consumed while using media tools.

References

1. Bibiloni M, Pich J, Cordova A, Pons A, Tur A. Association between sedentary behaviour and socioeconomic factors, diet and lifestyle among the Balearic Islands adolescents. BMC Public

Health 2012; 12: 718.
2. Craig LC, McNeill G, Macdiarmid JI, Masson LF, Holmes BA. Dietary patterns of school-age children in Scotland: association with socio-economic indicators, physical activity and obesity. Br J Nutr 2010; 103: 319-34.
3. Chaput JP, Klingenberg L, Astrup A, Sjödin AM. Modern sedentary activities promote overconsumption of food in our current obesogenic environment. Obes Rev 2011; 12: e12-20.
4. Coon KA, Goldberg J, Rogers BL, Tucker KL. Relationships between use of television during meals and children's food consumption patterns. Pediatrics 2001; 107: E7.
5. Von Post-Skagegard M, Samuelson G, Karlström B, Mohsen R, Berglund L, Bratteby LE. Changes in food habits in healthy Swedish adolescents during the transition from adolescence to adulthood. Eur J Clin Nutr 2002; 56: 532-8.
6. Bickham D, Blood EA, Walls CE, Shrier LA, Rich M. Characteristics of screen media use associated with higher BMI in young adolescents. Pediatrics 2013; 131:935-41.
7. Scully M, Wakefield M, Niven P et al. Association between food marketing exposure and adolescents' food choices and eating behaviors. Appetite 2012; 58: 1-5.
8. de Onis M, Blossner M, Borghi E. Global prevalence and trends of overweight and obesity among preschool children. Am J Clin Nutr 2010; 92:1257-64.
9. Sağlık Bakanlığı Sağlık Araştırmaları Genel Müdürlüğü. Hacettepe Üniversitesi Sağlık Bilimleri Fakültesi Beslenme ve Diyetetik Bölümü. Ankara Numune Eğitim ve Araştırma Hastanesi. Türkiye Beslenme ve Sağlık Araştırması 2010: Beslenme Durumu ve Alışkanlıklarının Değerlendirilmesi Sonuç Raporu. Ankara: Sağlık Bakanlığı Sağlık Araştırmaları Genel Müdürlüğü; 2014, Rapor No: 931.
10. Ogden CL, Caroll MD, Kit BK, Flegal KM. Prevalence of childhood and adult obesity in the United States, 2011-2012. Jama 2014; 311: 806-14.
11. Sahoo K, Sahoo B, Choudhury AK, Sofi NY, Kumar R, Bhadoria AS. Childhood obesity: causes and consequences. J Family Med Prim Care, 2015; 4: 187-92.
12. Baysal A, Aksoy M, Bozkurt N et al. Diyet El Kitabı. 5. baskı. Hatipoğlu Yayınevi. Ankara,2008.
13. Watson JT, Gayer M, Connolly MA, Epidemics after Natural Disasters. Emerging Infectious Diseases 2007;13: 1-5.
14. Rakıcıoğlu N, Tek NA, Ayaz A, Pekcan G. Yemek ve Besin Fotoğraf Kataloğu. Ata Ofset Matbaacılık. Ankara, 2006
15. Schmid M, Beslenme Bilgi Sistemi BeBis 7.0 paket programı. Entwickelt an der Universital Hohenheim, Stuttgart, 2011.
16. T.C. Sağlık Bakanlığı T.S.H.G.M. ve Hacettepe Üniversitesi Beslenme ve Diyetetik Bölümü. Türkiye'ye Özgü Beslenme Rehberi. Ankara, 2015.
17. Alpar R. Spor, Sağlık ve Eğitim Bilimlerinden Örneklerle Uygulamalı İstatistik ve Geçerlik-Güvenirlik. 3. Baskı. Detay Yayıncılık. Ankara, 2010.
18. Rennie KL, Johnson L, Jebb SA. Behavioural determinants of obesity. Best Pract Res Clin Endocrinol Metab 2005; 19: 343-58.
19. Steffen LM, Dai S, Fulton JE, Labarthe DR. Overweight in children and adolescents associated with TV viewing and parental weight: Project HeartBeat! Am J Prev Med 2009; 37: 50-5.
20. Stroebele N, de Castro JM. Television viewing is associated with an increase in meal frequency in humans. Appetite 2004; 42: 111-3.
21. Roberts DF, Christenson PG, Strange J. Prime-time teens. Burbank, CA: Mediascope, 2004.
22. Strasburger VC, Jordan AB, Donnerstein E. Children, adolescents, and the media: health effects. Pediatr Clin North Am 2012; 59: 533-87.
23. Borraccino A, Lemma P, Berchiala P et al. Unhealthy food consumption in adolescence: role of sedentary behaviours and modifiers in 11-, 13- and 15-year-old Italians. Eur J Public Health 2016; 26: 650-6.
24. American Academy of Pediatrics: Children, adolescents, and television. Pediatrics, 2001; 107: 423-6.
25. Türkiye Çocukluk Çağı (7-8 yaş) Şişmanlık Araştırması (COSI-TUR) 2013. Available from: http://beslenme.gov.tr/content/files/yayinlar/kitaplar/diger_kitaplar/cosi_temel_bulgular.pdf.
26. Neslisah R, Emine AY. Energy and nutrient intake and food patterns among Turkish university students. Nutr Res Pract 2011; 5: 117-23.
27. Bell MJ, Zeiler M, Herrero R et al. Healthy Teens @ School: Evaluating and disseminating transdiagnostic preventive interventions for eating disorders and obesity for adolescents in school settings. Internet Interventions 2018;16:65-75.
28. Fiorentino M, Landais E, Bastard G, Carriquiry A, Wieringa FT, Berger J. Nutrient Intake Is Insufficient among Senegalese Urban School Children and Adolescents: Results from Two 24 h Recalls in State Primary Schools in Dakar. Nutrients 2016; 8: E650.
29. de Assumpcao D, Dias MR, de Azevedo Barros MB, Fishberg RM, de Azevedo Barros Filho A. Calcium intake by adolescents: a population-based health survey. J Pediatr (Rio J) 2016; 92: 251-9.
30. Broniecka A,Wyka J, Bronkowska M, Piotrowska E, Biernat J. Assessment of intake of iron and nutrients that affect bioavailability of daily food rations of girls. Ann Agric Environ Med 2014; 21: 327-30.
31. Rakicioğlu N, Topçu AA, Javidipour I. Ankara'daki Bazi Fast-Food Restoranlarinda Satilan Hamburgerlerin Besin Öğesi İçeriği ve Beslenme Açisindan Değerlendirmesi. Deneysel Biyoloji Dergisi 2000;57: 7-12.
32. Falbe J, Willett WC, Rosner B, Gortmarker SL, Sonneville KR, Field AE. Longitudinal relations of television, electronic games, and digital versatile discs with changes in diet in adolescents. Am J Clin Nutr 2014; 100:1173-81.

Correspondence:
Neslişah Rakıcıoğlu, PhD
Hacettepe University, Faculty of Health Sciences, Department of Nutrition and Dietetics,
06100, Ankara, Turkey
E-mail: neslisah@hacettepe.edu.tr

Progress in Nutrition 2020; Vol. 22, N. 1: 68-74 DOI: 10.23751/pn.v22i1.8243

Original article

Assessment of microbiological quality of ready-to-eat foods in institutions providing mass feeding

Ayhan Dağ

Department of Nutrition and Dietetics, Faculty of Health Science, Cyprus International University, Lefkoşa, Northern Cyprus, E-mail: adag@ciu.edu.tr

Summary. *Objective:* This study was designed to evaluate microbiological quality of ready-to-eat foods in institutional mass feeding and to determine their risk for food poisoning. In the study, 275 ready-to-serve food samples were evaluated in institutional feeding. The food groupings in the Turkish Food Codex Regulation Supplementary Document I and parameters established in food safety criteria in Supplementary Document I and III were adopted. The samples were analyzed for parameters about coagulase-positive Staphylococci (TS EN ISO 6888-1/A1), *B. cereus* (TS EN ISO 7932), *E. coli* (TS ISO 16649-2), Staphylococcal enterotoxins (AOAC OMA) 2007.06 - VIDAS SET 2), *Salmonella spp*. (TS EN ISO 6579-1), *L. monocytogenes* (TS EN ISO 11290-1) and mold - yeast (TS ISO 21527-1/2) by reference methods. Of the samples analyzed, 250 samples (90.9%) were found appropriate for consumption while 25 samples (9.1%) as inappropriate according to Microbiological Criteria Regulation. It was concluded that meats served warm carry risk for *L. monocytogenes, B. cereus* and *E. coli* parameters while salads-appetizers served cool carry risk for *L. monocytogenes* and *Salmonella spp*. and deserts, particularly cream cakes, carry risk for *E. coli* and mold - yeast parameters.

Key words: ready-to-eat food, hygiene, microbiological quality, public health

Introduction

Nutrition, providing basis for maintaining life, is a process that involves intake, digestion, absorption and metabolizing nutrients required for function of body with foods (1, 2). Mass feeding defined as feeding of individuals out of home by foods and beverage planned and prepared from a center has become increasingly important (3-5).

There are several organizations serving food out of home. Food services previously given in restaurants and eating houses have evolved to mass feeding industry through provision of food services to employees with own kitchen and dining hall by institutions where substantial number of individuals employed and emergence of food factories providing food services to these institutions (4-6).

The demand for mass feeding is increased by increasing number of working individuals, participation of women into professional life, acceleration of urbanization, alterations in standards, economic and sociocultural environment, need for and interaction with comfort and ready-to-eat food (6-8).

The incidence of food poisoning is higher in institutions providing mass feeding when compared to home conditions since substantial amounts of food is produced in institutions providing mass feeding on contrary to home conditions (7, 8).

Products marketed in food stores and ready to consume instantly are termed as ready-to-eat food (9, 10). These foods are ready to consume as raw or cooked, cold or hot, or without additional heat treatment (9).

Based on data from previous studies, more than 70% of foodborne diseases are linked to food service or

catering sectors. Microorganisms that causes contamination during a cascade of processes ranging from food production to delivery to consumers can lead impaired sensory properties, economical losses and foodborne diseases by growing rapidly in case of suitable conditions (11-13). In this process, staff hygiene is one of the most important steps of chain of hygiene. In addition, other contamination sources include chopping boards, slicers, mixers, grinders, water and air used in the stages of food production and processing as well as waste, insects, rodents and pets that should not be in the production media (12).

In a study by Aksu et al., it was suggested that food poisoning caused by ready-to-eat foods mainly occurred in hotels, restaurants, school or student residence which provide food services (14).

Lack of microbiological safety of food remains to be a global public health issue. The attempts to take novel measures to improve food safety have been made in response to increase in foodborne diseases globally. Some arrangements have been made in regulations to establish a "Food Safety Management System" for manufacturers in the field of agriculture; as such, it is aimed to prevent foodborne outbreaks (15). In our country, the most recent regulation for this purpose is Turkish Food Codex Microbiological Criteria Regulation established by Republic of Turkey, Agriculture and Livestock Ministry (16).

The aim of this study is to investigate the microbiological quality of ready-to-eat meals offered in mass feeding institutions and to determine the risk of food poisoning.

Materials and Methods

In this study, 275 food samples were taken from ready-to-eat foods served in 15 institutions providing mass feeding in Ankara between December, 2018 and February 2019. Samples of ready-to-eat meals were taken from non commercial (school, workplace, etc.) and commercial nutritional institutions (restaurants, cafes, etc.).

Food samples (100 g each) were taken into sterile containers in aseptic conditions and transferred to laboratory in transport vessels containing pack ice within 4 hours. The samples were analyzed within the same day by taking parameters established in food safety criteria in Turkish Food Codex Microbiological Criteria Regulation Document I and III into account. In our study, the ready-to-eat food samples analyzed were grouped according to section in 1.13 Ready-to-Eat Foods in Supplementary Document I of Turkish Food Codex Microbiological Criteria Regulation: 1.13.1. all kinds of ready-to eat cooked meat and vegetables etc. (grilled chicken thighs served with saffron rice, mexican chicken, meat sauteed, okra with meat, green peas with meat, beef barbecue, chicken schnitzel, roast beef, chicken thighs with broccoli and carrots, kebab with mashed potatoes, roasted meatballs); 1.13.2. all kinds of ready-to eat salads, delicatessen products and cold appetizers etc. (potato salad, amasra salad, coleslaw salad, olive oil bean, iceberg salad, mediterranean salad); 1.13.3. all kinds of ready-to-eat cooked bakery products including all kinds of pastry, pancake with spicy meat filling, pita, pizza, Turkish ravioli etc. (phyllo pastry stuffed with cheese, pasta with yogurt, pasta with basil sauce, rice pilaf, baked pasta with béchamel sauce and cheese, noodle with walnuts and cheese); 1.13.4 all kinds of ready-to-eat cooked deserts including pudding, milk pudding, cream cake, Noah's pudding and etc. (fruit cup, chocolate cake, tiramusu, eclair pie, pudding, rice pudding, semolina dessert with milk and chocolate). The samples were analyzed according to limits and parameters described in food safety criteria in Turkish Food Codex Microbiological Criteria Regulation Supplementary Document I and III. The parameters included coagulase-positive Staphylococci, *B. cereus, E. coli,* Staphylococcal enterotoxins, *Salmonella spp., L. monocytogenes* and mold - yeast. Reference methods were used for analyses, including TS EN ISO 11290-1 for *L. monocytogenes,* TS ISO 21527-1/2 for mold - yeast, TS EN ISO 6888-1/A1 for coagulase positive staphylococcus, TS EN ISO 7932-Mannitol Yolk Polymyxin (MYP) agar for *B. cereus* count, TS EN ISO 6579-1-Mini Vidas method for *Salmonella spp.* Count, Association of Official Analytic Chemists Official Methods of Analysis (AOAC OMA) 2007.06-VIDAS SET for Staphylococcal enterotoxins and TS ISO 16649-2 for *E. coli* count (16). Microbiological analyzes of ready-to-eat meals were carried out once.

In the microbiological analysis, the change in units (MPN/g or cfu/g) resulted from analytical methods. The methods were determined according to limits in Turkish Food Codex. If limit value was low (for example, <3 for E. coli), EMS method was used.

Results

Table 1 presents distribution of 275 ready-to-eat foods sampled for microbiological analyzes according to food groups. Of 275 samples, 64 (23.63%) were all kinds of ready-to eat cooked meat and vegetables etc. whereas 90 (30.72%) were all kinds of ready-to eat salads, delicatessen products and cold appetizers etc.; 25 (9.10%) were all kinds of ready-to-eat bakery products including all kinds of pastry, pancake with spicy meat filling, pita, pizza, Turkish ravioli and etc; and 79 (28.73%) were all kinds of ready-to-eat deserts including pudding, milk pudding, cream cake, Noah's pudding etc. (16).

Table 2 presents groupings of ready-to-eat foods according to Turkish Food Codex Microbiological Criteria Regulation (16) and their appropriateness. Of the food samples analyzed, 250 samples (90.9%) were found appropriate for consumption while 25 samples (9.1%) as inappropriate according to Microbiological Criteria Regulation.

It was found that, of 25 ready-to-eat food product found to be inappropriate for consumption, 3 (4.6%) were cooked meat and vegetable dishes whereas 8 (8.9%) were salads, delicatessen products and appetizers; 2 (8.0%) were cooked bakery products) 11 (13.9%) were deserts (milk puddings, cream cakes, pudding etc.) and one (6.25%) were other foods (sauces, kashar cheese).

No coagulase positive Staphylococci or Staphylococcal enterotoxin was detected in 275 ready-to-eat food samples analyzed in our study. Table 3 presents distribution of 25 food samples found to be inappropriate for consumption according to food groups and microbiological parameters. Of these samples, 5 were found inappropriate according to L. monocytogenes while 2 according to *Salmonella spp*., 10 according to *E. coli,* one according to *B. cereus* and 7 according to mold - yeast parameters. Of samples found to be inappropri-

Table 1. Distribution of ready-to-eat food samples according to food groups

Food Group	Number of samples	%
All kinds of ready-to eat cooked meat and vegetables etc.	65	23.63
All kinds of ready-to eat salads, delicatessen products and cold appetizers etc.	90	32.72
All kinds of ready-to-eat cooked bakery products including all kinds of pastry, pancake with spicy meat filling, pita, pizza, Turkish ravioli etc	25	9.10
All kinds of ready-to-eat cooked deserts including pudding, milk pudding, cream cake, Noah's pudding and etc.	79	28.73
Other	16	5.82
Total	275	100.0

Table 2. Assessment of food groups according to results of analyses

Food Group	Sample	Appropriate		Inappropriate	
	Number	Number	%	Number	%
All kinds of ready-to eat cooked meat and vegetables etc.	65	6	95.4	3	4.6
All kinds of ready-to eat salads, delicatessen products and cold appetizers etc.	90	82	91.1	8	8.9
All kinds of ready-to-eat cooked bakery products including all kinds of pastry, pancake with spicy meat filling, pita, pizza, Turkish ravioli etc	25	23	92.0	2	8.0
All kinds of ready-to-eat cooked deserts including pudding, milk pudding, cream cake, Noah's pudding and etc.	79	68	86.1	11	13.9
Other	16	15	93.75	1	6.25
Total	275	250	90.9	25	9.1

Table 3. Distribution of inappropriate microbiological parameters in ready-to-eat foods according to food groups

Microbiological Parameter		***L. Monocytogenes***	***Salmonella Spp.***	***E.coli***	***B. cereus***	***Mold - yeast***
Food Group	**Sample Number**	**Analysis result/ Acceptable limit (in 25 g)**	**Analysis result/ Acceptable limit (in 25 g)**	**Analysis result/ Acceptable limit *<10 cfu/g - <3 MPN/g**	**Analysis result/ Acceptable limit 1.000 cfu/g**	**Analysis result/ Acceptable limit 1.000 cfu/g**
All kinds of ready-to eat cooked meat and vegetables etc.	3	Positive	-	10 cfu/g	3.800	-
All kinds of ready-to eat salads, delicatessen products and cold appetizers etc.	8	Posİtİve Posİtİve Posİtİve Positive	Posİtİve	320 cfu/g 30 cfu/g 30 cfu/g	-	-
All kinds of ready-to-eat cooked deserts including pudding, milk pudding, cream cake, Noah's pudding and etc.	11	-	Posİtive	3.6 MPN/g 7.4 MPN/g 9.2 MPN/g 23 MPN/g 20 MPN/g 1.100 MPN/g		>15.000 >15.000 >15.000 >15.000
All kinds of ready-to-eat cooked bakery products including all kinds of pastry, pancake with spicy meat filling, pita, pizza, Turkish ravioli etc	2	-	-	-	-	3.000 >15.000
Other (sauces, kashar cheese	1	-	-	-	-	9.600
Total (Number-%)	25 %100	5 20%	2 8%	10 40%	1 %4	7 %28

ate according to *E.coli;* 6 were desert samples while 3 were salad samples. Of samples found to be inappropriate for *L. monocytogenes* parameters, 4 were salad and appetizer samples while one were meat samples. Again, of the samples found to be inappropriate for mold - yeast parameters, 4 were desert samples while 2 were pasta-rice samples.

Overall, *L. monocytogenes* was found to be positive in 5 samples including one cooked meat sample and 4 salad-appetizer samples, which is not allowed in 25 g of food according to Turkish Food Codex Microbiological Criteria Regulation (16).

The *E. coli* count was found as 1.100 MPN/g in the tiramusu sample among 6 desert samples found to be inappropriate according to *E. coli* parameter. Among deserts, *E. coli* growth was detected particularly in cream cakes such as tres leche cake, chocolate chestnut cake and chocolate cream cake while no growth was detected in milky puddings and semolina desert. Of 3 salad-appetizer samples found to be inappropriate to consumption according to *E. coli* parameter, *E. coli* count was found as 320 cfu/g in potato salad. This was the highest *E. coli* burden in ready-to-eat foods. While preparing potato salad in stripping stage, staff with contaminated hand by microorganisms, particularly by coliform bacteria and coagulase positive Staphylococci, can cause contamination in potato salad.

Salmonella spp. was found to be positive in salad-appetizer (chickpea salad) and desert (chocolate brownie) samples.

B. cereus was found as 3,800 cfu/g in sautéed meat. Mold - yeast growth was found as >15,000 cfu/g in 4 deserts including chocolate cake, eclairs, and tiramisu samples whereas >15,000 cfu/g and 3,000 cfu/g in 2 bakery products including sandwiches and 9,000 cfu/g in a ready-to-eat sauce.

Discussion and Conclusion

In our study, it was found that proportion of ready-to-eat food with appropriate microbiological

quality was 90.9% while proportion of those found to be inappropriate was 9.1% in institutions providing mass feeding. It was also found that bacterial growth detected differed according to type of food. In a study by Ergül et al., it was shown that microbiological quality was good in ready-to-eat foods served in distinct places (92%). Authors reported that proportion of products inappropriate to consumption was 8% and that bacterial growth differed based on type of food (17). In our study, microbiological quality of ready-to-eat foods was found to be comparable to those reported in the study by Ergül et al.

In our study, it was concluded that ready-to-eat foods, particularly meat and vegetables, deserts and salads carried risk for *E. coli, Salmonella spp.,* and *L. monocytogenes* parameters. In addition, no coagulase positive Staphylococci, Staphylococcal enterotoxins and *S. aureus* was detected in foods analyzed, which was considered as favorable.

In several studies, it was seen that *Salmonella, E. coli 0157, L. monocytogenes* and *Campylobacter spp.* frequency was rather low in ready-to-eat foods (18, 19).

In a study by Yalçın and Can, 100 ready-to-eat foods were evaluated for parameters of *Salmonella spp., S. aureus, E. coli* and *B. cereus*. As similar to our study, it was reported that no *S. aureus* growth was detected in these samples (20). In that study, it was reported that there was *S. aureus* (1×10^2 - 4×10^2 cfu/g) in 8 samples, *B. cereus* (1×10^2 - 3×10^2 cfu/g) in 7 samples *E. coli* (1×10^2 - 2×10^2 cfu/g) in 6 samples analyzed. Of these foods, only those with *E. coli* growth showed incompliance to criteria defined in relevant regulation (20). In another study, microbiological features of 30 ready-to-eat doner kebap were investigated (21). In analyses, no *S. aureus* and sulfide-reducing anaerobic bacteria growth was detected in doner kebap samples while it was reported total aerobic mesophilic bacteria count ranged between 10^3 and 10^4 cfu/g, reaching up 10^6 cfu/g in some samples. In the same study, 36.6% of doner kebap samples was found to be negative for *Enterobacteriaceae* while 56.6% for *E. coli*. Bacteria counts were reported to be 10^4 and 10^3 cfu/g in samples with *Enterobacteriaceae* and *E. coli* growth, respectively. In both studies, it was shown that meat dishes can comprise risk for health due to poor hygiene applications despite they are served after cooking. In our study, *E. coli* was detected in a sample from meat meal and bacteria number was found as 10 cfu/g.

In a study by Arıcı et al. (22), it was found that coliform bacteria and *E. coli* were detected in most of ready-to-eat salad samples analyzed and that bacteria number were 10^2-9.2×10^6 and 25-10^4 cfu/g, respectively. In addition, authors reported that *S. aureus* number ranged between 1.2 and 2.8×10^3 cfu/g. In our study, *E. coli* was detected in samples from salad group and bacteria number was found to range from 30 cfu/g to 320 cfu/g. In our study, *L. monocytogenes* was found in 4 and *Salmonella spp.* in one of 90 samples. The high bacterial burden and consequent hygiene risks are well-known in raw vegetables and salads using these vegetables. Chopping and rasping of vegetables during salad preparation will not only promote growth of already present microorganisms but also lead contamination with microorganisms. Thus, salads could be considered as risky foods in term of public health.

In a study by Can and Yalçın (20), microbiological evaluation was performed in 50 cake samples (23). Based on analysis, no *Salmonella spp*. and *L. monocytogenes* was detected in the samples. In our study, no *L. monocytogenes* was detected in cake samples in the desert group and *Salmonella spp*. was found to be positive in only one sample. In our study, 11 desert samples were found to be inappropriate, including cake, eclairs, tiramisu, chocolate brownie, tres leche cake and chocolate chestnut cake. In that study, it was reported that *E. coli* was detected in 4 of cake samples and that bacteria number ranged from 9 to 21 cfu/g. In our study, *E. coli* was detected in 6 of cake samples in the desert group and microorganism number was found as 3.6-1,100 MPN/g.

In the study by Hilal Çolak et al. (24), coliform bacteria was detected in 28 (30.4%) of 92 samples including 7 (28%) of meat meal samples, 3 (20%) of 15 meat-free vegetable meal samples, 6 (30%) of 20 rice samples, 3 (20%) of pasta samples and 9 (52.9%) of 17 mashed potato samples with levels ranging from 5.5×10^2 to 6.2×10^4 cfu/g (24). In our study, *E. coli* was detected 10 (3.6%) of 275 samples including one (1.5%) 65 meat meal sample, 6 (7.6%) of 79 desert samples and 3 (3.3%) of 90 salad-appetizer samples with levels of 10 cfu/g -320 cfu/g and 3.6 MPN/g-1,100 MPN/g. in our study, it was seen that proportion of ready-to-eat food in which *E. coli* was detected was lower. This may

be due to differences in hygiene procedures of institutions and level of knowledge of staff about hygiene.

In a study by Ildız and Çiftçioğlu (25), *E. coli* was detected in 4 (7.69%) of 52 soup samples and 8 (15.09%) of 53 meat meal sample. In our study, ineligibility rate was found as 4.6% in meat dish group, 8.9% in salad-appetizer group, 8% in bakery product group and 13.9% in desert group when assessed regarding ineligibility to all parameters. The ineligibility rates in our study were lower than those reported in the study by Ildız and Çiftçioğlu.

In a study by Aksu (14), coliform bacteria was detected in 15 rice samples (<10-5.4 x 10^4 cfu/g) and 5 pasta samples (3.6 x 10^2 cfu/g -1.6 x 10^3 cfu/g) while *E. coli* was detected in one rice sample containing meat and vegetable. In that study, it was reported that *E. coli* was isolated up to 30% of meat-rich products. In our study, mold - yeast was found as >15,000-3,000 cfu/g in 2 samples from bakery products but no *E. coli* was detected. This may be due to fact study by Aksu dated previous period and novel industrial hygiene and disinfection products are being used to ensure food and personal hygiene during this period. Presence of coliform microorganisms in ready-to-eat food is considered as a marker for failure of heat treatment or re-contamination following heat treatment. In addition, coliform microorganisms can also be found in these foods as a result of inappropriate sanitation procedures (26). Presence of *E. coli* in ready-to-eat foods is an indicator for fecal contamination.

In our study, *S. aureus* was detected in none of samples analyzed. Lack of *S. aureus* in the samples was considered as beneficial for public health. In the contamination of foods with *S. aureus*, the most important is food processing in poor conditions and it generally contaminates foods via staff involved in food services and surfaces having contact with foods (chopping board, knife etc.) (27). Another important way is cross-contamination between raw and cooked foods (26).

In our study, *B. cereus* was detected in one (0.36%) of 275 samples in meat meal group at a level of 3,800 cfu/g. In a study, Ayçiçek et al. (27) reported that *B. cereus* was detected in none of ready-to-eat food samples from different food groups. In our study, rate for *B. cereus* was lower than those reported by Aksu and comparable to those reported by Ayçiçek et al.

In a study on microbiological quality of ready-to-eat raw seafood such as sashimi and sushi, analyses were performed by 8 foodborne pathogen (*B. cereus, E. coli O157: H7, Listeria monocytogenes, Salmonella spp., S. aureus, Vibrio cholerae, V. parahaemolyticus* and *Vibrio vulnificus*) and unacceptable growth levels were detected. Ready-to-eat raw seafood such as Sashimi and sushi can be readily contaminated by bacteria originated from water environment and human reservoirs, comprising risk for food poisoning (28).

In conclusion, it was found that microbiological quality is generally sufficient (90.9%) in ready-to-eat foods analyzed. It was seen that type of parameters in which ready-to-eat foods were found to be ineligible varied according to food type.

In our study, ready-to-eat foods marketed and served hot carried risk for *L. monocytogenes, B. cereus* and *E. coli* parameters while products served cool such as salad-appetizer carried risk for *L. monocytogenes* and *Salmonella spp*. parameters and cream cakes in desert group carried risk for *E. coli* and mold - yeast parameters. Presence of *E. coli, Salmonella spp., L. monocytogenes* and *B. cereus* in ready-to-eat foods at varying levels is considered as risk for public health.

To eliminate these risks, one should adopt measures of food and personnel hygiene and adequate disinfection and cleaning of equipments and tools used in kitchen should be performed. The awareness of hygiene should be improved by training kitchen staff about hygiene.

Core temperature should reach up to 75˚C - 80˚, which is considered as safe temperature during cooking phase. In re-heated foods, core temperature should also reach up to 75˚C - 80˚ which must be maintained for 2 minutes. Chafing food for warm service can be maintained maximum 2 hours and temperature should not fall below 63˚C. In foods undergoing cooling process, temperature should be cooled rapidly as being 21˚C within 2 hours and <4˚C within 4 hours.

References

1. Baysal, A. Beslenme. Hatipoğlu Yayınevi, 18. Baskı, Ankara, 2018, p.9-12.
2. Kutulay, Merdol, T., Dağ, A., Baş, M. et al. Genel Beslenme.

T.C. Anadolu Üniversitesi Yayını No:2768. 3. Edition. 2018, p.45-60.
3. Brown, J.E. Nutrition Now. West Publishing Company, 8 th Edition, 2017, p.1.3-1.7.
4. Kutluay, Merdol. T. Temel Beslenme ve Diyetetik. Güneş Tıp Kitapevleri.2015, p. 423-425.
5. Ninemeier, J. D. Planning and Control for Food and Beverage Operations. Eighth Edition, Educational Instıtute American Hotel and Motel Association, 2013, p.11-13.
6. Davis, B., Lockwood, A., Pantelidis, İ., Alcott, P. Food and Beverage Management. 6th Edition. 2018, p.1-2
7. Beyhan, Y. Toplu Beslenme Sistemlerinin Yönetim ve Organizasyonu. Nobel Tıp Kitapevleri, 2018, p.383-384
8. Palacio, P.J., Theis, M. Food Service Managemet Principles and Practices.13th Edition. 2016, p.4, 38-39, 65-67.
9. Alyaaqoubi SJM, Sani NA, Abdullah A, Rahman RDA. Microbiological quality of selected ready-to-eat food at Hulu Langat district, Malaysia. Prosiding Seminar Kimia Bersama UKM-ITB VIII 9-11 June 2009, Pp. 422.
10. Oranusi US, Braide W. A study of microbial safety of ready-to-eat foods vended on highways: Onitsha- Owerri, South East Nigeria. Int Res J Microbiol, 2012; 3(2): 66-71.
11. Bilgin B, Erkan ÜC. Bir Hazır yemek işletmesinde HACCP sisteminin kurulması. Tekirdağ Zir Fak Derg, 2008; 5(3): 268-81.
12. Fidan F, Ağaoğlu S. Ağrı bölgesinde bulunan lokantaların hijyenik durumu üzerine araştırmalar. YYÜ Vet Fak Derg, 2004; 15(1-2): 107-14.
13. Afshin J, Reza Z, Saeid S. Microbiological study of cocktail sausage during shelf life. Middle-East J Sci Res, 2011; 7(6): 1056-1056.
14. Aksu H. İstanbul'da tüketime sunulan bazı hazır yemeklerin mikrobiyolojik kalitesi üzerine araştırmalar. V. Ulusal Halk Sağlığı Kongresi Kitabı, İstanbul, 1996.
15. Jacxsens L, Kussaga J, Luning PA, Van der Spiegel M, Devlieghere F, Uyttendaele M. A microbial assessment scheme to measure microbial performance of food safety management systems. Int J Food Microbiol, 2009; 134: 113–25.
16. Anonymous. Türk Gıda Kodeksi Mikrobiyolojik Kriterler Yönetmeliği. Ankara: T.C. Gıda Tarım ve Hayvancılık Bakanlığı, 2011.
17. Ergül Ş. S, Sarı H, Ertaş S, Berberoğlu U, Cesaretli Y, Irmak H. Tüketime sunulan çeşitli hazır yemek ürünlerinin mikrobiyolojik kalitesinin belirlenmesi. Türk Hij Den Biyol Derg, 2015; 72(3):199-208.
18. Hosein A, Muñoz K, Sawh K, Adesiyun A. Microbial load and the prevalence of Escherichia coli, Salmonella spp. and Listeria spp. in ready-to-eat products in Trinidad. The Open Food Sci J, 2008; 2: 23-8.
19. Anonymous. Guidelines for assessing the microbiological safety of ready-to-eat foods placed on the market. http://www.hpa.org.uk/ webc/hpawebfile/ hpaweb_c/1259151921557. 31.12.2013.
20. Yalçın H, Can ÖP. Tüketime hazır bazı et yemeklerinin mikrobiyolojik kaliteleri. Erciyes Üniv Vet Fak Derg, 2013; 10(1): 1-6.
21. Bostan K, Yılmaz F, Muratoğlu K, Aydın A. Pişmiş döner kebaplarda mikrobiyolojik kalite ve mikrobiyel gelişim üzerine bir araştırma. Kafkas Univ Vet Fak Derg, 2011; 17(5): 781-6.
22. Arıcı M, Gümüş T, Şimşek O. Hazır salataların hijyenik durumu. Gıda, 2003, 28(6): 571-7.
23. Can ÖP, Yalçın H. Mersin'de tüketime sunulan kremalı pastaların mikrobiyolojik kalitelerinin değerlendirilmesi. Gıda Teknol Elektr Derg, 2011; 6(3): 42-8.
24. Çolak H, Ulusoy B, Bingöl B, Hampikyan H, Muratoğlu K. Tüketime sunulan bazı hazır yemeklerin mikrobiyolojik kalitelerinin incelenmesi. Turk Mikrobiyol Cem Derg, 2007; 37(4): 225-33.
25. Ildız F, Çiftçioğlu G. Toplu tüketim amacıyla üretilen gıdaların patojen mikroorganizmalar yönünden incelenmesi. İ.Ü. Veteriner Fak Derg 1997;23(2):405-412
26. Uğur M, Nazlı B, Bostan K. Gıda Hijyeni. İstanbul: Teknik Yayınları, 2002.
27. Aycicek H, Sarimehmetoğlu B, Cakiroğlu S. Assessment of the microbiological quality of meals sampled at the meal serving units of a military hospital in Ankara, Turkey. Food Control 2004; 15: 379- 384.
28. Kim H.W., Hong Y J., Jo J I., Ha S.D., Kim S.H., Lee H.J., Rhee M.S. Raw ready-to-eat seafood safety: microbiological quality of the various seafood species available in fishery, hyper and online markets. Lett Appl Microbiol. 2017 Jan;64(1):27-34.

Correspondence:
Ayhan Dağ
Department of Nutrition and Dietetics,
Faculty of Health Science, Cyprus International University, Lefkoşa, Northern Cyprus
E-mail:adag@ciu.edu.tr

Progress in Nutrition 2020; Vol. 0, N. 0: 75-83 DOI: 10.23751/pn.v22i1.7801

Original article

Does mediterranean diet correlate with cognitive performance among elderly? A cross-sectional study from Cyprus

Nezire Ince[1], *Pınar Gelener*[2], *Halit Tanju Besler*[1]

[1] Eastern Mediterranean University, Faculty of health Sciences, Department of Nutrition and Dietetic [2] University of Kyrenia, Faculty of Medicine, Neurology Department; Near East University of Medicine, Department of Neurology - E-mail: nezireince@hotmail.com;

Summary. *Purpose:* Today, the importance of the Mediterranean Diet is emphasized due to its beneficial effects on chronic and neurodegenerative diseases, which represent a serious health problem affecting an increasing number of elderly people. Our objective was to investigate whether MeDi influences cognitive functions in the elderly. *Methods:* 541 participants over 50 years of age were selected by "Stratified random sampling" method. Adherence to a Mediterranean Diet was measured using MeDi Adherence Screener. Neuropsychological tests were evaluated by Mini Mental State Examination (MMSE) and Subjective Cognitive Complaints Scale (SCC). Anthropometric measurements like body analysis, handgrip strength, waist and mid-upper arm circumference were also assessed. *Results:* Out of 541 participants, 25.3% had high, 68.4% had medium and 6.3% had low adherence to MeDi. The MMSE scores showed that 79% of the participants were normal, 20% had mild dementia and 1% had severe dementia. The SCC showed that 42% had good, 52% had moderate and 6% had poor subject memory. A weak and positive correlation was found between higher MeDi scores and higher MMSE scores. This correlation was particularly observed in attention-calculation, language and recall. There was a positive correlation between the consumption of canned tuna fish, legumes, dark green leafy vegetables, olives, olive oil and MMSE scores. Additionally age, right hand grip strength and Adherence to MeDi scores were significantly predictors of MMSE scores ($p<0.05$). *Conclusion:* In this study, higher adherence to MEDi is correlated with better cognitive functions. In addition to these results, right hand grip strength, which is an objective measure for frailty, is also correlated with MMSE scores.

Key words: Mediterranean diet, nutrition, cognitive performance

Introduction

Today, the importance of the Mediterranean Diet is emphasized due to its beneficial effects on chronic and neurodegenerative diseases, which represent a serious health problem affecting an increasing number of elderly people (1,2).

There is growing evidence in epidemiologic studies and randomized controlled trials suggesting that adherence to a Mediterranean Diet (MeDi) may be protective against chronic diseases, particularly on cardiovascular diseases and dementia (3).

The Mediterranean Diet is an antioxidant-rich dietary pattern which includes high consumption of unrefined cereals, fruit, vegetables, legumes and olive oil, moderate consumption of dairy products and alcohol and low meat consumption. The primary source of fat is the monounsaturated fatty acids in the form of olive oil (4,5).

Bioactive constituents, including dietary phenolic compounds, have antioxidant activity which reduces tau aggregation and neuroinflammation and interacts with intracellular signaling pathways (6). Oxidative stress and inflammation in particular have a significant effect on cognitive decline and brain ageing (7). Due to the high content of fruits, vegetables and olive oil, MeDi reduces oxidative stress and lipid peroxidation, which may lead to DNA damage

and neuronal death. Tocopherols and polyphenols are the antioxidant compounds of olive oil (8). Another mechanism is the anti-inflammatory effect via from fish and olive oil (3,9). Furthermore the MeDi provides a combination of vitamins E, B_6, B_{12}, folate, carotenoids, which are the other dietary antioxidants which exhibit synergitic neuroprotection against frontal and subcortical systems via memory tasks (8). Also, MeDi has been shown to decrease vascular risk factors, which decreases the risk of dementia (3).

Another possible mechanism is that MeDi increases neurotropic factors, neurotransmission, synaptic plasticity and leads to the elimination of beta amyloid from the brain (10).

Although the causes of dementia and cognitive decline in the elderly are multifactorial, research on modifiable risk factors like MeDi, physical activity and physical strength are important (11). In addition to these modifiable risk factors, frailty, which is a clinical syndrome characterised by an age-related decrease in physical functioning, is associated with cognitive decline, low physical activity, poor muscle strength and low adherence to MeDi (12).

The aim of this study was to investigate the relationship between adherence to a Mediterranean Diet and cognitive functions among elderly people living in North Cyprus.

Methods

Study Population and Design

The study population consisted of 541 male and female Turkish Cypriots aged 50 and over (mean age 60.42±8.71). Eligible participants were selected by stratified random sampling method from different cities (Nicosia, Famagusta, Kyrenia, Guzelyurt, Iskele) in North Cyprus between the years 2016-2017.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures were approved by the Eastern Mediterranean University Scientific Research and Publication Ethics Committee (project number: ETK00-2016-0042). Written informed consent was obtained from all subjects.

Participants were face to face interviewed at home by a specifically trained dietician. Exclusion criteria included any type of diagnosed dementia, also conditions that cause any kind of cognitive impairement such as psychiatric condition, brain injury and using medications known to influence cognition.

The study questionnaire is divided into four sections that cover; general information, Mini Mental State Examination (MMSE) (13), Quantitative Food Frequency Questionnaire (QFFQ) and Mediterranean Diet Adherence Screener (MEDAS) (14).

Assesment of adherence to a Mediterranean Diet

Adherence to a Mediterranean Diet is measured using the validated 14-point Mediterranean Diet Adherence Screener. MEDAS consists of 14 questions, 12 of them was designed to evaluate the frequency of major ingredients of MeDi (olive oil, vegetables, fresh fruits, legumes, fish, nuts) and two items that were related to eating habits (primary oil and meat choice). Zero or one point was assigned to each of the 14 items, one point was given for using olive oil as the principal source of fat for cooking, preferring white meat over red meat, or for consuming: a) four or more tablespoons (1 tablespoon = 10 gram) of olive oil (including that used in frying, salads, meals eaten away from home, etc.); b) two or more servings of vegetables/day; c) three or more pieces of fruit/day (1 piece = 100 gram); d) < 1 serving of red meat or sausages/ day (1 serving = 90 gram); e) < 1 serving of animal fat/day (1 serving = 12 gram); f) < 1 cup (1 cup = 200 mL) of sugar-sweetened beverages/day; g) seven or more servings of red wine/week; h) three or more servings of legumes/week (1 serving = 150gram); i) three or more servings of fish/week (1 serving= 100g); j) fewer than two commercial pastries/ week; k) three or more servings of nuts/week (1 serving = 30gram); or l) two or more servings/week of a dish with a traditional sauce of tomatoes, garlic, onion, or leeks sautéed in olive oil. If the condition was not met, 0 points were recorded for the category. As a result, total MEDAS score could range from 0 to 14 points and was classified as Low (≤ 7points), Moderate (8-9 points) and High (≥ 10 points) Degree of Adherence.

Quantitative Food Frequency Questionnaire (QFFQ)

Dietary information was assessed by the validated quantitative food frequency questionnaire almost every season to consider seasonal differences. The number of servings per frequency was expressed in natural units (for example, slice of bread), household measures (for example, cup or spoon) or grams (cooked vegetables or meat). Pictures of foods were also shown to participants along with common household measuring tools to help participants estimate portion sizes. For all foods, the amount reported by the trained dietician and then was multiplied by the frequency variable to obtain the amount of consumed food item per day (in grams or mL). Frequency of consumption categories considered were: no consumption, once a month, two times per month, once or twice a week, three to four times per week, five to six times per week, every day and every meal. This questionnaire consists of minimum of 130 foods that belong to 5 food groups; milk and dairy products, meat-egg-legumes, fruits and vegetables, bread and cereals and fats-sugar-desserts-beverages. In Table 4, only the foods that are related to the Mediterranean Diet are represented.

Anthropometric Measurements

Among the anthropometric measurements; body weight (BW), body fat percentage and total body water were evaluated by using the Tanita TBF 300 scale to the nearest 0.1 kg without shoes and 0.5-1 kg deducted for clothes. Body Height (BH) while standing against a wall without shoes, waist and mid-upper arm circumference were measured by plastic measuring tape to the nearest 0.1 cm. Finally, hand grip strength was measured using a Takei hand dynamometer. Body mass index (BMI) was calculated as BW/BH2. The test was repeated three times and a mean value was calculated. All measurements were taken by the same trained dietician.

Physical Activity Level

Physical activity level was determined by recording 24-hour physical activity. Mean MET values based on specific activities within corresponding categories were used. Physical activity levels (MET×h) for specific activities were estimated by multiplying reported time (h) by MET (kcal/kg×h). Participants were categorised according to Physical Activity Levels as follows; Sedentary-Low Active (PAL: 1.40-1.69), Moderate Active (PAL: 1.70-1.99) and High Active (PAL: 2.00-2.40).

Cognitive Functions

The Turkish validated version of Mini Mental State Examination (MMSE) (15) was used as a cognitive screening tool, which is a standardised test assesing global cognitive functions. It measures orientation, attention, calculation, recall and language. The cut-point for cognitive function was as follows: 24-30 points: normal range, 23-18: mild dementia,17-0: severe dementia.

Subjective memory complaints were also investigated. The Subjective Cognitive Complaints Scale (SCCs) was used to assess subjective memory problems and forgetfulness. The scores were categorised as follows: 0: no symptoms (good subjective memory), 1-3: low symptoms (moderate subjective memory), 4-6: high symptoms (poor subjective memory).

All cognitive tests were performed and evaluated by trained neurologists.

Statistical Analysis

All statistical analysis was performed using Statistical Package for Social Sciences (SPSS) 21.0.

Descriptive statistics were used for the evaluation of the MMSE, SCCs and Adherence to MeDi scores.

Data was tested for normal distribution using the Kolmogorov-Smirnov, Q-Q plot and skewness-kurtosis tests. As the data showed normal distribution, parametric tests were used.

In order to determine the correlation between the MMSE, SCCs and Adherence to Mediterranean Diet scores, the Pearson correlation coefficient was used.

To test for any independent associations between both demographic-anthropometric factors and MMSE scores, Multivariate analyses were conducted using hierarchical regression, where age, BMI, Mid-Upper Arm Circumference, handgrip strength and physical activity were treated as the main predictors of MMSE scores and adjusted for socio demographic factors. All models were performed by multivariate linear regression. All statistically significant differences were set at $p<0.05$.

Results

The study population consisted of 541 participants (377 female, 164 male) aged over 50 years. The mean age was 60.42±8.71 years. None of the participants had been diagnosed with dementia. The general characteristics of the participants have been displayed in Table 1.

According to the MeDi scores; 25.3%, 68.4% and 6.3% of participants showed high, medium and low adherence to MeDi respectively (Fig.2).

The MMSE scores showed that 79% of the participants were in the normal range (MMSE score >24 points), 20% had mild dementia (MMSE 18-23 points) and 1% had severe dementia (MMSE score ≤17 points) (Fig.1a).

Out of 541 participants, 42% of them had no symptoms (good subject memory), 52% had moderate symptoms (moderate subject memory) and 6% had high symptoms (poor subject memory) according to the Subjective Cognitive Complaint scale (Fig.1b).

When we compared the correlation between the MMSE scores and MeDi, a weak and positive correlation was found between higher MeDi scores and higher MMSE scores. This correlation was particularly in attention-calculation, language and recall ($p<0.05$) (Table 2). Also, a weak and negative correlation was found between higher MeDi scores and SCCs scores ($p<0.05$) (Table 2).

The results of the hierarchical regression analyses are given in Table 3, where age, BMI, Mid-Upper Arm Circumference, handgrip strength and physical activity are treated as the main predictors of MMSE scores. According to Table 3, age ($\beta=-0.24$, $p<0.05$), right hand grip strength ($\beta=0.18$, $p<0.05$) and Adherence to Mediterranean Diet scores ($\beta=0.09$, $p<0.05$) were significant predictors of Mini Mental State scores. Resultantly, a one unit rise in age causes a 0.24 unit decrease in MMSE scores, while a one unit rise in right hand grip strength causes a 0.18 unit rise in MMSE scores and a one unit rise in the MeDi adherence score increases the MMSE scores by 0.09. Furthermore, BMI, mid-upper arm circumference, left hand grip strength and physical activity were not significant predictors of the Mini Mental State scores ($p>0.05$).

Table 1. Socio-Demographic and Health Characteristics of Participants (n=541)

Characteristics	**Number (n)**	**Percentage (%) p<0.05**
Age (Group)		
50-54 years	146	26.99
55-59 years	116	21.44
60-64 years	111	20.52
65 years and over	168	31.05
Gender		
Male	164	30.31
Female	377	69.69
Education		
None	34	6.28
Primary School	202	37.34
Completed high school	170	31.42
University and Postgraduate	135	24.95
Marital Status		
Married	468	86.51
Single or divorced	73	13.49
Work Status		
Working	160	29.57
Not working	381	70.43
Cigarette Smoking		
None	286	52.87
Ex-smoker	158	29.21
Current Smoker	97	17.93
Physical Activity Level		
Sedentary or Low Active	503	93.00
Moderate Active	33	6.00
High Active	5	1.00
Health Status		
Have at least one chronic disease	245	45.29
Healthy	296	54.71
Diseases (n=245)		
Hypertension	176	71.84
Diabetes	67	27.35
Cholesterol	56	22.86
Thyroid	52	21.22
Cardiovascular	37	15.10
Renal	2	0.82

Besides the Mediterranean dietary pattern as a whole, the consumption of different food groups were also investigated and correlated with the

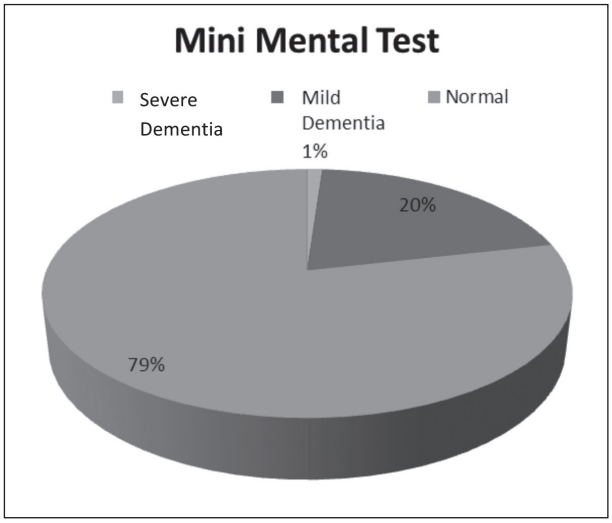


Figure 1a. The distribution of MMSE Scores

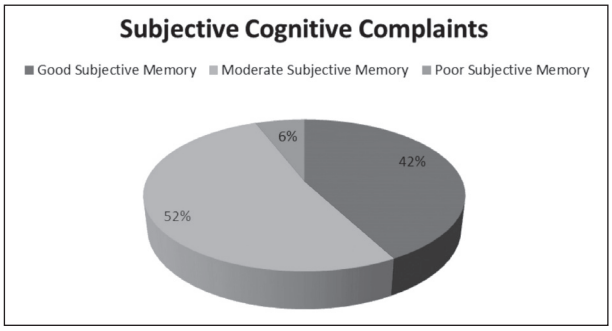


Figure 1b. The distribution of SCC Scores

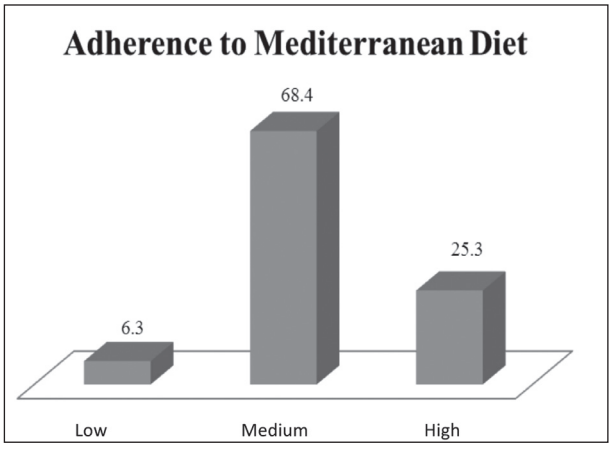


Figure 2. The Adherence to MeDi

MMSE scores. There was a positive correlation between the consumption of canned tuna fish (r:0.09, p:0.03), legumes (r:0.12, p:0.00), dark green leafy vegetables (r:0.20, p:0.00), olives (r:0.13, p:0.00) and olive oil (r:0.09, p:0.03) and MMSE scores. No correlation was found between fish (r:0.05, p:0.22) and nut (r:0.06, p:0.18) consumption and MMSE scores (Table 4).

Table 2. Correlation between mini mental test score, subjective cognitive complaints and adherence to Mediterranean Diet (n:541)

		Subjective Cognitive Complaints	Adherence to Mediterranean Diet
Orientation	r	-0.09	0.01
	p	0.04*	0.79
Registration	r	-	-
	p	-	-
Attention and Calculation	r	-0.16	0.09
	p	0.00*	0.03*
Recall	r	-0.05	0.08
	p	0.26	0.05*
Language	r	-0.12	0.10
	p	0.00*	0.02*
Mini Mental Test Total Score	r	-0.17	0.11
	p	0.00*	0.01*

**p<0.05*

Discussion

Numerous studies suggested that adherence to the MeDi is associated with improved cognitive performance and can be protective against dementia and Alzheimer's disease.

Studies published in the Mediterranean region, show encouraging results in relation to cognitive functions (1,7, 16). This study was the first and largest study that was held among elderly population in North Cyprus and its results are consistent with the literature.

Our findings suggest that there was a positive correlation between higher MeDi scores and higher MMSE scores. This correlation was particularly in attention-calculation, language and recall ($p<0.05$) (Table 2). Also, a negative correlation was found between higher MeDi scores and SCCs scores ($p<0.05$).

The protective effect of MeDi was also supported by the review of Aridi et al. and Hardman et al. that investigate the association between MeDi and cognition (9,16). Similar results were also found in two dif-

Table 3. Hierarchical regression model to predict MMSE Score according to age, BMI, physical activity, adherence to Mediterranean Diet score

Model	Estimates	Unstandardized coefficients		Standardized coefficients	t	p
		B	Std. Error	Beta		
Model 1	(Constant)	31.95	0.93		34.30	0.00*
	Age	-0.10	0.02	-0.28	-6.68	0.00*
Model 2	(Constant)	29.46	1.07		27.61	0.00*
	Age	-0.09	0.02	-0.24	-5.73	0.00*
	Right hand grip strength	0.06	0.01	0.19	4.51	0.00*
Model 3**	(Constant)	28.37	1.21		23.36	0.00*
	Age	-0.09	0.02	-0.24	-5.68	0.00*
	Right hand grip strength	0.06	0.01	0.18	4.40	0.00*
	Adherence to MeDi	0.13	0.06	0.09	2.09	0.04*

**p<0.05; ** R^2=0.12*

ferent large-scaled cohort studies, which indicated that the Mediterranean Diet is protective against dementia and decreases the risk of Mild Cognitive Impairment (MCI) and Alzheimer's disease (AD) (17,18).

According to one systematic review and meta-analysis, subjects in the highest MeDi score tertile had 33% less risk of cognitive impairment (MCI or AD) in comparison to the lowest tertile (19). In a similar way, the results of another study suggested that individuals with medium and high adherence to MeDi had a 15 to 21% and 39 to 40% reduction in Alzheimer's disease risk, respectively. Additionally, for every one-unit rise in the participants' MeDi adherence score, the risk of developing MCI decreased by 8% (2).

In the HELIAD study conducted in Greece, adherence to MeDi was associated with a decrease in dementia and better cognitive performances in language, visuospatial perception and particularly in memory. Additionally, in the same study, each unit increase in Mediterranean dietary score was associated with a 10% decrease in the odds for dementia (20).

Our finding is also consistent with these results. According to the result of our study; one unit rise in the MeDi adherence score increases the MMSE scores by 0.09.

Additionally, prospective studies investigating the relationship between MeDi, cognitive performance and the risk of dementia and also in the review of Roy J Hardman et al., high adherence to MeDi was found to be associated with slower cognitive decline. As a result, the risk for dementia, mainly AD, and conversion from MCI to AD were reduced (1,5, 21).

The results of many studies support that the Mediterranean Diet might be protective against cognitive decline as it comprises several foods and nutrients that are shown to be protective against cognitive dysfunction or dementia, such as fish (n:3 PUFA), olive oil (MUFA), fruits and vegetables, nuts and seeds (PUFA), folate, polyphenols and antioxidants (vitamin E, vitamin C, carotenoids, flavonoids) (1,21,22).

Besides the adherence to MeDi, our study also investigate the correlation between dietary components such as fish, vegetables, dairy products, olive oil consumptions and MMSE scores. It was found that there was positive correlation between the consumption of canned tuna fish (r:0.09, p:0.03), legumes (r:0.12, p:0.00), dark green leafy vegetables (r:0.20, p:0.00), olives (r:0.13, p:0.00) and olive oil (r:0.09, p:0.03) and MMSE scores. No correlation was found between fish (r:0.05, p:0.22) and nut (r:0.06, p:0.18) consumption and MMSE scores (Table 4).

Olive oil is one of the major components of MeDi. In a prospective, 4-year follow up Three-City Study conducted in France, a weak association was described between increased olive oil intake which and the risk of cognitive decline. However in an Italian Longitudinal Study on Aging involving an 8 year follow up, greater intake of monounsaturated fatty acids, mainly olive oil, was found to be related to better cognitive functions (23,24). Additionally, in a RCT by Martin-

Table 4. The correlation between Mediterranean Diet related food consumption, MMSE Scores and SCC Scores (n=541)

		MMSE Scores	SCC Scores
Fresh Fish	r	0.05	-0.03
	p	0.22	0.51
Canned Tuna	r	0.09	0.05
	p	0.03*	0.26
Legumes	r	0.12	-0.01
	p	0.00*	0.90
Nuts	r	0.06	0.02
	p	0.18	0.59
Dark Green Leafy Vegetables	r	0.20	-0.09
	p	0.00*	0.03
Fresh Fruits	r	-0.01	-0.02
	p	0.81	0.59
Olive	r	0.13	-0.09
	p	0.00*	0.04*
Olive Oil	r	0.09	-0.04
	p	0.03*	0.34

**p<0.05*

ez Lapiscina et al, MeDi supplemented with EVOO or mixed nuts (1 lt/week EVOO or 30 g/day mixed nuts) showed better cognitive performance versus control in all cognitive domains, particularly fluency and memory tasks (25).

Legume consumption of participants was also found to be correlated with MMSE scores in our study. In a recent study consisting of 214 elderly people aged 65 years and over, a positive association was found between MMSE scores and the participants' legume consumption one year after the start of the investigation (26).

Fish consumption, which is one of the main elements of MeDi and a good source for EPA and DHA (n:3 PUFA), was found to be the only predictor of dementia in Anastasiu et al.'s study. Fish consumption was negatively associated with dementia (20). In parallel to our study, according to the Women's Health study by Kim et al., consumption of tuna and dark meat fish once a week or higher was found to be associated with a lower decline in verbal memory, although total seafood consumption was not related with changes in global cognition over a 4-year period (27).

Leafy vegetables are also a good source of folate and B vitamins, which are effective for reducing homocysteine levels that are a risk factor for age-related cognitive decline (21). According to the result of our study, green leafy vegetables were correlated with MMSE scores. In the prospective study held by Morris et.al; it was shown that green leafy vegetables help to slow cognitive decline with ageing (28).

In our research, handgrip strength was also found to be associated with MMSE scores. Handgrip strength is easy and safe to evaluate in the elderly and is used as a measure of whole-body muscular strength. It is a proxy tool for the assessment of nutritional status, physical functions and frailty (29). Accumulating evidence suggests that higher levels of physical function are associated with better cognitive abilities. According to the study of Jang and Kim, greater handgrip strength was found to be associated with higher cognitive function in cognitively normal elderly Korean subjects (30).Similarly, from the population-based longitudinal Swedish Adoption/Twin Study of Aging, grip strength performance was associated with a positive change in the four cognitive abilities after 65 years of age (Verbal ability, Spatial ability, Processing speed, Memory) (31).

Conclusion

Our data suggests that MeDi is correlated with better cognitive functions based on the MMSE scores, particularly in the attention-calculation, language and recall domains in the elderly population living in North Cyprus. This correlation was also significant among the diet components olives, olive oil, green leafy vegetables and legumes.

Another important outcome was the significant positive correlation between cognitive functions and right hand grip strength test, which is an objective predictor of frailty and physical strength.

Among the lifestyle-related modifiable risk factors, optimising dietary behaviour is crucial to prevent or delay cognitive decline that is associated with normal aging and frailty. Due to the increasingly aging world population, we believe that increasing scientific and public recognition on the beneficial effects of MeDi is important and deserves further attention.

All the authors declared that "there is no conflict of interest".

References

1. Feart C, Samieri C, Rondeau V, et al. Adherence to a Mediterranean diet, cognitive decline, and risk of dementia. Jama. 2009; 302 (6):638-648.
2. Sousa MJ, Guimarães J. Prevention of Alzheimer's disease: The role of the Mediterranean diet. Revista de Nutrição. 2015;28 (6):691-703.
3. Petersson SD, Philippou E. Mediterranean Diet, Cognitive Function, and Dementia: A Systematic Review of the Evidence. Advances in Nutrition. 2016;7 (5):889-904.
4. Bach-Faig A, Berry EM, Lairon D, Reguant J, Trichopoulou A, Dernini S, Medina FX, Battino M, Belahsen R, Miranda G, et al. Mediterranean diet pyramid today. Science and cultural updates. Public Health Nutr. 2011;14 12A:2274–84.
5. Féart C, Samieri C, Barberger-Gateau P. Mediterranean diet and cognitive function in older adults. Curr Opin Clin Nutr Metab Care. 2010;13 (1):14-8.
6. Martínez-Huélamo M, Rodríguez-Morató J, Boronat A, de la Torre R. Modulation of Nrf2 by Olive Oil and Wine Polyphenols and Neuroprotection. Antioxidants (Basel). 2017;6 (4).
7. Beilharz JE, Maniam J, Morris MJ. Diet-induced cognitive deficits: the role of fat and sugar, potential mechanisms and nutritional interventions. Nutrients. 2015;7 (8):6719-38.
8. Panza F, Solfrizzi V, Colacicco AM, D'Introno A, Capurso C, Torres F, Del Parigi A, Capurso S, Capurso A. Mediterranean diet and cognitive decline Public Health Nutrition. 2004;7 (7)959–963.
9. Aridi YS, Walker JL, Wright ORL. The Association between the Mediterranean Dietary Pattern and Cognitive Health : A Systematic Review. Nutrients. 2017;28:9 (7).
10. Valls-Pedret C, Sala-Vila A, Serra-Mir M, Corella D, de la Torre R, Martínez-González MÁ, Martínez-Lapiscina EH, Fitó M, Pérez-Heras A, Salas-Salvadó J, Estruch R, Ros E. Mediterranean Diet and Age-Related Cognitive Decline: A Randomized Clinical Trial. JAMA Intern Med. 2015;175 (7):1094-103.
11. Rakesh G, Szabo ST, Alexopoulos GS, Zannas AS. Strategies for dementia prevention: latest evidence and implications. Therapeutic advances in chronic disease. 2017;8 (8-9):121-36.
12. Rahi B, Ajana S, Tabue-Teguo M, Dartigues JF, Peres K, Feart C. High adherence to a Mediterranean diet and lower risk of frailty among French older adults community-dwellers: Results from the Three-City-Bordeaux Study. Clinical Nutrition. 2017 May 31.
13. Folstein MF, Folstein S, Mc Hugh PR. "Mini Mental State" A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res. 1975;12:189-198.
14. Martínez-González MÁ, Corella D, Salas-Salvadó J, Ros E, Covas MI, Fiol M, et al. Cohort profile: design and methods of the PREDIMED study. International journal of epidemiology. 2012;41 (2):377-385.
15. Güngen C, Ertan T, Eker E, Ya ar R, Engin, F. Standardize mini mental test'in Türk toplumunda hafif demans tanısında geçerlik ve güvenilirliği. Türk Psikiyatri Dergisi. 2002;13: 273-281.
16. Hardman RJ, Kennedy G, Macpherson H, et al. Adherence to a Mediterranean-style diet and effects on cognition in adults: a qualitative evaluation and systematic review of longitudinal and prospective trials. Front Nutr. 2016;3:22.
17. Kaur N, Koley S. An association of nutritional status and hand grip strength in female labourers of North India. The Anthropologist. 2010;12 (4):237-43.
18. Lee JE, Kim KW, Paik NJ, Jang HC, Chang CB, Baek GH, Lee YH, Gong HS. Evaluation of factors influencing grip strength in elderly Koreans. Journal of bone metabolism. 2012;19 (2):103-10.
19. Singh B, Parsaik AK, Mielke MM, Erwin PJ, Knopman DS, Petersen RC, Roberts RO. Association of mediterranean diet with mild cognitive impairment and Alzheimer's disease: a systematic review and meta-analysis. Journal of Alzheimer's disease. 2014;39 (2):271-82.
20. Anastasiou CA, Yannakoulia M, Kosmidis MH, Dardiotis E, Hadjigeorgiou GM, Sakka P, Arampatzi X, Bougea A, Labropoulos I, Scarmeas N. Mediterranean diet and cognitive health: Initial results from the Hellenic Longitudinal Investigation of Ageing and Diet. PLoS One. 2017;12 (8):e0182048.
21. Hardman RJ, Meyer D, Kennedy G, Macpherson H, Scholey AB, Pipingas A. The association between adherence to a Mediterranean style diet and cognition in older people : The impact of medication. Clin Nutr. 2017. pii: S0261-5614 (17)31388-2. doi: 10.1016/j.clnu.2017.10.015.
22. Zamroziewicz MK, Barbey AK. Nutritional cognitive neuroscience: innovations for healthy brain aging. Frontiers in neuroscience. 2016;10:240.
23. Berr C, Portet F, Carriere I, Akbaraly TN, Feart C, Gourlet V, Combe N, Barberger-Gateau P, Ritchie K. Olive oil and cognition: results from the three-city study. Dementia and geriatric cognitive disorders. 2009;28 (4):357-64.
24. Solfrizzi V, Colacicco AM, D'Introno A, Capurso C, Torres F, Rizzo C, Capurso A, Panza F. Dietary intake of unsaturated fatty acids and age-related cognitive decline: a 8.5-year follow-up of the Italian Longitudinal Study on Aging. Neurobiology of aging. 2006;27 (11):1694-704.
25. Martinez-Lapiscina EH, Clavero P, Toledo E, et al. Virgin olive oil supplementation and long-term cognition: the PREDIMED-NAVARRA randomized, trial. J Nutr Health Aging. 2013;17 (6):544–552.
26. Mazza E, Fava A, Ferro Y, Moraca M, Rotundo S, Colica C, Provenzano F, Terracciano R, Greco M, Foti D, Gulletta E. Impact of legumes and plant proteins consumption on cognitive performances in the elderly. Journal of translational medicine. 2017;15 (1):109.
27. Kim DH, Grodstein F, Rosner B, Kang JH, Cook NR, Manson JE, Buring JE, Willett WC, Okereke OI. Seafood types and age-related cognitive decline in the Women's Health Study. J Gerontol A Biol Sci Med Sci. 2013;68 (10):1255-62.
28. Morris MC, Wang Y, Barnes LL, Bennett DA, Dawson-

HughesB, Booth SL. Nutrients and bioactives in green leafy vegetables and cognitive decline: Prospective study. Neurology. 2018;90 (3):e214-e222.
29. Whiting SJ, Cheng PC, Thorpe L, Viveky N, Alcorn J et al. Hand Grip Strength as A Potential Nutritional Assessment Tool in Long-Term Care Homes. Journal of Aging Research And Healthcare -2016;1 (2):1-11.
30. Jang JY, Kim J. Association between handgrip strength and cognitive impairment in elderly Koreans: a population-based cross-sectional study. Journal of physical therapy science. 2015;27 (12):3911-5.
31. Sternäng O, Reynolds CA, Finkel D, Ernsth-Bravell M, Pedersen NL, Dahl Aslan AK. Grip strength and cognitive abilities: associations in old age. Journals of Gerontology Series B: Psychological Sciences and Social Sciences. 2015;71 (5):841-8.

Correspondence:
Nezire Ince
Eastern Mediterranean University, Faculty of health Sciences, Department of Nutrition and Dietetic
Famagusta, Cyprus
Phone 00903926301084 - 00903926301081
E-mail: nezireince@hotmail.com / nezire.ince@emu.edu.tr

Progress in Nutrition 2020; Vol. 22, N. 1: 84-89 DOI: 10.23751/pn.v22i1.7742

Original article

Teachers' self-efficacy is related to their nutrition teaching methods

N. Unusan, H. Yalcin

[1]KTO Karatay University, Nutrition and Dietetics Department, Konya/Turkey - E-mail: nurhan.unusan @karatay.edu.tr; [2]KTO Karatay University, Child Development Department, Konya/Turkey

Summary. *Background:* Teacher-led nutrition education is a practical method for informing children about healthy eating at an early age. Commonly reported teacher barriers include self-efficacy and use of teaching methods. *Objective:* This study is the first to evaluate preschool teachers' self-efficacy in teaching nutrition and their preferred teaching methods. *Material and Method:* Researchers recruited preschool teachers from 133 preschools in Konya. At the baseline, teachers were asked to provide demographic information, including their age, sex, level of education, teaching experience, previous involvement in teaching nutrition and resources used to plan nutrition lessons. The completed survey data were entered into SPSS 24. The data were analysed using descriptive statistical methods, and each question was analysed for frequencies. Kendall's Tau-b was used throughout the analysis to determine the relationships among the variables (self-efficacy and teaching methods). *Results:* All of the teachers were female. The baseline score for self-efficacy was relatively high, with an average of 74±65.6. Teachers who demonstrated decreases in self-efficacy were those who reported previous experience teaching nutrition, and those who showed increases in self-efficacy were those who reported no previous experience teaching nutrition. Kendall's Tau-b showed a statistically significant relationship between self-efficacy and teaching method and indicated a statistically significant relationship between the belief that teachers are doing a good job regarding the health of students and role playing, 0.194 ($p<0.05$). Teachers believe that students' engagement in regular physical activity and preference for well-balanced meals increased with increased numbers of field trips (Kendall Tau b 0.260 and 0.245 respectively). *Conclusions:* The study investigates teachers' self-efficacy in teaching nutrition and preferred methods of teaching nutrition. Role-playing, field trips and project-based learning methods of nutrition teaching are directly related to teachers' self-efficacy. This study provides information for teachers on how to improve their nutrition education efforts for the benefit of students. Teachers' self-efficacy and teaching methods for preschool children's nutrition education may have profound effects on the implementation of a nutrition education programme.

Key words: preschool teachers, self-efficacy, nutrition, nutrition education, teaching methods, role-playing, field trips, project-based learning, Kendall's Tau-b, questionnaire

Introduction

Nutrition is a key component of mental development, mood alteration, behaviour and physical activity. Children's general nutritional habits also affect teachers' teaching methods and self-efficacy. Obesity is a major and widespread public health problem throughout the world, and has substantial medical psychosocial and economic consequences. The prevalence of overweight and obesity has increased in the last two decades. For example, preschool obesity has become an important and alarming health issue in Turkey. An effective way to prevent childhood overweight and its consequences while maintaining adequate nutrition in later life is to promote and establish healthy eating behaviours and increased physical activity early in life (1). Many of the health-related attitudes and

behaviours developed at preschool ages are maintained throughout life and may have profound effects on chronic disease risk later in life. Food nutrition is also crucial for a child's normal growth and development. Healthy food choices thus contribute to academic success in addition to providing a foundation for good health.

There are no systematic national studies on the prevalence and trends of overweight and obesity in Turkish children. Studies have highlighted that body weight prior to 5 years of age is a significant predictor of health at older ages (2). The Turkish Ministry of Health addresses fighting overweight and obesity, and the Department of Obesity, Diabetes and Metabolic Diseases and the Head Office of Health Promotion have implemented policies such as, 'Turkey Nutrition Guide', 'Turkey Nutrition and Physical Activity Pyramid with Healthy Food Plate' and 'Movement to Combat Obesity'. The policies of the Ministry focus on nutrition attitudes and behaviour, and physical activities. A number of projects target children, such as, 'School Milk Program', 'Nutrition Friendly Schools Program' 'Control of School Canteens and Hygiene Rules to Conform' and 'Nutrition Recommendations and Menu Programs for Preschool and School Age Children'.

Obesity is a major problem among pre-school children in Turkey, 11 % of children under 5 years of age are overweight/obese (3) and later a recent research by Santas and Santas (4) showed that obesity is a major problem among pre-school children in Turkey: 8.6% of pre-school children are overweight and/or obese by height and 6.6% are overweight and/or obese by age. This finding demonstrates that effective interventions regarding obesity in Turkey should begin as early as infancy. Due to the complexities of individual, social and environmental factors on children's dietary choices, nutrition interventions often use theoretical models of behaviour change as frameworks to develop nutrition education interventions (5). Of the many models of behaviour change, social cognitive theory (SCT) has been most widely used in school-based interventions (6). One of the primary constructs of SCT is self-efficacy – a person's perceived ability to perform desired behaviours.

Schools can serve as an excellent vehicle for disseminating health and nutrition information. Individual teacher characteristics such as education, training and skills also play considerable roles in how teachers behave in the classroom. While nutrition is not a part of traditional preschool teacher training programmes, teacher-led nutrition education can potentially encourage children to adopt healthful eating practices in a supportive school environment. School-based nutrition education intervention has the potential to improve children's dietary behaviours (7), and the implementation of nutrition curricula in schools can be influenced by preschool teachers' self-efficacy and methods of teaching nutrition (8). Self-efficacy refers to beliefs in teacher's capabilities to organise and execute the courses of action vital to produce the given attainment (9). Teachers' perceived self-efficacy is supposed to affect behaviour in several ways. Perceived efficacy affects the choices that individuals make and the courses of action that they follow. Individuals tend to engage in tasks in which they feel skilled and avoid those in which they do not. Self-beliefs determine how much effort people will expend on an activity, how long they will persist when encountering obstacles and their resilience in the face of opposition. As teachers' efficacy increases, their effort, persistence and flexibility also increase. Pajares and Schunk (10) noted that efficacy beliefs influence thought patterns and emotional responses. Teachers with low self-efficacy may underestimate their abilities, which can foster stress, depression and less creativity in problem solving. In contrast, teachers with high self-efficacy are likely to feel calmer in approaching difficult tasks and activities.

Teacher-led nutrition education is a practical method for informing children about healthy eating at an early age. Commonly reported teacher barriers include self-efficacy and use of teaching methods. This study is the first to evaluate teachers' self-efficacy in teaching nutrition and their preferred teaching methods.

Material and methods

Research Group

Researchers recruited preschool teachers from 133 preschools in Karatay, Konya at the beginning of the 2016 academic year. At the baseline, teachers were asked to provide demographic information, including their age, sex, level of education, teaching experience, previous involvement in teaching nutrition and resourc-

es used to plan nutrition lessons. The researcher interviewed the Ministry of Education of Konya Karatay district to obtain information about nutrition education programmes implemented by teachers enrolled in this study. Meetings were conducted at the university at which the researcher was working during normal school hours. During the introductory meeting, the researcher presented the study's goals, methods and expectations.

Study Instrument

Section one explores the self-efficacy of teaching nutrition using a questionnaire that was administered before the preliminary meeting and after the completion of the preschool nutrition education curriculum. This questionnaire was revised from an earlier published and validated survey measuring self-efficacy in the teaching of health education (11). Fifteen questions relevant to nutrition education were chosen. Answer choices were provided using a five-point Likert scale (strongly disagree, somewhat disagree, neither agree nor disagree, somewhat agree, and strongly agree). Section two explores the methods that teachers use to present nutrition information and the extent to which nutrition education is integrated with other subject areas.

Statistical Analysis

The completed survey data were entered into SPSS 24. A professional statistician worked with the researcher to check data for errors. The data were analysed using descriptive statistical methods, and each question was analysed for frequencies (percentages or distributions). Kendall's Tau-b was used throughout

Table 1. Teachers' self efficacy of teaching nutrition

Self Efficacy	**Strongly Disagree n (%)**	**Somewhat Disagree n (%)**	**Neither agree nor disagree n (%)**	**Somewhat Agree n (%)**	**Strongly Agree n (%)**
I believe I can do a good job teaching students about health	2(6.1)	1(3)	-	2(6.1)	28(84.8)
I believe I can do a good job teaching students about nutrition	2(6.1)	-	-	2(6.1)	29(87.9)
I understand nutrition concepts well enough to be effective in teaching elementary nutrition education	1(3)	-	1(3)	4(12.1)	27(81.8)
I am able to stimulate students enough so they ask thoughtful questions about nutrition	2(6.1)	-	1(3)	4(12.1)	26(78.8)
I believe I can do a good job teaching student about physical activity	1(3)	2(6.1)	1(3)	1(3)	28(84.8)
Even if I try hard, I will not teach about nutrition as well as I will most other subjects	16(48.5)	7(21.2)	1(3)	4(12.1)	5(15.2)
I believe I can do a good job teaching student about nutrients	2(6.1)	2(6.1)	1(3)	2(6.1)	26(78.8)
I believe I can do a good job teaching student about recommendations for a healthy diet	1(3)	3(9.1)			29(87.9)
I believe if I do a good job teaching, the students I teach will be more knowledgeable about nutrition and health	2(6.1)	-	1(3)	2(6.1)	28(84.8)
Increased teaching time in nutrition produces significant changes in nutrition related behaviours of many students	1(3)	-		8(24.2)	24(72.7)
I believe that if I do a good job teaching, the students I teach will be more likely to engage in regular physical activity	1(3)	-	3(9.1)	2(6.1)	27(81.8)
I believe that if I do a good job teaching, the students I teach will be more likely to eat well-balanced meals	2(6.1)	2(6.1)	2(6.1)	5(15.2)	22(66.7)
I believe that if I do a good job teaching, the students I teach will be more likely to maintain a normal weight	3(9.1)		7(21.2)	8(24.2)	15(45.5)
I believe that if I do a good job teaching, the students I teach will be more knowledgeable about nutrients	1(3)		2(6.1)	9(27.3)	21(63.6)
I believe that if I do a good job teaching, the students I teach will be more knowledgeable about recommendations for a healthy diet	2(6.1)	2(6.1)	2(6.1)	10(30.3)	17(51.5)

the analysis to determine the relationships among the variables (self-efficacy and teaching methods).

Results

All of the teachers were female (male preschool teachers are very rare in Turkey); none had earned Master's degrees. The baseline score for self-efficacy was relatively high, with an average of 74±65.6. There were no significant differences in the change in self-efficacy between schools. Teachers who demonstrated decreases in self-efficacy were those who reported previous experience teaching nutrition, and those who showed increases in self-efficacy were those who reported no previous experience teaching nutrition (Table 1).

When asked about methods used to teach nutrition, teachers most often reported the use of demonstrations (97%) (Table 2). Guest speakers (84.8%), role playing (78.8%), field trips (72.7%), student projects (66.7%), collaborative work (51.5%) and hands-on learning (54.5%) were also used. Teachers in this study reported comparatively more limited use of lecturing, active classroom discussions and computers.

Kendall's Tau-b showed a statistically significant relationship between self-efficacy and teaching method (Table 3) and indicated a statistically significant relationship between the belief that teachers are doing a good job regarding the health of students and role playing, 0.194 ($p<0.05$). As the use of role playing as a preferred teaching method increased, so did the belief that teachers are doing a good job regarding the health of the students.

Teachers believe that students' engagement in regular physical activity and preference for well-balanced meals increased with increased numbers of field trips (Kendall Tau b 0.260 and 0.245 respectively). Additionally, teachers believe that increased project-

Table 2. Methods used by teachers to teach nutrition

Instruction method	Very Poor n (%)	Below Average n (%)	Average n (%)	Above Average n (%)	Excellent n (%)
Role playing	2(6.1)	-	1(3)	3(9.1)	26(78.8)
Lecturing	2(6.1)	14(42.4)	-	11(33.3)	5(15.2)
Active classroom discussion	2(6.1)	1(3)	2(6.1)	15(45.5)	12(36.4)
Guest speaker	4(12.1)	-	-	-	28(84.8)
Field trips	-	-	2(6.1)	6(18.2)	24(72.7)
Project based learning	1(3)	1(3)	-	8(24.2)	22(66.7)
Collaborative work	1(3)	5(15.2)	-	9(27.3)	17(51.5)
Computers	2(6.1)		3(9.1)	16(48.5)	11(33.3)
Hands on learning	2(6.1)	1(3)	-	11(33.3)	18(54.5)
Demonstrations	1(3)	7(21.2)	-	24(72.7)	32(97)

Table 3. Relationship between self efficacy and teaching method

Self-efficacy and teaching method	Kendall's tau b	p
I believe I can do a good job teaching student about health - Role playing	0.194	0.042*
I believe that if I do a good job teaching, the students I teach will be more likely to engage in regular physical activity - Field trips	0.260	0.014*
I believe that if I do a good job teaching, the students I teach will be more likely to eat well balanced meals - Field trips	0.245	0.049*
I believe if I do a good job teaching, the students I teach will be more knowledgeable about nutrition and health - Project based learning	0.272	0.017*
Increased teaching time in nutrition produces significant changes in nutrition related behaviours of many students - Project based learning	0.270	0.047*
I am able to stimulate students enough so they ask thoughtful questions about nutrition – Project based learning	0.033	0.004*

**(p<0,05)*

based learning resulted in increases in students' knowledge about nutrition and health, significant changes in nutrition-related behaviours and more questions from students about nutrition.

Discussion and Conclusions

The study investigates teachers' self-efficacy in teaching nutrition and preferred methods of teaching nutrition. Preschool teachers play a significant role in developing children's knowledge and skills to enable them to make informed, healthful choices about their diet (12, 13). Role-playing could also bring about better and deeper effects on nutrition by actively engaging preschoolers in educational sessions (14).

Teachers play a very important role in designing and organising trips to out-of-school environments to promote education, and they have to invest a great deal of time and effort in planning successful school trips (15). It is therefore important to train self-confident and well-informed teachers (16). Hence, instead of exclusively offering them theoretical knowledge, having them perform extra-curricular activities will help them become more well-equipped (17).

Project-based learning requires students to design and complete projects. Students learning by this method become creative and constructive; they interact more with their classmates and can thus better develop their understanding of scientific concepts (18). Role-playing, field trips and project-based learning methods of nutrition teaching are directly related to teachers' self-efficacy.

This study provides information for teachers on how to improve their nutrition education efforts for the benefit of students. The sample size of teachers was small, however, and this study should be replicated with larger samples. All of the data used in this study were self-reported. The nature of the survey was closely connected to the act and vocation of teaching, and participants may have had a tendency to respond in a personally favourable manner or to give perceived socially desirable responses, biasing the results (19). Teachers' self-efficacy and teaching methods for preschool children's nutrition education may have profound effects on the implementation of a nutrition education programme. It is therefore recommended that future studies investigate whether student outcomes are dependent on teacher self-efficacy and teaching method. Studies should also investigate whether these factors vary depending upon the number of hours of nutrition taught in the classroom. Nutrition professionals should push for more specific standards so that teachers have clear guidance and direction for teaching nutrition in their classrooms (20).

References

1. Erem, C., Prevalence of Overweight and Obesity in Turkey. IJC Metabolic & Endocrine, 2015. 8: p. 38-41.
2. Brophy, S., et al., Risk factors for childhood obesity at age 5: analysis of the millennium cohort study. BMC public health, 2009. 9(1): p. 467.
3. Hacettepe University Institute of Population Studies, et al., Turkey Demographic and Health Survey 2013. 2014, Hacettepe University Institute of Population Studies, Ministry of Health, Ministry of Development: Ankara, Turkey.
4. Santas, F. and G. Santas, Prevalence of pre-school children for overweight/obesity in Turkey. World Journal of Pediatrics, 2018. 14(1): p. 77-83.
5. Contento, I., Review of nutrition education research in the Journal of Nutrition Education and Behavior, 1998 to 2007. Journal of nutrition education and behavior, 2008. 40(6): p. 331-340.
6. Glanz, K., B.K. Rimer, and K. Viswanath, Health behavior and health education: theory, research, and practice. 2008: John Wiley & Sons.
7. Metos, J.M., K. Sarnoff, and K.C. Jordan, Teachers' Perceived and Desired Roles in Nutrition Education. Journal of School Health, 2019. 89(1): p. 68-76.
8. Unusan, N., Promoting dietary behavior and nutrition knowledge among fourth graders: evaluation of power point presentation and traditional lecture types. Ciência e Técnica Vitivinícola, 2017. 32(8).
9. Bandura, A., Self-efficacy: The exercise of control. 1997: Macmillan.
10. Pajares, F. and D. Schunk, The development of academic self-efficacy. Development of achievement motivation. United States, 2001. 7.
11. Telljohann, S.K., et al., Effects of an Inservice Workshop on the Health Teaching Self Efficacy of Elementary School Teachers. Journal of school health, 1996. 66(7): p. 261-265.
12. Jones, A.M. and S. Zidenberg-Cherr, Exploring nutrition education resources and barriers, and nutrition knowledge in teachers in California. Journal of nutrition education and behavior, 2015. 47(2): p. 162-169.
13. Kracht, C.L., et al., Early care and education teacher's role in obesity prevention and healthy development of young

American Indian children. Journal of Transcultural Nursing, 2019. 30(1): p. 75-85.
14. Savabi Esfahani, M., S. Kohan, and S. Ehsanpour, Promoting breastfeeding self-efficacy through role-playing in pregnant women. International Journal of Pediatrics, 2016. 4(7): p. 2061-2068.
15. Kete, R., & Horasan, Y. and F. Santas. Teacher candidates' efficiencies in applied (Nature Centered) Biology lessons. in VI. National Postgraduate Education Symposium, (10-11 May). 2013. Sakarya University, Sakarya, Turkey.
16. Bozdoğan, A.E., The practice of prospective science teachers regarding the planning of education based trips: Evaluation of six different field trips. Educational Sciences: Theory & Practice, 2012.
17. Bozdoğan, A.E., The effects of elective course designed with different contents on preservice science teachers'self-efficacy beliefs and knowledge about organizing curriculum based field trips. Journal of Baltic Science Education, 2018. 17(3).
18. Singer, J.E., R. Tal, and H.K. Wu, Students' understanding of the particulate nature of matter. School Science and Mathematics, 2003. 103(1): p. 28-44.
19. Van de Mortel, T.F., Faking it: social desirability response bias in self-report research. Australian Journal of Advanced Nursing, The, 2008. 25(4): p. 40.
20. Gehris, J., R. Gooze, and R. Whitaker, Teachers' perceptions about children's movement and learning in early childhood education programmes. Child: care, health and development, 2015. 41(1): p. 122-131.

Correspondence:
Prof. Dr. Nurhan Unusan*
KTO Karatay University
Nutrition and Dietetics Department
Konya/Turkey
E-mail: nurhan.unusan @karatay.edu.tr

Progress in Nutrition 2020; Vol. 22, N. 1: 90-95 DOI: 10.23751/pn.v22i1.8136

Original article

Nutritional habits of people with type two diabetes mellitus

Bjelanovic Jelena[1,2], Petric Teodora[1], Pavlica Tatjana[3], Rakic Rada[3], Dragic Natasa[1,2], Bijelovic Sanja[1,2], Bjelica Artur[1,4]

[1]University of Novi Sad, Faculty of Medicine, 21000 Novi Sad, Serbia - Email: jelena.bjelanovic@mf.uns.ac.rs; [2]Institute of Public Health of Vojvodina, 21000 Novi Sad, Serbia; [3] University of Novi Sad, Faculty of Natural Sciences and Mathematics, 21000 Novi Sad, Serbia; [4]Clinical Center of Vojvodina, 21000 Novi Sad, Serbia

Summary. *Background and aim of the work:* Prevalence of diabetes mellitus has reached pandemic proportions. Our aims were to recognize nutritional habits of people with type two diabetes mellitus, as well as the differences in respect of gender and nutritional state. *Methods:* The study encompassed 50 patients from Polyclinic for Endocrinology, Diabetes and Metabolic Diseases of the Clinical Center of Vojvodina. Data were collected based on a questionnaire specially designed for this study. *Results:* The majority of polled patients were overweight and obese (86%), and there were no undernourished subjects. The analysis of nutritional habits showed that the intake of fatty meat and offals by males was statistically significantly higher compared to females (p=0.0229). In the group of the same nutritional state, obese subjects consumed significantly more meat products compared to those with normal nitritional status (p=0.0097). No other differences were observed. *Conclusions:* It is a worrying finding that the majority of subjects with type two diabetes mellitus are overweight or obese. This indicates that the energy intake is not in proportion with the energy expenditure. It is necessary to carry out more detailed studies on a larger number of subjects, which would include the amounts of foodstuffs consumed, as well as the daily energy intake, accompanied by the analysis of the level of the subjects' physical activities.

Key words: diabetes melitus, diet, adults, attitude to health, Body Mass Index

Introduction

Diabetes melitus (DM) represents one of the greatest public health challenges in the 21st century. According to the data of the World Health Organization (WHO), there are presently about 422 million of adults with this disease (1). If no adequate preventive measures were taken, the prognoses indicate that in the period of twenty years this number will amount to 522 million. The majority (about 80%) of people with DM live in developing countries, where a highest increase of those with this disease could be expected in the future (2,3).

A similar situation is observed in Serbia. According to the data of the National Institute of Public Health there are currently 7.6% of adults with DM (4).

DM type 2 represents a group of similar metabolic disturbances that are manifested as hyperglycemia (elevated glucose blood level), caused by a complex interaction of genetic and external factors, as well as by lifestyle (5). Overweight, especially when accompanied with sedentary lifestyle and inappropriate diet, can be one of the major triggers for DM type 2 (6).

Nutritional therapy and appropriate physical activities represent the basis of DM type 2 treatment (7,8). Adequate changes in the patient's daily routines lead to better lifestyle quality and lessen the risk of cardiovascular, cerebrovascular disorders (6).

The aim of the study was to identify the nutritional habits of patients with type 2 DM, as well as the differences in respect to gender and nutritional status.

Material and methods

The study was carried out in October 2017 at the Polyclinic for Endocrinology, Diabetes and Metabolic Diseases of the Clinical Center of Vojvodina. It was a cross-sectional study. The sample included all hospitalized patients with DM type 2 at the Clicinal Centre at that period of time. There were 50 subjects of both genders.

The criteria for excluding certain patients from the study referred to their unwillingness to give a consent for taking part in the research or those whose condition (e.g. dementia) would prevent them from supplying valid answers, the information provided from the patients' doctors. None of the patients was exluded from the study.

The data for the study were collected from patients via face-to-face interviews, using a questionnaire[1] that was filled anonimously. The questionnaire was specially designed for the purpose of this study and had not been pilot-tested nor validated. It consisted of two parts. The first part contained questions concerning general data of the subjects - gender, age, duration of the disease, body height and body mass. The patients' height and weight were not measured during the study, as these values had been obtained when the patients came to the Clinical Centre. These were considered as valid self-reported data, as it has been the case in many other studies (9,10). The data for height and body mass were used to calculate the body mass index (BMI), which served as the basis for assessing the nutritional status of the patients (11).

The second part was concerned with the frequency of consumption of particular foodstuffs. The questions concerning nutrition were designed in such a way to provide information about the frequency of consuming particular foodstuffs in the last seven days. These questions encompassed both the foodstuffs that are recommended and not recommended by WHO in the nutritive therapy of type 2 DM (12). For each question, there were four proposed answers, from which the subjects could choose only one.

Ethics

This study was approved by the Ethical Committee of the Faculty of Medicine, University of Novi Sad.

Data Analysis

The respondents' answers were coded and entered into a Microsoft Excel table. They were analyzed using descriptive quantitative statistics and presented in the form of graphs and tables. The gender dependence of the obtained answers relating to nutrition was analyzed using the Mann-Whitney nonparametric test, whereas the comparison in respect to the BMI groups was performed using the Kruskall-Wallis nonparametric test. Both tests were carried out at the significance level of 0.05.

Results

The study group of subjects with type 2 DM consisted of 30 (60%) females and 20 (40%) males. The average age of the subjects was 60.02 ± 10.84. The youngest subject was 26 years of age, and the oldest one was 75 years old.

The total number of those with normal nutritional status was 7 (14%), the number of the overweight was overweight 28 (56%), whereas the group of obese (class I, II, and III) consisted of 15 (30%) subjects.

The average BMI value was 28.92 ± 3.99 kg/m^2: 29.20 and 28.77 kg/m^2, for females and males, respectively.

Table 1 shows the participants' responses concerning the foodstuffs recommended for patients with type 2 DM.

By analyzing the responses related to the foodstuffs recommended for nutrition of patients with type 2 DM, shown in Table 1, it can be seen that the majority of pa-

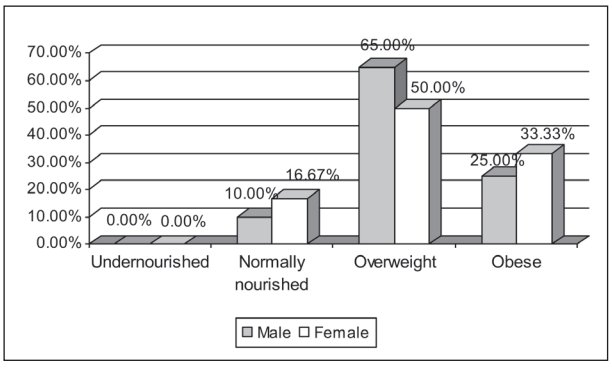


Figure 1. Gender dependence of the noursihment status based on BMI

1 The original questionnaires are available from the authors

Table 1. Intake of foodstuffs recommended for patients with type 2 DM.

Foodstuff	Every day		3-5 x weekly		1x weekly		Never	
	n	%	n	%	n	%	n	%
Whole-grain cereals	25	50.00	8	16.00	7	14.00	10	20.00
Citruses and apples	35	70.00	11	22.00	4	8.00	0	0.00
Nut fruits	4	8.00	4	8.00	23	46.00	19	38.00
Fresh vegetables	33	66.00	16	32.00	1	2.00	0	0.00
Low-fat dairy products	26	52.00	17	34.00	4	8.00	3	6.00
Young animals' lean meat	14	28.00	31	62.00	4	8.00	1	2.00
Fish	0	0.00	7	14.00	28	56.00	15	30.00

Table 2. Intake of foodstuffs that are not recommended for patients with type 2 DM.

Foodstuff	Every day		3-5 x daily		1x daily		Never	
	n	%	N	%	n	%	n	%
White-flour and fatty bakery products	4	8.00	25	50.00	14	28.00	7	14.00
Other fruit types (*grapes, bananas, figs,...)*	4	8.00	25	50.00	14	28.00	7	14.00
Dry fruits	0	0.00	2	4.00	15	30.00	33	66.00
Legumes and potato	11	22.00	33	66.00	5	10.00	1	2.00
High-fat dairy products	2	4.00	12	24.00	26	52.00	10	20.00
Fatty meat and offals	1	2.00	5	10.00	19	38.00	25	50.00
Meat products	9	18.00	17	34.00	20	40.00	4	8.00

tients consumed the recommended foodstuffs. However, a worrying fact is that that 30% of the subjects never consumed fish.

Table 2 gives the answers concerning the intake of the foodstuffs that are not recommended for patients with type 2 DM.

The data presented in Table 2 show the frequency of intake of foodstuffs that are not recommended for patients with type 2 DM, especially of white-flour products and fatty bakery products, fruits with high sugar content, high-fat dairy products, and meat products.

The comparison of the answers given by the subjects of different genders, using the Mann-Whitney nonparametric test, showed that there was a statistically significant difference only in respect to the intake of low-fat dairy products (p=0.0322) and intake of fatty meat and offals (p=0.02292) (Table 3). The other differences were not statistically significant.

The analysis of the answers of the subjects of different nutritional status using the Kruskall-Wallis nonparametric test showed that there was a statistically significant difference only in respect to the intake of meat products (p=0.0097) (Table 3).

The other differences were not statistically significant.

Discussion

There are numerous risk factors responsible for the onset of diabetes, but of special importance is the fact that some of them can be modified. These factors may prevent the beginning of the disease and its development and they are termed modifiable risk factors. On the other hand, there are some factors that cannot be influenced by lifestyle and these are called non-modifiable risk factors (race, gender, age, genetic predisposition, etc.) (13).

The group of modifiable factors includes obesity, nutritional habits, physical inactivity, etc. (13).

Obesity is one of the most important etiological factor in the development of type 2 DM, and it is significantly present in patients suffering from this disease (14-16).

In the present study, the average BMI value of the subjects was 28.92±3.99 kg/m^2, which indicates that it is higher than the recommended values, as well as the values reported in some similar investigations (17-19).

The analysis of nutritional status of the patients showed that there were only 7 (14%) of them with normal status values. The largest number of patients (28, 56%) were overweight, whereas none of them was underweight.

Table 3. Statistical significance of difference between the genders and between patients of different nutritional status in respect to the intake of particular foodstuffs

Foodstuff	Difference between the genders p-value	Difference between patients of different nutritional status p-value
Whole-grain cereal products	0.4838	0.4192
Citruses, apples	1	1
Nut fruits	0.823	0.7654
Fresh vegetables	1	1
Low-fat dairy products	0.0322	0.9524
Young animals' lean meat	0.2364	0.9404
Fish	0.2169	0.2466
White-flour and fatty bakery products	0.8089	0.1352
Other fruit types (*grapes, bananas, figs,...)*	0.1427	0.1584
Dry fruits	0.9135	0.6008
Legumes and potato	0.2364	0.9404
High-fat dairy products	1	0.0882
Fatty meat and offals	0.0229	0.4878
Meat products	0.5412	0.0097

As it has already been said, the basic principle of treating patients with type 2 DM is the appropriate diet aimed at preventing and curing the disease, as well as preventing development of acute and chronic complications (20). It is assumed that diabetes treatment should be a proper combination of correct calory intake and choice of foodstuffs with other therapy methods (oral antidiabetics, physical activity, reduction of body mass, etc.) (8, 21-23).

The analysis of nutritional habits of the patients encompassed by this study showed that even 73% of them consumed white-flour products and fatty bakery products once or several times a weak. This kind of diet poses serious problems to people with type 2 DM because it involves high concentrations of simple carbohydrates, along with saturated and trans fats, which directly influence the levels of blood glucose and LDL cholesterol. Also, it should be pointed out that fine white flours do not contain adequate concentrations of vitamins, minerals, and dietetic fibers from the grain husk, as all of these ingredients are highly recommendable to the patients. Hence, it is necessary to replace such foodstuffs with whole- grain bakery products (24-27).

Apart from whole-grain products, important sources of dietetic fibers, vitamins, and minerals make foodstuffs from the group of vegetables and fruits. People with type 2 DM should consume fresh raw vegetables and less sweet fruits (citruses, apples, berries, etc.) every day (24-27). The present study did not show statistically significant differences in respect to the intake of different flour products, vegetables and fruits, either related to the nutritional status or gender.

There was a gender-dependent statistically significant difference in respect to the intake of dairy products, as females more frequently consumed these products with lower fat content. On the other hand, the males appeared to be more frequent consumers of fatty meat and offals. No other gender differences were found.

As far as the intake of meat products is concerned, they were involved in diets of the majority of subjects, whereas only 4 (8%) never consumed them. Still, it was found that obese subjects consumed more meat products compared to the other subjects and this proved to be statistically significant difference. Apart from this, no other significant differences were observed in respect to the nutritional status. This study confirmed the results of similar investigations carried out in the surrounding countries and worldwide. The nutritional habits of patients with type 2 DM are most often in accordance with the pertinent recommendations, and are not often related to the nutritional status and gen-

der. Namely, there were more patients who frequently consumed the recommended foodstuffs than those who did not. In most cases these were the patients who previously obtained the knowledge from their family physicians (28, 29, 13).

A shortcoming of this study lies in the fact that it analyzed only one segment of therapy of type 2 DM and it included a small number of subjects, which appears as an impediment for drawing some more precise conclusions.

Conclusion

This investigation showed that, although patients to a significant extent followed the recommendations about dietetic regime, especially in respect to the recommended foodstuffs, their nutritional status was not in accordance with the recommendations for patients with type 2 DM. The recomendations strictly insist on reduced energy intake, more frequent consumption of recommended foodstuffs, and more limited intake of those foodstuffs that are not recommended in the nutritional therapy.

Further investigations should be focused on the analysis of the energy intake, amounts of foodstuffs consumed, as well as on the type and frequency of physical activity, along with the addiction to smoking and alcohol.

Acknowledgments

We sincerely thank all patients who participated in this study. This research received no specific grant from any funding agency in the public, commercial, or nonprofit sectors.

Statement on conflict of interests

The authors of the paper claim that the manuscript has not been published elsewhere as a whole or partly. We agree with the content of the manuscript and approve about its publication in ²Progress in Nutrition². Researching has been approved by institutional ethics committee. These are no financial problems that might lead to a conflict of interest.

References

1. WHO. Global report On Diabetes. World Health Organization, 2016
2. Koye D.N., Magliano D.J., Nelson R.G., Pavkov M.E. The Global Epidemiology of Diabetes and Kidney Disease. Adv Chronic Kidney Dis. 2018; 25(2):121-132
3. Milašinović G. Nacionalni vodič dobre kliničke prakse za dijagnostikovanje i lečenje diabetes mellitus-a, Agencija za akreditaciju zdravstenih ustanova Srbije, Beograd, 2013
4. IZJZS "M. J. Batut", Zdravstveno stanje stanovništva Srbije, 2013
5. Fauci A.S., Kasper D.L., Longo D.L., Braunweld E., Hauser S.L., Lameson J.L., et al, eds. Harrison's principles of Internal medicine, 17thed. New York-Toronto: The McGraw-Hill Companies, 2008
6. Skyler J.S., Bakris G.L., Bonifacio E., Darsow T., Eckel R.H. et all. Differentiation of Diabetes by Pathophysiology, Natural History, and Prognosis. Diabetes. 2017; 66(2):241-255
7. Maheri A., Asnaashari M., Joveini H., Tol A., Firouzian A.A., Rohban A.. The impact of educational intervention on physical activity, nutrition and laboratory parameters in type II diabetic patients. Electron Physician. 2017;9(4):4207-4214
8. Sami W., Ansari T., Butt N.S., Hamid M.R.A. Effect of diet on type 2 diabetes mellitus: A review. Int J Health Sci (Qassim). 2017;11(2):65-71
9. Moghani L.M., Assari S. Association between Actual and Perceived Obesity Weaker among Black than White Children. Behav Sci (Basel). 2018; 8(5):48
10. Roth L.W., Allshouse A.A., Lesh J., Polotsky A.J., Santoro N. The correlation between self-reported and measured height, weight, and BMI in reproductive age women. Maturitas. 2013;76:185-188.
11. http://www.euro.who.int/en/health-topics/disease-prevention/nutrition/a-healthy-lifestyle/body-mass-index-bmi
12. World Health Organization and International Diabetes Federation, Diabetes action now:An initiative of World Health Organization and International Diabetes Federation, 2004., (http://www.who.int/diabetes/actionnow/en/DANbooklet.pdf)
13. Sami W., Ansari T., Butt N.S., Hamid M.R.A. Effect of diet on type 2 diabetes mellitus: a review. Int J Health Sci. 2017;11(2):65-71.
14. Al-Goblan A.S., Al-Alfi M.A., Khan M.Z. Mechanism linking diabetes mellitus and obesity. Diabetes Metab Syndr Obes. 2014;7:587-91
15. Banjari I., Kenjerić D., Mandić M., Glavaš M., Leko J. Longitudinal Observational Study on Diet Quality during Pregnancy and Its Relation to Several Risk Factors for Pregnancy Complications and Outcomes. British Journal of Medicine & Medical Research. 2015; 7(2):145-154
16. Cai J., Ma A., Wang Q., et al. Association between body mass index and diabetes mellitus in tuberculosis patients in China: a community based cross-sectional study. BMC Public Health. 2017;17(1):228

17. Sujanitha V., Sivansuthan S., Selvakan P., Parameswaran P. Overweight, obesity and chronic complications of diabetes mellitus in patients attending Diabetic Centre, Teaching Hospital, Jaffna, Sri Lanka. Cezlon Med J. 2015; 60(3):94-96.
18. Gray N., Picone G., Sloan F., Yashkin A. Relation between BMI and diabetes mellitus and its complications among US older adults. South Med J. 2015;108(1):29-36
19. de Mutsert R., Sun Q., Willett W.C., Hu F.B., van Dam R.M. Overweight in Early Adulthood, Adult Weight Change, and Risk of Type 2 Diabetes, Cardiovascular Diseases, and Certain Cancers in Men: a Cohort Study. American Journal of Epidemiology. 2014;179:1353-1365
20. Demilew Y.M., Alem A.T., Emiru A.A. Dietary practice and associated factors among type 2 diabetic patients in Felege Hiwot Regional Referral Hospital, Bahir Dar, Ethiopia. BMC Res Notes. 2018;11(1): 434
21. Broadbent E., Donkin L., Stroh J., Psych D. Illness and treatment perceptions are associated with adherence to medications, diet, and exercise in diabetic patients. Diab Care. 2011;34:338-340
22. Colberg S.R., Sigal R.J., Fernhall B. et al. Exercise and type 2 diabetes. Diabetes Care. 2010; 33(12):2692-2696
23. Alhariri A., Daud F., Saghir S.A.M. Factors associated with adherence to diet and exercise among type 2 diabetes patients in Yemen. Diabetes Manag. 2017; 7(3):264-271
24. Evert A.B., Boucher J.L., Cypress M., et al. Nutrition therapy recommendations for the management of adults with diabetes. Diabetes Care. 2013;36(11):3821-42
25. Franz M.J. Diabetes Nutrition Therapy: Effectiveness, Macronutrients, Eating Patterns and Weight Management. The American Journal of the Medical Sciences. 2016; 351(4):374-379
26. American Diabetes Association. Nutrition Recommendations and Interventions for Diabetes. A position statement of the American Diabetes Association. Diabetes Care. 2008; 31(Supplement 1): S61-S78.
27. Vitale M., Masulli M., Calabrese I., et al. Impact of a Mediterranean Dietary Pattern and Its Components on Cardiovascular Risk Factors, Glucose Control, and Body Weight in People with Type 2 Diabetes: A Real-Life Study. Nutrients. 2018;10(8):1067
28. Senadheera S.P., Ekanayake S., Wanigatunge C. Dietary Habits of Type 2 Diabetes Patients: Variety and Frequency of Food Intake. J Nutr Metab. 2016;2016:7987395
29. Bazzano L.A., Li T.Y., Joshipura K.J., Hu F.B. Intake of fruit, vegetables, and fruit juices and risk of diabetes in women. Diabetes Care. 2008; 31(7):1311-1317

Correspondence:
Bjelanovic Jelena, MD, MS, PhD,
Specialist in Hygiene, Associate Professor
Futoška 121, 21000 Novi Sad, Serbia
Phone: +381 64/125 24 80
Email: jelena.bjelanovic@mf.uns.ac.rs

Progress in Nutrition 2020; Vol. 22, N. 1: 96-105 DOI: 10.23751/pn.v22i1.7944

Original article

Evaluation of nutritional status in patients with end-stage renal disease in hemodialysis using principal component analysis

Erika Saenz-Pardo-Reyes, Fatima Ezzahra Housni, Antonio López-Espinoza, Alma Gabriela Martínez Moreno, María del Rocio Padilla Galindo

Research Center in Food Behavior and Nutrition (CICAN by its acronym in Spanish). Centro Universitario del Sur. Universidad de Guadalajara. Guzmán, Jalisco. México - E-mail: antonio.lopez@cusur.udg.mx

Summary. The evaluation of nutritional status in patients with end-stage renal disease in hemodialysis is composed of a large number of measurements that complicate their execution. Therefore, the objective of this study is to reduce the number of variables through the principal component analysis (PCA). For this, a PCA was performed with 10 variables of the nutritional diagnosis in patients with hemodialysis: Energy Intake, Protein Intake, IBM,% UBW,% SBW,% MUAC, cAMA,% TCF, HGS and TLC as well as the age of the patients. The results show that PCA matrix with orthogonal rotation Varimex yielded four main components of the evaluation of the nutritional status of renal disease in patients with end-stage hemodialysis, whose value was greater than 0 and explains the 79.91% of the total variance. The first factor was called body composition status, which is composed of cAMA (r = 0.9138), IBM (r = 0.8755),% MUAC (r = 0.8681) and% SBW (r = 0.6238). In the second factor called nutritional risk, a correlation was observed with energy intake (r = -0.8934), protein intake (r = -0.8752) and %TCF (0.5040). The third component called functional status risk is composed of age (r = 0.9022) and HGS (r = 0.8508). The fourth factor, called body composition stability, was correlated with %UBW (r = 0.7456) and %TCF (r = 0.5825). The results of this study will allow reducing the number of variables for the preparation of a nutritional diagnosis in hemodialysis patients. From many to one of the four main components: 1) body composition status, 2) nutritional risk, 3) functional status risk or 4) body composition stability.

Key words: evaluation of nutritional status, principal component analysis, hemodialysis patients, end-stage renal disease

List of abbreviations

%MUAC: Percent of Mid-Upper Arm Circumference
%SBW: Percent of Standard Body Weight
%TSF: Percent of Triceps Skinfold Thickness
%UBW: Percent of Usual Body Weight
Alb: Albumin
AND: Academy of Nutrition and Dietetics
AW: Actual Weight
BMI: Body Mass Index
cAMA: Corrected Arm Muscle Area
CKD: Chronic Kidney Disease
ESRD: End-Stage Renal Disease
HD: Hemodialysis
HGS: Hand-Grip Strenght
ISRNM: International Society of Renal Nutrition and Metabolism
KDOQUI: Kidney Disease Outcomes Quality Initiative
KMO: Kaiser-Meyer-Olkin
LYM%: Lymphocyte percentage
MUAC: Mid-Upper Arm Circumference
NCPM: The Nutrition Care Process and Model
NHANESS II: The second National Health and Nutrition Examination Survey
NKF: National Kidney Foundation
PCA: Principal Component Analysis

PEM: Protein-Energy Malnutrition
PEW: Protein-Energy Wasting
SAH: Systemic Arterial Hypertension
SEGG: Spanish Society of Geriatrics and Gerontology
SENPE: Spanish Society of Parenteral and Enteral Nutrition
T2DM: Type 2 Diabetes mellitus
TLD: Total Lymphocyte count
TSF: Triceps Skinfold
WBC: White Blood Cells
WHO: World Health Organization

Introduction

The Academy of Nutrition and Dietitians (AND) places the evaluation of nutritional status as the first step of the Nutritional Care Process Model (NCPM) and describes it as a systematic method of collecting, comparing and interpreting data and information from different sources that allow us to write a nutritional diagnosis (1-3). In turn, over the years, various authors have differed in the number of components that make up the evaluation of nutritional status in patients with end-stage renal disease in hemodialysis, but have agreed that it requires several components for its application. In addition, it has been sought to group all the components by categories, establishing that the evaluation of nutritional status is composed mainly of anthropometric, biochemical, clinical and dietary parameters, usually referred as the A, B, C, D of the evaluation of nutritional status. However, each proposed category is made up of a large number of components that, when collected and interpreted together, complicate the execution of the evaluation of nutritional status and the writing of nutritional diagnosis (1-14,20,22-23).

For this reason, the objective of this study is to determine the main components of the evaluation of nutritional status that allow formulating a nutritional diagnosis in patients with end-stage renal disease (ESRD) in hemodialysis (HD). In such a way that the clinical health and nutrition professional can select the minimum components of the evaluation of nutritional status that make up the nutritional diagnosis in a group of patients.

Materials and methods

Cross-sectional, observational, descriptive and correlational study, in which the principal component analysis method was applied and the main indicators of nutritional status in hemodialysis patients were correlated. We evaluated 31 outpatients diagnosed with chronic renal failure (CKD) between 21 and 84 years who were in a hemodialysis program. The study was conducted according to the Helsinki declaration and the informed consent of all the patients was obtained before enrollment.

The evaluation of the nutritional status of patients on hemodialysis was made up of the following components: 1) anthropometric parameters, 2) biochemical parameters, 3) dietary parameters and 4) functional parameters.

Anthropometric parameters

The anthropometric measurements were made by a specialist in clinical nutrition with 10 years of experience in Care Process Certification & Medical Therapy in Renal Disease. The anthropometric parameters evaluated were: current weight (AW), body mass index (BMI), percent of usual body weight (% UBW), percent of standard body weight (% SBW), percent of triceps skinfold (% TSF), the percentage of the mid-upper arm circumference (% MUAC), the corrected mid-upper arm muscle area (cAMA).

Next, the method used for its interpretation is described. The AW was considered post-hemodialysis weight or dry weight. The BMI was interpreted in subjects > 20 and < 65 years of age using the cut-off points proposed by the World Health Organization (WHO) of 2006. For subjects > 64 years of age, the interpretation proposed by the Spanish Society of Parenteral and Enteral Nutrition (SENPE) and the Spanish Society of Geriatrics and Gerontology (SEGG) of 2007 was used (15-16).

The % UBW was calculated by comparing the AW against the usual body weight (UBW) by the following formula: %UBW=[(UBW-AW)]/UBW x100, considering weight loss > 7.5% in three months as a serious weight loss. To calculate the % SBW, the bone structure of the patient was first determined and classified with the tables of the Metropolitan Life Insurance Company. Subsequently, the standard body weight (SBW) was ob-

tained with the tables of the National Health and Nutrition Examination Survey (NHANESS II) and finally the variation between the patient›s AW with the SBW using the formula: %SBW=(AW/SBW)X100; being interpreted as malnutrition values < 95% and as excess weight at values > 115% (17-20).

To calculate and interpret the % TSF and the % MUAC, the TSF and the MUAC were first located in the Frisancho percentile tables and then the percentages of each were calculated with the following formulas: % TSF=[(TSF actual)/(TSF p50)]x100 y MUAC=[MUACactual/(MUAC p50)]x100. Finally, the TSF was interpreted as adipose tissue excess at values > 110% and deficit values <90%. Subsequently, the cAMA was calculated and according to the Frisancho percentile tables, the data located from p5 to p15 were interpreted as mild to moderate depletion of muscle tissue and the data <p5 as severe depletion of muscle tissue (17-20).

Height and current weight (AW) were taken in a single measurement. The triceps skinfold (TSF) and mid-upper arm circumference (MUAC) were performed in three measurements repeated by a single evaluator. Subsequently, we calculated the technical error (TEM) intra-evaluator of TSF and MUAC measurements for patients with end-stage renal disease in hemodialysis with the following equation: Absolute TEM=$\sqrt{(\sum D_i^2)/2n}$ and Relative TEM%=(Absolute TEM)/VAV*100. The relative TEM for intra-evaluator verification for TSF were 5.2% and for MUAC were 0.6%, this means that the human error for measurements in the study was acceptable. (21-22).

Biochemical parameters

The biochemical parameters analyzed for the evaluation of nutritional status were serum albumin (Alb) and total lymphocyte count (TLC). The TLC was calculated with the total leukocyte values (WBC) and the percentage of lymphocytes (LYM%) by the following formula: TLC=[LYM(%)*WBC(k/uL)]/100, considering as malnutrition the values ≤ 2000 lymphocytes / mL; and values ≤ 3.5 g/dL were interpreted as malnutrition by serum albumin. Serum phosphorus and potassium were also evaluated as metabolic markers related to the nutrition of patients on hemodialysis (11,23-25).

Dietary parameters

The energy and protein intake was evaluated by means of a 3-day food diary also called a food and beverage register. Prior to the delivery of the food diary, individual training was provided on the size of the portion in household measurements and grams by a specialist in clinical nutrition. In turn, the method of registering the food diary was taught and the patients were asked to record the consumption of two non-consecutive weekday days and one weekend day. Afterwards, the nutrient content of the ingested food and beverages of the three days was calculated in the Nutrimind© nutrition software and an average of the energy, protein, phosphorus, potassium, liquid and fiber intake of each patient was performed. Finally, the data obtained were compared with the values recommended by the KDOQUI Clinical Practice Guidelines for patients on hemodialysis. In the intake of phosphorus, potassium, liquids and fiber, an adjustment was made to be considered deficient intake at values lower than 90% compared to those recommended and an excessive intake at values higher than 110% compared to those recommended for each nutrient (4,7-9,24-26).

Functional parameters

The hand-grip strength (HGS) was evaluated by dynamometry, performing the measurement on the non-fistula side or hemodialysis catheter. The measurement was made before the hemodialysis session, with the arms in extension, parallel to the body and without support, indicating the patients to grasp the dynamometer with maximum force. The strength of the hand was measured three times with a recovery time of one minute, registering the maximum value with the muscle strength data. Values lower than the 10th percentile according to age and sex were interpreted as low muscular strength and values between the 10th percentile and the 25th percentile categorized as below average muscular strength (27).

Statistical analysis

A Principal Components Analysis (PCA) was performed using the Factor procedure in STATA (version 12.0). We use the 10 types of nutritional status

assessment in our models. In nutritional epidemiology, the most used method to derive the nutritional diagnosis is ACP with varimax rotation; therefore, the factors were rotated by an orthogonal transformation (varimax rotation function) to improve the difference between the loads, which allowed an easier interpretation. The number of factors to be retained was determined using the own values diagram (the Scree graph) and the interpretability of the factors.

Results

Of the 31 patients evaluated nutritionally, 39% (n = 12) corresponded to the female sex and 61% (n = 19) to the male sex; with a minimum age of 21 years, a maximum of 84 years and a mean of 61.1 ± 16.9 years. In addition to the diagnosis of CDK, 87% (n = 27) had pathological personal history of systemic arterial hypertension (SAH), 68% (n = 21) of diabetes mellitus type 2 (T2DM), 6% (n = 2) of hyperuricemia, 6% (n = 2) of alcoholism and 3% (n = 1) of dyslipidemias (Table 1). The results obtained from the evaluation of nutritional status in hemodialysis patients are described below:

Anthropometric parameters

The sample of patients on hemodialysis had a minimum AW of 41 kg and a maximum of 117.4 kg, with a mean of 70.9 ± 17.9 kg. Regarding the evaluation of nutritional status, the results are shown in Table 1, with a higher prevalence of normal BMI (54.8%), followed by overweight (19.4%) and obesity I (19.4) and without any diagnosis of thinness by BMI. However, in the evaluation of nutritional status by %UBW it is shown that 26% of the population has presented a serious loss of weight, that is, they lost more than 5% of weight in a month. Likewise, a high prevalence of malnutrition was shown by %SBW (58%), a high prevalence of adipose tissue deficit (39%) and a greater prevalence of muscle mass depletion as measured by %MUAC (45.3%) and cAMA (54.9 %).

Biochemical parameters

A higher prevalence of malnutrition was shown when evaluating nutritional status by means of CTL than by Alb levels; we observed a frequency of 72.4% of the diagnosis of malnutrition by CTL, in contrast

Table 1. Anthropometric parameters of nutritional assessment

	n	%
BMI (kg/m²)[1]		
-Severe thinness (<16 kg/m²)	0	0
-Moderate thinness (16 to 16.99 kg/m²)	0	0
-Mild thinness (17 to 18.49 kg/m²)	0	0
-Normal range (18.5 to 24.99 kg/m²)	17	**54.8**
-Overweight (25 to 29.99 kg/m²)	6	19.4
-Obese class I (30 to 34.99 kg/m²)	6	19.4
-Obese class II (35 to 39.99 kg/m²)	1	3.2
-Obese class III (≥ 40 kg/m²)	1	3.2
%UBW[1]		
-Severe weight loss (in 3 months a weight loss of 7.5%)	7	**23**
%SBW[1]		
-Severe malnutrition (<70%)	2	**6**
-Moderate malnutrition (70 to 85%)	8	**26**
-Mild malnutrition (85.1 to 95%)	8	**26**
-Normal (95 to 115%)	8	26
-Overweight (115.1 to 130%)	5	16
-Moderate obese (131 to 150%)	0	0
-Severe obese (>150%)	0	0
%TSF[1]		
-Severe deficit of adipose tissue (<70%)	8	**26**
-Moderate deficit of adipose tissue (70 to 80%)	1	**3**
-Mild deficit of adipose tissue (80 to 90%)	3	**10**
-Average adipose tissue (90 to 110%)	8	26
-Excess of adipose tissue (>110%)	11	35
%MUAC[1]		
-Severe muscle tissue deficit (<70%)	2	**6.5**
-Moderate muscle tissue deficit (70 to 80%)	6	**19.4**
-Mild muscle tissue deficit (80 to 90%)	6	**19.4**
-Average muscle tissue (90 to 110%)	15	48.4
-Excess of muscle tissue (>110%)	2	6.5
cAMA[1]		
-Severe depletion of muscle mass (<p5)	11	**35.5**
-Moderate depletion of muscle mass (p5 to p15)	6	**19.4**
-Average muscle mass (>p15)	14	45.2

[1]BMI: Body mass index; %USW: Percent usual body weight; %SBW: Percent standard body; %TSF: Percent triceps skinfold thickness; %MUAC: Percent mid-upper arm circumference; cAMA: Corrected arm muscle area

Table 2. Evaluation of nutritional biochemical parameters

	n	%
Alb[1] (n=23)		
-Severe malnutrition (<2.5 g/dL)	0	0
-Moderate malnutrition (2.5 - 2.9 g/dL)	1	4
-Mild malnutrition (3 - 3.49 g/dL)	2	9
-Normal (3.5 - 4.5 g/dL)	20	**87**
TLC[1] (n=29)		
-Severe malnutrition (<800 lymphocytes/mL)	4	**13.8**
-Moderate malnutrition (800-1999 lymphocytes/mL)	4	**13.8**
-Mild malnutrition (1200-1599 lymphocytes/mL)	13	**44.8**
-Normal (>1600 lymphocytes/mL)	8	27.6
Serum phosphorus levels (n=25)		
-Normal values (2.5 - 5 mg/dL)	12	48
-High (>5 mg/dL)	11	**44**
-Below (<2.5 mg/dL)	2	8
Serum potassium levels (n=28)		
-Normal values (3.5 - 5 meq/L)	8	29
-High (> 5 meq/L)	20	**71**

[1]Alb: Albumin; TLC: Total lymphocyte count

Table 3. Dietary parameters of nutritional assessment

	n	%
Energy intake		
-Inadequate energy intake	30	**97**
>60 years: <30 cal/kg		
<60 years: <35 cal/kg		
-Adequate energy intake	0	0
>60 years: 30-35 cal/kg		
<60 years: 35 cal/kg		
-Excessive energy intake	1	3
>60 years: >35 cal/kg		
<60 years: >35 cal/kg		
Protein intake		
-Inadequate protein intake (<1.1 g/kg)	26	**84**
-Adequate protein intake (1.1 - 1.2 g/kg)	3	10
-Excessive protein intake (>1.2 g/kg)	2	6
Phosphorus intake[1]		
-Inadequate phosphorus intake (<800 mg)	23	**74.2**
-Adequate phosphorus intake (800-1000 mg)	6	19.4
-Excessive phosphorus intake (>1000 mg)	2	6.5
Potassium intake[1]		
-Inadequate potassium intake (<40 mg/kg)	0	0
-Adequate potassium intake (40 mg/kg)	7	23
-Excessive potassium intake (>40 mg/kg)	24	**77**
Fluid intake[1]		
-Inadequate fluid intake (<1000 mL + diuresis)	21	**68**
-Adequate fluid intake (1000 mL + diuresis)	9	29
-Excessive fluid intake (>1000 mL + diuresis)	1	3
Fiber intake[1]		
-Inadequate fiber intake (<20 g)	23	**74**
-Adequate fiber intake (20 - 30 g)	8	26
-Excessive fiber intake (>30 g)	0	0

[1]Inadequate intake: <90% of recommended values; Excessive intake >110% of recommended values.

with 13% by Alb. On the other hand, the evaluation of the metabolic markers related to the nutrition of patients on hemodialysis showed high levels of serum phosphorus with a 44% prevalence and elevated levels of serum potassium in 71%.

Dietary parameters

The evaluation of nutritional status by dietary parameters showed that patients on hemodialysis have a deficient intake of energy (97%) and proteins (84%). In addition to a deficient intake of phosphorus (74.2%), liquids (68%) and fiber (74%); and an excessive intake of potassium (77%).

Functional parameters

There was a high prevalence of low muscular strength (92%, n = 24) measured by HGS in the hemodialysis patients evaluated. In addition, no patient showed average muscle strength levels for age and sex, and only 8% (n = 2) had below-average muscle strength levels.

Principal Component Analysis (PCA)

A PCA was performed with 10 variables of nutritional diagnosis in patients with hemodialysis: Energy Intake, Protein Intake, IBM, %UBW, %SBW, %MUAC, cAMA, %TCF, HGS and TLC as well as the age of the patients. First, we obtain a matrix of correlations between all the variables considered (r of Pearson). The basic assumption of factor analysis is that the correlation matrix expresses a pattern of relations between variables that can be deciphered. It can be seen in table 4, the significant correlation between the variables (p <0.005).

Table 4. Correlation matrix

Variable	Age	Energy Intake	Protein Intake	IBM	%UBW	%SBW	%MUAC	cAMA	%TCF	HGS	TLC
Age	1										
Energy Intake	0.8830	1									
Protein Intake	0.9060	**0.0000**	1								
IBM	0.6454	**0.0024**	**0.0004**	1							
%UBW	0.5772	**0.0150**	**0.0356**	0.0933	1						
%SBW	0.0913	**0.0005**	**0.0003**	**0.0000**	0.3235	1					
%MUAC	0.7449	**0.0325**	**0.0207**	**0.0000**	**0.0214**	**0.0366**	1				
cAMA	0.6931	**0.0011**	**0.0005**	**0.0000**	0.0625	**0.0000**	**0.000**	1			
%TCF	0.5315	0.0743	**0.0323**	**0.0190**	0.9663	**0.0136**	0.3589	0.0558	1		
HGS	**0.0006**	0.3787	0.4393	0.3970	0.5970	**0.0422**	0.6285	0.3284	0.5256	1	
TLC	0.4695	**0.0664**	0.1826	**0.0164**	**0.0397**	0.1163	0.0815	0.0531	0.6560	0.7862	1

Determinant of the correlation matrix P<0.005

With the generation of the correlation matrix, we obtain a series of statistical tests that indicated whether it is pertinent or not to carry out the factorial analysis with the available information. Then, it was found that for the Bartlett test, the variables are significantly correlated since a value of p-value = 0.0000 is obtained, so the adjustment of the variables by factor analysis is considered appropriate. In addition, for the Kaiser-Meyer-Olkin Coefficient (KMO) the value is greater than 0.6 (0.661), so it is considered an acceptable analysis (Table 5).

From the correlation of the main components (Table 6), four factors whose value is greater than 0 were determined and which explain 79.91% of the total variance. The first factor identified explains 43.85% of the variance, the second factor explains 59.74% of the variance, factor three the 70.69% of the variance and the last factor of 79.91% of the variance.

An orthogonal varimax rotation was performed, from which we obtain a matrix of rotated components that indicates the correlation between each of the variables and their corresponding factor. According to the matrix of analysis of principal components (PCA) with varimax orthogonal rotation, the first factor is made up of IBM, %SBW, %MUAC, cAMA. In such a way that IBM, %SBW, %MUAC, cAMA, present positive correlations indicating that an increase in IBM, %WBW, %MUAC involves an increase in cAMA. The second factor is formed by Energy Intake, Protein Intake and %TCF in such a way that when there is a decrease in energy and protein intake there is an increase in %TCF. The third factor is formed by age and HGS so that when age increases the measure of HGS decreases and the fourth factor is made up of %UBW and %TCF with a positive correlation (Table 7).

Table 5. Bartlett Test of Sphericity and Kaiser-Meyer-Olkin measure

Bartlett test	Chi-square	234.089
	Degrees of freedom	55
	p-value	**0.000**
Kaiser-Meyer-Olkin Measure of Sampling Adequacy	KMO	**0.661**

Table 6. Correlation of principal components

Component	Eigenvalue	Difference	Proportion	Cumulative
Component 1	4.82299	3.0749	43.85%	43.85%
Component 2	1.74809	0.543774	15.89%	59.74%
Component 3	1.20431	0.190118	10.95%	70.69%
Component 4	1.0142	0.258623	9.22%	79.91%
Component 5	.755573	.171305	6.87%	86.77%
Component 6	.584268	.182416	5.31%	92.09%
Component 7	.401852	137625	3.65%	95.74%
Component 8	.264227	.158208	2.40%	98.14%
Component 9	.106019	.036534	0.96%	99.10%
Component 10	.0694851	.0404956	0.63%	99.74%
Component 11	.0289895	.	0.26%	100%

Chi2(45) = 243.27 and P= 0.0000

Table 7. Matrix rotation

Variable	Matrix rotation			
	Factor 1	Factor 2	Factor 3	Factor 4
Age	-0.0587	0.1150	0.9022	0.0968
Energy Intake	-0.2714	-0.8934	0.0251	0.1679
Protein Intake	-0.3253	-0.8752	-0.0216	0.0576
IBM	0.8755	0.3602	-0.1096	0.0475
%UBW	-0.2207	-0.3774	0.0800	0.7456
%SBW	0.6238	0.4977	-0.3870	0.1914
%MUAC	0.8681	0.0774	0.1517	-0.2425
cAMA	0.9138	0.2979	-0.0853	-0.0441
%TCF	0.2922	0.5040	0.0671	0.5825
DINAMOTRY	0.0153	0.1970	-0.8508	0.0572
TLC	0.3953	0.2437	0.1898	-0.4588

After the varimax rotation, the correlation between the set of variables that make up the factor 1 present 29.25% of the total variance. The correlation between the variables in factor 2 is 52.38%, factor 3 represents 68.55% of the total variance and factor 3 of 79.91% (Table 8).

Discussion

First, it is important to discern the nomenclature and the diagnostic criteria used so far to evaluate the nutritional status in patients with CDK. The International Society of Renal Nutrition and Metabolism (ISRNM) "review and develop standard terminology and definitions related to wasting, cachexia, malnutrition, and inflammation in CDK and recommends the term protein-energy wasting (PEW) for the loss of body protein mass and fuel reserves" that has specific criteria for clinical diagnosis (28). For its part, the National Kidney Foundation (NKF) developed the K/DOQUI guidelines which have a section entitled "Evaluation of Protein-Energy Nutritional Status" in patients with dialysis, concluding that the nutritional status should be evaluated with a combination of different components, since there is no single measure that provides a complete indication of the protein-energy status. It is recommended to evaluate energy and protein intake, visceral protein reserves, muscle mass, other dimensions of the body composition and functional status to evaluate protein-energy malnutrition (PEM) (24).

Therefore, it is important to point out that the purpose of the evaluation of nutritional status is to identify problems related to nutrition and the pathology of study, which allow to plan and implement evidence-based strategies and clinical practice guidelines designed to address the identified nutritional problems (1-11,24). The objective of this study is to simplify the process of evaluation of nutritional status through the PCA.

This study shows that patients with ESRD in HD have a high prevalence of PEM diagnosed nutritionally by a severe weight loss determined by %UBW (26%), malnutrition by %SBW (58%), adipose tissue deficit by %TSF (39%), depletion of muscle tissue by

Table 8. Analysis principal component factors

Principal component	Names	Location	Variance	Difference	Cumulative
Factor 1	Body composition status	IBM	3.21	0.67356	29.25%
		%SBW			
		%MUAC			
		cAMA			
Factor 2	Nutritional risk	Energy Intake	2.54	0.76614	52.38%
		Protein Intake			
		%TCF			
Factor 3	Functional status risk	HGS	1.77	0.52851	68.55%
		Age			
Factor 4	Body composition stability	%SBW	1.24	.	79.91%
		%TCF			

Chi2(55) = 243.27 P= 0.0000

%MUAC (45.3%) and cAMA (54.9%), malnutrition by CTL (72.4%), deficient energy intake (97%), deficient protein intake (84%) and low HGS (92%). These results agree with cross-sectional analyzes performed in patients on HD who have found a prevalence of more than 60% of PEM and some authors have cited that the HD procedure is a general catabolic event *per se*, which decreases circulating amino acids and accelerates proteolysis rates muscle and body mass leading to PEM (29-32). It is also important to point out that in the sample studied, 68% (n = 21) of the patients with ESRD in HD had T2DM and it has been experimentally concluded that diabetes mellitus causes loss of muscle proteins by the activation of the ubiquitin-proteasome pathway with increased expression of the ubiquitin gene (33).

In addition to the above, it is relevant to note the high prevalence of low HGS (92%) present in patients with ESRD in HD and to contrast this fact with the results found by BMI and Alb; the BMI did not identify underweight (0%) as a nutritional problem that contributes to the nutritional diagnosis of PEM and a low prevalence of malnutrition (13%) was found by Alb. Some studies have found that low HGS is not influenced by dialysis variables and therefore can be used as a reliable nutritional marker in HD patients. A significant linear trend towards progressively lower values of HGS with the degree of malnutrition has also been shown (27,30). Therefore, we conclude that HGS can be considered an indicator of nutritional risk in patients with ESRD in HD independently of the results of anthropometric and biochemical parameters. Given that muscle strength is the first component of the evaluation of nutritional status that is affected, reflecting a decrease in functionality that will lead to a progressive loss of muscle mass that will affect the morbidity and mortality of this group of patients.

The objective of the analysis of main components was to reduce the number of variables used for the evaluation of nutritional status, losing as little information as possible. The results of this analysis showed that a nutritional diagnosis can be made in patients on hemodialysis by means of four main components of the evaluation of nutritional status. Also, the results obtained through the PCA show theoretical and practical logic that allowed us to assign names to the components. The first component was called the body composition status component, the second component was the nutritional risk component, the third component was functional status risk and the fourth component was body composition stability. In the rotation matrix (Table 7) the relation between the components and the indicators is observed, being in the first component called body composition status a correlation with cAMA (r = 0.9138), IBM (r = 0.8755), %MUAC (r) = 0.8681) and %SBW (r = 0.6238), that is, the higher the cAMA, IBM, %MUAC and %SBW, the greater or better body composition status. As previously mentioned, these results show theoretical and practical coherence, since the anthropometric parameters of weight and body mass are related to the body composition status (15-20) since several studies have shown that decreased levels in these parameters lead to a decrease in the quality of life and an increased risk of mortality related to PME (34-36).

In the second component called nutritional risk, a correlation was observed with energy intake (r = -0.8934), protein intake (r = -0.8752) and %TCF (0.5040), that is, the lower the energy and protein intake, the greater the nutritional risk, or in other words, the higher the energy and protein intake, the lower the nutritional risk. These results have already been demonstrated in several studies, recognizing as one of the main causes of malnutrition in patients on hemodialysis to deficient food intake. On the other hand, the correlation between the %TCF with this component shows that body fat is a nutritional risk factor, because it increases the risk of suffering cardiovascular disease (37-40).

In the third component called functional status risk, a correlation was found with age (r = 0.9022) and HGS (r = 0.8508), that is, higher values of HGS lower functional status risk, or said other way at lower values of HGS greater functional status risk. Likewise, the higher the age, the higher the functional status risk. These results agree with the information shown in other studies in patients on hemodialysis, in which, it has been found that a low HGS is related to a lower quality of life and nutritional status. Likewise, it has been pointed out that age is a risk factor for malnutrition and morbidity and mortality in patients on hemodialysis 30,41).

Finally, in the fourth component called body composition stability was correlated with the %UBW (r = 0.7456) and the %TCF (r = 0.5825), that is, the higher %USW and %TCF higher body composition stability. Concluding that the body composition stability is indispensable in patients with end-stage renal disease in hemodialysis.

To conclude: The number of variables used for the evaluation of nutritional status in the end-stage renal disease in hemodialysis was reduced by the PCA to four main components called: 1) body composition status, 2) nutritional risk, 3) functional status risk and 4) body composition stability.

Ultimately, this manuscript provides scientific evidence for health and nutrition professionals to make decisions about the selection and interpretation of the main minimum components for the assessment of nutritional status in the end-stage renal disease in hemodialysis. These conclusions facilitate the medical and nutritional care of greater efficiency and quality in this group of patients.

References

1. Lacey K, Pritchett E. Nutrition care process and model: ADA adopts road map to quality care and outcomes management. J Am Diet Assoc. 2003; 8:1061-72.
2. Writing Group of the Nutrition Care Process/Standardized Language Committee. Nutrition care process and model part I: the 2008 update. J Am Diet Assoc. 2008;108(7):1113-7.
3. Writing Group of the Nutrition Care Process/Standardized Language Committee. Nutrition care process part II: using the international dietetics and nutrition terminology to document the nutrition care process. J Am Diet Assoc. 2008;108(8):1287-93.
4. Pasticci F, Fantuzzi AL, Pegoraro M, McCann M, Bedogni G. Nutritional management of stage 5 chronic kidney disease. J Ren Care. 2012;38(1):50-8.
5. Moise C, Thomas LK. Nutrition Care Process: Screening and assessment. In: Thomas LK, Bohnstadt JO, editors. Nutrition therapy for chronic kidney disease. New York: CRC Press; 2012. p. 93-106.
6. DiBenedetto P, Brommage D. Nutrition assessment in chronic kidney disease. In: Byham-Gray L, Stover J, Wiesen K, editors. A clinical guide to nutrition care in kidney disease. 2nd Ed. US: Academy of Nutrition and Dietetics; 2013. p. 7-24.
7. Hutson B, Stuart N. Nutrition management of adult hemodialysis patient. In: Byham-Gray L, Stover J, Wiesen K, editors. A clinical guide to nutrition care in kidney disease. 2nd Ed. US: Academy of Nutrition and Dietetics; 2013. p. 53-68.
8. Beto JA, Ramirez WE, Bansal VK. Medical nutrition therapy in adults with chronic kidney disease: integrating evidence and consensus into practice for the generalist registered dietitian nutritionist. J Acad Nutr Diet. 2014;114(7):1077-87.
9. Moore L. Dietary Assessment in Kidney Disease. In: Byham-Gray LD, Burrowes JD, Glen MC, editors. Nutrition in Kidney disease. 2nd Ed. US: Humana Press; 2014. p. 25-48.
10. Dumbler F. Anthropometric assessment in kidney disease. In: Byham-Gray LD, Burrowes JD, Glen MC, editors. Nutrition in Kidney disease. 2nd Ed. US: Humana Press; 2014. p. 49-56.
11. Cockram D. Biochemical nutritional assessment in chronic kidney disease. In: Byham-Gray LD, Burrowes JD, Glen MC, editors. Nutrition in Kidney disease. 2nd Ed. US: Humana Press; 2014. p. 57-68.
12. Suverza A, Haua. El ABCD de la evaluación del estado de nutrición. México: Mc Graw Hill, 2010.
13. Gibson R. Principles of nutritional assessment. New York: Oxford University Press, 1990.
14. Gibson R. Principles of nutritional assessment. 2nd Ed. New York: Oxford University Press, 2005.
15. World Health Organization. Physical status: the use and interpretation of anthropometry. Report of a WHO Expert Committee. World Health Organ Tech Rep Ser. 1995; 854:1-452.
16. Sociedad Española de Nutrición Parenteral y Enteral (SENPE), Sociedad Española de Geriatría y Gerontología (SEGG). Valoración nutricional en el anciano. Recomendaciones prácticas de los expertos en geriatría y nutrición. España: Galénitas-Nigra Trea, 2007.
17. Blackburn GL, Bistrian BR, Maini BS, Schlamm HT, Smith MF. Nutritional and metabolic assessment of the hospitalized patient. JPEN J Parenter Enteral Nutr. 1977; 1:11-22.
18. Frisancho AR. Anthropometric standards for the assessment of growth and nutritional status. Ann Arbor, Michigan: University of Michigan Press, 1990.
19. Frisancho AR. New standards of weight and body composition by frame and size and height for assessment of nutritional status of adults and the elderly. Am J Clin Nutr. 1984; 40(4): 808-19.
20. Osuna IA. Proceso del Cuidado Nutricional en la Enfermedad Renal Crónica. México: Manual Moderno, 2016.
21. Perini TA, Oliveira GL, Ornellas J, Oliveira FP. Technical error of measurement in anthropometry. Rev Bras Med Esporte. 2005; 11(1): 81-85.
22. Geeta A, Jamaiyah H, Safiza MN, Khor GL, Kee CC, Ahmad AZ, Suzana S, Rahmah R, Faudzi A. Reliability, technical error of measurements and validity of instruments for nutritional status assessment of adults in Malasyia. Singapore Med J. 2009; 50(10): 1013-8.
23. Ignacio de Ulíbarri J, González-Madroño A, de Villar NG, González P, González B, Mancha A, Rodríguez F, Fernández G. CONUT: a tool for controlling nutritional status. First validation in a hospital population. Nutr Hosp. 2005; 20:38-45.
24. National Kidney Foundation: K/DOQUI. Clinical practice guidelines for chronic kidney disease: Nutrition in chronic renal failure: adult guidelines. Am J Kidney Dis. 2000;35(6S2): S17-S104.

25. National Kidney Foundation: K/DOQUI. Clinical practice guidelines for bone metabolism and disease in chronic kidney disease. Am J Kidney Dis. 2003;42(4S3):S63-S64,S77-S78.
26. Nutrimind. Software de Nutrición. 2018. https://www.nutrimind.net
27. Leal VO, Stockler-Pinto MB, Farage NE, Aranha LN, Fouque D, Anjos LA, Mafra D. Handgrip strength and its dialysis determinants in hemodialysis patients. Nutrition. 2011; 27(11-12):1125-1129.
28. Fouque D, Kalantar-Zadeh K, Kopple J et al. A proposed nomenclature and diagnostic criteria for protein-energy wasting in acute and chronic kidney disease. Kidney Int. 2008;73(4):391-8.
29. Kalantar-Zadeh K. Protein-Energy Wasting. In: Byham-Gray LD, Burrowes JD, Glen MC, editors. Nutrition in Kidney disease. 2nd Ed. US: Humana Press; 2014. p. 219-230.
30. Qureshi AR, Alvestrand A, Danielsson A, Divino-Filho JC, Gutierrez A, Lindholm B, Bergström J. Factors predicting malnutrition in hemodialysis patients: a cross-sectional study. Kidney Int.1998;53(3):773–82.
31. Guiterrez A, Alvestrand A, Wahren J, Bergström, J. Effect of in vivo contact between blood and dialysis membranes on protein catabolism in humans. Kidney Int. 1990;38(3):487-94.
32. Ikizler TA, Pupim LB, Brouillette JR, Levenhagen DK, Farmer K, Hakim RM, Flakoll PJ. Hemodialysis stimulates muscle and whole-body protein loss and alters substrate oxidation. Am J Physiol Endocrinol Metab. 2002; 282: E107-16.
33. Price SR, Bailey JL, Wang X, Jurkovitz C, England BK, Ding X, Phillips LS, Mitch WE. Muscle wasting in insulinopenic rat's results from activation of the ATP-dependent, ubiquitin-proteasome proteolytic pathway by a mechanism including gene transcription. J Clin Invest. 1996;98(8):1703-08.
34. Allen KL, Miskulin D, Yang G, Dwyer JT, Frydrych A, Leung J, Poole D. Hemodialysis (HEMO) Study Group. Association of nutritional markers with physical and mental health status in prevalent hemodialysis patients from HEMO study. J Ren Nutr. 2002;12(3):160-9.
35. Chumlea WC, Dwyer J, Bergen C, Burkat J, Paranandi L, Frydrych A, Cockram DB, Kusek JW, McLeroy S. Hemodialysis Study Group. Nutritional status assessed from anthropometric measures in the HEMO study. J Ren Nutr. 2003;13(1):31-8.
36. Fung F, Sherrard DJ, Gillen DL, Wong C, Kestenbaum B, Seliger S, Ball A, Stehman-Breen C. Increased risk for cardiovascular mortality among malnourished end-stage renal disease patients. Am J Kidney Dis. 2002;40(2):307-14.
37. Dwyer JT, Cunniff PJ, Maroni BJ, Kopple JD, Burrowes JD, Powers SN, Cockram DB, Chumlea WC, Kusek JW, Makoff R, Goldstein DJ, Paranandi L. The hemodialysis pilot study: nutrition program and participant characteristics at baseline. The HEMO Study Group. J Ren Nutr. 1998;8(1):11-20.
38. Bossola M, Muscaritoli M, Tazza L, Panocchia N, Liberatori M, Giungi S, Tortorelli A, Rossi Fanelli F, Luciani G. Variables associated with reduced dietary intake in hemodialysis patients. J Ren Nutr. 2005;15(2):244-252.
39. Bergström J. Why are dialysis patients malnourished? Am J Kidney Dis. 1995;26(1):229-41.
40. González E, Díez JJ, Pérez Torres A, Bajo MA, Del Peso G, Sánchez-Villanueva R, Grande C, Rodríguez O, Coronado M, Gómez Candela C, Díaz-Almirón M, Iglesias P, Selgas R. Body composition analysis and adipocytokine concentrations in hemodialysis patients: abdominal fat gain as and additional cardiovascular risk factor. Nefrologia. 2017;37(2):138-148.
41. Chauveau P, Combe C, Laville M, Fouque D, Azar R, Cano N, Canaud B, Roth H, Leverve X, Aparicio M. French Study Group for Nutrition in Dialysis. Am J Kidney Dis. 2001;37(5):997-1003.

Correspondence:
Antonio López Espinoza
Av. Enrique Arreola Silva 883, Col. Centro. C.P. 49000. Cd. Guzmán, Jalisco. México.
Phone: (+52) 341 5752222 ext. 46123
E-mail: antonio.lopez@cusur.udg.mx

Progress in Nutrition 2020; Vol. 22, N. 1: 106-111 DOI: 10.23751/pn.v22i1.9091

Original article

Determination of difference between freshman and senior-level of psychology students in terms of the eating attitudes and psychological symptoms

Saban Karayagiz
Department of Psychology, Nuh Naci Yazgan University, Kayseri/Turkey - E-mail: skarayagiz@nny.edu.tr

Summary. *Aim*: Eating disorder (ED) is a deviation in eating habits that are based on insufficient or overeating and mental influences, causing serious problems such as anxiety. In recent years, some physical appearances have become more prominent in social settings such as TV and social media. The purpose of this study was to investigate the impacts of appearance with body dissatisfaction, perfectionism levels, and psychological symptoms of the university students on the eating attitude enrolled at the department of psychology. Particularly, it focused on whether any changes in the attitudes towards eating habits could change after psychology education in college. *Method*: Data was collected from the psychology students at their freshman in 2015 and senior years in 2019 by utilizing a longitudinal study methodology. Data collection tools contained Positive and Negative Perfectionism Scale (PNPS), Social Appearance Anxiety Scale (SAAS), Brief Symptom Inventory (BSI), and the Eating Attitudes Test (EAT). *Study Group*: A total of 53 college students were included in the study. Most of them (N=40, 75.6%) were female and few (N=13, 24.5%) were male. The average ages of the students were found 20.6(2015 sd parameter 1.8, 2019 sd parameter 1.7) years old during the data collection process. *Results and Discussion*: The findings showed that male students achieved lower scores than females in terms of eating habits (EAT) and psychological symptoms (BSI), and higher on appearance anxiety levels (SAAS) based on the results of the pretest and posttest sessions.

Key words: eating behaviors, appearance anxiety, perfectionism.

Introduction

Eating disorder (ED) is one of the most common problems among teenagers and commonly considered to be affected by their attitudes towards social and physical appearances, and perfectionism (especially physically) as well as having any forms of psychological symptoms (1-3).

Several studies have been conducted regarding eating habits and disorder cases at different age stages and groups concerning other significant factors such as perfectionism, body image dissatisfaction, gender issues, eating behavior, personality, and parental bonding (1-9).

In a related study, Eisenberg (10) investigated the prevalence, correlation, persistence, and treatment-related issues of the eating disorder among a group of college students, randomly selected. The researchers collected data from students through an internet survey at a large university in 2005 and 2007. The SCOFF(Sick, Control, one stone, Fat, Food) questionnaire was utilized to measure ED symptoms of the participants. The SCOFF basically describes five essential questions for ED 'Sick, Control, one stone, Fat, Food'. A total of 2822 students, 56% of the group asked to participate, completed initial survey questions at baseline. The students were required to be at least 18 years old. The prevalence of positive ED screens was

found 13.5% women and 3.6% for men. Some of the students with positive screens (20%) stated that they had previously received mental health treatment. In 2007, out of the initial group, 753 students participated in the follow-up survey. The results at baseline initial group were used to predict symptoms 2 years later. The findings of the study showed prevalent and persistent ED symptoms for the participants. The study also suggested that a brief screen such as SCOFF can identify students with ED symptoms.

In another study, ethnic differences in eating disorder symptoms among college students were explored (11). They studied the importance of body mass index (BMI). A reliable method was utilized to collect data from Hispanic, Asian, and white college women from two different groups. Controlling their BMI levels, the effects of ethnic differences in eating disorder and concern about weight and shape were analyzed. The results revealed that no statistical differences existed among the participants. A total of 407 freshmen students (sample 1) and 123 college students (sample 2) at a large university were selected for the participants. In the first sample, Hispanic students were more concerned than Asian and White students regarding weight and shape concerns. However, when BMI is controlled the differences disappeared. Besides, the participants showed that the more weight a person has, the higher levels of concerns appear. Therefore, it is important to lose weight to treat eating disorders and reduce the symptoms.

The main purpose of this study was to explore how much certain attitudes towards physical and social appearances and phycological anxieties affect eating behaviors among a group of college students. The findings would be important for the researchers to overview the correlations and regressions between those variables. Also, it is especially valuable to study the evolution of teenagers before and after psychology education at college in a longitudinal research methodology.

Materials and Methods

This study was designed as a quasi-experimental with pretest and posttest methodology. For the study ethics committee approval received on 03/04/2019 from Nuh Naci Yazgan University. The participants initially responded to the items on the questionnaires in their freshmen years in 2015. After four years of university education in 2019, the same participants were asked to answer the same questions to identify the changes in their attitudes and behaviors about eating habits. Data was collected in their classrooms and students were promised extra points for participation in the study.

Participants

The participants were composed of university students enrolled in the department of psychology at a private university in Turkey. The average ages of the students were found 20.6 years old during the data collection process. 40 (76%) participants were female and 13 (24%) were males. Students are required to take psychology courses during their course of undergraduate education such as introductory psychology, social psychology, developmental psychology, health psychology, psychopathology.

In 2015, 67 students agreed to participate in the study at their freshmen year and completed data collection tools. After four years, the same students were found and asked to continue the study by filling out items in the same inventories. 60 of them participated in the second half of the study. After reviewing their responses for inconsistencies, errors, and incompleteness, responses from 53 participants for both data collection sessions were included in the study for data analysis.

Data Collection Tools

The study data was collected via four different questionnaires in addition to a sociodemographic form. Positive and Negative Perfectionism Scale (PNPS) was developed by Oguzhan Kirdok to measure the features of perfectionism in 2004 (12). It was designed with the uses of three forms of scales designed to measure depression (13), anxiety (14), and self-respect (15) inventories. It assesses both positive and negative perfectionism of individuals with 17 items on the inventory. The reliability value (Cronbach alpha) in the original study was found 0.81.

Social Appearance Anxiety Scale (SAAS) was the second data collection tool developed to measure anxiety by Spielberger in 1973 (14). The original version

of the SAAS consists of 17 items with 5-point Likert scale response options.

Brief Symptom Inventory (BSI) is an instrument designed to identify nine dimensions of physical symptoms in adolescents and adults (16). It consists of 53 items that use a 5-point Likert scale. The BSI is generally used to gather information regarding treatment procedures, patient progress, and psychological assessment. It has an average internal reliability score of 0.70.

The Eating Attitudes Test (EAT), developed by Garner and Garfinkel (17) in 1979, was another data collection tool implemented in the study. It is a 40-item inventory to determine whether a person might have an eating disorder required for professional attention. The EAT items are answered with a 6-point Likert scale from always (6) to never (0) options. The reliability (Cronbach alpha) of the EAT in the original study was found around 0.87.

Hypothesis

This study was guided by the seven hypotheses regarding how college students' eating habits, appearance anxiety, perfectionism, and psychological disturbances evolve during their college education. It was necessary to investigate these hypotheses to provide a general perspective of their eating habits.

H1: There is a significant difference between the eating attitudes of college students in their freshman and senior years.

H2: There is a positive correlation between the number of psychological symptoms and deterioration in eating attitudes among college students in the freshman and senior years.

H3: There is a positive correlation between eating attitudes and the social appearance anxiety among college students in the freshman and senior years.

H4: There is a significant difference between deterioration in eating attitudes and positive-negative perfectionism levels among college students in the freshman and senior years.

H5: The eating habit positively correlates with psychology symptoms.

H6: The eating habit positively correlates with appearance anxiety.

H7: The eating habit positively correlates with perfectionism.

Results

It is important to investigate college students' eating attitudes before and after achieving psychology education. In this study, initially, the participants' sociodemographic factors were analyzed. Sociodemographic issues included gender and ages of the students concerning four important features provided from EAT, PNSP, SAAS, and BSI inventories. Table 1 illustrates the relationship between sociodemographic factors and features related to eating attitudes.

Gender

Average scores of the data collection inventories in regard to gender differences were summarized in Table 1. According to the table 1, most of the participants (N=40, 75.6%) were female and few (N=13, 24.5%) were male students. Moreover, females showed higher levels of social appearance disturbance (SAAS) and psychological symptoms (BSI).

Following four years of psychology education, female students scored dramatically lower on the eating habits (EAT), physical symptoms (BSI) and improved on social appearance (SAAS). Both male and female participants scored approximately same levels of perfectionism (PNSP). No further elaboration wasn't discussed on this aspect since the number of female and male students were not equivalent.

Factors Affecting Eating Behaviors

In the second part of the study, we investigated how some particular factors including the attitudes towards

Table 1. The relationship between gender and EAT, PNSP, SAAS, and BSI scores of the participants

		EAT		PNSP		SAAS		BSI	
Gender	**N (%)**	**Pre-EAT**	**Post-EAT**	**Pre-PNSP**	**Post-PNSP**	**Pre-SAAS**	**Post-SAAS**	**Pre-BSI**	**Post-BSI**
Male	13 (24.5)	85.9	81.8	30.6	30.7	16.6	20.8	48.3	32.8
Female	40 (75.6)	85.4	57.2	28.7	27.2	20.5	23.1	71.4	39.5

perfectionism, physical symptoms, and social appearance affect the eating behaviors of the college students at different levels. Similarly, the findings were compared to test the hypothesis. Data analysis included statistical tests including regression analysis, correlational analysis, ANOVA. The results also revealed that certain important relationships between college students' eating behaviors evolve during their psychology education.

During data analysis in terms of the above study purposes, it was suggested using three data combinations. First, eating attitudes were compared between freshmen and senior year students. As shown in Table 2, freshmen and senior scores showed a low positive correlation (0.27) for eating habits. Besides, there exists a statistically significant difference between freshman and senior-level students (p-value < 0.05) for eating habits. This outcome supported hypothesis 1. These results refer to the effectiveness of psychology education towards eating habits.

Table 2 also illustrated that positive low correlation between freshmen and senior students according to PNSP (0.29), SAAS (0.24), and BSI (0.17) test scores. Moreover, there existed a statistically significant difference among them in the case of psychological symptoms (BSI). That shows that a strong and effective psychology education has an impact on students' psychological development. Secondly, Pre-EAT scores were compared with the initial scores of the PNSP, SAAS, and BSI collected in their freshman year. Later, Post-EAT scores were compared with the scores of the PNSP, SAAS, and BSI for their freshman year. Lastly, we compared Pre-EAT scores with the PNSP, SAAS, and BSI results collected in both their freshman and senior years. The results of the three comparisons are illustrated in Table 3. Table 3 presented that statistically significant differences were found for eating attitudes for freshmen students.

R-square scores (correlation coefficient) were shown between eating attitudes among freshmen students, between freshmen and seniors. Also, eating attitudes (EAT), social/physical appearance anxiety (PNPS, SAAS), perspectives of perfectionism (BIS) freshmen and seniors were also summarized.

Table 2. Comparison of the freshmen and senior students' scores of EAT, PNSP, SAAS, and BSI inventories

	EAT	**PNSP**	**SAAS**	**BSI**
Correlation	0,27	0,29	0,24	0,17
P-value	0,0000	0,3587	0,2712	0,0001

Table 3. Regression statistics and ANOVA results and significant statistical figures

P-VALVE	Freshmen-EAT	Senior-EAT	Freshmen & Senior-ALL
R Square	0.24	0.06	0.35
Sig. F	0.003	0.39	0.002
Freshmen-PNPS	0.094	0,284	0,060
Freshmen-SAAS	l 0.916	0,577	0,733
Freshmen-BSI	0.009	0,188	0.025
Senior-PNPS			0,355
Senior-SAAS			0.013
Senior-BSI			0,704

Pre- and Post-tests (EAT, PNPS, SAAS, BIS) scores were compared with each other. P-values, r-squared, and significant F scores are illustrated in table 3. Some of the P-values were insignificant since their values were higher than the boundary (0.05) for statistical difference.

The first important result was that eating attitude of freshmen and social/physical appearance anxiety (PNPS, SAAS), perspectives of perfectionism (BIS) for both freshmen and senior students had statistically significant differences. The correlations coefficients were also found to be low positive levels, and such as result supported hypothesis 2, 3, and 4.

The scores of the eating attitude and perspectives of perfectionism for freshmen is correlated with a low positive relationship (0.24) with each other. Also, eating attitudes and perfectionism for freshmen students were found to have a statistically significant difference (p-value < 0.05). That result supported hypothesis 5.

On the other hand, the eating attitude was not correlated with social/physical appearance anxiety (PNPS, SAAS) among freshman and senior levels. Therefore Hypothesis 6 and 7 were not supported by this finding. Table 4 summarizes the findings of hypothesis testing.

Discussion

Identifying eating disorders and eating behaviors of young adults (college students) is important to de-

Table 4. Results of the hypothesis tests

		Accept	Reject
H1	There is a significant difference between the eating attitudes of college students in their freshman and senior years.	√	
H2	There is a positive correlation between the psychological symptoms and deterioration in eating attitudes among college students in the freshman and senior years.	√	
H3	There is a positive correlation between eating attitudes and the social appearance anxiety among college students in the freshman and senior years.	√	
H4	There is a significant difference between deterioration in eating attitudes and perfectionism levels among college students in the freshman and senior years.	√	
H5	Eating habit positively correlates with psychological symptoms.	√	
H6	Eating habit positively correlates with appearance anxiety.		√
H7	Eating habit positively correlates with perfectionism.		√

velop methods and approaches towards the treatment process. Also, the findings could be essential for designing courses and curriculum for college students especially psychology majors.

The findings from freshman students revealed that males scored higher on eating habits (EAT) and perfectionism (PNSP), however, lower on social appearance disturbance (SAAS) and psychological symptoms (BSI) than females. These results supported previous findings in the literature (18-21) at some certain degrees.

As found in earlier similar studies (22-23), a statistically significant difference was found between freshman and senior-level students (p-value < 0.05) in terms of the eating attitudes and psychological symptoms.

Other related important features including perfectionism and social/physical appearance for them also provide important results in terms of eating attitudes and its evolution during college education.

More detailed and extensive studies could be conducted as a qualitative research methodology to generate deeper and specific consequences. A mixed methodology could also be a good approach to identify more detailed understandings of the situations.

Any improvement in training and eating attitudes tests could be an idea that the appropriate education for the young generation might change the deceptive perception of society created by the advertisements. For this reason, it is important that institutions such as ministries of national education, the higher education council, other education- related authorities should offer and support such courses that contain topics regarding eating attitudes and importance of balanced diet chapters are important for the physical and social development of the students.

Conclusion

This study was constructed to investigate the revolutions of university students' eating habits, perfectionism, psychological symptoms, and appearance anxiety that were affected by psychology education for four years in college. It is important to identify such patterns and tendencies among these four variables for creating treatments and providing important methodological approaches for college students. Also, it would help university decision-makers to design a more appropriate and useful curriculum for the psychology department. Finally, the results are expected to provide great inputs for future researches.

References

1. Cochrane, C. E., Brewerton, T. D., Wilson, D. B., & Hodges, E. L. (1993). Alexithymia in the eating disorders. International Journal of Eating Disorders, 14(2), 219-222.
2. Lipson, S. K., & Sonneville, K. R. (2017). Eating disorder symptoms among undergraduate and graduate students at 12 US colleges and universities. Eating behaviors, 24, 81-88.
3. Mann, T., Nolen-Hoeksema, S., Huang, K., Burgard, D., Wright, A., & Hanson, K. (1997). Are two interventions worse than none? Joint primary and secondary prevention of eating disorders in college females. Health Psychology, 16(3), 215.
4. Nelson, W. L., Hughes, H. M., Katz, B., & Searight, H. R. (1999). Anorexic eating attitudes and behaviors of male and female college students. Adolescence, 34(135), 621-633.
5. Rosen, J. C., Compas, B. E., & Tacy, B. (1993). The relation among stress, psychological symptoms, and eating disorder symptoms: A prospective analysis. International Journal of Eating Disorders, 14(2), 153-162.

6. Minarik, M. L., & Ahrens, A. H. (1996). Relations of eating behavior and symptoms of depression and anxiety to the dimensions of perfectionism among undergraduate women. Cognitive therapy and research, 20(2), 155-169.
7. Mintz, L. B., & O'Halloran, M. S. (2000). The Eating Attitudes Test: validation with DSM-IV eating disorder criteria. Journal of personality assessment, 74(3), 489-503.
8. Furnham, A., Badmin, N., & Sneade, I. (2002). Body image dissatisfaction: Gender differences in eating attitudes, self-esteem, and reasons for exercise. The Journal of psychology, 136(6), 581-596.
9. Bulik, C. M., Sullivan, P. F., Fear, J. L., & Pickering, A. (2000). Outcome of anorexia nervosa: eating attitudes, personality, and parental bonding. International Journal of eating disorders, 28(2), 139-147.
10. Eisenberg, D., Nicklett, E. J., Roeder, K., & Kirz, N. E. (2011). Eating disorder symptoms among college students: Prevalence, persistence, correlates, and treatment-seeking. Journal of American College Health, 59(8), 700-707.
11. Arriaza, C. A., & Mann, T. (2001). Ethnic differences in eating disorder symptoms among college students: The confounding role of body mass index. Journal of American College Health, 49(6), 309-315.
12. Kırdök, O. (2004). Olumlu ve olumsuz mükemmeliyetçilik ölçeği geliştirme çalışması. Yayım-lanmamış Yüksek Lisans Tezi, Çukurova Üniversitesi, Adana.
13. Kovacs, M., & Beck, A. T. (1977). An empirical-clinical approach toward a definition of childhood depression. Depression in childhood: Diagnosis, treatment, and conceptual models, 1-25.
14. Spielberger, C. D. (1973). State-trait anxiety inventory for children. Consulting Psychologists Press.
15. Coopersmith, S. (1967). The antecedents of self-esteem. San Francisco: W. H. Freeman &Co.
16. Derogatis, L.R.(1993). BSI Brief Symptom Inventory: Administration, Scoring, and Procedure Manual (4th Ed.). Minneapolis, MN: National Computer Systems.
17. DM Garner, PE Garfinkel. The Eating Attitudes Test: An Index of the Symptoms of Anorexia Nervosa. 9 Psychological Medicine 273-279. 1979.
18. Reyes-Rodríguez, M. L., Franko, D. L., Matos-Lamourt, A., Bulik, C. M., Von Holle, A., Cámara-Fuentes, L. R., & Suárez-Torres, A. (2010). Eating disorder symptomatology: prevalence among Latino college freshmen students. Journal of Clinical Psychology, 66(6), 666-679.
19. Aruguete, M. S., DeBord, K. A., Yates, A., & Edman, J. (2005). Ethnic and gender differences in eating attitudes among black and white college students. Eating Behaviors, 6(4), 328-336.
20. Bas, M., Asçi, F. H., Karabudak, E., & Kiziltan, G. (2004). Eating attitudes and their psychological correlates among Turkish adolescents. Adolescence, 39(155), 593.
21. Phares, V., Steinberg, A. R., & Thompson, J. K. (2004). Gender differences in peer and parental influences: Body image disturbance, self-worth, and psychological functioning in preadolescent children. Journal of Youth and Adolescence, 33(5), 421-429.
22. Nelson, W. L., Hughes, H. M., Katz, B., & Searight, H. R. (1999). Anorexic eating attitudes and behaviors of male and female college students. Adolescence, 34(135), 621-633.
23. Clarke, M. G., & Palmer, R. L. (1983). Eating attitudes and neurotic symptoms in university students. The British Journal of Psychiatry, 142(3), 299-304.

Correspondence:
Saban Karayagiz
Department of Psychology, Nuh Naci Yazgan University
Kayseri/Turkey
(90) 352-324-0000
ORCID ID: 0000-0002-6914-4630
E-mail: skarayagiz@nny.edu.tr

Progress in Nutrition 2020; Vol. 22, N. 1: 112-121 DOI: 10.23751/pn.v22i1.9277

Orignal article

The relationship between university students' quality of life and nutrition

İbrahim Dalbudak[1], Taner Yılmaz[2], Şıhmehmet Yiğit[3]

[1]Atabey Vocational School, Isparta University of Applied Sciences / Isparta, Turkey - E-mail: dalbudakibo@hotmail.com; [2]Turkey Usak University sports science faculty /Uşak, Turkiye; [3]School of Physical Education and Sports Namık Kemal University / Tekirdağ, Turkiye

Summary. The aim of this study is to investigate whether there is a difference in nutrition habits of the students in different departments of a vocational school in a university, depending on the life quality of them, and to compare by various variables. A total of 205 students, as 92 female and 113 male in Atabey Vocational School in Isparta University of Applied Sciences in the 2018-2019 academic year, participated in this study. Students who took or did not take the lesson of healthy life and nutrition, participated in the study. As a data collection tool in the research; the turkish version of The World Health Organization Quality of Life Scale short form questionnaire and, Three-factor Eating Questionnaire (TFEQ) were applied. And The Personal information Form was used to obtain the demographic information. In this study, SPSS 22.00 Programme was used which is used in quantitative research methods. The data were summarized by giving the percentage and frequency tables. Statistically, there are both significant ($p < .05$) and nonsignificant ($p > .05$) differences in students between life quality and its subfactors by age, gender, being engaged in sports, taking the lesson of healthy life and nutrition and income status. Since age and gender variables have no effect on any of the nutrition scale snd its sub-factors, there is no significant difference between them ($p > .05$). There is a significant difference between the students' income level, the status of being actively involved in sports, the status of taking the lesson of healthy life and nutrition, and the nutrition scale and its sub-factors ($p < .05$). Statistically, there was found a significant relationship intensive and in a positive way between individuals' quality of life and their nutrition habits ($p < .05$). As the total score of the life quality scale or the total score of the nutrition scale increases, other one increases, as well. As a result, an increment in a significance level is observed in the quality of life and nutritional levels of the university students taking the lesson of healthy life and nutrition. When the lesson of healthy life and nutrition becomes a compulsory course and its length gets increased, we can say that there will be difference in the levels of the quality of life and nutrition. As a result of ensuring the participation in the class of healthy life and nutrition, we can say that it will be helpful for individuals in terms of protecting against disesases and gaining health life and nutrition behaviors by struggling against the factors that affect the life negatively. Since it will be useful to repeat this study in different fields as more inclusively, we can say that this study will shed light on future studies.

Key words: quality of life, nutrition, university students

Introduction

"Healthy life and nutrition" is one of the indispensable facts in the quality of life of human, in other words in the life of human. Mankind needs to pay attention to the quality of life, in order to keep living. The human, by nature, is a whole with his/her physical and psychological aspects. People who can balance between their physical and psychological aspects, and who can protect and improve their physical and mental health, can live a happy and healthy life (1). According to the definition of World Health Organization (WHO), "Health is not only the absence of disease or disability, but the status of a full well-being, physically

and mentally (2)". WHO has also defined health, as awareness of breathing, meeting the needs, changing or coping the environment (3). And the healthy lifestyle includes taking responsibility of health behaviors, balanced nutrition, adequate and regular exercise, being a nonsmoker, having health responsibility, taking hygiene measures, establishing positive interpersonal relationships and stress management (4). In the process starting with birth, people aimed to maintain a long and healthy life by giving importance to the issues such as improving the quality of life, being resistant to physiological and psychological factors and healthy nutrition (5). Quality of life is the perception of life about how individuals feel well themselves within the values of the society where they live (6). Quality of life analyses the material and non-material aspects of the individual. The concept of quality of life can be defined as not only being in a good state of health, but a full well-being state containing physical, social and spiritual concepts. The target of the quality of life is to determine how much the individuals feel pleased or displeased because of their physical, socio-economic and spiritual functions (7). When the studies conducted are examined, researchers have used the term quality of life, as synonym with the terms such as pleasure, happiness and life satisfaction (8).

Adequate and balanced nutrition is one of the basic conditions for the society and individuals to live healthy and strong, improve themselves economically and socially and increase the level of welfare (9). Many studies conducted up to the present have revealed the importance of nutrition on various subjects and many definitions have been made. Nutrition is the use of macro and micronutrients which are taken by the organism for the vital activities. As a result of a number of chemical processes, macro and micronutrients are absorbed by the body through the intestines and used for vital activities through circulation (10). In another definition, nutrition is the status that the body utilizes the food eaten, in order to grow, perform the body functions and maintain the life as healthy and happy (11). According to the reports of the World Health Organization (2), it was explained that the most important risks of non-communicable disease are high blood pressure, high cholesterol concentrations in blood, insufficient intake of fruits and vegetables, being overweight or obese, physical inactivity and tobacco use. Insufficient nutrition is an important risk factor in non-communicable diseases such as cardiovascular diseases, diabetes and some types of cancer (12). Balanced nutrition is formed by taking the required food from different food components in order to meet one's energy and nutritional needs (13). Therefore, diet and physical activity have an impotant effect on health protection and disease prevention. Physical activity is any bodily movements performed by skeletal muscle that result in energy expenditure (14). Researches states that the physical activity shows protective and curative effects in many diseases (15). Physical activity is not limited to sports activities only. Physical activity is in every part of life, like individuals' home, work and transportation (16).

As a result, the education of healthy life and nutrition changes the quality of life of individuals significantly, when it starts to be given first in the family, and then at the universities regularly. The education individuals received in this regard, causes the quality of life to change.

Materıal And Method

In this research, as the data collection tools; "Personal Information Form" which was prepared by the researcher and Three-Factor Eating Questionnaire (TFEQ) which was prepared by Kıraç et al. (16) were used in order to determine the nutritional habits of individuals, and the turkish version of The World Health Organization Quality of Life Scale Short Form (WHOQOL-BREF-TR) were used in order to determine the quality levels of life. The validity and reliability of the WHOQOL-BREF scale for the Turkish language and society was confirmed and the scale was found suitable fort he Turkish society. When evaluating the validity of the scale; construct validity, concurrent validity, distinctive validity and the importance of the areas in explaining the overall health and quality of life were evaluated and the scale was found valid in these areas. Regarding the evaluation of the reliability, internal consistency (Cronbach alpha) of all sections and areas of the WHOQOL-BREF scale was calculated and it was found between 0.53 and 0.83. These

values show that the reliability of the scale is high (18). It has been translated into more than 20 languages including Turkish. >Adaptation of the scale to Turkish was done by Eser et al. (19). The question 27 which was added to the scale evaluates the national environmental area. Field scores range from 0 to 20. As the score increases, the quality of life increases, as well.

Data Analysis

World Health Organızatıon - "Lıfe Qualıty Scale"

- The total score of the life quality scale is calculated from 26 items. The minimum score that can be obtained on the scale is 26 and the maximum score that can be obtained is 130.
- Subscales of the Quality of Life Scale;
  - Factor 1: General health condition (items 1-2) (minimum 2, maximum 10 points can be obtained)
  - Factor2: Physical health condition (items 3-4-10-15-16-17-18) (minimum 7, maximum 35 points can be obtained)
  - Factor 3: Psychological condition (items 5-6-7-11-19-26) (minimum 6, maximum 30 points can be obtained)
  - Factor 4: Social relations (items 20-21-22) (minimum 3, maximum 15 points can be obtained)
  - Factor 5: Environment (items 8-9-12-13-14-23-24-25) (minimum 8, maximum 40 points can be obtained)
- Looking at the "Cronbach's Alpha if Item Deleted" column in the "Item-Total Statistics" table, it is seen that the cronbach's alpha value increased from 0.988 to 0.996 when the item 3 was removed from the survey. The answers given by the participants to this item decrease the reliability of the questionnaire. Looking at the "Corrected Item-Total Correlation" column, -0.959 is seen, but all the rest is positive. This shows that it has a completely opposite consistency with other items.

Three Factor Nutrıtıon Questıonnaıre

Important Remınder - The name of the questionnaire is "three-factor nutrition survey", but since one more factor (hunger sensitivity level) has been added to the survey, four-factor nutrition questionnaire was analyzed.

- The total score of the "Three-Factor Nutrition Survey" is calculated from 18 items. The minimum score that can be obtained on the scale was 18, while the maximum was 72. Questions 1-2-3-4-5-6-7-8-9-10-11-12-13 were scored from top to bottom from 4 to 1. Questions 14-15-16-17 are listed from 1 to 4 from top to bottom. In the 18th question, those who marked 1-2 were scored as "1", those who marked 3-4 were scored "2", those who marked 5-6 were scored as "3" and those who marked 7-8 were scored as "4".
- Three-factor nutrition questionnaire subscales;
  - Factor 1: Restricting eating (items 1-7-13-14-17) (minimum 5, maximum 20 points can be obtained)
  - Factor 2: Level of uncontrolled eating (items 3-6-10) (minimum 3, maximum 12 points can be obtained)
  - Factor 3: Degree of eating at emotional times (items 2-11-12-15-16-18) (minimum 6,
  - maximum 24 points can be obtained)
  - Factor 4: Hunger sensitivity level (items 4-5-8-9) (minimum 4, maximum 16 points can be obtained)

Table 1. Statistics for the Total Scores of Quality of Life Scale and its Subscales

	Minimum	Maximum	Average	Std. Deviation	Skew
Factor 1	2	10	5.9220	3.2949	-0.057
Factor 2	11	31	21.0244	7.5260	-0.037
Factor 3	7	30	17.2732	9.5081	0.096
Factor 4	5	15	9.3073	3.9603	0.232
Factor 5	10	34	20.8780	10.3478	0.070
Quality of life scale	40	117	74.4049	34.1238	0.096

Table 2. Cronbach's Alpha Values for Total Scores of Life Quality Scale and Sub-Scales

	Cronbach's Alpha
Factor 1	0.967
Factor 2	0.837
Factor 3	0.990
Factor 4	0.990
Factor 5	0.983
Quality of life scale	0.988

Table 3. Statistics of Four-Factor Nutrition Survey and Sub-Factor Total Scores

	Minimum	Maximum	Average	Std. Deviation	Skew
Factor 1	11	16	13.2537	2.0541	0.260
Factor 2	3	12	8.0049	4.0481	-0.139
Factor 3	14	18	15.2976	0.8069	-0.367
Factor 4	5	16	10.7756	5.1590	-0.005
4-Factor Nutrition Survey	34	60	47.3317	11.7622	-0.005

Table 4. Cronbach's Alpha Values for four factor nutrition scale and Sub-Factor Total Scores

	Cronbach's Alpha
Factor 1	0.976
Factor 2	0.993
Factor 3	0.988
Factor 4	0.981
Four factor nutrition scale	0.823

- Looking at the "Cronbach's Alpha if Item Deleted" column in the "Item-Total Statistics" table, it is seen that when the items numbered 2-11-12-14-17 are removed from the questionnaire, the cronbach's alpha value is higher than 0.823. The answers given by the participants to these items decrease the reliability of the questionnaire. looking at the "Corrected Item-Total Correlation" column, negative correlation values are seen, but all the rest are positive. This shows that it has a completely opposite consistency with other items.

Findings

When the participants were examinerd in terms of demographic characteristics, the following findings were reached.

- 205 people participated in the questionnaire. 70 people of them (34.1%) were between 0-19 years old, 106 people of them (51.8%) were between 20-29 years old, and 29 people of them (14.1%) were 30 or older. While the youngest participant was 14 and the oldest participant was 48 years old. The average age of 205 participants was found as 23.2341 and the standard deviation was found as 6.0959.
- 113 (55.1%) of 205 participants were male and 92 (44.9%) were female.

Table 5. Data Distribution by Demographic Characteristics

Class	**Frequency**	**Percentage (%)**	**Cumulative Percentage (%)**
Age			
	70	34.1	34.1
	106	51.8	85.9
	29	14.1	100.0
Total	205	100	
Gender			
Male	113	55.1	55.1
Female	92	44.9	100.0
Total	205	100	
Active Sports			
Doing	100	48.8	48.8
Not-doing	105	51.2	100.0
Total	205	100	
Lesson of Healthy Living and Nutrition			
Taken	100	48.8	48.8
Not-taken	105	51.2	100.0
Total	205	100	
Income status			
0 – 500	55	26.8	26.8
501 – 1000	75	36.6	63.4
1001 – 2000	75	36.6	100.0
Total	205	100	

- Among the 205 people prticipated in survey, 100 (48.8%) people were actively involved in sports, while 105 (51.2%) were not actively involved in sports.
- 100 (48.8%) of the participants took "healthy life and nutrition lessons" while 105 (51.2%) did not take this lesson.
- 55 (26.8%) of the 205 people surveyed were between 0-500, 75 (36.6%) were between 501-1000 and 75 (36.6%) were between 1001-2000.
- There were obvious differences between "quality of life" and "its sub-factors" according to demographic findings.
- As the ages of the participants increased, it was observed that the "physical health conditions" decreased and the "social relations" decreased. Additionally, according to the general scale, statistically the quality of life was found significantly decreased as the ages of the people increased.

Table 6. Findings Regarding the Quality of Life and Sub-Scale Scores, according to Participants' Age, Gender, Active Athletics, Healthy Life and Nutrition Lesson and Income Status

	Age	Gender	Involved in Active Sports	Lesson of Healthy Life and Nutrition	Income Status
General Health Condition	0.118	0.001	0.000	0.000	0.000
Physical Health Condition	0.006	0.015	0.000	0.000	0.000
Psychological Condition	0.183	0.000	0.000	0.000	0.000
Social relations	0.007	0.051	0.000	0.000	0.000
Environmental Conditions	0.091	0.526	0.000	0.000	0.000
Quality of Life	0.016	0.148	0.000	0.000	0.000

- The "gender" of the individuals did not have any effect on their general quality of life, but there were significant differences found in some sub-factors. General health and psychological conditions of females were better than males. Physical health conditions of males were better than females.
- The quality of life of people who were actively involved in sports and the people o taken healthy living and nutrition lessons is significantly higher than those who were not involved in sports and who did not take healthy living and nutrition lessons. Statistically significant differences were obtained for all sub-scales and general scale.
- Again, when looking at all sub-factors and general quality of life scale, statistically significant differences were obtained for all of them. According to the results obtained, it was seen that as the income level of the individuals increased for all the sub-factors and the quality of life scale, the quality of life significantly increased.
- It was observed that age and gender variables had no effect on any of the "Four-Factor Nutrition Survey" and related "sub-factors".
- "Degree of restriction to eating" is higher in people who were actively involved in sports and who took a healthy lifestyle and nutrition lessons, comparing others. At the
- same time, "uncontrolled eating levels", "degree of eating at emotional times" and "hunger sensitivity level" in these people were lower than those who do not do sports and take a healthy lifestyle and nutrition lesson.
- A significant correlation was found between the total scores of 4-factor nutrition questionnaire and its sub-factors with the individuals' income status. According to the results obtained, as the inco This means that as people's income increases, their eating habits become better. They pay attention to what they eat and avoid uncontrolled consumption that will cause obesity.me status of individuals increases, total scores of general scale and sub-scales increase.

Relatıonshıp Betweeη The Lıfe Qualıty Scale And Three Factor Nutrıtıonal Survey

The Pearson Correlation coefficient takes values ranging from -1 to +1. A positive value indicates the same directional relationship between the two variables, and a negative value indicates an inverse relationship between the two variables. As the correlation value approaches -1 and +1, the severity of the rela-

Table 7. Findings Regarding Nutrition and its Sub-Scale Scores of the Participants, According to their Age, Gender, Active Athletics, Healthy Life and Nutrition Lesson and Income Status

	Age	Gender	Being and active athlete	Lesson of healthy life and Nutrition	Income status
Degree of Restriction to Eating	0.053	0.067	0.000	0.000	0.001
Uncontrolled Eating Level	0.051	0.055	0.000	0.000	0.000
Degree of Eating in Emotional Times	0.078	0.334	0.000	0.000	0.000
Hunger Sensitity Level	0.051	0.055	0.000	0.000	0.000
Four-Factor Nutrition Survey	0.056	0.060	0.000	0.000	0.001

Table 8. Pearson Correlation Coefficient of the Relationship between the scores of Quality of Life Scale and the Four-Factor Nutrition Scale

	Quality of Life Scale	Four-factor Nutrition Survey
Quality of Life Scale	1.000	0.971 (0.000)
Four-factor Nutrition Survey	0.971 (0.000)	1.000

tionship between them increases. A correlation coefficient of 0 indicates that there is no relationship between the two variables. As it approach 0, the severity of the relationship decreases.

- A very strong and positive relationship was found statistically significant between the quality of life of individuals and their eating habits. Pearson's correlation coefficient was found as 0.971. As the total score of the quality of life scale or total score of the four-factor nutrition questionnaire increases, the other increases, as well.

Dıscussıon and Conclusıon

Within the scope of the research, it was aimed to investigate whether there are difference between the nutrition habits and the quality of life of students in different departments of the vocational school in the university and to compare with various variables. Discussions and conclusions related the research findings are given below.

It was observed that as the age of the participants increased, their "physical health condition" went back and their "social relations" decreased. In addition, according to the general life scale, it was found statistically significant that the quality of life decreased as the ages of the people increased ($p < .05$). In psychological condition, there is no statistically significant difference between the environmental conditions and age ($p > .05$). No studies have been found about the relationship between, according to the age variable. We can say that, as the age gets older, the changes in individuals' body structure and health, emotions and thoughts can cause changes in individuals' life and health.

The "genders" of individuals have no effect on their general quality of life, social relations, and environmental conditions ($p > .05$). However, significant differences were found in some sub-factors. Females' general health and psychological conditions are better than males. Physical health status of males is better than females ($p < .05$). When the studies on different sample groups were examined according to the gender, Salıcı (20) stated that there is was no statistically significant difference found in terms of gender in the study of the quality of life of the university students. İlhan (21), in his study examining healthy lifestyle behaviors of athlete students in university teams, In terms of total scores of healthy lifestyle behaviors, it

was concluded that there was no significant difference between male and female students. In the study conducted by Kangal (22) on university students about their quality of life, he found a significant difference in favor of female students in terms of gender. Erdal (23), in his study titled the effect of university students' physical activity levels on their quality of life levels and social participation levels, when the sub-dimensions of the gender variable and the quality of life scale were examined, the psychological status of the female participants received higher scores and in all other sub-dimensions male participants received higher scores. As a result of the studies conducted, it is thought that the reasons for the relationship between the quality of life and the gender variable shows difference or not; can be the sample groups' specific characteristics, the environment they live, the university they study, their economical status and their cultural characteristics.

The general health, physical health, psychological state, social relations, environmental conditions and quality of life of those who are actively involved in sports are significantly higher than those who do not do sports. There is a significant difference between those who do sports and those who do not ($p < .05$). Ayhan (24) obtained the results that the individuals who are in physical activity have high quality of life. In the study conducted by Yılmaz and Karaca (7), it was determined that healthy life and nutrition were effective on university students who do sports and do not do sports. Blair (25) in his study on the benefits of regular physical activity; found that exercise improves quality of life, prevents diseases and injuries, and reduces mortality. Also, Hawk et al. (26), in their study about healthy lifestyle and behavior of those who do

regular sports, found higher results. As a result of the studies, we can say that sports or physical activity is extremely effective on healthy life and individuals who do sports are conscious of this issue.

The general health status, physical health status, psychological state, social relations, environmental conditions, quality of life of the people who take the lesson of healthy life and nutrition are significantly higher than those who do not take the lesson healthy life and nutrition. Statistically significant differences were obtained for all subscales and general scale ($p < .05$). Jones et al. (27) stated that the nutritional knowledge levels of students who took nutrition lessons were significantly higher than others. In his study, Kavas and Kavas (28) stated that the veradge scores of the students who received nutrition education were higher compared to the average scores of students who did not received nutrition education. In their study, Yılmaz and Karaca (7) stated that there is a significant difference between university students who took nutrition classes and who did not take nutrition classes. As a result of the studies, we can say that the reason for the difference between those who take and do not take the healthy life and nutrition lesson was that the lesson of healthy life and nutrition is effective on the students.

When all sub-factors and general quality of life scale regarding income level were analyzed, statistically significant differences were obtained for all of them ($p < .05$). According to the results obtained, for all the sub-factors and the quality of life scale, it was seen that the quality of life increased significantly as the income level of the people increased. There is no study we found, investigating income status. Healthy living is directly proportional to income status. The increase in income will cause individuals to change the quality of life. As the quality of life changes, the individual will take care of himself in better health and better conditions. So, we can say that income status has an impact on the quality of life.

It was observed that the age variables had no effect on any of the "Nutrition Survey" and the related "sub-factors". Since the general nutrition and subscale items (the degree of restriction on eating, the level of uncontrolled eating, the degree of eating at emotional times, the level of sensitivity to hunger) had no effect on age, there is no significant difference between them ($p > .05$). We can say that mankind knows the nutritional conditions at any age. Because of the education received by the university students in the study we have done and because they know the nutrion for any range of age, we can state that age is not effective on nutrition. We can say that nutrition is not effective on age due to the characteristics of individuals such as education, cultural characteristics, environment they live in, increased communication, nutrition programs on television, increased participation in regular physical activities, and their awareness.

It was observed that gender variables had no effect on any of the "Four Factor Nutrition Survey" and related "sub-factors". Since the general nutrition and subscale items (the degree of restriction on eating, the level of uncontrolled eating, the degree of eating at emotional times, the level of sensitivity to hunger) had no effect on gender, there is no significant difference between them ($p > .05$). In the nutrition study conducted by Kıraç et al. (17), some items were found similar, while some items did not differ. In the study conducted by Aksoydan and Çakır (29), it was stated that male students' diet was better than girls, but there was no significant difference between the groups. Nutrition is important for every individual. In order to sustain the life in a healthy and peaceful manner, mankind should be fed balanced and regularly. Therefore, because nutrition affects male and female, we can say that there is no difference between them.

"Restriction of eating" was higher in people who are actively involved in sports compared to others. At the same time, in these people, "uncontrolled eating level", "degree of eating at emotional times" and "hunger sensitivity level" are lower than those who do not do sports. It means, there is a significant difference in nutrition and its subscales between the people who do sports and those who do not ($p < .05$). Individuals doing sports eat a balanced diet and they know well the diet. They have a programmatic diet. They arrange their diet according to that programme and do not go out of that programme. They are strong-willed about feeding them selves. They know what to eat and drink very well. They take good care of themselves. Their diet is very different, according to individuals who do not do sports. Otherwise they can not do sports. We can say this is the reason of the difference.

People who took the lesson of healthy life and nutrition have a higher degree of "restriction on eating" than others who did not take this lesson. At the same time, "uncontrolled eating level", "degree of eating at emotional times" and "hunger sensitivity level" in these people were lower than those who did not take healthy living and nutrition lessons. Shortly, there is a significant difference in nutrition and its subscales between those who take healthy life and nutrition lesson and those who do not take this lesson ($p < .05$). In the study conducted by Yılmaz and Karaca (7), it was found that there was a significant difference in favor of students who took a nutrition lesson compared to those who did not take a nutritional lesson. When the nutritional attitudes were analyzed, it was found that there was a significant difference between the students who took a nutritional lesson and those who did not take it. We can say that the healthy life and nutrition lessons taking in schools is effective on individuals. Individuals will be able to learn and apply healthy eating and living through their healthy life and nutrition lesson. Through the healthy life and nutrition lessons, individuals will be able to learn healthy eating and living and apply them to their lives. This way, they will know what to eat and what to drink and how to do the physical activities. The individuals who eat properly and do the regular physical activities, will be able to sustain their lives in a healthy way. We can say that healthy life and nutrition are directly proportional to long life. By applying the healthy life and nutrition lesson in preschool education, we can raise healthier generations.

A significant correlation was found between the income status and the total scores of nutrition and its sub-factors by ($p < .05$). According to the results obtained, as the income status of individuals increases, total scores of general scale and the sub-factors increase. This means thatt as the people's income increases, their eating habits become better. They pay attention to what they eat and avoid uncontrolled consumption that will cause obesity. Healthy eating, in parallel with the economic situation of the family, varies to the access to the healthy food (Tanriverdi et al., 2011.30). In the determination of the factors affecting the nutrition and exercise behavior in adolescents, Kalay and Türkmen (2015.31) concluded that healthy nutrition-exercise behavior of the students with good economic status is better than the students with poor economic status. This finding is similar to the literature. In our study, we stated that the nutrition and sub-factors show increasement in the students with good economic status while thees factors show decreasement in the students with poor economic status. We can say that as the income status increases, the diet changes and individuals can take care of themselves in better conditions.

A very strong and positive relationship was found statistically significant between the individuals' quality of life and their eating habits ($p < .05$). As the total score of the quality of life scale or the total score of the nutrition scale increases, the other increases as well. In a study by Yılmaz and Karaca (2019.7), they found a significant difference between the total scores of nutrition and the total scores of quality of life. Nutrition increases the quality of life. Considering these, individuals should be made conscious about healthy life and nutrition. As the quality of life increases, a significant increase is observed in nutrition scores. In this context, since as the nutrition habits will be regulated as the quality of life will increase, it is thoght that the individuals will be able to do physical activity or sports, take care of their health, be conscious of diseases and take necessary precautions, have information about nutrition, know their healthier and longer lives, and increase the quality of life as they will eliminate the factors that negatively affect their life. In order to maintain the life, mankind needs to be fed regularly. Otherwise, he/she cannot survive. If the human body cannot get the necessary nutrients, he/she will endanger his/her development and will be open to all kinds of dangers that may arise outside. We can say that the stronger body structure means the stronger human against the outside. If the immune system of individuals is strong, we can say that they are healthy. We can state that the strong immune system depends on the quality living conditions.

As a result, a significant increase is observed in the quality of life and nutritional levels of university students taking the lesson of healthy life and nutrition. We can say that when the healthy life and nutrition lessons are compulsory at universities and as the duration of the existing lessons increases, the quality of life and nutrition levels will differ in a positive way. As a result, ensuring the participation of the healthy

life and nutrition lesson will be helpful about learning to live healthy, protecting against diseases and gaining healthy living and nutrition behaviors by strugling with factors affecting life negatively. We can say that this study will shed light on future studies, since it is thought to be useful to repeat this study more broadly in different areas.

References

1. Bozdağ, B. (2019). Üniversite Öğrencilerinin Fiziksel Aktivite Düzeyleri İle Yaşam Kaliteleri Arasındaki İlişkinin İncelenmesi. Gazi Üniversitesi Eğitim Bilimleri Enstitüsü, Doktora Tezi, Ankara
2. World Health Organization: The world health report. (2002). Reducing risks, promoting healthy life Geneva: WHO.
3. World Health Organization(WHO) Health Promotion(1986). A discussion document on the concept and principles, Public Health Rev; 14: 245-254.
4. Bahar, Özvarış, Ş. (2006). Sağlık eğitimi ve sağlığı geliştirme. Ankara. Hacettepe Üniversitesi Yayınları, 1133.
5. Zorba, E. (2010). Yaşam kalitesi ve fiziksel aktivite. 10. Uluslararası Spor Bilimleri Kongresi, 01, Bolu.
6. Baydur, H. (2010). İş sağlığı ve yaşam kalitesi. 3. Ulusal Sağlıkta Yaşam Kalitesi Kongresi Özet Kitabı (İzmir), 82-84.
7. Yılmaz, G., & Karaca, S. (2019). Spor Yapan ve Sedanter Üniversite Öğrencilerinin Beslenme Bilgi Tutum ve Yaşam Kalitelerinin İncelenmesi, Niğde Üniversitesi Beden Eğitimi ve Spor Bilimleri Dergisi Cilt 13, Sayı 3.
8. Bayrak, M. (2011). Sporu bırakmış profesyonel futbolcuların depresyon düzeylerinin yaşam kalitesi açısından değerlendirilmesi. Doktora Tezi, Samsun On Dokuz Mayıs Üniversitesi, Sağlık Bilimleri Enstitüsü, Samsun.
9. Soylu, M.(2006). Sağlıklı beslenme-sağlıklı süt. I. Ulusal Sağlığı Geliştirme ve Sağlık Eğitimi Kongresi Özet Kitabı. Marmaris-Muğla, 9-12 Kasım 2006: 37.Erişim: (http://www.wacc.mu.edu.tr/ozet%20kitabi.pdf) Erişim tarihi: 20.12.2019.
10. Baysal, A. (2015). Beslenme. Hatipoğlu Yayınevi, Ankara.
11. Kavas, A.(2003). Sağlıklı Yaşam İçin Doğru Beslenme. 3. Basım, İstanbul: Literatür Yayıncılık, 6-15.
12. Skof, B., & Kozjek, N.R. (2015). A comparison of dietary habits between recreational runners and a randomly selected adult population in Slovenia. Zdrav Var, 54(3), 212-221.
13. Padavinangadi, A., Xuan, L.Z., Chandrasekaran, N., Johari, N., Kumar, N., & Jetti, R. (2017). The impact of eating and exercise frequency on weight gain - a cross-sectional study on medical undergraduate students. Journal of Clinical and Diagnostic Research, 11(2), 1-3.
14. Scott, S. (2008). Able bodies balance training. New York: Human Kinetics.
15. Donnelly, J. E., Blair, S. N., Jakicic, J. M., Manore, M. M., Rankin, J. W., & Smith, B. K. (2009). American College of Sports Medicine Position Stand. Appropriate physical activity intervention strategies for weight loss and prevention of weight regain for adults. Medicine and Science in Sports and Exercise, 41(2), 459-471.
16. Edwards, P., & Tsouros, A. D. (2006). Promoting physical activity and active living in urban environments: the role of local governments. WHO Regional Office Europe.
17. Kıraç, D., Kaspar E. Ç., Avcılar, T., Çakır, Ö.K., Ulucan, K., Kurtel, H., Deyneli, O., & Güney, A.İ. (2015). Obeziteyle ilişkili beslenme alışkanlıklarının araştırılmasında yeni bir yöntem "Üç Faktörlü Beslenme Anketi" , Marmara Üniversitesi, Sağlık Bilimleri Enstitüsü Dergisi, Cilt: 5, Sayı: 3.
18. Fidaner, H., Elbi, H., Fidaner, C., Eser, S. Y., Eser, E., & Göker, E. (1999). Psychometric properties of whoqol-100 and Whoqol-Bref. Psychiatr Psychol Psychopharmacol, 7(2), 23-40.
19. Eser, S. Y., Fidaner, H., Fidaner, C., Elbi, H., Eser, E., & Göker, E. (1999). Yaşam kalitesinin ölçülmesi. WHO-QOL-100 ve WHOQOL-Bref. 3P dergisi, 7(2), 5-13.
20. Salıcı, O. (2010). Beden Eğitimi ve Spor Yüksekokulu öğrencilerinin üniversitedeki yaşam kalitesine ilişkin algılarının demokratik yaşam kültürü çerçevesinde değerlendirilmesi. Yüksek Lisans Tezi, Çukurova Üniversitesi, Adana
21. İlhan, E. L. (2012). Examination on the healthy life style behaviors of the student-athletes in university teams in Turkey. Energy Educatıon Scıence And Technology Part BSocıal And Educatıonal Studıes, 4(3), 1511-1522.
22. Kangal, A. (2009). Üniversite Yaşam Kalitesi Ölçeğinin Psikometrik Özelliklerinin İncelenmesi Ve Türk Üniversite Öğrencilerine Uyarlanması. Yüksek Lisans Tezi, Akdeniz Üniversitesi Sosyal Bilimler Enstitüsü, Antalya.
23. Erdal, M. (2018). Üniversite öğrencilerinin fiziksel aktivite düzeylerinin yaşam kaliteleri ve toplumsal katılım düzeylerine etkisi. Yüksek Lisans Tez, Samsun On Dokuz Mayıs Üniversitesi, Samsun.
24. Ayhan, C. (2017). Aktif sporcuların rekreatif etkinliklere katılımlarına engel oluşturabilecek faktörlerin yaşam tatmini ve yaşam kalitesi üzerine etkisi. Yüksek Lisans Tezi, Sakarya Üniversitesi, Sakarya.
25. Blair, S. N. (2003). Physical activity epideniology, public health and the american collage of sport medicine. Medicine and Science in Sports and Exercise, 35(9), 1463-6.
26. Hawks, RS., Madanat, HN., Merill, RM., Goudy, MB., & Miyagwa, TA.(2002). Cross-Cultural Comparison of Health promoting Behaviours Among College Students. The Journal of Health Education , 5: 84-92.
27. Jones, A.M., Lamp, C., Neelon, M., Nicholson, Y., Schneider, C., Wooten, SP., & Zidenberg, C.S. (2015). Reliability and validity of nutrition knowledge questionnaire for adults. Journal of nutrition education and behavior. 47(1), 69-74.
28. Kavas, A., & Kavas, A. (1985). Üniversite Öğrencilerinin Beslenme Bilgi Düzeyi, Beslenmeye Karşı Tutumları ve Beslenme Durumları Üzerine Bir Araştırma. Beslenme ve

Diyet Dergisi. 14, 63-73.
29. Aksoydan, E., & Çakır, N., (2011). Adölesanların beslenme alışkanlıkları, fiziksel aktivite düzeyleri ve vücut kütle indekslerinin değerlendirilmesi, Gülhane Tıp Derg, 53(4): 264-270.
30. Tanrıverdı, D., Savas, E., Gonulluoglu, N., Kurdal, E., & Balık, G., (2011). Lise öğrencilerinin yeme tutumları, yeme davranışları ve benlik saygılarının incelenmesi, Gaziantep Tıp Dergisi, 17(1): 33-39.
31. Kalay, R., & Türkmen, A.S. (2015). Factors Affectıng The Nutrıtıon and Exercıse Behavıours Of Adolescents, International Peer-Reviewed Journal of Nutrition Research, 05:1-14.

Correspondence:
İbrahim Dalbudak
Atabey Vocational School, Isparta University of Applied Sciences
Isparta, Turkey
E-mail: dalbudakibo@hotmail.com

Progress in Nutrition 2020; Vol. 22, N. 1: 122-126 DOI: 10.23751/pn.v22i1.8117

Original article

Effects of rewards and pedometer-feedback on children's physical activity: a school-based intervention study

Ahmad R Allafi

Department of Food Science and Nutrition, College of Life Sciences, Kuwait University, Kuwait. E-mail: ahmadallafi@yahoo.com

Summary. *Background:* Current prevalence estimates for child obesity in Arabian Gulf countries are some of the world's highest. The study aims to evaluate the effect of rewarding and pedometer- feedback on increasing the children's physical activity and decrease obesity rates. *Methods:* A sum of 225 participants (110 boys - 115 girls) ranging from 9 to 11 years old from six public elementary schools in Kuwait City, Kuwait took part in the study. Three different groups were created; feedback (FB), feedback with rewards (FD+R), and control group (C). Children were assigned to one of the three groups randomly. In the FB group, participants received information about the function of pedometer only wherein the FB+R group received information about the function of the pedometer and were asked for a 3000 counts milestone to get ten stickers. The control group participants haven't received any information about the function of the pedometer. Pedometer counts were taken from all participants through five physical education classes. *Results:* The average step counts for the groups were; 2091±483 for the control group, 2655±577 for the FB group, and 3429±458 for the FB+R group. A significant difference was found in the counts among the three groups ($p < 0.05$). Post-hoc Tuky analysis indicated a specific significant difference between the FB+R group and the two other groups ($P < 0.00$). *Conclusion:* The results of the study showed that encouraging children with rewards will sustainably increase their physical activity.

Key words: children, physical activity, rewards, pedometer

Background

Facing the growing rates of child obesity is an important research area as it has been increasing vastly in many countries all over the world (1-3). Literature shows that the Food Dude program which is simply a peer-modeling and rewarding has increased children's fruits and vegetables' consumption (4, 5). The Food Dude program is based on a group of fictional characters presented to children in a video series. Food dude stickers and pencils were awarded daily to children as they achieve a certain number of fruit and vegetable virtues. Previous studies indicate that the poor eater's consumption noticeably increased over the others. Also, the combination of both the video modeling and the daily rewards gave better results than using each separately (6-8).

This study investigates the influence of the previously discussed model in increasing the children's physical activity. While a large percentage of children in Kuwait do not take the recommended daily physical activity hours (9), studies show that Food Dude program may also be effective when it comes to changing the children's behavior for a better level of physical activity. Children and adolescents are evident to be influenced by significant other in their social environment. For instance, an observational study that was conducted in a school playground indicated that children were motivated to be more active from their peers (10). Also, it has been indicated through research

that higher levels of physical activity were tied to peer encouragement, supports, and participation (11).

When it comes to increasing children's physical activities, It is evident that rewards become more effective when they are tangible items like baseball tickets and stickers and when awarded after clear and explicit goals (12). So, in this study, children need to have the ability to monitor their own progress through the task that leads to the reward. To achieve that, activity monitors, like the pedometer, need to be used. The pedometer is an activity monitor that provides a count of movements and steps which will help in setting activity goals and help children follow their own performance to achieve the goal according to the given instructions. Goldfiel et al. (2006) instructed obese children to set a goal of either 750 steps or 1500 steps in a 20 lab session to get 10 minutes of television viewing in return. Results indicated that the 1500 steps group was more active than the 750 steps group, which was more active than the control group. Over a six week period, this approach has shown to be effective at increasing physical activity of sedentary children (13). A follow-up study showed that obese children achieved more physical activity than children who had physical activity monitors but no rewards in returns. Findings accumulatively indicate that a combination of activity monitoring and tied rewards has a broader effect than just activity monitoring (14).

No experimental studies have been conducted in Kuwait to investigate the effectiveness of rewards on increasing children's physical activity up till today. This study evaluated the effect of the effectiveness of a combined pedometer-feedback and rewards versus feedback alone on increasing physical activity.

Methods

Participants

A total of 225 children participated in the study (110 boys, 115 girls). The age ranged from 9 to 11 years old. Children attended six different public schools in Kuwait City, Kuwait. Participants were selected from this age because pre-adolescent has been identified as a high-risk age for developing obesity (15). Studies also showed that physical activity noticeably declined with age during elementary school (16). Also, the age is appropriate as children would have the cognitive abilities to absorb the process of setting up the pedometer and self-monitoring. The consent form for the study was approved by the department of food science and nutrition at Kuwait University. A written consent form was signed by the parent of each child.

Experimental design and procedures

Three different groups were created; feedback (FB), feedback with rewards (FD+R), and control group (C). Children were assigned to one of the three groups randomly. In the FB group, participants received information about the function of pedometer only wherein the FB+R group received information about the function of the pedometer and were asked for a 3000 counts milestone to get ten stickers. The control group participants haven't received any information about the function of the pedometer. Cross-contamination was minimized through assigning children to groups based on the school they attended. A well-validated pedometer (Yamax Digiwalker SW-200, Tokyo, Japan) that displays a digital count of physical activity was used. Physical activity was measured through 5 physical activity sessions.

Measures

Pedometers were given to participants at the beginning of every session. All pedometers were set to zero and participants were instructed to keep wearing them during the whole session (50 minutes). The pedometer given to the control group was sealed with a tape. Step counts were not revealed to the control group and the researcher would simply say thank you to the child at the end of a session. Children's Body mass (to the nearest 0.1 kg) and height (to the nearest 0.1 cm) were measured without shoes using a Hanson electronic scale and a tape measure attached to a vertical wall, respectively. Each child's body mass index (BMI) was then computed.

Statistical analysis

Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) version 24 was used for data analysis. Differences in average values and p-values were tested by analysis of variance (ANOVA) with a 95% confidence interval.

Results

Characteristics of the participants are shown in (table 1), the average weight was 38.7±12.4 kg, the average height was 137.4±7.1 cm and the average BMI was 27.9±7.9 kg/m2. It is also important to notice that the number of girls was a little bit more than boys, 115 to 110. The average weight of boys was 37.5±11.6 kg, while the average weight for girls was 39.9 ± 13.1. Girls had a higher average height than boys, 137.9±7.8 cm to 136.9±6.4 cm). The average BMI for boys was 27.2±7.6 kg/m2 while the average BMI for girls was 28.6±8.1 kg/m2. According to ANOVA, there was no significant difference between boys and girls in the average age ($p = 0.43$), step counts ($p = 0.16$), height ($p = 0.13$), mass ($p = 0.15$), or BMI ($p = 0.15$).

The average step counts for the groups, shown in (table 2), were; 2091±483 for the control group, 2655±577 for the FB group, and 3429±458 for the FB+R group. A significant difference was found in the step counts among the three groups. ($p < 0.05$). Post- hoc Tuky analysis indicated a specific significant difference between the FB+R group and the two other groups ($p < 0.00$).

Discussion

Results claimed the efficacy of pedometer-feedback and rewards combination in increasing the physical activity of 9 to 11 years old children. During the study, the FB children showed a sustainable increase (2655 steps) while the FB+R group showed an increase of 3429 steps which is a great progress over the control group that had an average of 2091 steps.

No significant difference was noticed between the two sexes in the step counts ($p = 0.16$). Although Vandongen et al., Sallis et al., 1997 found the girls are more responsive to physical activities than boys (17), further research is required to explore the validity of gender differences. Generally, an active lifestyle needs to be mentioned over long period of time so children can experience its health benefits.

Table 1. Descriptive data for the total sample and within sex groups

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	Sig.
						Lower Bound	Upper Bound			
steps	Boys	110	2639	666	63	2513	2765	1108	4494	0.16
	Girls	115	2775	786	73	2629	2920	273	4679	
	Total	225	2708	731	49	2612	2804	273	4679	
weight	Boys	110	37.5	11.6	1.1	35.3	39.7	21.0	71.0	0.15
	Girls	115	39.9	13.1	1.2	37.4	42.3	22.0	96.0	
	Total	225	38.7	12.4	0.8	37.1	40.3	21.0	96.0	
height	Boys	110	136.9	6.4	0.6	135.7	138.1	123.0	153.0	0.31
	Girls	115	137.9	7.8	0.7	136.4	139.3	119.0	159.0	
	Total	225	137.4	7.1	0.5	136.4	138.3	119.0	159.0	
BMI	Boys	110	27.2	7.6	0.7	25.8	28.6	16.0	47.3	0.17
	Girls	115	28.6	8.1	0.8	27.1	30.1	16.4	60.4	
	Total	225	27.9	7.9	0.5	26.9	29.0	16.0	60.4	

Table 2. Mean step counts for the control, feedback and feedback plus reward groups

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	Sig.
					Lower Bound	Upper Bound			
Control	72	2091	483	57	1977	2204	273	3251	0.00
FB	85	2655	557	60	2535	2775	1349	4494	0.00
FB+R	68	3429	458	56	3318	3540	2212	4679	0.00
Total	225	2708	731	49	2612	2804	273	4679	

The use of intervention and pedometer step target to promote physical activity behavior change is what is unique about this intervention. The literature showed many studies using self-reporting to assess physical activity while there is a widespread concern about the validity of such measures (18).

The current study is the first test in this pilot intervention. Strong effects were accomplished, although the study was short in duration (only 5 sessions). Basic learning principle suggests that longer exposure to such circumstances may result in better consistency of effect. For future research, longer periods are recommended. It also appears that it might be more useful to include parents in the process due to the importance of parental support and encouragement (19). In this study, parents were involved only by via home pack and information letters, but in future research, the role of parents should be promoted to maybe giving parents their own pedometers with a set of step count goals in order to promote modeling. Also, the pedometer data were collected at school which means that the study missed measuring the physical activity on the weekends. Many studies have shown a significant difference between physical activity on weekends and weekdays (20), so it is recommended that weekends should be included in future studies of this intervention.

Future research should also investigate the effects of this intervention on children of different weight status as it was indicated that pre-adolescence is a high-risk age for developing obesity which calls for programs that aim to prevent it from a very young age.

Conclusion

The current study indicates, for the first time, the effectiveness of rewards and pedometer feedback in increasing habitual activity among 9 to 11 years old children in Kuwait city, Kuwait. Even though, some modifications in the process might be necessary to ensure long-term maintenance of behavioral change in children. The findings of this study are considered an initial step towards a physical activity intervention program in Kuwait which can lead up to the reduction of child obesity if implemented widely.

Acknowledgements

The authors are grateful for the participated schools. Also thankful for Ala'a Ahmed Bu-Abbas, Nouf Essa Al- Shatti, Zahraa Ahmad Al Haddad, and Lama Basem Saffouri, for their assistance with data collection.

References

1. Fitzgerald MP, Hennigan K, O'Gorman CS, McCarron L. Obesity, diet and lifestyle in 9-year-old children with parentally reported chronic diseases: findings from the Growing Up in Ireland longitudinal child cohort study. Ir J Med Sci. 2019; 188(1):29–34.
2. Hardy LL, Jin K, Mihrshahi S, Ding D. Trends in overweight, obesity, and waist-to-height ratio among Australian children from linguistically diverse backgrounds, 1997 to 2015. International Journal of Obesity 2019; 43(1):116.
3. Bramante CT, Thornton RLJ, Bennett WL, Zhang A, Wilson RF, Bass EB, et al. Systematic Review of Natural Experiments for Childhood Obesity Prevention and Control. American Journal of Preventive Medicine 2019; 1:56(1):147–58.
4. Horne P, Tapper K, Lowe C, Hardman C, Jackson M, Woolner J. Increasing children's fruit and vegetable consumption: a peer-modelling and rewards-based intervention. Eur J Clin Nutr 2004; 58:1649–1660.
5. Lowe C, Horne P, Tapper K, Bowdery M, Egerton C. Effects of a peer modeling and rewards-based intervention to increase fruit and vegetable consumption in children. Eur J Clin Nutr 2004; 58:510–522.
6. Dohle S, Diel K, Hofmann W. Executive functions and the self-regulation of eating behavior: A review. Appetite 2018; 1:124:4–9.
7. Boots SB, Tiggemann M, Corsini N. Pumpkin is "yucky"!: A prospective study of overt and covert restriction in the development of young children's food preferences. Appetite 2019; 1:135:54–60.
8. Luesse HB, Paul R, Gray HL, Koch P, Contento I, Marsick V. Challenges and Facilitators to Promoting a Healthy Food Environment and Communicating Effectively with Parents to Improve Food Behaviors of School Children. Matern Child Health J. 2018; 1:22(7):958–67.
9. Al-Haifi A, Al-Fayez M, Al-Athari B, Al-Ajmi F, Allafi A, Al-Hazzaa H, Musaiger A. Relative contribution of physical activity, sedentary behaviors, and dietary habits to the prevalence of obesity among Kuwaiti adolescents. Food and Nutrition Bulletin 2003; 34(1): 6–13.
10. Carr KA, Epstein LH. Influence of sedentary, social, and physical alternatives on food reinforcement. Health Psychology 2018; 37(2):125–31.
11. Ek KE, Miltenberger RG, Valbuena D. Promoting physical activity among school-age children using feedback, goal setting, and rewards. Behavior Analysis: Research and Practice 2016; 16(1):41–6.

12. Lubans DR, Plotnikoff RC, Miller A, Scott JJ, Thompson D, Tudor-Locke C. Using Pedometers for Measuring and Increasing Physical Activity in Children and Adolescents: The Next Step. American Journal of Lifestyle Medicine 2015; 1:9(6):418–27.
13. Goldfield G, Mallory R, Parker T, Cunningham T, Legg C, Lumb A. Effects of open-loop feedback on physical activity and television viewing in overweight and obese children: a randomized, controlled trial. Pediatrics 2006; 118: e157–e166.
14. Short KR, Chadwick JQ, Cannady TK, Branam DE, Wharton DF, Tullier MA, et al. Using financial incentives to promote physical activity in American Indian adolescents: A randomized controlled trial. PLOS ONE 2018; 1:13(6):e0198390.
15. Pozuelo-Carrascosa DP, Cavero-Redondo I, Herráiz-Adillo Á, Díez-Fernández A, Sánchez-López M, Martínez-Vizcaíno V. School-Based Exercise Programs and Cardiometabolic Risk Factors: A Meta-analysis. Pediatrics 2018; 142(5):e20181033.
16. Springer A, Kelder S, Hoelscher D. Social support, physical activity and sedentary behavior among 6th-grade girls: a cross-sectional study. Int J Behav Nutr Phys Act 2006; 3(8).
17. Wardle J, Henning Brodersen N, Cole T, Jarvis M, Boniface D. Development of adiposity in adolescence: five year longitudinal study of an ethnically and socioeconomically diverse sample of young people in Britain. BMJ 2006; 332: 1130-1135.
18. Park J, Ishikawa-Takata K, Lee S, Kim E, Lim K, Kim H, et al. Comparison of daily physical activity parameters using objective methods between overweight and normal-weight children. Journal of Sport and Health Science 2018; 1:7(2):210–7.
19. Dagger RM, Davies IG, Mackintosh KA, Stone GL, George KP, Fairclough SJ, et al. The CHANGE! Project: Changes in Body Composition and Cardiorespiratory Fitness in 10- to 11-Year-Old Children After Completing the CHANGE! Intervention. Pediatric Exercise Science 2017; 10:30(1):81–9.
20. Pozuelo-Carrascosa DP, García-Hermoso A, Álvarez-Bueno C, Sánchez-López M, Martinez-Vizcaino V. Effectiveness of school-based physical activity programmes on cardiorespiratory fitness in children: a meta-analysis of randomised controlled trials. Br J Sports Med. 2018; 1:52(19):1234–40.

Correspondence:
Ahmad R Allafi
Department of Food Science and Nutrition, College of Life Sciences, Kuwait University
P.O.Box: 5969, Safat 13060, Kuwait
Tel: (965)2498-3161
Fax: (965)2251-3929
Email: ahmadallafi@yahoo.com

Progress in Nutrition 2020; Vol. 22, N. 1: 127-136 DOI: 10.23751/pn.v22i1.7790

Original article

The relationship between BMI, WHR and serum vitamin B12, folic acid and ferritin levels in adults

Sabiha Zeynep Aydenk Koseoglu[1], Dilek Toprak[2], Nurhan Dogan[3]

[1]Istanbul Zaim University, Faculty of Health Sciences, Nutrition and Dietetics Dept. Halkali Cad. No:2 - E-mail: sabiha.koseoglu@izu.edu.tr; [2]Tekirdag Namik Kemal University, Faculty of Medicine, Family Medicine Dept. Namik Kemal Mah. Kampus Cad. No:1 Suleymanpasa, [3]Afyon Kocatepe University, Faculty of Medicine, Biostatistics and Medical Informatics. Alicetinkaya Kampusu, Izmir Yolu

Summary. *Aim:* The evaluation of relationship between serum vitamin B12, folic acid, ferritin levels and anthropometric measurements (Body Mass Index and Waist-Hipp Ratio) in 18-64 years Turkish citizens. *Material and Method:* The questionnaire including demographic features, health status, biochemical parameters (vitamin B12, folic acid, ferritin) was applied to the participants who were 18-64 years. Moreover their weight were measured by a delicate scales (Tanita HB 418) and height were taken by stadiometer. Waist – Hipp measurements were made by using a flexible tape recorder and all measurements were recorded. Their biochemical values were done by Sisli Hamidiye Etfal, Education and Resarch Hospital Lab. in Istanbul. *Results:* The findings in this study were obtained from 251 healthy volunteers. 72,9% (n:183) of 251 subjects were female and 27,1% (n:68) were male and mean age was 36,85 ± 12,17. According to findings there was found a significant relationship between gender with BMI (body mass index) and WHR (waist-hipp ratio) (p=0,000). Additionally the waist-hipp ratio (WHR) measurements were higher in male than female participants and it was statistically significant. According to gender vitamin B12 deficiency was more male than female. Besides the blood levels of hemoglobin (Hb), ferritin, folic Acid (PA) were lower in female than male (respectively, p=0,053; p=0,000; p=0,431). In this study, according to age BMI were observed and it was found meaningful relationship between them (p=0,000). *Conclusion:* The measurements of BMI and WHR of subjects can be related with serum folic Acid, ferritin and vitamin B12 levels. As a result there is more scientific research for supporting our study.

Key words: BMI, WHR, vitamin B12 level, folic acid level, ferritin level, adult

Introduction

High body mass index (BMI) can be an indicator of high body fatness and it can be used to screen weight categories that may lead to health problems but it is not diagnostic for the health of an individual (1).

Some common conditions related to overweight and obesity include: premature death, cardiovascular diseases, high blood pressure, osteoarthritis, some cancers and diabetes (2).

BMI does not measure body fat directly, but research has shown that it is moderately correlated with more direct measures of body fat obtained from skinfold thickness measurements, bioelectrical impedance, densitometry (underwater weighing), dual energy x-ray absorptiometry (DXA) and other methods (1, 3, 4).

As a water-soluble vitamin, vitamin B12 is naturally found in fish, meat, poultry, eggs, milk and milk products and it is required for proper blood cell forma-

tion, neurological function, and DNA synthesis. The main causes of vitamin B12 deficiency include vitamin B12 malabsorption from food, pernicious anemia, postsurgical malabsorption, and dietary deficiency (5).

Folic acid, also known as folate is found naturally in foods, is one of the B-group vitamins. Folic acid has several important functions like working together with vitamin B12 to form healthy red blood cells and to help to reduce the risk of central nervous system defects, such as neural tube defects (NTDs), in unborn babies. Folic acid deficiency could lead to anaemia. Folate can be found in many foods. Good sources include: broccoli, brussels sprouts, liver, spinach, asparagus, peas, and chickpeas fortified breakfast cereals (6).

Ferritin is a compound composed of iron molecules bound to apoferritin, a protein shell. Stored iron represents about 25% of total iron in the body, and most of this iron is stored as ferritin. Ferritin is found in many body cells, but especially those in the liver, spleen, bone marrow, and in reticuloendothelial cells. Ferritin is found in serum in low concentrations and is directly proportional to the body's iron stores (7).

This study aimed to identify relationships between BMI measuements and the level of "Vitamin B12", "Folic Acid", and "Ferritin" among Turkish citiziens.

Material And Methods

The survey was performed with face to face interviews by the researchers over 300 adults, aged between 18-64 years. Measurements of weight, height, body mass index (BMI), waist and hip circumferences and blood analysis of 251 adults were recorded.

Population and Sampling

The population for the survey was defined as adults aged between 18 -64 years. The study population was randomly selected fromTurkish citiziens who applied to Family Medicine Polyclinics of Sisli Hamidiye Etfal Training and Research Hospital. 251 of the subjects gave permission for the research. Pregnant, women who were in postpartum period, vegetarians or vegans, people who were taking H2 receptor inhibitors, vitamins, metphormin or were taken antibiotics in the last week, who had diagnosed as gastritis, peptic ulcus, anemia, Chronic Renal Deficiency or Chronic Heart Disease and people who had (known) excessive homocysteine were excluded from the study.

Data collection

A questionnare structered by us was applied to all subjects and socio-demographical features (gender, age) health status.

From the general questionnaire, we extracted information on sex, age, smoking, and alcohol consumption. Blood sampling and biochemical determinations during the normal life condition over the 8 hours fasting period venous blood samples were obtained from subjects in a sitting position.

Respondents were chosen from the volunteer patients who applied to the Family Medicine Polyclinics of Sisli Hamidiye Etfal Hospital in Istanbul in Turkey for any complain, in 6 months and who were between the age of 18 -64 years, in both gender. After the face to face interviews, height, weight, BMI, waist, and hip circumferences were measured. Socio-demographic and medical histories were recorded. Serum levels of vitamin B12, folic acid and Ferritin levels that had been obtained in the last 3 months were evaluated retrospectively.

Measurements

Height and weight were measured; BMI was calculated from measured height and weight. Height was measured with a steel anthropometer. Weight was measured on a Tanita bathroom digital scale. The waist was measured at the smallest horizontal trunk circumference and the hip was measured at the largest horizontal circumference around the hip and buttocks, with nonstretching fiberglas. Three trained fieldworkers acquainted with standardized methods took all anthropometric measurements. All measurements were performed in duplicate, and the average was used for analysis, or metal tapes. BMI was expressed as weight (kg) divided by height (m) squared and also waist / hip ratio was calculated.

Statistical Analysis

All statistical analyses were performed using the survey analysis method except for factor analysis and correlation. In defining statistics of datas had been used average, standard deviation, median, minumum, maximum, rate and frequency values. Distribution of variables had been controlled by kolmogorov and simimov test. In the analysis of quantitative datas had been used independent-sampling t test and mann-whitney u test. In analysis of qualitative datas had been used chi-square test. In analysis of correlation according to distribution of datas had been used correlation analysis of pearson and spearman. Analyses were conducted using SPSS 16.0.

Study Delivery

Study protocols were piloted and refined. Protocols were given ethical approval by the Research Ethics Committee of the Sisli Hamidiye Etfal Hospital Istanbul in Turkey. Study interviews were conducted by trained researchers.

Results

The findings reported in this research are collected from the 251 eligible healthy volunteer participants. Of 251 participants 72,9% (n=183) were female and 27,1% (n=68) were male with the mean age of 36.85±12.17 (min:18; max:64 years). 99,6% (n=250) of participants were lived in downtown. A total of 50,6% (n=127) were married, 33,1% (n=83) were active smokers, 74,9% (n=188) were working and 34,1% (n=61) had chronic disease.

While a total of 16,3% (n=41) had at least one children, 57,8% (n=145) had no children. Of 251 participants, 69,7% (n=175) had mid level income.

Table 1. Demographical Characteristics

	n	%
Gender		
Female	183	72,9%
Male	68	27,1%
Marital status		
Married	127	50,6%
Single	105	41,8%
Divorced	19	7,6%
Educational status		
Low	56	22,3%
High	195	77,7%
Working status		
Yes	188	74,9%
No	63	25,1%
Child number		
Have no child	145	57,8%
Have one or more than one child	106	42,2%
Income status		
Low	26	10,4%
Medium	174	69,7%
High	50	19,9%
Using alcohol		
Yes	70	27,9%
No	181	72,1%

45.8% (n=115) of the participants were low educated and 54,2% (n=136) were high educated.

Consumption rate of alcohol were 27,9% (n=70) in total participants (Table 1).

There was a significant relationship between gender and BMI and WHR values. According to gender, BMI values were higher among women (p=0,000). On the other hand weist/height ratio (WHR) values were higher in men than women (p=0,013) (Table 2).

According to gender, vitamin B12 deficiency were more common in men (n=17; 25,0%) than women (n=36; 19,7%) (p=0,358). On the other hand, Hemo-

Table 2. Anthropometry measurements according to gender

Gender	WHR		BMI		
	Lower than 0,85	Upper than 0,85	<25	25≤BMI≤30	≥30
Female	146 (79,8%)	37 (20,2%)	115 (62,8%)	38 (20,8%)	30 (16,4%)
Male	44 (64,7%)	24 (35,3%)	27 (39,7%)	32 (47,1%)	9 (13,2%)
P	0,013		0.000		

globin, ferritin, and folic acid levels were lower in women than men (p=0,053, p=0,000, p=0,431) (Table 3).

While no relationship was found between WHR, BMI, and vitamin B12, folic acid values, there was a significant relationship between BMI and Ferritin levels (p=0,013) (Table 4).

Regarding the age 28,4% (n=29) of people who were ≥40 age group had high BMI (≥30kg/m2) and it was 6,7% (n=10) among 18-39 age group (p=0,000)

There was a significant relationship between age group and WHR measurements (p=0,010) that WHR measurements were higher as 35,3% (n=36) in ≥40 age group in comparison with 18-39 age group (n=25; 16,8%) (Table 5).

The results of serum vitamin B12, hemoglobin, folic acid, and ferritin levels. Regarding age were shown in Table 6.

No relationship was found between education level and vitamin B12, folic acid, Hb and Ferritin values (p>0.05)

There were significant relationship between education level and BMI values and WHR measurements (p=0,000, p=0,000). BMI measurements were 9,7% (n=19) in high educated people and 35,7% (n=20)in low educated subjects (p=0,000). According to education level, WHR measurements were lower in high educated people (n=37; 19,0%) than in low educated people (n=24; 42,9%) (p=0,000) (Table 7, 8).

There was a significant relationship between having children and BMI and WHR measurements (p=0,000, p=0,000). BMI measurement was higher in people who has got one or more children (n=30; 28,3%) than participants who haven't got any children

Table 3. Biochemistry values according to gender

Gender	Vitamin B12		Folic Acid		Ferritin	
	126,5 pg/mL	**505 pg/mL**	**3,1 ng/mL**	**19,9 ng/mL**	**11 ng/mL**	**306,8 ng/mL**
Female	36 (19,7%)	147 (80,3%)	11 (6%)	172 (94%)	52 (28,4%)	131 (71,6%)
Male	17 (25%)	51 (75%)	6 (8,8%)	62 (91,2%)	1 (1,5%)	67 (98,5%)
P	0,38		0,431		0.000	

Table 4. Biochemical values according to Anthropometric measurements

WHR	Vitamin B12		Folic Acid	
	126.5 pg/mL	**505 pg/mL**	**3.1 ng/mL**	**19.9 ng/mL**
n (lower than 0,85)	42	148	15	175
n (upper than 0,85)	11	50	2	59
P	0,498		0,212	
BMI	**Vitamin B12**	**Folic Acid**	**Ferritin**	
<25	333,6	8,3	28,1	
25≤BKI<30	339,8	8,7	43,5	
≥30	323,2	9,5	40,4	
P	0,666	0,176	0,013	

Table 5. Anthropometry measurements according to age

Year	WHR		BMI		
	Lower than 0,85	**Upper than 0,85**	**<25**	**25≤BMI≤30**	**≥30**
18-39 (n=149)	124 (83,2%)	25 (16,8%)	105 (70,7%)	34 (22,8%)	10 (6,7%)
40 and upper (n=102)	66 (64,7%)	36 (35,3%)	37 (36,3%)	36 (35,3%)	29 (28,4%)
P	0,001		0.000		

Table 6. Biochemical measurements according to age

Year	Vitamin B12		Folic Acid		Ferritin	
	126.5 pg/mL	**505 pg/mL**	**3.1 ng/mL**	**19.9 ng/mL**	**11 ng/mL**	**306.8 ng/mL**
18-39 (n=149)	30 (20,1%)	119 (79,9%)	15 (10,1%)	134 (89,9%)	42 (28,2%)	107 (71,8%)
40 and upper (n=102)	23 (22,5%)	79 (77,5%)	2 (2%)	100 (98%)	11 (10,8%)	91 (89,2%)
P	0,645		0,012		0,001	

Table 7. Education and Anthropometric measurements

Education	WHR		BMI		
	Lower than 0,85	**Upper than 0,85**	**<25**	**25≤BMI≤30**	**≥30**
Low	32 (57,1%)	24 (42,9%)	23 (41,1%)	13 (23,2%)	20 (35,7%)
High	158 (81%)	37 (19%)	119 (61%)	57 (29,2%)	19 (9,7%)
P	0.000		0.000		

Table 8. Education and Biochemical values

Education	Vitamin B12		Folic Acid		Ferritin	
	126,5 pg/mL	**505 pg/mL**	**3,1 ng/mL**	**19,9 ng/mL**	**11 ng/mL**	**306,8 ng/mL**
Low	10 (17,9%)	46 (82,1%)	2 (3,6%)	54 (96,4%)	10 (17,9%)	46 (82,1%)
High	43 (22,1%)	152 (77,9%)	15 (7,7%)	180 (92,3%)	43 (22,1%)	152 (77,9%)
p	0,498		0,279		0,498	

(n=9; 6,2%) while in subjects who haven't got any children had lower WHR measurements (n=23; 15,9%) than people who has got one or more children (n=38; 35,8%). There was no significant relationship between having children and biochemical parameters (p >0.05) that vitamin B12, hemoglobin, ferritin, and folic acid deficiences were more common in people who had no child.

While the relationship between family income and BMI was significant (p=0,011) there was no relationship between family income and biochemical parameters (p>0,05).

The relationship between BMI, WHR, alcohol consumption, and serum biochemical parameters were shown in Table 9 and Table 10. In addition to the results there was a limited significant relationship between cardiovaskular disease and serum folic acid levels.(p=0,066)

Discussion

According to our study results, there was a statistically significant relationship between gender and

Table 9. Alcohol and anthropometric measurements

Alcohol Type	BMI			WHR	
	<25	**25≤BMI≤30**	**≥30**	**Lower than 0,85**	**Upper than 0,85**
yes (n)	24	4	4	22	10
yes (%)	75%	12,5%	12,5%	68,8%	31,3%
beer, vine (fermented) n	27	5	2	27	7
fermented (%)	79,4%	14,7%	5,9%	79,4%	20,6%
raki, wisky, vodka (not fermented) n	11	5	1	13	4
not fermented (%)	64,7%	29,4%	5,9%	76,5%	23,5%
P	0,136			0,793	

anthropometric measures BMI (p=0,000) and WHR (p=0,000). BMI was found high in female subjects, while WHR in men was higher than in women. Noh J.W. *et al*. (8), Reas L.D. *et al*. (9) and Fan M. *et al*. (10) reached conclusions that support our study. On the other hand, McKinnon E.J. *et al*. (11) did not find significant correlation between gender and anthropometric measurements in that study. The results we achieved in our study show similarities to those of most scientific studies.

According to gender biochemistry findings, there was not statistically significant relationship between gender and serum vitamin B12 and serum folic acid levels, but only between gender and serum ferritin level (p=0,358, p=0,431, p=0,000, respectively).

Serum ferritin level was found to be higher in females than males in our study but Ellidag H.Y. *et al*. (12), McNamee T. *et al*. (13), McKinnon E.J. *et al*. (11), Rushton D.H. & Barth J.H. (14) and Cafolla A. *et al*. (15) found significantly lower levels of serum ferritin in women than in men. In our study, the presence of lower serum ferritin levels in men suggests that it may be due to more caffeine consumption in men's diet (16). We could not observe a statistically significant difference between gender and serum vitamin B12 level in our study. Likewise, Baltacı D. *et al*. (17) and Cafolla A. *et al*. (15) did not correlate gender and vitamin B12 levels. Shams M. *et al*. (18) and Fakhrzadeh H. *et al*. (19) observed significant differences between gender and serum vitamin B12 levels. Shams M. *et al*. (18) found serum vitamin B12 levels lower in males, while Fakhrzadeh H. *et al*. (19) found higher levels. No statistically significant correlation was observed between gender and serum folic acid level in our study. Shams M. *et al*. (18) and Fakhrzadeh H. *et al*. (16) found that serum folic acid levels were lower in males in their studies, while Cafolla A. *et al*. (15) found no relationship between gender and serum follicle, which supports our study.

When we examine the relationship between anthropometric measurements and bio-chemical values of the participants (serum vitamin B12, serum folic acid, serum ferritin); there was no significant correlation between BMI and serum vitamin B12 and serum folic acid (p=0,666; p=0,176) while BMI and serum ferritin levels were found to be statistically significant (p=0,013). There was no significant relationship between WHR and biochemical parameters (p=0,498 for vitamin B12, p=0,212 for folic acid).

Arshad M. *et al*. (20), Abu-Samak M. *et al*. (21) (p<0,001) and Baltacı D. *et al*. (17) (p<0,001) found a statistically significant relationship between BMI and serum vitamin B12 in their studies. Abu-Samak M. *et al*. (21) and Baltacı D. *et al*. (17) (p=0,673) did not reach a statistically significant relationship between BMI and serum folic acid, similar to our study. Alam F. *et al*. (22) (p<0,001) and McKinnon E.J. *et al*. (11) (p<0,001) found a statistically significant relationship between BMI and serum ferritin, like our study. However, Baltacı D. *et al*. (17) and Ghadiri-Anaria A. *et al*. (23) did not find statistical significance between BMI and serum ferritin (p=0,132).

Regarding the relationship between haemoglobin (Hb) and BMI; Hemamalini J. (24) showed that serum Hb level increases as BMI increases. In a study to find the relationship between Hb and WHR, Jamshidi L. *et al*. (25) found that serum Hb levels were low in people with normal WHR. Statistically significant differences were found between them (p<0,0005).
The relationship between Hb and BMI was observed by Ghadiri-Andri A. *et al*. (23), and they found no statistical significance in their study.

Anthropometric measurements (BMI and WHR) by age were evaluated in our study, and we reached a statistically significant relationship between age and BMI, age and WHR, respectively (p=0,000; p=0,01). In other words, we observed that as the age advances, there is an increase in BMI and WHR as expected.

In studies conducted by Dalvand S. *et al*. (26) and Gillum R.F. (27), BMI and WHR increased as age advanced. Moreover, according to the study by Gillum R.F. (27), WHR was found to increase with age. These two studies support our work in terms of the results they found.

Reas L.D. *et al*. (9) reported that the correlation between age and BMI was negative and significant (p<0,001). In the study by Akhlaghi M. *et al*. (28), however, the relationship between age and BMI was not statistically significant.

When the relationship between age and biochemical parameters (vitamin B12, folic acid, ferri-

tin) was examined in our study, only the relationship between serum vitamin B12 and age was insignificant (p=0,645), while the relationship between serum folic acid and serum ferritin and age was statistically significant (p=0,012; p=0,001).

Heilmann E. & Bartling K. (29) showed that serum vitamin B12 decreased with age and there was a negative correlation between them with statistical significance (p=0,001). Fakhrzadeh H. *et al*. (19) reported in their study that the lowest level of vitamin B12 was between 35-44 years for women and 45-54 years for men. Andres E. *et al*. (30) found a negative correlation between aging and vitamin B12. In our study, the relationship between age and vitamin B12 was not statistically significant. We think that this is due to the unequal number of women and men in the sample group that we included in the study.

Fakhrzadeh H. *et al*. (19) found similar results with ours, that is, as the age increases a decrease in the serum folic acid value is observed, with a significant relationship found between them.

In a study in women, Ellidag H.Y. *et al*. (12) found that serum ferritin levels increases with age. Gillum R.F. (27) observed a decrease in serum ferritin with age, similar to our results. We think that the reason Ellidag H.Y. *et al*. (12) reached a conclusion that is the contradicting to ours is that because they only included women in their study.

In our study, the relationship between education and BMI and WHR was examined and the correlation was found to be significant respectively (p=0,000; p=0,000). According to our study, as the level of participants' education increases, BMI and WHR also increase.

According to studies by Kroeger R.A. (31) and Hermann S. *et al*. (32), as education increases, BMI levels decrease. Maddah M. *et al*. (33) observed that in women, the level of BMI was found to be low as education increased, whereas in adults, this was the opposite. Fan M. *et al*. (10) reported that the level of education increased as the level of BMI increased (p=0,030). Garcia-Mendizabal M.J. *et al*. (34) observed an increase in BMI as the level of education decreased in the study they conducted. Maddah M. *et al*. (33) and Hermann S. *et al*. (32) found a negative relationship between WHR and education level.

As the reason why the levels of BMI and WHR were found to increase with the level of education in our study, can be attributed to the sedentary lifestyle as a consequence of the socioeconomic change in the society, and to the fact that the study was conducted in a metropolis with inadequate facilities for physical activity, and to the difficulties in accessing healthy food. The relationship between education and biochemical parameters (serum vitamin B12, serum folic acid, serum ferritin) of the subjects was statistically insignificant (p=0,498, p=0,279, p=0,498 respectively). Studies explaining the relation between education and biochemical parameters were searched but no relevant data about the topic were found.

The relationship between alcohol and anthropometric measurements (BMI, WHR) was statistically insignificant in our study (p=0,136; p=0,793 respectively). The reason for such a result can be attributed to the fact that participants did not use alcohol or the consumption of alcohol was small in quantity and frequency.

Noh J.W. *et al*. (8) found a positive relationship between alcohol intake and BMI (p=0,004).

Freshmen M.A.N. *et al*. (35) stated that individuals who use alcohol had a higher BMI and that there was a statistically significant relationship between alcohol intake and BMI (p<0,01). French M.T. *et al*. (36) observed a positive relationship between alcohol intake and BMI (p<0,01). Liangpunsakul S. *et al*. (37) stated that there was a significant positive significant relationship between alcohol intake and WHR. Kelly N. *et al*. (38) found a statistically significant association between alcohol consumption and visceral adiposity and WHR (p=0,025). While Lukasiewicz E. *et al*. (39) found a significant relationship between alcohol consumption and WHR in both genders, women who consumed alcohol at low levels found to have lower WHR than women who did not consume alcohol or consumed excessive alcohol. In men, that was the opposite, and the relationship between alcohol intake and BMI was significant (p=0,05).

In our study, the relationship between alcohol consumption and biochemical parameters (serum vitamin B12, serum folic acid, serum ferritin) was statistically insignificant (p=0,161, p=0,947, p=0,244 respectively). The reason why our study resulted in this

way is that the number of alcohol users and alcohol use rates in the sample group taken for the study were low. Green P.H. (40) observed in a study that vitamin B12 level decreased with alcohol consumption. Cravo M.L. *et al*. (41) found that serum vitamin B12 increased due to alcohol consumption. Lieb M. *et al*. (42) and Eichner E.R. & Hillman R.S. (43) found no significant association between alcohol consumption and vitamin B12 in their studies. Eichner E.R., & Hillman R.S. (43) and Green P.H. (40) found low serum folic acid levels in alcohol users, whereas Cravo M.L. *et al*. (41) observed that the level of serum folic acid in alcohol consumers was high. Lieb M. *et al*. (42) found no significant relationship between alcohol intake and serum folic acid level. While McKinnon E.J. *et al*. (11) and Eichner E.R., & Hillman R.S. (43) found no significant relationship between alcohol consumption and serum ferritin, Lieb M. *et al*. (42) observed a high serum ferritin level in alcohol users.

Conclusion

All of the findings we have obtained in our study are approximately in line with similar studies and we think that the differences may be due to the selection of the participants in the study in a cross-sectional and randomized manner. In our work, we wanted to multiply the parameters to observe different results. For that reason, in some parameters we did not obtain exactly the same results as those of similar studies that had been conducted before. In the discussion part, information related to this topic is provided. Studies like ours, which was conducted in a metropolitan area, should be made in rural areas as well and be compared with groups with different socioeconomic and sociocultural characteristics so that we can shed light on the public health problems.

Acknowledgements

As a research team, we thank the interviewers.

Thanks to Laboratory's staff at Sisli Hamidiye Etfal Hospital Istanbul for managing the collection and analysis of blood.

Information from blood test was interpreted for feedback to respondents.

Finally, we thank the Family Physician Polyclinics of Sisli Hamidiye Etfal Hospital for the opportunity to conduct this survey.

We hope that the research will practice in the pursuit of good health.

References

1. Centers for Disease Control and Prevention (CDC), (May 15, 2015) Health Weight; Assessing Your Weight; Body Mass Index (BMI). Available: https://www.cdc.gov/healthyweight/index.html (Accessed 10.02.2018)
2. World Health Organization (WHO), Body Mass Index BMI. Available: http://www.euro.who.int/en/health-topics/disease-prevention/nutrition/a-healthy-lifestyle/body-mass-index-bmi (Accessed 20.03.2018)
3. Garrow J.S, & Webster J. Quetelet's index (W/H2) as a measure of fatness. Int. J. Obes. 1985; 9(2): 147-153.
4. Freedman D.S, Horlick M, & Berenson G.S. A comparison of the slaughter skinfold-thickness equations and BMI in predicting body fatness and cardiovascular disease risk factor levels in children. Am. J. Clin. Nutr. 2013; 98(6): 1417–24.
5. National Institutes of Health (NIH), Vitamin and Mineral Supplement Fact Sheets. Available: https://ods.od.nih.gov/factsheets/list-VitaminsMinerals (Accessed 20.03.2018)
6. National Health Service (NHS), Vitamins and minerals. Available: https://www.nhs.uk/Conditions/vitamins-minerals/Pages/Vitamin-B.aspx (Accessed 20.03.2018)
7. National Health Service (NHS), Ferritin. Available: https://www.southtees.nhs.uk/services/pathology/tests/Ferritin (Accessed 20.03.2018)
8. Noh J.W, Kim J, Park J, Oh I.H, Kwon Y.D. Age and gender differential relationship between employment status and body mass index among middle-aged and elderly adults: A cross-sectional study. BMJ Open 2016; 6(11): e012117. doi: 10.1136/bmjopen- 2016-012117.
9. Reas D.L, Nygard J.F, Svensson E, Sorensen T, Sandanger I. Changes in body mass index by age, gender, and socioeconomic status among a cohort of Norwegian men and women (1990–2001). BMC Public Health 2007; 7: 269.
10. Fan M, Su M, Tan Y, Liu Q, Ren Y, Li L, Lv J. Gender, age and education level modify the association between body mass index and physical activity: A cross-sectional study in Hangzhou, China. PloS One 2015; 10(5): e0125534. doi: 10.1371/journal.pone. 0125534.
11. McKinnon E.J, Rossi E, Beilby J.P, Trinder D, & Olynyk J.K. Factors that affect serum levels of ferritin in Australian adults and implications for follow-up. Clinical Gastroenterology and Hepatology 2014; 12: 101–108.e4. doi: 10.1016/j.cgh.2013.07.019.
12. Ellidag H.Y, Eren E, Akdag M, Giray O, Kiraz K, & Yilmaz N. The relationship between serum ferritin levels and serum lipids and HDL function with respect to age and gender. Ukr Biochem J. 2016; 88(6): 76-86. doi: 10.15407/ubj88.06.076.

13. McNamee T, Hyland T, Harrington J, Cadogon S, Honari B, Perera K, Fitagerald A.P, Perry I.J, & Cahill M.R. Haematinic deficiency and macrocytosis in middle-aged and older adults. PLoS One 2013; 8(11). doi: 10.1371/journal.pone.0077743.
14. Rushton D.H, & Barth J.H. What is the evidence for gender differences in ferritin and haemoglobin? Crit Rev Oncol Hematol 2010; 73(1): 1-9. doi: 10.1016/j.critrevonc.2009.03. 010.
15. Cafolla A, Dragoni F, Girelli G, Tosti M.E, Costante A, Pastorelli D, Bedogni G, & Scott S. Folate status in Italian blood donors: relation to gender and smoking. Haematologica 2000; vol: 85(7): 694-698.
16. Khorshid L, & Sari D. Caffeine Consumption in Staff in University. Ege Üniversitesi Hem irelik Fakültesi Dergisi 2013; 29(1): 45-59. Naklen: Köro lu E. Mental bozuklukların tanısal ve sayımsal el kitabı. DSM-IV. Amerikan Psikiyatri Birli i, Hekimler Yayın Birli i 1994; 262-266.
17. Baltaci D, Kutlucan A, Turker Y, Yilmaz A, Karacam S, Deler H, Ucgun T, & Kara I.H. Association of vitamin B12 with obesity, overweight, insulin resistance and metabolic syndrome, and body fat composition; primary care-based study. Medicinski Glasnik 2013; volume 10: number 2.
18. Shams M, Homayouni K, & Omrani G.R. Serum folate and vitamin B12 status in healthy Iranian adults. East Mediterr Health J. 2009; 15(5): 1285-92.
19. Fakhrzadeh H, Ghotbi S, Pourebrahim R, Nouri M, Heshmat R, Bandarian F, Shafaee A, & Larijani B. Total plasma homocysteine, folate and vitamin B12 status in healthy Iranian adults: The Tehran homocysteine survey (2003-2004)/a cross-sectional population based study. BMC Public Health 2006; 6: 29.
20. Arshad M, Jaberian S, Pazouki A, Riazi S, Rangraz M.A, & Mokhber S. Iron deficiency anemia and megaloblastic anemia in obese patients. Rom J Intern Med. 2017; 55(1): 3-7. doi: 10.1515/rjim-2016-0046.
21. Abu-Samak M, Kuhazie R, Abu- Hasheesh M, Jaradeh M, & Fawzi M. Relationship of vitamin B12 deficiency with overweight in male in Jordanian youth. Journal of Applied Sciences 2008; 8 (17): 3060-3063.
22. Alam F, Memon A.S, & Fatima S.S. Increased body mass index may lead to hyperferritenemia irrespective of body iron stores. Pak J Med Sci. 2015; 31(6): 1521-6. doi: 10.12669/pjms. 316.7724.
23. Ghadiri-Anari A, Nazemian N, & Vahedian-Ardakani H.A. Association of body mass index with hemoglobin concentration and iron parameters in Iranian population. Hindawi Publishing Corporation ISRN Hematology 2014. doi: 10.1155/2014/525312.
24. Hemamalini J. Anemia in relation to body mass index and waist circumference among Andhra Pradesh women. J Obes Weight Loss Ther 2013; 3: 3.
25. Jamshidi L, Karimi L, Seif A, & Vazini H. The relationship between anthropometric factors and iron deficiency anemia factors. Novelty in Biomedicine 2017; 2: 59-64.
26. Dalvand S, Koohpayehzadeh J, Karimlou M, Asgari F, Rafei A, Seifi B, Niksima S.H, & Bakhshi E. Assessing factors related to waist circumference and obesity: application of a latent variable model. J Environ Public Health 2015; 893198. doi: 10.1155/2015/893198.
27. Gillum R.F. Association of serum ferritin and indices of body fat distribution and obesity in Mexican American men - The Third National Health and Nutrition Examination. International Journal of Obesity 2001; 25: 639-645.
28. Akhlaghi M, Kamali M, Dastsouz F, Sadeghi F, & Amanat S. Increased waist-to-height ratio may contribute to age-related increase in cardiovascular risk factors. Int J Prev Med. 2016; 7: 68.
29. Heilmann E, & Bartling K. The vitamin B12 serum level at various ages. Folia Haematol Int Mag Klin Morphol Blutforsch 1997; 104(4): 569-74.
30. Andres E, Loukili N.H, Noel E, Kaltenbach G, Abdelgheni M.B, Perrin A.E, Noblet-Dick M, Maloisel F, Schlienger J.L, & Blickle J.F. Vitamin B12 (cobalamin) deficiency in elderly patients. CMAJ 2004; 171(3): 251-9.
31. Kroeger R.A. Union Status, Educational Attainment, and Body Mass Index Among Emerging Adults. Emerg Adulthood 2017; 5(5): 357-363. doi: 10.1177/2167696817701601.
32. Hermann S, Rohrmann S, Linseisen J, May A.M, Kunst A, Besson H, Romaguera D, Travier N, Tormo M.J, Molina E, Dorronsoro M, *et al*. The association of education with body mass index and waist circumference in the EPIC-PANACEA study. BMC Public Health 2011; 11: 169. doi: 10.1186/1471-2458-11-169.
33. Maddah M, Eshraghian M.R, Djazayery A, & Mirdamadi R. Association of body mass index with educational level in Iranian men and women. Eur J Clin Nutr. 2003; 57(7): 819-23.
34. Garcia-Mendizabal M.J, Carrasco J.M, Perez-Gomez B, Aragones N, Guallar-Castillon P, Rodriguez-Artalejo F, Lopez-Abente G, & Pollan M. Role of educational level in the relationship between body mass index (BMI) and health-related quality of life (HRQL) among rural Spanish women. BMC Public Health 2009; 9: 120. doi: 10.1186/1471-2458-9-120.
35. Freshmen M.A.N. *et al*. Relationship of body mass index to alcohol consumption in college. The Scientific World Journal 2012. doi: 10.1100/2012/849018.
36. French M.T. *et al*. Alcohol consumption and body weight. Health Econ. 2010; 19(7): 814-32. doi: 10.1002/hec.1521.
37. Liangpunsakul S. *et al*. Relationship between alcohol intake, body fat, and physical activity - A Population - based study. Ann Epidemiol 2010; 20(9): 670-5. doi: 10.1016.
38. Kelly N, Coleman I, Cawley S, Mullaney L, O'Higgins A, Turner M.J, & McCartney D. Changing dietary behaviour: Physiology through to practice associations between alcohol intake and anthropometric measurements in an Irish obstetric cohort. Irish Section Meeting, 18-20 June 2014. Proceedings of the Nutrition Society 2014; 73(OCE2): E104.
39. Lukasiewicz E, Mennen L.I, Bertrais S, Arnault N, Preziosi P, Galan P, & Hercberg S. Alcohol intake in relation to body mass index and waist-to-hip ratio: the importance of

type of alcoholic beverage. Public Health Nutr. 2005; 8(3): 315-20.
40. Green P.H. Alcohol, nutrition and malabsorption. Clin. Gastroenterol 1983; 12(2): 563-74.
41. Cravo M.L, Gloria L.M, Selhub J, Nadeau M.R, Camilo M.E, Resende M.P, Cardoso J.N, Leitao C.N, & Mira F.C. Hyperhomocysteinemia in chronic alcoholism correlation with folate, vitamin B-12, and vitamin B-6 status. Am J Clin Nutr. 1996; 63(2): 220-4.
42. Lieb M, Palm U, Hock B, Schwarz M, Domke I, & Soyka M. Effects of alcohol consumption on iron metabolism. Am J Drug Alcohol Abuse 2011; 37(1): 68-73. doi: 10.3109/00952990.2010.535584.
43. Eichner E.R, & Hillman R.S. Effect of alcohol on serum folate level. J Clin Invest. 1973; 52(3): 584-91.

Correspondence:
Sabiha Zeynep Aydenk Koseoglu,
Istanbul Zaim University, Faculty of Health Sciences,
Nutrition and Dietetics Dept. Halkali Cad. No:2
Halkali Kucukcekmece, 34303, Istanbul, Turkiye.
Tel: +90 444 9798,
E-mail: sabiha.koseoglu@izu.edu.tr; szaydenk@gmail.com

Progress in Nutrition 2020; Vol. 22, N. 1: 137-145 DOI: 10.23751/pn.v22i1.7688

Original article

Effect of dairy products intake in women with premenstrual syndrome: a randomized controlled trial

Merve Yurt[1], *Seyit Mehmet Mercanlıgil*[1,2], *Seray Kabaran*[1]

[1]Department of Nutrition and Dietetics, Faculty of Health Sciences, Eastern Mediterranean University, Famagusta, North Cyprus via Mersin 10, Turkey - E-mail: merve.yurt@emu.edu.tr; [2] Cyprus International University, Faculty of Health Sciences, Nutrition and Dietetics Department, Nicosia, Cyprus

Summary. *Aim:* This study was planned and conducted to investigate the effects of adequate dairy products, at least 3 portions, and calcium intake on Premenstrual Syndrome (PMS) symptoms in women with PMS who have inadequate calcium intake. *Methods:* Thirty-one women, aged between 20-28 years who were diagnosed with PMS had participated. All women had inadequate calcium intake and regular menstrual cycles. These participants were randomly allocated into two groups, an intervention (n=16) group and a control (n=15) group. It was ensured that the intervention group consumed foods containing at least 1000 mg calcium for two months. Turkish *kasseri* cheese (50 g) which is made from cow's milk was provided to the intervention group and they were informed to consume at least 400 ml of milk and 150 g of yogurt every day for two months. No specific diet was recommended to the control group. The Premenstrual Syndrome Scale (PMSS) and the Short Form of Quality of Life Scale were administered at the baseline and after the study. Independent t-test and Mann-Whitney-U test were used for group comparisons according to data normality. ***Results:*** No significant difference in PMSS scores, physical functions and mental health scores on the SF-36 quality of life scale and daily calcium intake between the intervention (500.9±114.6 mg) and the control groups (511.3±149.2 mg) at baseline assessment ($p>0.05$). The intervention group's total PMSS post-intervention (104.8±21.5) score was found to be significantly lower than their baseline scores (151.9±15.4) ($p<0.05$). The intervention group's physical functions (47.9±8.0) and mental health (48.6±6.9) post-intervention scores on the SF-36 quality of life scale were found to be significantly higher than their baseline scores (41.2±10.7 and 45.0±10.7 respectively) ($p<0.05$). ***Conclusion:*** These results indicate that sufficient dairy and calcium intake affects women's PMS symptoms and improves the quality of life.

Keywords: premenstrual syndrome, calcium, quality of life

Introduction

Premenstrual syndrome (PMS) is characterized by physical, behavioral and psychological symptoms during the luteal phase of menstrual cycle, which ends with onset menstrual flow (1,2). Prevalence of PMS among the women of reproduction age varies between 30% and 73.7% (2-4). PMS not only affects women's mental health and social relationships but also reduces their efficiency at work, family, friends and hobbies due to its physical and behavioral symptoms. PMS, considered a community health problem, is common among most women of reproduction age (5).

PMS is considered a psychoneuroendocrine disorder. Instabilities of the ovarian hormones such as estrogen and progesterone, and neurotransmitters such as serotonin and γ-amino butyric acid (GABA) can be seen in the pathophysiology of PMS. Estrogen, increasing in the fourteenth day of menstruation, may decrease the amount of calcium in the luteal phase

and increase the parathyroid hormone (hyperparathyroidism). It may also develop PMS symptoms such as mood swings, muscle cramps, tension and migraine (6,7). Therefore, nutritional, hormonal and neurotransmitters such as serotonin treatments are administered to PMS patients (8). The guide published by the Royal College of Obstetrics and Gynecology (RCOG) in 2007 indicates that B6 vitamin supplements, oral progesterone (10mg/day) and selective serotonin reuptake inhibitors should be administered in the luteal phase or continually to reduce PMS symptoms (9).

Formation process of PMS is affected by some nutrients. Insufficient intake of calcium, copper, potassium, iron and group B vitamins, alcohol consumption, smoking and sedentary behaviors increase PMS risk (6,10). Estrogen increases with ovulation, causing fluctuations in calcium, parathyroid hormone and vitamin D levels. Fluctuating calcium levels cause hyperparathyroidism in the luteal phase and PMS symptoms (6,7,11). It was found in the Nurses' Health Study-II that women who consume low amounts of calcium have a higher risk of PMS. Women who consumed high amounts of dietary calcium (1283 mg/day on average) and those who consumed low amounts of calcium (529 mg/day) were compared and it was found that high calcium consumption decreases PMS risk by 30%. Fat free or low-fat milk consumption was also found to be related to lower risk of PMS (12). The symptoms of women with PMS were found to decrease in many studies that calcium supplements were used (13-16). Thys-Jacobs et al. (17,18) reported that a daily supplement of 1200 mg of calcium decreased PMS symptoms by 48%, and a daily supplement of 1000 mg of calcium reduced PMS pains and reduced edema.

Dairy products are good source of riboflavin, niasin and vitamin B_{12} and calcium, magnesium, phosphorus and zinc. Previous studies indicating the effect of calcium, zinc, vitamin B_6, D and iron supplements on PMS symptoms exist, but there is no study on the effectiveness of dietary dairy and calcium intake. The aim of this study is to examine if increasing dairy product and dietary calcium consumption improves the symptoms of PMS in women with inadequate calcium intake.

Subjects and methods

This study was planned as a randomized controlled trial consisting of intervention and control groups. Resource screening and background processes began in April, 2014. The sample consists of voluntary students studying at the Eastern Mediterranean University who meet the study inclusion criteria. This study was conducted between January 2015 and January 2016 with thirty-one women between 20 and 28 years of age who were consuming less than the recommended dietary allowance (RDA) of calcium and had been diagnosed with PMS by a doctor. This study is registered at Trials.Gov (NCT02809066).

All of the voluntary students were diagnosed with PMS and had been consuming less calcium than the RDA. The inclusion criteria for the intervention and control groups include: regular menstrual cycle (lasting for three to eight days between the range of 22 to 35 days), non-smoker, no systemic diseases (diabetes, chronic renal failure, hypertension, hyperlipidemia, PCOS, coronary heart disease, hyperthyroid, renal or hepatic diseases), no use of oral contraceptives, antidepressant and vitamin/mineral supplements, and no history of hormonal treatment. These criteria were observed while selecting the sample. The research procedures were approved by the Clinical Research Ethics Committee of Eastern Mediterranean University and written informed consent was obtained from all participants (2015/10-03).

The sample size was calculated using G*Power software 3.1.9 and assuming a nonparametric test was used in comparison of two groups with 0,05 alpha value, 0,80 power and Cohen *d*:1 effect size, total sample size was calculated as at least 15 for each group. With an expected drop out, 20 participants per group was considered adequate.

The participants were allocated into two groups randomly by simple randomization using random number charts, an intervention group of twenty and a control group of twenty. It was ensured that the intervention group consumed the RDA of 1000 mg of calcium (70-80% minimum from dairy products, 20-30% from other food groups) for two months. Turkish *kasseri* cheese (50 g) which is made from cow's milk was given to the intervention group every day for two months, and they were informed to consume at least

400 ml of milk and 150 g of yogurt each day. The nutrition composition of Turkish *kasseri* cheese is summarized in Table 1. The calcium consumption of the control group was not altered.

Interviews were conducted with the participants and a questionnaire was administered including questions about their general backgrounds, nutritional status and physical activities, the Premenstrual Syndrome Scale (PMSS) and the short form of the Quality of Life Scale (SF-36). The Premenstrual Syndrome Evaluation Scale is a five-point Likert type scale with forty-four items that was developed in 2006 by Gençdoğan using DSM-III and DSM-IV-R. This scale was administered to the participants both at the beginning and at the end of the study. The PMSS has nine subscales: depressive mood, anxiety, tiredness, anger bursts, depressive thoughts, pain, appetite changes, sleep disturbances and abdominal bloating. Exceeding half of the maximum score on the subscale of this scale determines whether an individual has PMS (19). Total scores on the PMSS and its nine subscales were calculated. The SF-36 quality of life scale was administered to the participants at the beginning and end of the study (20). The participants were followed up for two months, and at the end of two months 31 participants finished the study: 16 in the intervention group and 15 in the control group.

Dietary assessment

3-day dietary record was recorded twice, once at the beginning of the study and once at the end. Dietary assessments were recorded using the Photography Catalogue of Food and Dishes: Portion Sizes and Amounts (21). Portions were calculated using the Standard Recipes book (22). After specifying the daily amounts of the consumed foods, daily energy and macro- and micronutrient intakes were calculated using the Nutritional Information Systems Package Software (BEBİS) 7.2 professional edition (23). It was evaluated whether energy and nutrient intakes met the RDA (24).

Anthropometric Measures

Anthropometric measurements such as waist and hip circumference, height, weight and body composition (body fat mass, fat-free mass, total body water and abdominal fat volume) were performed by the researcher at the beginning and end of the study. The body weights of the participants were measured using the Jawon X Scan Plus 2 bioelectrical impedance analyzer. The measurements were carried out in the fasting state. They were wearing light clothes and no shoes.

Table 1. Kasseri cheese nutrition composition of edible portion (23).

Nutrient	Value per 100 g
Energy (kcal)	452.2
Protein (g)	19.1
Total lipid (fat) (g)	39.1
Carbohydrate (g)	0.0
Minerals	
Calcium (mg)	600.0
Magnesium (mg)	50.0
Phosphorus (mg)	400.0
Potassium (mg)	100.0
İron (mg)	0.4
Zinc (mg)	3.0
Vitamins	
A (µg)	468.0
E (mg)	1.2
B_2 (mg)	0.4
B_3 (mg)	6.4
B_6 (mg)	0.1
B_{12} (µg)	2.0

Table 2. Demographic and anthropometric characteristics of participants at baseline

	Intervention Group (n=16)	Control Group (n=15)	p
	Mean±SD (% 95 CI)	Mean±SD (% 95 CI)	
Age (years)	22.3±1.84	22.9±2.06	0.425
Weight (kg)	57.0± 9.40	54.9±5.65	0.621
BMI (kg m^{-2})	21.3±3.08	21.0±2.13	0.767
Menarche age (years)	13.5±1.03	13.4±1.12	0.902
Menstrual duration (day)	5.4±1.09	5.1±0.74	0.529
Body composition			
Lean body mass (kg)	42.8±4.66	41.2±2.62	0.251
Soft lean mass (kg)	39.5±4.18	38.1±2.34	0.220
Skeletal muscle mass (kg)	18.9±4.06	17.3±2.25	0.363
Body fat (kg)	14.2±5.4	13.7±3.7	0.906
Total body water (kg)	30.8±3.35	29.4±2.5	0.206

p value was calculated Mann-Whitney U test

Statistical Analysis

Statistical analysis of the data obtained from the study was performed using SPSS (Statistical Package for Social Sciences) Version 20.0. The Kolmogorov-Smirnov test was used to see whether the numerical data had a normal distribution. The data with normal distributions were evaluated with parametric tests, and those that did not were evaluated using non-parametric statistical tests.

To compare the numerical data of the intervention and control groups, the independent two-sample t-test was used when the parametric conditions were met, and the Mann-Whitney U test was used when they were not. Baseline and post-intervention results were compared using dependent two-sample t-test when the parametric conditions were met, and the Wilcoxon test was used when they were not. For the categorical data and comparisons between the intervention group and control group, 2*2 and 2*3 chi-square tests were used. P value could not be specified when sufficient frequency was not ensured in the categorical variables. The threshold for significance was $p<0.05$ in all statistical test results. *P*-value was adjusted for potential confounders, such as the baseline values of daily intake of energy and macro- and micro-nutrients in Table 3 and Table 4.

Table 3. Evaluation of the premenstrual syndrome scale (PMSS) total subscale mean scores and quality of life (SF-36) scale

	Intervention Group (n=16)			Control Group (n=15)				
	Baseline	Post-intervention	p	Baseline	Post-intervention	p	p^a	p^b
	Mean±SD (% 95 CI)			Mean±SD (% 95 CI)				
PMSS subtitles								
Depressive mood	24.7±3.9 (22.6-26.7)	17.5±4.5 (15.2-19.8)	<0.001*	23.1±4.0 (21.0-25.2)	23.0±4.5 (20.6-25.4)	0.688	0.306	0.003*
Anxiety	19.0±3.3 (17.3-20.8)	13.2±3.9 (11.2-15.2)	0.001*	20.0±3.3 (18.2-21.8)	20.0±3.9 (18.0-21.1)	0.951	0.446	<0.001*
Tiredness	21.1±3.9 (19.1-23.2)	14.7±4.6 (12.3-17.0)	0.001*	21.7±3.9 (19.6-23.8)	22.4±4.6 (20.0-24.8)	0.688	0.725	<0.001*
Angry	20.5±3.3 (18.8-22.2)	12.1±4.5 (9.8-14.4)	0.001*	17.5±3.3 (15.7-19.3)	19.0±4.5 (16.6-21.3)	0.674	0.026*	0.001#
Depressive thoughts	22.3±4.7 (19.8-24.7)	15.7±4.5 (13.4-18.0)	<0.001*	22.9±4.7 (20.3-25.4)	24.2±4.5 (21.8-26.6)	0.680	0.744	<0.001*
Pain	11.6±2.9 (10.0-13.1)	6.6±2.5 (5.3-7.9)	0.001*	9.2±3.0 (7.6-10.8)	11.1±2.5 (9.8-12.5)	0.078	0.047*	<0.001#
Appetite changes,	11.9±3.5 (10.1-137)	8.8±3.5 (7.0-10.6)	0.007*	10.1±3.5 (8.2-11.9)	10.9±3.5 (9.0-12.8)	0.031*	0.188	0.119
Sleep disturbances	10.3±2.4 (9.0-11.6)	7.6±2.5 (6.3-8.8)	0.003*	10.4±2.5 (9.1-11.7)	10.9±2.5 (9.6-12.3)	0.783	0.898	0.001*
Abdominal bloating	10.8±2.2 (9.6- 11.9)	7.7±2.9 (6.2-9.2)	0.001*	10.1±2.2 (9.0-11.3)	10.6±3.0 (9.1-12.2)	0.506	0.453	0.014*
PMSS total scores	151.9±15.4 (143.9-159.8)	104.8±21.5 (93.8-115.8)	<0.001*	145.0±15.5 (136.8-153.2)	151.1±21.5 (139.7-162.5)	0.320	0.254	<0.001*
SF-36 quality of life scale								
Physical functions	41.2±10.7 (35.7-46.7)	47.9±8.0 (43.8-51.9)	**0.041***	45.0±10.7 (39.2-50.7)	40.9±8.0 (36.7-45.1)	0.222	0.364	**0.027***
Mental health	40.3±9.2 (35.6-45.0)	48.6±6.9 (45.0-52.1)	**0.002***	37.6±9.2 (32.7-42.5)	38.7±6.9 (35.0-42.3)	0.078	0445	**0.001***

**p < 0.05; p value was calculated Wilcoxan test; p^a: Comparing the early data of the Intervention and control groups; (p-value was adjusted for baseline protein, carbohydrate, vitamin E and zinc intake); p^b: Comparing the post-intervention data of the Intervention and control groups (p-value was adjusted for post-intervention potassium intake; # p-value was adjusted for baseline scores and post-intervention potassium intake*

Table 4. Individuals' daily intake of energy and macro- and micro-nutrients

	Intervention Group (n=16)			Control Group (n=15)				
	Baseline	Post-intervention	P	Baseline	Post-intervention	p	p^a	p^b
	Mean±SD (% 95 CI)			Mean±SD (% 95 CI)				
Energy†	1608.2±371.9 (1410.0-1806.3)	1733.0±319.5 (1562.8-1903.3)	0.034*	1313.6±218.3 (1192.7-1434.5)	1351.1±349.3 (1157.8-1544.8	0.955	0.09	<0.001*
Protein (g)†	54.1±9.3 (49.1-59.1)	69.7±9.6 (64.8-74.6)	<0.001*	47.5±7.6 (43.3-51.7)	47.6±9.6 (42.5-52.7)	0.650	0.040*	<0.001#
Carbohydrate (g)†	200.2±57.6 (169.5-230.8)	173.9±34.7 (156.1-191.7)	0.088	160.7±41.0 (137.9-183.4)	164.7±34.8 (146.3-183.2)	0.532	0.037*	0.488#
Fat (g)†	63.2±19.2 (52.9-73.4)	77.1±21.1 (65.9-88.4)	0.013*	51.1±11.6 (44.6-57.5)	54.3±17.2 (44.8-63.9)	0.570	0.078	0.003*
Vit. A (µg)•	653,32 (339,5-1383,5)	838,59 (584,8-2426,9)	0,005*	697,16 (330,56-1174,44)	659,49 (343,57- 954,13)	0,088	0,626	0,003*
Riboflavin (mg)†	1.1±0.2 (0.9-1.2)	1.8±0.2 (1.7-1.9)	<0.001*	1.0±0.3 (0.8-1.1)	1.0±0.3 (0.8-1.2)	0.513	0.299	<0.001*
Niacin (mg)†	19.7±5.2 (16.9-22.5)	23.8±5.7 (20.8-26.9)	0.009*	18.2±4.4 (15.7-20.6)	16.7±5.1 (13.9-19.6)	0.307	0.338	0.001*
Vitamin B6 (mg)•	1.4±0.4 (1.2-1.7)	1.5±0.4 (1.3-1.7)	0.623	1.3±0.4 (1.1-1.6)	1.3±0.5 (1.1-1.6)	0.463	0.175	0.313
Vitamin B12 (µg)†	2.8±0.8 (2.4-3.3)	5.3±1.3 (4.6-6.1)	<0.001	2.1±1.3 (1.4-2.8)	2.7±1.5 (1.9-3.5)	0.177	0.066	<0.001*
Vitamin E (mg)•	16,0±4.7 (7.4-24.6)	14,9±7.9 (10.9-18.9)	0,877	12.7±4.8 (6.9-25.9)	15,9±7.9 (11.9-20.0)	0.394	0.027*	0.,751#
Potassium (mg)•	2216.1±554.8 (1920.5-2511.7)	2630.1±613.7 (2303.4-2957.5)	0.003*	2156.9±699.1 (1769.7-2544.0)	2054.1±762.2 (1632.0- 2476.2)	0.570	0.379	0.016*
Calcium (mg)†	500.9±114.6 (439.9-562.0)	1284.8±140.3 (1210.1-1359.5)	<0.001	511.3±149.2 (428.7-593.9)	496.7±158.3 (409.1-584.4)	0.570	0.953	<0.001*
Magnesium (mg)†	236.7±61.6 (203.9-269.6)	271.4±55.8 (241.6-301.1)	0.015*	214.3±63.2 (179.4-249.3)	197.8±96.3 (144.4-251.1)	0.394	0.216	0.009*
Phosphorus (mg)†	927.7±147.9 (848.9-1006.6)	1387.2±140.8 (1312.2-1462.2)	<0.001*	841.6±160.1 (712.9-930.2)	825.5±254.1 (584.8-966.3)	0.570	0.163	<0.001*
Iron (mg)•	9.5±2.3 (8.3-10.7)	8.6±2.7 (7.1-10.1)	0.215	8.5±2.9 (6.9-10.1)	7.8±3.5 (5.9-9.7)	0.334	0.318	0.213
Zinc (mg)•	7.1±1.6 (6.3-8.0)	8.7±1.7 (7.8-9.5)	0.001*	6.0±1.6 (5.2-6.9)	6.7±1.7 (5.8-7.5)	0.977	0.030*	0.003#

**p<0.05; p value was calculated Wilcoxan test; p^a: Comparing the early data of the Intervention and control groups; p^b: Comparing the post-intervention data of the Intervention and control groups; † The data were distributed normally. p value was calculated Two- sample t-test, • The data were not distributed normally. p value was calculated Mann-Whitney U test; # p-value was adjusted for baseline scores*

Results

Thirty-one women with PMS, 16 in the intervention group and 15 in the control group, participated in the study. A Consolidated Standards of Reporting Trials (CONSORT) flow chart of the randomization procedure can be seen in Figure 1. Their ages ranged from 20 to 28, and the mean ages of the intervention and control groups were found to be 22.3±1.8 and 22.9±2.1, respectively. The menarche ages of the intervention and control groups are 13.5±1.0 and 13.4±1.1, respectively. The menstruation durations of the intervention group and the control group were 5.4±1.1 days and 5.1±0.7 days, respectively. There was no signifi-

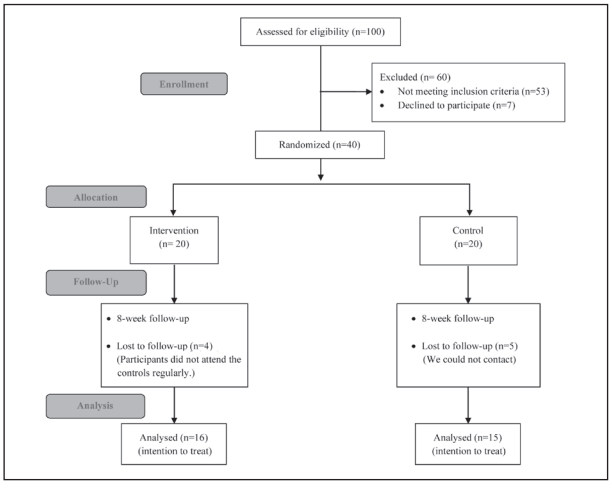


Figure 1. CONSORT flow diagram of the study

cant difference between the ages, menarche ages and menstruation durations of the intervention and control groups (p>0.05) (Table 2).

The baseline mean Body Mass Index of the intervention and control groups were 21.3±3.1 kg/m^2 and 21.0±2.1 kg/m^2, respectively. They were 21.5±3.1 kg/m^2 and 21.1±2.1 kg/m^2 at the end of the study. There was no significant difference between the BMIs of the intervention and control groups at the beginning and end of the study (p>0.05). A statistically significant increase was determined in the BMIs of the participants in the intervention group (p<0.05). A statistically significant increase was determined in the baseline (42.8±4.6) and post-intervention (43.1±4.6) fat-free body masses of those in the intervention group (p<0.05). There were no differences in the baseline and post-intervention body composition values of the control group (Table 2).

Dairy products are sources of calcium, B2, B3, B12, magnesium, zinc, and phosphorus. In addition, the recommended consumption of milk and dairy products to the intervention group resulted in increased energy, protein and fat consumption of individuals. For this reason, PMSS total score, PMSS sub-scale scores and SF-36 quality of life scores were adjusted at the baseline and post- intervention.

PMSS score and sub-scale scores except the appetite change score were found to have decreased between the baseline and post-intervention in the intervention group (p<0.05). There is no significant difference between the baseline and post-intervention PMSS scores and PMSS sub-scale scores of the control group (p>0.05). The intervention group's baseline and post-intervention physical functions and mental health scores on the SF-36 quality of life scale were increased (p<0.05) (Table 3). The changes of the baseline and post-intervention PMSS scores of the intervention and control groups are shown in Figure 2.

At the end of the study, daily calcium intakes of the intervention and control groups were 1284.81±140.28 g and 496.74±158.34 g respectively (p<0.05) (Table 4). Post-intervention intake of the intervention group's daily energy, protein, fat, riboflavin, niacin, B_{12}, potassium, calcium, magnesium, phosphorus, zinc, and magnesium was found to be higher than that of the control group (p<0.05) (Table 4).

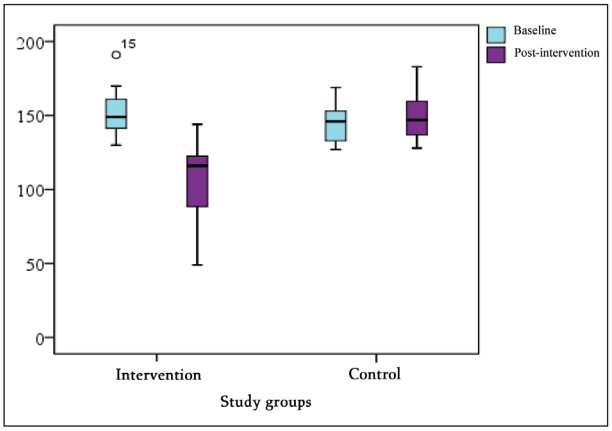


Figure 2. Distribution of the total baseline and post-intervention PMSS scores of the intervention and control groups

Discussion

The intervention group received significantly higher amounts of energy, protein, fat, riboflavin, niacin, vitamin B12, potassium, calcium, magnesium, phosphorus, and zinc due to macro and micro-nutrients having positive effects on PMS symptoms and quality of life.

Vitamins and minerals play an active role in the hormonal and neurotransmitter metabolisms related to PMS. Therefore, consuming less than the RDA of vitamins and minerals is a risk factor for PMS (6, 25).

Insufficient dietary intake of minerals is related to depressive symptoms (26, 27). Davison et al. (26) found that insufficient intake of minerals such as calcium, potassium, phosphorus, magnesium and iron, and vitamins such as pentatonic acid, B_2, B_3, B_6, B_{12}, and folate is related to depression and mood disorders. Chocano-Bedoya et al. (25) found that group B vitamins (thiamine, niacin, riboflavin, folic acid, B_6, and B_{12}) might have a role in PMS development by affecting neurotransmitters such as serotonin and GABA. The participants in this study consumed less than the RDA of thiamine and folic acid, which play a role in PMS. Vitamin B_6 serves as a cofactor in the transformation of tryptophan amino acid to serotonin, and riboflavin is necessary for B6 activation (25). The participants' baseline and post-intervention dietary assessments revealed that they consumed sufficient vitamin B_6. Both groups' baseline riboflavin intake was less than the RDA. After the study, an increase in ribofla-

vin intake was found only in the intervention group. This increase in the riboflavin intake is thought to reduce some of the PMS symptoms such as depressive feelings, depressive thoughts, and anger bursts.

Chronic zinc deficiency reduces zinc in the hippocampus and glucocorticoid secretions, causing neurological symptoms (28, 29). The Nurses' Health Study-II data indicate that those diagnosed with PMS consume less zinc than those who are not diagnosed. Insufficient zinc intake is reported to increase PMS risk (6). Our study found that all the participants had low baseline zinc intakes. The intervention group's post-intervention zinc intake increased significantly. This increase may have caused a significant decrease in their PMSS score and PMSS sub-scale scores, and a significant increase in their SF-36 quality of life scores. The control group's post-intervention zinc intake was found to be significantly lower than their baseline results. Iron is the cofactor of tryptophan hydrolysis enzyme that plays a role in the formation of preliminary serotonin substances (30). Especially the parts of the brain that receive signals from GABA are rich in iron. Low intake of iron causes depression arising from the changes in the ovarian hormones (31). The baseline and post-intervention iron intakes of all the participants were lower than the RDA. Insufficient iron intake may have affected their PMS.

Magnesium reduces menstrual pains, relaxes muscles and ensures vasodilation by inhibiting prostaglandin F2 (PGF2) (32). The intervention group's baseline magnesium intake was lower than the RDA. The post-intervention increase in their magnesium intake may have reduced their PMS symptoms and increased their physical functions scores on the SF-36 quality of life scale.

Excessive increase of estrogen in the ovulation and insufficient calcium intake can cause hypocalcaemia. Bocchieri et al. (33) stated that hypocalcaemia is one of the reasons of PMS is seen particularly in luteal phase. Hypocalcaemia can cause similar symptoms that arise from PMS like anxiety, emotional fluctuations, and depression. (34, 35). Also, these symptoms arise with a hypocalcaemia trigger during PMS (34). The body increases parathormone secretion to prevent hypocalcaemia if dietary calcium intake is low. Borer et al. (33) stated that hyperparathyroidism causes neuropsychiatric disorders such as tiredness, concentration deficit, stress and sadness. Moreover, calcium plays a role in the synthesis of serotonin. The level of serotonin synthesis decreases with hypocalcaemia. King et al. (34) found that hypocalcaemia might lead to depression. Sufficient dietary calcium intake in the intervention group caused the decrease in total PMSS score and increase in quality of life score.

Penland and Johnson (37) compared the effect of the daily intake of 1336 mg of calcium (calcium lactate) and 5.6 mg of manganese (manganese sulphate) on PMS symptoms to that of 587 mg of calcium and 1.0 mg of manganese. They found that increased serum calcium concentration affected symptoms such as pain, behavioral symptoms, and positive mood. Thys-Jacobs et al. (6) noted that serum calcium concentration decreases in the luteal phase, as a result of this, PMS symptoms increase. Calcium supplementation inhibits the decline of serum calcium concentration in the luteal phase.

Bae and Kim found daily intake of calcium to be related to depression (36). Miki et al. (37) compared the calcium intakes of those with and without depressive symptoms and found that low intake of (220 mg/1000 kcal/day) calcium increased the depressive symptom development by 36%. Participants' calcium intakes were low in our study, so this might have increased the depressive mood.

Sutariya et al. (38) found that a 500 mg/day calcium supplement (calcium carbonate or glutamate) reduced symptoms such as mood disorder, depression, anger bursts, crying attacks, edema, abdominal cramps and abdominal bloating, back pain, headaches, appetite, acne, and desire for sweets. Dairy products consumption rates were increased in this study to enhance the daily calcium intakes of the participants in the intervention group. Moreover, depending on this increase, there was an increase in the intake of calcium, phosphorus, B_2, and B_{12}. This may have reduced the intervention group's PMSS sub-scale scores for depressive mood, anxiety, anger bursts, and depressive thoughts, and increased their SF-36 mental health scores.

Obesity changes the neurotransmitter functions that affect estrogen and progesterone hormones (39). Bertone-Johnson et al. (40) noted that every increase

of 1 kg/m^2 in BMI increased PMS risk by 3%. In another study, a significant relation was found between a BMI higher than 30 kg/m^2 and PMS occurrence (39). The participants in this study had intense PMS symptoms although their BMIs were in the normal range. Insufficient consumption of the nutrients related to PMS may have caused PMS symptoms.

Limitations of our study are as follows:

Individuals who were consuming low amounts of dietary calcium and using no supplements were included in the study to see the effect of dairy intake on PMS symptoms. However, it was not possible to determine whether participants had hypocalcaemia since their blood findings such as serum calcium, ionized calcium, vitamin D, and parathormone were not examined.

In conclusion, sufficient and balanced nutrition is always important, as it is for PMS, too. Dairy products are sources of calcium, B_2, B_3, B_{12}, magnesium, zinc, phosphorus. The previous studies showed that these vitamins and minerals could decrease the PMS symptoms. We concluded that daily sufficient intake of calcium from food sources has positive effects on depression, anxiety, fatigue, anger, depressive thoughts, pain, appetite chances, sleep disorders and swelling of extremities symptoms in women with PMS whose calcium intakes are lower than recommended.

Acknowledgments

The authors are thankful to the valuable support of Ceren Gezer and Halit Tanju Besler, Faculty of Health Sciences, Nutrition and Dietetics Department.

References

1. Biggs WS, Demuth RH. Premenstrual syndrome and premenstrual dysphoric disorder. Am Fam Physician 2011;8:918-24.
2. Erbil N, Karaca A, and Kırış T. Investigation of premenstrual syndrome and contributing factors among university students. Turk J Med Sci 2010;4:565-573.
3. Baker LJ, O'Brien PM. Premenstrual syndrome (PMS): a peri-menopausal perspective. Maturitas 2012;2:121-5.
4. Duenas JL, Lete I, Bermejo R et al. Prevalence of premenstrual syndrome and premenstrual dysphoric disorder in a representative cohort of Spanish women of fertile age. Eur J Obstet Gynecol Reprod Biol 2011;1:72-7.
5. Maharaj S, Trevino K. A Comprehensive Review of Treatment Options for Premenstrual Syndrome and Premenstrual Dysphoric Disorder. J Psychiatr Pract 2015;5:334-50.
6. Thys-Jacobs S, McMahon D, Bilezikian JP. Cyclical changes in calcium metabolism across the menstrual cycle in women with premenstrual dysphoric disorder. J Clin Endocrinol Metab. 2007; 8: 2952-9.
7. Saha S, Goswami R. Menstruation associated hypocalcemic symptoms and serum calcium in patients with idiopathic hypoparathyroidism. BMC Endocr Disord. 2014; 14: 28.
8. Panay N. Management of premenstrual syndrome. J Fam Plann Reprod Health Care, 2009; 3:187-94.
9. Arrigo T, De Luca F, Antoniazzi F et al. Menstrual cycle pattern during the first gynaecological years in girls with precocious puberty following gonadotropin-releasing hormone analogue treatment. Eur J Pediatr. 2007; 1: 73-4.
10. Chocano-Bedoya PO, Manson JE, Hankinson SE et al. Intake of selected minerals and risk of premenstrual syndrome. Am J Epidemiol 2013;10:1118-27.
11. Thys-Jacobs S. Micronutrients and the premenstrual syndrome: the case for calcium. J Am Coll Nutr 2000;2:220-7.
12. Bertone-Johnson ER, Hankinson SE, Bendich A, Johnson Sr, Willett WC, Manson JE. Calcium and vitamin D intake and risk of incident premenstrual syndrome. Arch Intern Med 2005;11:1246-52.
13. Shobeiri F, Araste FE, Ebrahimi R, Jenabi E, Nazari M. Effect of calcium on premenstrual syndrome: A double-blind randomized clinical trial. Obstet Gynecol Sci 2017;60:100-5.
14. Ghanbari Z, Haghollahi F, Shariat M, Foroshani AR, Ashrafi M. Effects of calcium supplement therapy in women with premenstrual syndrome. Taiwan J Obstet Gynecol 2009;2:124-9.
15. Masoumi SZ, Ataollahi M, Oshvandi K. Effect of Combined Use of Calcium and Vitamin B6 on Premenstrual Syndrome Symptoms: a Randomized Clinical Trial. J Caring Sci 2016;1:67-73.
16. Shehata NA. Calcium versus oral contraceptive pills containing drospirenone for the treatment of mild to moderate premenstrual syndrome: a double blind randomized placebo controlled trial. Eur J Obstet Gynecol Reprod Biol 2016;1:100-4.
17. Thys-Jacobs S, Ceccarelli S, Bierman A, Weisman H, Cohen MA, Alvir J. Calcium supplementation in premenstrual syndrome: a randomized crossover trial. J Gen Intern Med 1989;3:183-9.
18. Thys-Jacobs S, Starkey P, Bernstein D, Tian J. Calcium carbonate and the premenstrual syndrome: effects on premenstrual and menstrual symptoms. Premenstrual Syndrome Study Group. Am J Obstet Gynecol 1998;2:444-52.
19. Gençdoğan B. A New Scale for Premenstrual Syndrome. Turk Psikiyatri Derg 2006;2:82-87.
20. Demiral Y, Erdor G, Unal B et al. Normative data and discriminative properties of short form 36 (SF-36) in Turkish urban population. BMC Public Health 2006;6:247-55.

21. Rakıcıoğlu N, Tek NA Ayaz A, Pekcan G. Photograph Catalog of Food and Dishes: Porsition Sizes and Amounts. Ankara: Hazırlık Ofset Pub; 2015.
22. Kutluay TM. Standart Recipes for Catering Establishments. Ankara: şahin Ofset Pub; 2003.
23. Bebis (Beslenme Bilgi Sistemi). Nutrition Data base Software Data base: The German Food Code and nutrition Data Base with additions from USDA-sr and other sources. İstanbul; 2004.
24. NAS. IOM. Food and Nutrition Board(1997). Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride. https://fnic.nal.usda.gov (accessed September 2018)
25. Chocano-Bedoya PO, Manson JE, Hankinson SE et al. Dietary B vitamin intake and incident premenstrual syndrome. Am J Clin Nutr 2011;5:1080-6.
26. Davison, KM, Kaplan BJ. Nutrient intakes are correlated with overall psychiatric functioning in adults with mood disorders. Can J Psychiatry 2012;2:85-92.
27. Kaplan BJ, Crawford SG, Field CJ, Simpson JS. Vitamins, minerals and mood. Psychological bulletin. 2007; 5: 747.
28. Sowa-Kucma M, Legutko B, Szewczyk B et al. Antidepressant-like activity of zinc: further behavioral and molecular evidence. J Neural Transm 2008;5:1621-8.
29. Takeda A, Tamano H. Insight into zinc signaling from dietary zinc deficiency. Brain Res Rev 2009;1:33-44.
30. Toxqui L, De Piero A, Courtois V, Bastida S, Sanhez-Muniz F, Vaquero MP. Iron deficiency and overload. Implications in oxidative stress and cardiovascular health. Nutr Hosp 2010;25:350-65.
31. Albacar G, Sans T, Martin-Santos R et al. An association between plasma ferritin concentrations measured 48 h after delivery and postpartum depression. J Affect Disord 2011;131:136-42.
32. Seifert B, Wagler P, Dartsch S, Schmit U, Nieder J. Magnesium-a new therapeutic alternative in primary dysmenorrhea. Zentralbl Gynakol 1989;11:755-60.
33. Bocchieri E, Thys-Jacobs S. Role of calcium metabolism in premenstrual syndrome. Expert Rev Endocrinol Metab 2008;3:645-55.
34. King RD, Wiest MC, Montague PR. Extracellular calcium depletion as a mechanism of short-term synaptic depression. J Neurophysiol 2001;5:1952-9.
35. Bae YJ, Kim SK. Low dietary calcium is associated with self-rated depression in middle-aged Korean women. Nutr Res Pract 2012;6:527-33.
36. Borer MS, Bhanot VK. Hyperparathyroidism: neuropsychiatric manifestations. Psychosomatics 1985; 7: 597-601.
37. Penland JG, Johnson PE. Dietary calcium and manganese effects on menstrual cycle symptoms. Am J Obstet Gynecol 1993;5:1417-23.
38. Miki T, Kochi T, Eguchi M et al. Dietary intake of minerals in relation to depressive symptoms in Japanese employees: the Furukawa Nutrition and Health Study. Nutrition 2015;5:686-90.
39. Sutariya S, Talsania N, Shah C, Patel M. An Intervention Study (Calcium Supplementation & Health Education) On Premenstrual Syndrome- Effect On Premenstrual And Menstrual Symptoms. Natl J Community Med 2011;1:100-104.
40. Masho SW, Adera T, South-Paul J. Obesity as a risk factor for premenstrual syndrome. J Psychosom Obstet Gynaecol 2005;1:33-39.
41. Bertone-Johnson ER, Hankinson SE, Walter CW, Susan RJ, JoAnn EM. Adiposity and the development of premenstrual syndrome. J Womens Health (Larchmt) 2010;11:1955-62.

Correspondence:
Merve Yurt
Eastern Mediterranean University, Faculty of Health Sciences, Nutrition and Dietetics Department, Mersin 10, Turkey
E-mail: merve.yurt@emu.edu.tr

Progress in Nutrition 2020; Vol. 22, N. 1: 146-152 DOI: 10.23751/pn.v22i1.7835

Original article

The effect of different term swimming exercise in rats on serum leptin levels

Hakan Acar[1], *Erkut Tutkun*[2]

[1] Physical Education and Sport Department, Zonguldak Bülent Ecevit University, Zonguldak/Turkey; [2] Faculty of Sport Science, Uludag University, Bursa/Turkey - E-mail: erkuttutkun@uludag.edu.tr

Summary. The aim of this study was to investigate the effect of serum leptin in rats performing various duration swimming exercises. In the study, 30 healthy albino wistar male rats with an average weight of 180-220 grams were divided into 5 groups including; control, water exercises, 15, 30 and 60 minutes swimming groups. Animals were swim-exercised for 90 days. At the end of 90 days, after performed urethane anesthesia, blood samples were taken by intracardiac way. Collected blood was analysed according to procedures. Data were analyzed by using SPSS 15. One-way anova and tukey multiple comparison test were performed in the study. The homogeneity of the variances was examined by Levene's statistic. Results showed that the leptin levels were as follows: control (1480,00 pg/ml), water exercise (705,83 pg/ml), 15 (602,33 pg/ml), 30 (396,67 pg/ml) and 60 (435,83 pg/ml). Statistically significant difference was found between control and 30 minutes swimming group (p=0,012) and control and 60 minutes swimming group (p=0,017) (p<0,05). It is determined that 3 months of different terms (15, 30, 60 minute) of swimming exercise reduces the levels of leptin.

Key words: swimming exercise, rat, leptin

Introduction

Biochemical parameters differ in terms of the type, intensity, period and continuity of the exercise. The effect mechanism of recently discovered leptin hormone which has significant roles in energy homeostasis and the association between leptin and exercise have been assessed with various acute and chronic exercise practices (1-18). In these studies, the dominant thought is that short-term (<12 weeks) acute exercises do not influence leptin levels or have a very little effect (1, 6, 7, 9, 13-15), while long term (>12 weeks) and long term chronic exercises influence serum leptin levels (4, 5, 8, 10, 12, 16, 18). However, there are studies which show that leptin levels decrease as a result of acute exercises (2, 3, 11, 17).

Several investigators reported that exercise may result in reductions depending on the duration and calorie expenditure whereas others have reported no change in leptin concentrations. Exercise doesn't generate decrease in leptin concentrations; Torjman et al. (1999), Weltman et al. (2000), Kraemer et al. (2002). Besides, exercise that generated decrease in leptin concentrations; Zafeiridis et al. (2003). In general, the decrease in leptin concentration after a long-term exercise (≥ 60 min) has been attributed to diurnal reduction in circulating leptin and hormonal changes induced by exercise. Exercises of very long duration that generated a sufficient energy imbalance (kilocalorie intake versus kilocalorie expenditure) suppress the amplitude of the diurnal rhythm of leptin (21).

Information regarding the response of serum leptin to a single bout of resistance exercise is limited. In contrast to continuous running of moderate intensity, heavy resistance exercise is a potent nonoxidative stimulus that produces differential neural, metabolic,

and neuroendocrine responses (9). Exercise is another physiological stress that may alter leptin secretion. In rats, ob mRNA levels were found to be decreased immediately and 3 h after a prolonged exercise bout to exhaustion (22). Significant reductions in serum leptin levels were observed after exercise training, but these were explained by changes in fat mass. One study performed in animals has shown that acute exercise in rats was associated with a 30% reduction of the expression of the ob gene in the adipose tissue (23).

According to some authors, low leptin concentration was probably due to weight reduction. Thus, short-term training (< 12 weeks) and long-term training (≥ 12 weeks) have disparate findings concerning leptin concentration. The reduction of leptin has been attributed to alteration in energy balance, improvements in insulin sensitivity, alteration in lipid metabolism and lipid concentration and unknown factors (21). Within this framework, a significant relationship is thought to exist between serum leptin level and type of exercise. Further, the characteristics of a sport affect the serum leptin levels of an athlete who does that sport (24). Physical activity is important for long-term regulation of body weight, partly because it increases the resting metabolic rate. Weight reduction after physical exercise is correlated with reductions in plasma leptin concentrations in obese middle-aged women. However, results regarding the effects of exercise on plasma leptin concentrations, independent of fat mass, are conflicting (25).

Leptin levels and body mass index have a positive relationship (26, 27). Leptin has a critical role on regulation of body weight and also body fat mass. Given the key role of leptin on regulation of body weight and prevention of obesity, it seemed that leptin levels were decreased during the elevation of body weight. But most obese humans have higher circulations of leptin. It has been indicated that obesity might induce state of leptin resistance (28). Moreover, Leptin is one of a growing number of adipocytokines that play an important role in the regulation of body weight by coordinating metabolism, feeding behavior, energy balance, fertility and neuroendocrine responses. Originally thought to be exclusively produced by adipocytes, it is now evident that leptin is expressed, in lesser amounts, in many fetal and adult tissues including: placenta stomach, mammary gland, skeletal muscle, pancreas, and bone (29).

Leptin, which is the product of obesity gene (30), regulates weight and food intake in both animals and people, suppresses hunger (31) and decreases food intake (32), increases energy use and prevents fat accumulation (25, 9, 33, 34, 35). The most important effect of leptin on energy expenditure is providing increase in thermogenesis (17). In addition, leptin has been found to have a great number of metabolic and endocrine functions (36, 37, 38, 39) and also have a role in obesity, bone development, wound healing (40), fetus development (41), start and development of adolescence in children (37), hypertension and heart diseases (42, 16), and the regulation of gastrointestinal functions and glucose metabolism (42, 33, 43).

The association of leptin, which is found to have a great number of functions for human metabolism, with exercise is still not clear. We can say the effects of physical exercise on leptin are still being discussed and currently controversial. When studies conducted so far are examined, the effect of different durations of chronic exercise on leptin has not been researched yet, to the best of our knowledge. Thus, the purpose of this study is to show the effects of long and different durations of swimming exercise (15, 30, 60 minutes for 3 months) on the leptin levels of rats.

Materials and Methods

Adult male Wistar rats weighing 180–220 gr. were used throughout this study after at least 1 week of acclimatization. All described procedures were approved by the local ethics committee. Animals were housed in groups of 5 and were allowed free access to food and water, except for the short time that the animals were removed from their cages for the experiments. All animals were kept in a temperature controlled (22±1°C) environment on a 12-h light/dark cycle.

Experimental Design

Rats were assigned to the following experiments and groups: (Group 1) control group; (Group 2) 15 minutes-trained for 90 days; (Group 3) 30 minutes-trained for 90 days; (Group 4) 60 minutes- trained for

90 days (Group 5) adapted to the water. Each animal group was composed of six rats.

The adaptation to the water proceeded during experimental period. The purpose of the adaptation to water was to reduce stress without promoting a physical training adaptation (44, 45).

Exercise training program

The swimming was performed in water at a temperature of 32-33ºC between 11.00-13.00 A.M. Training period lasted 90 days and consisted of 15, 30, and 60 minutes of daily sessions for seven days/week without workload. Exercise was performed by swimming in three training glass tanks (length 100 cm, width 50 cm, depth 50 cm) containing tap water.

At the end of 90 days, after performed urethane (1.25 g/kg) anesthesia, blood samples were taken by intracardiac way. Blood samples were collected into EDTA bottles for hormonal assay.

Analyzes were made in Samsun Veterinary Control Institute Biology Laboratory. In leptin analysis; Sigma Rat Leptin Elisa Kit was used. All reagents and samples were taken to room temperature (18–25˚C) before use.

All standards and samples were repeated twice. 100 ml of each standard and samples were added into appropriate wells. Well were covered and incubated for 2.5 hours at room temperature with gentle shaking by Heidolph Tiramax 1000 orbitel shaker. The solution was discarded and washed 4 times with 1x Wash Solution. Each well was washed and filled with 1x Wash Buffer (300 ml) using a multichannel pipette. At each step removal of liquid was completed for good performance. Biotinylated antibody was prepared and added 100 ml of 1x prepared to each well and was incubated for 1 hour at room temperature with gentle shaking. The solution was discarded and washed 4 times with 1x wash solution. Each well was washed and filled with 1x Wash Buffer (300 ml) using a multichannel pipette. 100 ml of prepared Streptavidin solution was added to each well and incubated for 45 minutes at room temperature with gentle shaking. The solution was discarded and washed 4 times with 1x Wash Solution. Each well was washed and filled with 1x Wash Buffer (300 ml) using a multichannel pipette. 100 ml of TMB One-Step Substrate Reagent (Item H) to each well was added and incubated for 30 minutes at room temperature in the dark with gentle shaking. 50 ml of Stop Solution was added to each well and was read at 450 nm immediately. The mean absorbance of each data was calculated. On the sigma plot software, the standards were plotted on the curve and the curve was drawn at these points. By using this standard curve, the leptin concentrations of the samples were calculated in pg/ml.

Data were analyzed using SPSS 15 (Statistical Package for the Social Sciences) package and the error rate was determined as 5%. One-way anova was used to determine the differences between the groups, and the Tukey's Multiple Comparison Test was used to determine which groups the difference was in when the difference was found. The homogeneity of the variances was examined by Levene's statistic.

Results

Serum leptin levels of rats which were trained with swimming exercises of different durations (15, 30, 60 minutes for 3 months) are given in the tables below respectively.

In the study, the highest average leptin values were found in the control group, while the lowest average leptin values were found in the 30 minutes swimming group.

Statistically significant difference was found between control and 30 minutes swimming group (p=0,012) and control and 60 minutes swimming group (p=0,017) (p<0,05). Differences were found between groups (p=0,010).

Discussion

In the present study, statistically significant difference was found between control and 30 minutes swimming group and control and 60 minutes swimming group when rats which were trained for 15, 30 and 60 minutes of swimming for three months were examined.

No statistically significant differences were found between the leptin levels of the control and water adaptation group. Short exercises during the one-week

Table 1. Post-swimming exercise serum leptin values and One Way Anova results

Groups	n	$\bar{x}$ pg/ml	sd	F	p
Control	6	1480,00	870,05		
Water adaptation	6	705,83	513,98		
15 Minutes of swimming	6	602,33	491,48	4,179	**0.010**
30 Minutes of swimming	6	396,67	269,48		
60 Minutes of swimming	6	435,83	242,54		
p<0,05					

Table 2. Post-swimming exercise serum leptin values and paired comparisons

Groups	n	$\bar{x}$ pg/ml	ss	Control p	Water adaptation p	15 min. swimming p	30 min. swimming p	60 min. swimming p
Control	6	1480,00	870,05		0,114	0,057	**0,012**	**0,017**
Water adaptation	6	705,83	513,98			0,997	0,846	0,900
15 Minutes of swimming	6	602,33	491,48				0,960	0,981
30 Minutes of swimming	6	396,67	269,48					1,000
60 Minutes of swimming	6	435,83	242,54					
p<0.05								

long process of water adaptation were not found to create stress-induced leptin changes. Although not significant, decreases in leptin levels of the water adaptation group when compared with the control group decreased leptin levels of one-week long water exercises; however, this decrease was not sufficient and supports our thought that stress-induced leptin decreases are not experienced.

Statistically significant difference was found between the control group and the 30 minute swimming group in terms of leptin levels. In their study they applied a training of 9 weeks treadmill exercise-every day for the first two weeks and twice a day for the following 7 weeks, which started with 30 minutes and got 2 minutes longer every day, Zhao et al. (2011) found a significant decrease in the leptin levels of rats (n=16) (35). In another study, Kondo et al. (2006) found decrease in leptin levels as a result of exercises they applied on 8 obese women for 7 months-4-5 days a week and 30-60 minutes a day (12). In a study conducted on women between the ages of 40 and 49 who were in premopausal period and who were not doing regular exercise (n=40)**,** Gözlükaya (2008) found significant changes in the leptin hormone levels of the quick tempo group as a result of 54-minute walking exercise which started as 30 minutes a day and increased 3 minutes a week for five days a week in 10 weeks (46). Another study showed that 30 min of swimming exercise caused a 30% reduction in leptin levels in lean but not obese (fa/fa) Zucker rats (22). Zafeiridis et al. (2003) controlled the effects of maximum strength, muscular hypertrophy and resistance exercise protocols on serum leptin concentrations. Leptin concentrations significantly decreased 30-minute into recovery after exercise protocols compared with the respective baseline values. While decreases are found in leptin levels in all studies as a result of exercises, data from investigations examining single exercise bouts suggest that serum leptin concentrations are unaltered by short duration (41 minutes or less), non-exhaustive exercise, but may be affected by short duration, exhaustive exercise (47). Weltman et al. (2000) found that 30 min

of exercise at various intensities and caloric expenditure (from 150 ± 11 to 529 ± 45 kcals) in 7 healthy young men did not cause modifications in leptin levels during the exercise and during the recovery (3.5 hours) (20). Exercise programs preferred in studies have been planned as 9,10 and 12 weeks, for periods of 30 minutes and more. According to the results of this study, it can be said that at least 9-10 week-long regular exercise programs with unit periods of 30 minutes and more can cause significant changes in leptin levels.

In the present study, statistically significant difference was found between the control group and the 60-minute swimming group in terms of leptin levels. Benatti et al. (2008) applied swimming exercises on rats (n=18) which lasted 40 minutes for 5 days in the first week and continued as 5 days a week and 60 minutes a day for the following 8 weeks. They found that 9-week long swimming exercises decreased the serum leptin levels of rats irrespective of their body fat mass. In their study they applied a diet for 4 months and an hour long moderate exercise 3-4 days a week on obese men (n=15) (48). Pasman et al. (1998) found with blood samples taken from the study group at months 0, 2, 4, 10 and 16 that exercise decreased plasma leptin levels irrespective of body fat change. In their study they examined the association between diet, exercise and obesity (4). Murakami et al. (2007) found that as a result of 60-minute long aerobic exercise for three days a week during 12 weeks decreased leptin levels (49). Moazami et al. (2013) found that 24-week long aerobic exercise (three days a week, for 60 minutes) decreased serum leptin levels in obese women (n=15) (50). While the results of the aforementioned studies are similar to the results of our study, the study period of our study is 12 weeks, as in Murakami et al. (2007)'s study; however, the frequency of their exercise is different from our study. The fact that the results are similar in all these studies although different exercises were applied in different intensities and frequencies shows that the type, content, intensity or frequency of exercise is not effective on the change in leptin levels and the change can occur as a result of regular and long-term exercises. Benatti et al. (2008)'s results showed that 9 week-long exercise can cause significant decreases in leptin levels and this shows that 9 week and longer regular exercises which last longer than 30 minutes can be enough.

In the present study, no statistically significant difference was found when the results between control group and 15-minute swimming group and water adaptation group and 15-minute swimming group measurement results were compared. Akbarpour (2013) applied a running exercise program on obese men (n=16) for 12 weeks as three days a week. Following 10 minute long warm up, the running period was 15 min for the first session, and every two sessions 1.5 min was added to the running period in a stepwise manner until the running period reached 30 min. The running exercise program was found to decrease serum leptin levels significantly (p=0,003); there were decreases in the leptin levels in interval measures conducted at the end of 6th week; however, the decrease was not found to be significant (51). This was thought to occur as a result of insufficient training period and intensity. These two studies show the significance of both unit exercise duration and long-term chronic exercises to have significant decreases in leptin. In our study, it was found that 15-minute long exercises decreased leptin levels; however, this decrease was not significant, while 30 and 60-minute long exercises caused significant decrease. According to these results, it can be said that to obtain significant decreases in leptin levels, unit exercise durations are as important as long-term exercises and 15-minute long exercises are not sufficient. The fact that the average leptin levels of the 15-minute swimming group were higher than both 30 and 60-minute swimming group supports this thought.

As a conclusion, while swimming exercises for 30 and 60 minutes during 12 weeks decreased the serum leptin levels of rats significantly, this significant decreasing was not found in 15-minute exercises. There are no experimental animal studies in literature conducted with parameters similar to ours in literature. Studies conducted in this field with different procedures and different approaches will give healthier information.

Acknowledgements

In order to conduct the study, B.30.2.ODM.0.20. 09.00-050.04–08 numbered Ondokuz Mayıs University Animal Experiments Local Ethical Board report was taken on 30.01.2013.

References

1. Hickey MS, Considine RV, Israel RG, Mahar TL, McCammon MR, Tyndall GL, Houmard JA, Caro JF. Leptin is related to body fat content in male distance runners, Am J Physiol. 1996; 271: 938-40.
2. Tuominen JA, Ebeling P, Laquier FW, Heiman ML, Stephens T, Koivisto VA. Serum leptin concentrations and fuel homeostasis in healthy men. Eur J Clin Invest 1997; 27:206-211.
3. Leal-Cerro A, Garcia-Luna PP, Astorga R, Parejo J, Peino R, Dieguez C, Casanueva FF. Serum leptin level in male marathon athletes before and after the marathon run, J Clin Endocrinol Metab, 1998; 83, 2376-2379.
4. Pasman W J, Westerterp-Plantenga MS, Saris WHM. The effect of exercise training on leptin levels in obese males. Am. J. Physiol. 1998; 274: 280-286.
5. Gutin B,Ramsey L,Barbeau P,Cannady W,Ferguson M,Litaker M,Owens S, Plasma leptin concentrations in obese children: Changes during 4-mo periods with and without physical training. AmJClin Nutr.1999; 69: 388-94.
6. Kraemer RR, Kraemer GR, Acevedo EO, Hebert EP, Temple E,Bates M,Etie A,Haltom R,Quinn S, Castracane V D. Effects of aerobic exercise on serum leptin levels in obese females. Eur J Appl Physiol. 1999; 80: 154-158.
7. Houmard JA, Cox JH, MacLean PS, Barakat HA. Effect of short-term exercise training on leptin and insulin action. Metabolism. 2000; 49: 858-61.
8. Kraemer RR, Chu H, Castracane VD. Leptin and exercise. Exp Biol Med (Maywood). 2002; 227: 701-708.
9. Zafeiridis A, Smilios I, Considine RV, Tokmakidis SP. Serum leptin responses after acute resistance exercise protocols. J Appl Physiol. 2003; 94: 591-597.
10. Miyatake N, Takahashi K, Wada J, Nishikawa H, Morishita A, Suzuki H, Kunitomi M, Makino H, Kira S, Fujii M. Changes in serum leptin concentrations in overweight japanese men after exercise. Diabetes Obes. Metab. 2004;6:332-337.
11. Christ ER, Zehnder M, Boesch C, Trepp R, Mullis PE, Diem P, Décombaz J. The effect of increased lipid intake on hormonal responses during aerobic exercise in endurance-trained men. Eur J Endocrinol. 2006; 154:397-403.
12. Kondo T, Kobayashi I, Murakami M. Effect of exercise on circulating adipokine levels in obese young women. Endocr J. 2006; 53: 189-195.
13. Pomerants T, Tillmann V, Karelson K, Jürimäe J, Jürimäe T. Ghrelin response to acute aerobic exercise in boys at different stages of puberty. Horm Metab Res. 2006; 38: 752-757.
14. Kraemer RR, Castracane VD. Exercise and humoral mediators of peripheral energy balance: and adiponectin. Exp Biol Med. 2007; 232:184-194.
15. Kyriazis GA, Caplan JD, Lowndes J, Carpenter RL, Dennis KE, Sivo SA, Angelopoulos TJ. Moderate exercise-induced energy expenditure does not alter leptin levels in sedentary obese men. Clin J Sport Med. 2007;17(1):49-51.
16. Nia F R, Hojjati Z, Rahnama N, Soltani B. Leptin, heart disease and exercise. World Journal of Sport Sciences. 2009; 2 (1): 13-20.
17. Tsao T-H, Hsu C-H, Yang C-B, Liou T-L. The effect of exercise intensity on Serum leptin and C-reactive protein levels J Exerc Sci Fit, 2009; 7(2): 98-103.
18. Bjersing, J. L., Larsson, A., Palstam, A., Ernberg, M., Bileviciute-Ljungar, I., Löfgren, M., Gerdle B, Kosek E., Mannerkorpi, K. Benefits of resistance exercise in lean women with fibromyalgia: involvement of IGF-1 and leptin. BMC Musculoskeletal Disorders, 2017; 18, 106. http://doi.org/10.1186/s12891-017-1477-5.
19. Torjman MC, Zafeiridis A, Paolone AM, Wilkerson C, Considine RV. Serum leptin during recovery following maximal incremental and prolonged exercise. Int J Sports Med, 1999; 20: 444-450.
20. Weltman A., Pritzlaff C.J., Wideman L., Considine R.V., Fryburg D.A., Gutgesell M.E., Hartman M.L., Veldhuis J.D. Intensity of acute exercise does not affect serum leptin concentrations in young men. Medicine and Science in Sports and Exercise,2000; 32, 1556-1561.
21. Bouassida A.,Zalleg D.,Bouassida S.,Zaouali, M., Feki Y.,Zbidi A.,Tabka Z. Leptin, its implication in physical exercise and training: a short review, Journal of Sports Science and Medicine 2006; 5, 172-181.
22. Sandoval DA.,Davis SN. Leptin metabolic control and regulation, journal of diabetes and its complications 2003;17; 108– 113.
23. Russe, Lp., Collıe,G., Gagnon,J., Leon,As., Rao,Dc.,Skınner, Js., Wılmore,Jh., Nadeau,A., Zımmet,Pz., Bouchard, C., Acute and Chronic Effects Of Exercise On Leptin Levels İn Humans, Journal of Applied Physiology, 1997; 83(1), 5-10.
24. Doğan E.,Tasmektepligil MY., Cankaya S. An Investigation of the leptin levels of footballers and wrestlers in terms of aerobic capacity, Anthropologist, 2015; 19(2): 355-359.
25. Reseland JE, Anderssen SA, Solvoll K, Hjermann I, Urdal P, Holme I, Drevon C. Effect of long-term changes in diet and exercise on plasma leptin concentrations. Am J Clin Nutr. 2001; 73: 240 –245.
26. Ibarra-Reynoso L del R, Pisarchyk L, Pérez-Luque EL, Garay-Sevilla ME, Malacara JM. Dietary restriction in obese children and its relation with eating behavior, fibroblast growth factor 21 and leptin: a prospective clinical intervention study. Nutr Metab (Lond). 2015;12:31.
27. Lewis KA, Brown SA. Searching for Evidence of an Anti-Inflammatory Diet in Children: A systematic review of randomized controlled trials for pediatric obesity ınterventions with a focus on leptin, ghrelin, and adiponectin. Biol Res Nurs. 2017;19(5):511-530.
28. Izadi,V.,Saraf-Bank,S.,Azadbakht,L. Dietary intakes and leptin concentrations. ARYA Atherosclerosis, 2014; 10(5), 266–272.
29. Morash,BA.Imran A.,Wilkinson D.,Ur E.,Wilkinson M., Leptin receptors are developmentally regulated in rat pituitary and hypothalamus, Molecular and Cellular Endocrinology 2003; 210, 1–8.
30. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L,

Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature 1994:372(6505);425-32.
31. Fantuzzi G, Faggioni R. Leptin in the regulation of immunity, inflammation, and hematopoiesis. J Leukocyte Biol, 2000; 68, 437-446.
32. Cooper JA, Watras AC, Paton CM, Wegner FH, Adams AK, Schoeller DA. Impact of exercise and dietary fatty acid composition from a high-fat diet on markers of hunger and satiety. Appetite. 2011; 56(1):171-178.
33. Ziylan Y Z, Baltacı A K, Moğulkoç R. Leptin transport in the central nervous system cell biochemistry and function Cell Biochem Funct. Review Article.2009;27:63-70.
34. Bouassida A, Chamari K, Zaouali M, Feki Y, Zbidi A, Tabka Z. Review on leptin and adiponectin responses and adaptations to acute and chronic exercise. Br J Sports Med 2010; 44: 620-630.
35. Zhao J, Tian Y, Xu J, Liu D, Wang X, Zhao B. Endurance exercise is a leptin signaling mimetic in hypothalamus of wistar rats, Lipids Health Dis. 2011;10:225.
36. Tsolakis C, Vagenas G, Dessypris A. Growth and anabolic hormones, leptin, and neuromuscular performance in moderately trained prepubescent athletes and untrained boys. J Strength Cond Res. 2003;17(1):40-46.
37. Ünal M, Ünal DÖ, Baltacı AK, Moğulkoç R, Kayserilioğlu A. Investigation of serum leptin levels in professional male football players and healthy sedentary males Neuroendocrin Lett. 2005; 26(2): 148-151.
38. Baltacı AK, Moğulkoç RA, Öztürk A. Testosterone and zinc supplementation in castrated rats: Effects on plasma leptin levels and relation with LH, FSH and testosterone, Life Sciences 2006; 78: 746-752.
39. Bozan G. Serum leptin and bone metabolism markers in obese children. Eskisehir Osmangazi University Faculty of Medicine, Department of Pediatrics, Speciality Thesis in Department of Pediatrics, Eskisehir, 2010; 1-45.
40. Werner S, Grose R. Regulation of wound healing by growth factors and cytokines.Physiol Rev.2003; 83: 835-870.
41. Hoggard N, Hunter L, Duncan JS, Williams LM, Trayhurn P, Mercer JG. Leptin and leptin receptor mRNA and protein expression in the murine fetus and placenta. Proceedings of the National Academy of Sciences of the United States of America 1997; 94(20):11073-11078.
42. Ahima, RA.,Dushay J., Flier, SN., Prabakaran, D., Flier,JS. Leptin accelerates the onset of puberty in normal female mice, J. Clin. Invest. 1997; 99:3, 391–395.
43. Özcan A. Sıçanlarda kronik egzersiz sonrası leptin, ghrelin, resistin düzeyleri ve bu düzeylere fluvastatin ve kafeik asit fenetil ester in (cape) etkisi Konya Selçuk Üniversitesi Meram Tıp Fakültesi Biyokimya Anabilim Dalı. Uzmanlık Tezi. 2009; 7-68.
44. Souza MA, Oliveira MS, Furian AF, Rambo LM, Ribeiro LR, Lima FD, Dalla Corte LC, Silva LF, Retamoso LT, Dalla Corte CL, Puntel GO, de Avila DS, Soares FA, Fighera MR, de Mello CF, Royes LF. Swimming training prevents pentylenetetrazol-induced inhibition of Na+, K+-ATPase activity, seizures, and oxidative stress. Epilepsia, 2009; 50: 811–823.
45. Yi X, Cao S, Chang B, Zhao D, Gao H, Wan Y, Shi J, Wei W, GuanY. Effects of acute exercise and chronic exercise on the liver leptin – Ampk - Acc signaling pathway in rats with type 2 Diabetes. Journal of Diabetes Research. 2013;4. Article ID 946432, 9 pages, 2013. doi:10.1155/2013/946432.
46. Gözlükaya F. Premenapozal dönemdeki bayanlarda 10 haftalık farklı yürüyüş hızında yapılan antrenmanların adiponektin, ghrelin ve leptin hormonları üzerine etkisi. Celal Bayar Üniversitesi, Sağlık Bilimleri Enstitüsü.Manisa. Unpublished master's thesis. 2008; 11-21.
47. Hulver MW and Houmard JA. Plasma leptin and exercise: recent findings. Sports Med. 2003; 33(7): 473-82.
48. Benatti FB, Polacow VO, Ribeiro SML, Gualano B, Coelho DF, Rogeri PS, Costa AS, Lancha Junior AH. Swimming training down-regulates plasma leptin levels, but not adipose tissue ob mrna expression. Braz J Med Biol Res. 2008; 41: 866-871.
49. Murakami T, Horigome H, Tanaka K, Nakata Y, Katayama Y, Matsui A. Effects of dietwith or without exercise on leptin and anticoagulation proteins levels in obesity, Blood Coagul Fibrin 2007;18: 389-394.
50. Moazami M, Bijeh N, Gholamian S. The response of plasma leptin and some selected hormones to 24-weeks Aerobic exercise in inactive obese women. International Journal of Sport Studies. 2013; 3 (1): 38-44.
51. Akbarpour M. The Effect of aerobic training on serum adiponectin and leptin levels and ınflammatory markers of coronary heart disease in obese men. Biology of sport; 2013; 30(1): 21-27.

Correspondence:
Erkut Tutkun,
Faculty of Sport Science, Uludag University,
16058 Nilüfer/Bursa/Turkey.
Tel: + 90-224-2940685
Fax: + 90-224-2940997
Gsm: +90-5326587696
E-mail: erkuttutkun@uludag.edu.tr

Progress in Nutrition 2020; Vol. 22, N. 1: 153-160 DOI: 10.23751/pn.v22i1.8743

Original article

The effects of weight class athletes' nutrient consumptions and eating habits on their depression-anxiety-stress levels

Deniz Özge Yüceloğlu Keskin, Menderes Kabadayı, Özgür Bostancı, Levent Bayram, Deniz Günay Derebaşı

Ondokuz Mayıs University, Yaşar Doğu Faculty of Sport Sciences - E-mail: levent.bayram@omu.edu.tr

Summary. Diet is important in sports in terms of having an influence on bodily and mental health besides sportive performance. The objective of this study is to find out the effects of dietary habits and eating attitudes on moods of weight class athletes. The study was conducted with a total of 60 athletes -33 wrestlers who participated in Turkey Men's Greco-Roman Wrestling National Team Preparation Camp, 18 wrestlers who participated in Turkey Men's Freestyle National Team Preparation Camp and 9 judokas who participated in Turkey Men's Judo National Team Preparation Camp during the years 2013 and 2014. Athletes' demographic characteristics and information about their three-day eating habits were found, eating attitudes test (EAT) and Depression, Anxiety and Stress Scales (DASS-42) were applied and some of their anthropometric measurements were found. Student-t, Mann Whitney U, Anova and Kruskal tests were used for the statistical analysis of data. Statistically significant difference was found between the EAT scores of Greco-roman wrestlers and freestyle wrestlers and EAT scores of Greco-roman wrestlers were found to be higher ($p<0,05$). Average carbohydrate intake and stress scores of athletes whose stress scores were higher than normal were found to be significantly higher when compared with the group with normal stress scores ($p<0,05$). Unhealthy diets which athletes follow to keep body weight under control both damage the athletes' diet and negatively affect their moods.

Key words: diet, wrestling, judo, sport

Introduction

Sports nutrition has recently become an excessively researched and increasingly interesting area of science. It has become an area in which sport scientists and also trainers, athletes and athlete families should have sufficient and correct information and this nutritional information should be received from expert dieticians (1).

Appropriate and balanced diet is essential for young athletes not only in terms of sportive achievement but also for their growth, development and general health conditions (2). An appropriate diet is important especially for developing performance, maintaining condition and protecting from post-exercise recovery and injuries (3). Mostly, individuals cannot allocate time for eating due to various reasons and thus, they cannot be nourished properly (4). It is certainly a truth to say that it is impossible to expect high performance from an athlete whose diet is not proper and whose health is deteriorated (5).

Psychological factors such as perfectionism, anxiety, mood, mood disorders, disrupted bodily image and self-respect have a role in the multi-factor etiology of eating disorders (6,7). According to American Psychiatry Association (APA)'s DSM-V (Diagnostic and Statistical Manual of Mental Disorders-V, 2014), eating disorders (ED) include anorexia nervosa (AN), bu-

This article is extracted from Deniz Özge Yüceloğlu Keskin' doctorate dissertation entitled "The effects of nutrition habits and eating attitudes of wei ght-class athletes on mood states", (PhD Dissertation, Ondokuz Mayıs University, Samsun/Turkey, 2015).

limia nervosa (BN) and other eating disorders (APA, 2014). Significant associations have been found between eating habits and anxieties about body shape, social anxiety, depression and body mass index (8).

In addition, it is also stated that eating habits and behaviors can be damaged as a result of exercise and also eating habits and behaviors can cause mood disorders and consequently depression (9-12).

In all sports branches based on weight class, athletes limit their daily energy consumption and thus by decreasing their weight, they get advantage when compared with other contestants (13).

Diet is important in sports in terms of having an influence on bodily and mental health besides sportive performance. The objective of this study is to find out the effects of dietary habits and eating attitudes on moods of weight class athletes.

Material and Method

The universe of the study consists of athletes who participated in TM wrestling (Greco-roman and Freestyle) and judo NTPC as part of 2013-2014 activity program. A total of 60 voluntary national team athletes – 33 athletes who participated in İstanbul Mersinli Ahmet Wrestling Camp Training Center Men's Greco-roman NTPC, 18 athletes who participated in Ankara Elmadağ Wrestling Camp Training Center Men's Freestyle NTPC and 9 judokas who participated in Samsun Atatürk Sports Hall Turkey Olympics Preparation Center Men's Judo NTPC- were included in the study. Of the national team athletes who were included in the study from wrestling, 17 had medals in European Championship, 27 had medals in World Championship and 7 had medals in Olympic Games. The athletes were informed about the questionnaires and measurements. Athletes who had injuries or health problems were not included in the study. Elite weight class athletes who are in camp train for an average of 5 days a week and 4 hours a day. In this study, the athletes' demographic features and information about their three-day food consumption were collected through questionnaire form and EAT and DASS-42 were applied and some anthropometric measurements were taken.

Demographic Information Form

A form which included 12 questions to get information about the athletes' demographic features was prepared.

Nutritional Status

Nutritional status was found by recording the dietary consumption of athletes in three consequent days. The amount of nutrients in individuals' one course were calculated by using the amounts determined by the individuals and by using Kutluay's book entitled "Standard Recipes for Catering of Institutions"(14). Energy and macro nutrient analyses of these consumptions were calculated by using diet information systems (BEBİS 6) package program and their averages were taken.

Anthropometric Measurements

All the participants' weights and body compositions (body fat mass and percentage, lean tissue mass, total bodily fluid) were measured. Tanita make BC 418 model BİA device was used to find out body components. The subjects were measured with shorts on and bare feet.

Body mass index

BMI is an easily calculated (Weight [kg]/Height^2 [m]) criterion which is accepted as a good indicator of total body fat (15).

Depression, Anxiety, Stress Scales (DASS-42)

The validity and reliability of this scale developed by Lovibond and Lovibond (1995) was conducted by Akın and Bayram (2007) (16,17). Each item in the scale has 4 Likert type grading of "0": did not apply to me at all, "1": applied to me to some degree or for some of the time, "2": applied to me to a considerable degree or for a good part of time and "3": applied to me very much or most of the time. The adaptation validity scores of DASS were .87 and .84, respectively. Cronbach Alpha internal consistency reliability coefficient was .89, while item total correlations were found to be between .51 and .75. The test retest and split-half reliability scores of the scale were found as .99 and .96. These results show that DASS has high level of reliability and validity. The participants are asked to choose

the item that applies most to them. The questions in the Depression and Anxiety category are grouped in three different groups and assessed according to the total scores of the individual from each group. According to these, a score of 0-9 taken from the depression category, a score of 0-7 taken from the anxiety category and a score of 0-14 taken from the stress category are interpreted as "normal" values. Higher values of these scores in each category show the increase in depression, anxiety and stress levels. Since we did not have too many participants, we did not grade them as normal, mild, fair and advanced level according to depression, stress and anxiety average scores, instead grouped in two as those within normal range and those over normal range.

The Eating Attitude Test- EAT: It is a self-assessment scale developed by Garner and Garfinkel (1979) to assess the disorders in eating attitudes and behaviors. The scale was adapted into Turkish by Savaşır and Erol (1989) (18,19). The cut-off score of EAT scale was found as 30. Savaşır and Erol (1989) found the Cronbach alpha reliability coefficient of the scale as 70. Individuals are asked to choose the choice that fits them best by thinking about their eating habits (20). In terms of pathology, 3 points are given for each end response and 2 and 1 points are given for the other choices. Total score is obtained by adding up the grading (21).

Statistical Analysis: Statistical analysis of all the data obtained within the context of the research was assessed through SPSS (Statistical Package for Social Sciences for Windows) 17.0 statistic program. Student-t, Mann Whitney U tests were used for the statistical assessment of the data. In the comparison of more than two independent groups, the groups which were normally distributed were compared with Anova test. Tukey multiple comparison test was used for paired comparisons. The groups which were not normally distributed were compared with Kruskal Wallis test.

Results

In Table 1, no statistically significant difference was found between the groups which participated in the study p(>0.05).

In Table 2, as a result of the calculation of energy and nutrient elements of daily average food consumption of the athletes who participated in our study, carbohydrate intake percentage of the wrestlers was found as 37,98±12,42, while their protein intake percentage was found as 18,58±6,42 and their fat intake percentage was found as 42,46±16,48. Carbohydrate intake percentage of free-style wrestlers was found as 44,50±16,74, their protein percentage was found as 18,13±5,68 and their fat percentage was found as 36,31±12,98. As a result of judo energy and nutrient element calculations, judokas' carbohydrate intake percentage was found as 38,33±1,56, their protein intake percentage was found as 19,97±4,24 and their fat intake percentage was found as 40,89± 9,39.

In Table 3, the athletes' depression score average was found as 6,72 ±5,69, their stress score average was found as 11,78± 6,20, and their anxiety score average was found as 7,33 ±5,56.

In Table 4, depression anxiety, stress and eating attitudes of the athletes in the study were examined as normal and above normal. Depression score was found as above normal in 21 (35%) participants and as normal in 39 (65%) participants. Stress score was found as above normal in 23 (38.3%) participants and as normal in 37 (61.7%) participants. Anxiety score was found as above normal in 24 (40%) participants and as normal in 36 (60%) participants. When eating attitude scores were examined, 10% of the athletes were found to have eating disorder.

In Table 5, statistically significant difference was found between eating attitude test score averages of Greco-Roman wrestlers and free style wrestlers (p<0.05). No statistically significant difference was found between groups when depression anxiety, stress score averages were examined (p>0.05).

When the athletes' EAT scores were compared between groups in terms of their eating attitude, anxiety, stress and depression scores, a statistically significant difference was found between EAT scores of Greco-Roman and Freestyle wrestlers (p<0.05).

In Table 6, average carbohydrate intake of athletes who had above normal stress scores was found to be higher. This difference was found to be statistically significant (p<0.05). No statistically significant difference was found between other nutrient elements and stress levels (p>0.05).

Table.1. Descriptive Information about the athletes

Parameter	Group	N	Ave ±Sd	Min.	Max.	p
Age (years)	Greco Roman	33	24,39 ±3,45	17	31	,096
	Freestyle	18	26,06 ±2,96	21	31	
	Judo	9	23,22 ± 3,90	18	31	
	General	60	24,71 ±3,46	17	31	
Height (cm)	Greco Roman	33	176,03±7,10	164	190	,080
	Freestyle	18	172,50±8,08	155	192	
	Judo	9	170,67±5,59	165	183	
	General	60	174,17±7,42	155	192	
Weight (kg)	Greco Roman	33	85,91±18,38	62,1	123,8	,117
	Freestyle	18	79,68±16,78	63	118,6	
	Judo	9	73,43±8,16	63,5	89,2	
	General	60	82,17±17,17	60	123,8	
BMI (kg/m²)	Greco Roman	33	27,41±4,11	22,3	37	.648
	Freestyle	18	26,53±3,23	21,3	32,2	
	Judo	9	25,38±1,71	22,5	27,7	
	General	60	26,84±3,62	21,3	37	
Fat (%)	Greco Roman	33	11,05±4,85	4,4	23,6	0,277
	Freestyle	18	11,28±4,88	2,7	20,1	
	Judo	9	8,38±3,83	3,3	14,2	
	General	60	10,72±4,76	2,7	23,6	

p<0.05

Table 2. Comparison of the athletes' average carbohydrate, protein and fat intake in percentages according to groups

Group	Carbohydrate intake	Protein intake	Fat intake
	Ave ±Sd	Ave ±Sd	Ave ±Sd
Greco Roman(%)	37,98±12,42	18,58±6,42	42,46±16,48
Freestyle(%)	44,50±16,74	18,13±5,68	36,31±12,98
Judo(%)	38,33±1,56	19,97±4,24	40,89± 9,39
Total (%)	39,85 ±13,39	18,66±6,02	40,52±15,35

Table 3. Depression, Stress and Anxiety Average Scores of the Athletes

Variable	N	Ave. ± S.d.	Min.	Max.
Depression score	60	6,72 ±5,69	0	19
Stress score	60	11,78± 6,20	0	23
Anxiety score	60	7,33 ±5,56	0	21

Table 4. Depression, Stress, Anxiety and Eating Attitudes of the Athletes

Variable	Group	N	%
Depression Score	Higher than normal	21	35
	Normal	39	65
	Total	60	100
Stress Score	Higher than normal	23	38.3
	Normal	37	61.7
	Total	60	100
Anxiety Score	Higher than normal	24	40
	Normal	36	60
	Total	60	100
Eating Attitude (EAT) Score	30>	6	10
	30<	54	90
	Total	60	100

Table 5. Comparison of groups' eating attitude, anxiety, stress and depression scores in terms of branches

Variable	Group	N	Ave. ± S.D.	Min.	Max.	p
Eating Attitude (EAT)	Greco-Roman	33	13,84 ± 8,289[b]	4	48	0,034*
	Freestyle	18	26,67 ±2,43[a]	4	81	
	Judo	9	15,33 ±10,75[ab]	6	40	
	Total	60	17,92 ±15,21	4	81	
Anxiety Score	Greco-Roman	33	7,51 ± 5,79	0	19	0.618
	Freestyle	18	7,83 ± 5,52	1	21	
	Judo	9	5,67 ± 5,05	0	15	
	Total	60	7,33 ± 5,56	0	21	
Stress Score	Greco-Roman	33	11,72 ± 6,20	0	23	0.993
	Freestyle	18	11,78 ± 5,49	2	20	
	Judo	9	12,00 ±8,08	0	20	
	Total	60	11,78 ±8,08	0	20	
Depression Score	Greco-Roman	33	7,30 ±5,79	0	19	0.245
	Freestyle	18	7,11 ±5,84	0	19	
	Judo	9	3,78 ±4,52	0	12	
	Total	60	6,72±5,69	0	19	

Table 6. Comparison of average total energy, carbohydrate, protein and fat intakes in terms of branches

Mood	Nutrients	Level	N	Ave. ± S.d	Min	Max	p
Anxiety	Carbohydrate	Normal	24	237,05 ± 82,94	121,7	410,5	0,633
		Higher than normal	36	227,15 ± 70,27	30,5	385,2	
	Protein	Normal	24	105,61 ± 34,27	21	174	0,638
		Higher than normal	36	109,98 ± 35,67	30	186,9	
	Fat	Normal	24	104,38 ± 42,21	25,9	182,1	0,991
		Higher than normal	36	104,50 ± 38,35	34,4	212,9	
Depression	Carbohydrate	Normal	39	233,03 ± 88,24	30,5	410	0,768
		Higher than normal	21	227,56 ± 54,53	121,7	334	
	Protein	Normal	39	108,36 ± 34,82	30	186,9	0,968
		Higher than normal	21	107,99 ± 35,88	21	168,3	
	Fat	Normal	39	396,84 ± 180,46	34,4	163,6	0,975
		Higher than normal	21	386,79 ± 201,93	25,9	212,9	
Stress	Carbohydrate	Normal	21	257,28 ± 70,25	158,7	410,5	**0,038***
		Higher than normal	39	214,85 ± 78,47	30,5	379,5	
	Protein	Normal	21	111,97 ± 29,08	52,7	174	0,517
		Higher than normal	39	105,90 ± 38,260	21	186,9	
	Fat	Normal	21	107,73 ± 34,82	41,9	182,1	0,617
		Higher than normal	39	102,41 ± 42,62	25,9	212,9	

p<0.05

Anxiety scores of the athletes who participated in our study were normal and the groups which had higher than normal anxiety scores were compared in terms of some nutrients. Average carbohydrate intakes of athletes who had higher than normal stress scores were found to be higher. This difference was found to be statistically significant (p<0,05).

Discussion

The objective of our study was to compare the effects of eating habits and eating attitudes of weight athletes on their mood. The total body fat percentage average of the athletes who participated in our study was found as 10,72±4,76%. Groups' body fat percentages were found to be 11,05±4,85 % for Greco-roman wrestlers, 11,28±4,88% for freestyle wrestlers and 8,38±30% for judokas (p>0.05). Various studies have shown average body fat values of wrestlers to be between 6% and 15% (22). In Roemmich and Sinning's (1996) study, BMI of adolescent wrestlers was found to be 7,8%, while in Zorba's (2006) study, BMI of elite Turkish wrestlers was found to be 10,92±5,3% and this result was in parallel with the results of our study (23,24).

As a result of energy and nutrient calculations of athletes' daily average food consumption, carbohydrate percentage was found as 39,85 ±13,39, protein percentage was found as 18,66±6,02% and fat percentage was found as 40,52±15,35. While it is sufficient for healthy adults to take 55-60% of their daily energy from carbohydrates, 12-15% from proteins and 25-30% from fats for a sufficient and balanced diet, for athletes, the contribution of nutrients to daily energy is different in terms of the athletes' branches. It has been reported that in sports branches such as boxing, wrestling, judo, karate, taekwondo, it is sufficient for 50% of the energy to come from carbohydrates, 20% from proteins and 30% from fats (25,26,27,28). It was found that the weight class athletes in our study had low intake of daily carbohydrate and protein, while they had higher values of fat intake than the levels recommended. When it is taken into consideration that the measurements in our study were conducted 3 days before competition and weigh-in, it can be seen that as a method of adjusting weight, athletes tend to get away from nutrients that have carbohydrate and protein and specific weight that can influence weight-in. This has shown a state that can be assessed negatively in general eating habits.

In our study, depression (6,72±5,69) and stress (11,78±6,20) scores of elite athletes were found to be within normal values and it was though that this could occur as a result of sport's positive effect on depression and stress. A great number of studies have examined the association between depression and anxiety and shown that a regular sport is useful for such disorders (29). In their study they compared the depression levels of elite wrestlers and taekwondo athletes, Kumartaşlı et al (2015) found that depression levels of elite wrestlers and taekwondo athletes were in intermediate levels (30).

Anxiety scores of our athletes were found to be above normal (7,33±5,56). In their study conducted with 131 male judokas and wrestlers participating in Universities Turkey Championship competitions, Civan et al. (2010) reported that athletes had average level of anxiety (31). When the sportive careers of the athletes in the study are taken into consideration, the size and significance of the competitions they participate in are understood better. The fact that they are participating in the biggest and most important competitions of the world and the national pressure they feel can cause increase in the anxiety levels of athletes.

Carbohydrate intake of the group with normal stress scores was found to be higher than the group with stress scores higher than the normal (p<0.05). This result related with stress is also present for depression scores, though not statistically significant. High amounts of carbohydrate or alcohol intake stimulates dopamine use in the brain (32). High amounts of food intake artificially triggers the reward center in the brain, provides dopaminergic activation and creates a kind of therapeutical effect (33). A diet in favor of carbohydrates at the same time eases tryptophan's transition to the brain and transformation into serotonin (34, 35). It is known that the serotonin level in the brain is effective on mood and the decreases in serotonin level are known to contribute to the etiology of depression in some individuals. The decreases in tryptophan levels which are necessary for serotonin synthesis also influence the decreases in serotonin

level. This situation results in a decrease in the moods of some individuals, though not in all (36).

It was found that the 10% of the athletes in our study got scores of 30 cut-off point and higher from EAT, while 90% got scores of lower than 30 cut-off point. In their study they conducted to examine the eating disorders of 446 high school students with an average age of 16,07, Tanrıverdi et al. (2011) found that 17.3% of the students got scores of 30 cut-off point and higher from EAT (37). In their study they conducted on 372 university students between the ages of 17–30, Toker (2008) found that 4,83% got scores of 30 cut-off point and higher from EAT (38). While some of the results of our study were in parallel with other studies, it was found that unlike some studies, the percentage of those who got scores of EAT cut-off point and higher were higher than the percentage of other groups.

When the EAT scores of the athletes who participated in our study were examined, a statistically significant difference was found between the EAT scores of Greco-roman wrestlers and freestyle wrestlers, with EAT scores of freestyle wrestlers being higher ($p<0,05$). There is an insignificant but noticeable difference between anxiety levels of groups. The average anxiety levels of Freestyle wrestlers were found to be higher than the other groups. Eating behavior is considerably influenced by mood. Various studies have found that the frequency of courses, the amount eaten and what is eaten are associated with psychological needs and it has been accepted that there is a strong association between these. In a study which examined the association between food intake amount and different kinds of emotion, while excessive food intake was found to be associated with distress, depression and fatigue, small quantities of food intake was associated with fear, tension and pain (39).

It can be interpreted that high levels of anxiety leads these athletes to have less balanced and consistent behaviors of eating.

Athletes may follow unhealthy diets from time to as weight control is a significant factor for weight class athletes, and as a result some essential nutrients which are not taken sufficiently can contribute to the development of depression and anxiety.

For the optimal performance of elite athletes, an experienced nutritionist who plans and follows the dietary habits and food consumptions of athletes not only during camp periods but also before and after camp periods and also a psychological advisor and regular training programs can make information more permanent and cause it to become a life style. We are of the opinion that increasing the number of athletes who participate in such studies can lead to healthier scientific information about the subject.

In weight athletes, body weight has significant effects on performance. Inappropriate diets to control body weight on the one hand disrupt the athlete's nutrition and on the other hand have a negative effect on their mood. It can be said that insufficient and unbalanced nutrition and negative mood will have a negative effect on the general health of athletes and also on their sportive success.

References

1. Nazni P, Vimala S.Nutrition knowledge, attitude and practice of college sportsmen. Asian journal of sports medicine, 2010;1(2), 93.
2. Cotugna N, Connie EV, McBee S. Sports nutrition for young athletes. The Journal of School Nursing. 2005; 21(6):323-328.
3. Aoi W, Naito Y, Yoshikawa T. Exercise and functional foods. Nutrition Journal. 2006; 5(15):1-5.
4. Clark N. Sports Nutrition Guidebook, America. Human Kinetics. 1997.
5. Pehlivan A. Sporda beslenme. İstanbul. Yaylacık Matbaası. 2005.
6. Markey MA, Vander Wal JS. The role of emotional intelligence and negative affect in bulimic ymptomatology. Compr Psychiatry. 2007; 48:458–64.
7. Sassaroli S, Ruggiero GM. The role of stress in the association between low self-esteem, perfectionism, worry and eating disorders. Int J Eat Disord. 2005; 37:135–41.
8. Liu LL, Li BM, Yang J, Wang YW. Does dopaminergic reward system contribute to explaining comorbidity obesity and ADHD? Med Hypotheses. 2008; 70:1118-1120.
9. Vardar E. Egzersiz bağımlılığı. Arşiv Kaynak Tarama Dergisi. 2012; 21(3):163-173.
10. Bamber DJ, Cockerill IM, Rodgers S, Carroll D. Diagnostic criteria for exercise dependence in women. British Journal of Sports Medicine. 2003; 37(5):393-400.
11. Öztürk O, Uluşahin A. Ruh Sağlığı ve Bozuklukları. Ankara. Nobel Tıp Kitapevleri. 2008.
12. Karamustafalıoğlu O, Yumrukçal H. Depresyon ve anksiyete bozuklukları. Şişli Eftal Hastanesi Tıp Bülteni. 2011; 45:65-74.
13. Hall C, Lane A. Effects of rapid weight loss on mood and

performance among amateur boxers.. Br J Sports Med. 2001; 35:390-5.
14. Kutluay T. Toplu Beslenme Yapılan Kurumlar İçin Standart Yemek Tarifleri. Ankara. Hatipoğlu Yayınevi. 2003.
15. Sarria A, Moreno LA, Garcia-Llop LA, Fleta J, Morellón MP, Bueno M. Body mass ındex, triceps, skinfold and waist circumference in screening for adiposity in male children and adolescents. Acta Paediatr. 2001; 90(4):387-92.
16. Lovibond PF, Lovibond SH. The structure of negative emotional states: comparison of the Depression Anxiety Stress Scale (DASS) with the Beck Depression and Anxiety Inventories. Behaviuor Research and Therapy. 1995; 33:335-343.
17. Akın A, Çetin B. Depresyon anksiyete stres ölçeği (DASÖ): geçerlik ve güvenirlik çalışması. Kuram ve Uygulamada Eğitim Bilimleri / Educational Sciences: Theory & Practice. 2007; 7(1):241-268.
18. Garner DM, Garfinkel P E. The eating attitudes test: An index of the symptoms of anorexia nervosa. Psychological Medicine . 1979; 9:273-279 .
19. Savaşır I, Erol N. Yeme tutum testi: anoreksiya nervoza belirtileri indeksi. Psikoloji Dergisi. 1989; 7:19-25.
20. Aydemir Ö, Köroğlu E. Psikiyatride Kullanılan Klinik Ölçekler. Ankara. Medico Graphics Maatbası. 2007;153-161.
21. Öner N, Le Comte A. Durumluk Kaygı Envanteri El Kitabı, İstanbul. Boğaziçi Üniversitesi Yayınları. 1983.
22. Houtkooper LB, Going SB. Body composition: how should it be measured? Does it affect sport performance? Sports Sci Exchange. 1994; 7:1-8.
23. Roemmich JN, Sinnig WE. Sports-seasonal changes in body cmposition, growth, power and strength of adolescent wrestlers. International Journal of Sports Medicine. 1996; 17(2):92-99.
24. Zorba E. Vücut Yapısı. İstanbul. MMP Baskı Tesisleri. 2006; 186–189.
25. Baysal A. Beslenme. Ankara, Hatiboğlu Yayınevi, 13. Baskı. 2011.
26. Baysal A, Aksoy M, Besler T, Bozkurt N, Keçecioğlu S, Merdol KT, Pekcan G, Mercanlıgil S, Yıldız E. Diyet El Kitabı. 6.baskı. Ankara, Hatiboğlu Yayınevi. 2011.
27. McArdle WD, Katch FI, Katch VL, Sport and Exercise Nutrition. Third Ed: PA-USA. Lippincott Williams & Wilkins. 2005.
28. Geisserler C, Powers H. Human Nutrition. 11th Ed. UK. Elseveir Churchill Livingston. 2005.
29. Byrne A, Byrne DG. The effect of exercise on depression, anxiety and other mood states-A review. J Psychosom Res 1993; 37:565-574.
30. Kumartaşlı M, Alp M, Yılmaz E, Gökbel S. Comparison of depression levels of elite wrestling and taekwondo athletes. Turkish Journal of Sport and Exercise. 2015; 17(1): 67-70.
31. Civan A, Arı R, Görücü A, Özdemir M. Bireysel ve takım sporcularının müsabaka öncesi ve sonrası durumluluk ve sürekli kaygı düzeylerinin karşılaştırılması. Uluslararası İnsan Bilimleri Dergisi. 2010; 7(1):193-206.
32. Liao Y, Knoesenb NP, Castleb DJ, Tanga J, Dengd Y, Bookunb R, Chena X, Haoa W, Menga G, Liua T. Symptoms of disordered eating, body shape, and mood concerns in male and female Chinese medical students. Comprehensive Psychiatry. 2010; 51:516–523.
33. Solanto MV. Dopamine dysfunction in AD/HD: integrating clinical and basic neuroscience research. Behav Brain Res. 2002; 130:65-71.
34. Berry EM, Growdon JH, Wurtman JJ, Caballero B, Wurtman RJ. A balanced carbohydrate: protein diet in the management of Parkinson's disease. Neurology 1991; 41:1295–1297.
35. Yokogoshi H, Wurtman RJ. Meal composition and plasma amino acid ratios: effects of various proteins or carbohydrates, and of various protein concentrations. Metab Clin Exp 1986; 35:637– 642.
36. Thomas B. Mental Illness. Manual of Dietetic Practice. 3rd ed. Oxford: Blackwell Publishing. 2001; 571-9.
37. Tanrıverdi D, Savaş E, Gönüllüoğlu N, Kurdal E, Balık G. Lise öğrencilerinin yeme tutumları, yeme davranışları ve benlik saygılarının incelenmesi. Gaziantep Tıp Dergisi. 2011; 33-39.
38. Toker ED. K.T.Ü. Trabzon Sağlık Yüksek Okulu öğrencilerinde yeme tutumu ve aile yapısı arasındaki ilişki. Trabzon. Karadeniz Teknik Üniversitesi, Sağlık Bilimleri Enstitüsü. Sağlık Psikolojisi Anabilim Dalı. Yüksek Lisans Tezi, 2008.
39. Sevinçer GM, Konuk N. Emosyonel yeme. Journal of Mood Disorders. 2013; 3(4);171-8.

Correspondence:
Levent Bayram
Ondokuz Mayıs University, Yaşar Doğu Faculty of Sport Sciences
E-mail: levent.bayram@omu.edu.tr

Progress in Nutrition 2020; Vol. 22, N. 1: 161-166 DOI: 10.23751/pn.v22i1.9209

Original article

Acute effects of static and dynamic stretching exercises on isokinetic strength of hip flexion-extension in male handball players

Mahmut Alp

Süleyman Demirel University, Faculty of Sport Sciences, Department of Physical Education and Sports, Isparta, Turkey - E-mail: mahmut.alp@windowslive.com

Summary. This study aimed to examine and compare the acute effects of static stretching (SS) and dynamic stretching (DS) on the hip flexor and extensor concentric (CON) isokinetic peak torque (PT) at 60°/s and 180°/s angular speeds in well-trained male handball players. A total of 14 male handball players (mean age 20.28±1.06 years; handball experience 11.50±1.45 years; height 180.64±6.34 cm; weight 73.35±6.60 kg; body mass index 22.49±1.90 kg/m2) who train at least 4 days a week, 120 min a day, were recruited in this study. Players were tested for hip flexor and extensor isokinetic PT at 60°/s and 180°/s angular speeds before and 4 min after three different stretching exercise sessions, namely, non-stretching (NS), SS, and DS, with 48-h rest intervals in a randomized crossover study design. Statistical analysis revealed that no significant difference ($p>0.05$) was observed among the stretching exercises in hip CON isokinetic PT values at 60°/s and 180°/s. The findings of our study are that SS exercises do not have a tension deficit in PT; DS exercises showed that PT did not increase the hip flexor and extensor CON isokinetic muscle movement more than SS exercises in male handball players. Based on previous studies, the findings suggest that athletes who are accustomed to static or dynamic stretching movements in male handball players may be less susceptible to stretch-induced power loss.

Key words: handball, static stretching, dynamic stretching, isokinetic strength

Introduction

Submaximal aerobic running is the main component of classical warm-up that increases the body temperature (1). Besides, SS is believed as an essential component in warm-up which degrade the risk of injury and improve performance (2). Although, latest researches stated that pre-exercise SS induced the performances of maximal strength output, jump height, sprint, agility compared to dynamic stretching (DS) (3). Negative effects of SS are based on neural variation such as changes in reflex sensitivity and induced motor unit activation as proofed by reductions in electromyography and mechanical factors (e.g., changes in muscle tendon unit stringency) (EMG) (4). Owing to the negative effects of SS previous to strength and power works, many researchers have proposed the DS exercise (5-7). Yamaguchi et al. (7) stated that development in muscle performance after DS could be related with two factors: the incident of post-activation potentiation (PAP) and the rise of muscle temperature. PAP includes the phosphorylation of myosin regulatory light chains, which improve actin–myosin interaction. This coaction is caused by the optional contraction of the muscle antagonist to target the stretched muscle (8).

Considering at the beginning and during the movement in sports, the explosive strength is highly important. Strength can extend the opponent's decision time as speed increases and increase the likelihood of making mistakes (9). Therefore, some researchers signified to a stretching protocol which will not cause reduces in

strength performance. Power and condition trainers have perceived active and DS protocols to make the athletes ready for competitions (10, 11). Kim et al. (12) lined up with that measuring the extensor and flexor strength is a major indicator in observing training effects.

Conflicting results regarding the effects of SS and DS on isokinetic strength were also reported. Some scientists advocated the detrimental effects of SS on isokinetic strength (13-16), while others stated that after SS there were no strength isokinetic deficiencies (17-19).

In the light of these information about literature, the current study aimed to examine and compare the acute effects of SS and DS exercises on isokinetic strength of hip flexor and extensor CON isokinetic peak torque (PT) at 60˚/s and 180˚/s angular speeds in male handball players.

Materials and Methods

Participants

A total of 14 male handball players (mean age 20.28±1.06 years; handball experience 11.50±1.45 years; height 180.64±6.34 cm; weight 73.35±6.60 kg; body mass index 22.49±1.90 kg/m^2) who train at least 4 days a week, 120 min a day, were recruited in this study.

Study Design

The inclusion criteria were as follows: *(i)* age between 18 and 22 years, *(ii)* male gender, *(iii)* active handball player for at least 5 years, and *(iv)* received specific training period for handball for at least 12 weeks without interruption. The exclusion criteria were determined as follows: *(i)* history of orthopedic problems, such as gluteus maximus-medius-minimus, hamstring, piriformis, adductor magnus-longus-brevis, psoas, iliacus, sartorius, gracilis injuries; fractures, surgery, or pain in the spine or gluteus maximus-medius-minimus, hamstring, piriformis, adductor magnus-longus-brevis, psoas, iliacus, sartorius, gracilis muscles over the past 3 months, *(ii)* missing a testing session during the data collection period, and *(iii)* using ergogenic aid that would affect the isokinetic test. The participants were verbally informed about the study method used as well as the purpose and risks of the study, and written informed consent was obtained from all participants.

Experimental Protocol

Participants began with a 5-min standardized warm-up (cycling at 90 W at 60–70 rpm) for NS, SS and DS sessions.

Non-stretching (NS) session: Participants had no stretching exercises.

SS session: This session consisted of rear foot elevated, the couch, glute and sumo squat stretch exercises for 3×30 sec with 20 sec resting intervals between the repetitions in the same position. Each stretch was executed to the point of discomfort, which was subjectively determined.

DS exercises: This session consisted of straight leg kick, butt kick, groin and rear foot elevated stretch exercises. The participants were instructed to execute the exercises in a repetitive manner and as fast as they could. In this session, exercises were executed at each joint for 3×30 sec with 20 sec resting intervals between repetitions.

Isokinetic tests were performed before and after each stretching session.

Isokinetic testing

The study data were collected using a Cybex-Humac Norm-brand Isokinetic Dynamometer. Isokinetic tests were conducted according to the study of Manoel et al. (20). Tests included a total of 3 days with at least 48-h resting intervals. The order of NS, SS, and DS sessions was randomized for each participant in a crossover study design. The tests were applied in dominant leg for 60˚/s and 180˚/s angular speeds in a CON manner. Each session began with a 5-min standardized warm-up (cycling at 90 W at 60–70 rpm), then 2.5-min rest was given. The dynamometer's seat was positioned in the supine position with zero degree in every ankle before the test start. The participants who randomized systematically before, were subjected to pre-test. Then, participants performed applying NS, SS and DS sessions. 4-min rest was given. In tests which 3 sub-maximal trials were followed by 3 maximal efforts, 2-min of relaxation intervals were given between the angular speeds. The PT values of the participants were recorded as "Newton meter (Nm)" in all pre- and post-tests. Table 1 presents the detailed flowchart of the study.

Statistical analysis

The statistical analysis was initially performed using the "Shapiro–Wilk" normality test, and all variables presented with normal distribution. Therefore, statistical differences between the pre- and post-test at each session were evaluated using the "Paired t-test." Statistical differences among post-tests after three stretching exercises were evaluated using "Repeated Measures ANOVA" according to the 95% reliability. So findings are presented as descriptive analyses, ANOVA results and an alpha level of $p<0.05$ considered statistically significant for all analyses. All data analyses were conducted using the statistical package program.

Results

Table 1. Flowchart of the study

NS	SS	DS
5 min submaximal warm-up 2.5 min resting 3 submaximal trial		
60°/sec isokinetic pre-test		
2 min resting 3 submaximal trial		
180°/sec isokinetic pre-test		
No Stretching	**Static Stretching**	**Dynamic Stretching**
4 min resting 3 submaximal trial		
60°/sec isokinetic post-test		
2 min resting 3 submaximal trial		
180°/sec isokinetic post-test		
Cool Down		

Table 2. Descriptive Statistics of Players' Demographic Information

	Minimum	Maximum	Mean	SD
Age (years)	18.00	22.00	20.28	1.06
Handball Experience (years)	9.00	14.00	11.50	1.45
Height (cm)	172.00	191.00	180.64	6.34
Weight (kg)	62.00	85.30	73.35	6.60
BMI (kg/m²)	18.48	25.89	22.49	1.90

Table 3. Comparison of Isokinetic PT at 60°/sec from Different Stretching Sessions

Stretching Type (Nm)	Test Sequence	Mean±SD	F	p
NS	Pre-test	281.34±8.88	10.09	.190
	Post-test	283.66±9.07		
SS	Pre-test	283.33±9.01		
	Post-test	281.63±8.08		
DS	Pre-test	298.70±8.54		
	Post-test	301.50±7.81		

Table 4. Comparison of Isokinetic PT at 180°/sec from Different Stretching Sessions

Stretching Type (Nm)	Test Sequence	Mean±SD	F	p
NS	Pre-test	186.14±22.51	8.33	.280
	Post-test	188.40±25.03		
SS	Pre-test	185.92±25.68		
	Post-test	184.00±24.20		
DS	Pre-test	191.92±23.70		
	Post-test	195.64±24.09		

Discussion

As the main findings of the current study were analyzed, SS exercises did not find stretch-related power deficit in PT, and DS exercises were not higher in handball players than SS exercises in increasing the hip joint CON isokinetic muscle strength. Prior to the study, we assumed that SS may have strain-related PT deficits in the hip joint at both angular velocities of 60 ° / s and 180 ° / s compared to DS. Based on the results of this study, we were unable to confirm our hypothesis due to the lack of previous studies describing the acute effects of SS and DS on hip flexor and extensor isokinetic PT in handball players.

For that reason, this study could support ours Kapo et al. (21). Researchers reported in their study that different and better results on PT at 60°/s and 180°/s in favor of DS compared with proprioceptive neuromuscular facilitation in 50 male athletes from different sports disciplines, such as karate, taekwondo, box, football, and athletic sprint.

Similar to the results of our study, Egan et al. (19) reported that SS did not affect the isokinetic PT at 60°/s and 300°/s angular speeds during any of the post-stretching intervals. In contrast with our study, ekir

et al. (22) defined that SS significantly decreased the muscle strength in eccentric (ECC) and CON modes at 60˚/s and 180˚/s angular speeds in well-trained track and field athletes.

On the contrary, some researchers reported that SS exercises have generally no stretching-induced PT loses in muscles (4, 14, 17, 18). Ayala et al. (17) reported that short pre-exercise SS and DS of stretching routine do not elicit stretching-induced loses or improvements on isokinetic CON and ECC strength and power at different speeds (60˚/s, 180˚/s, and 240˚/s). However, Marek et al. (15) and Miyahara et al. (13) reported different results than the previously mentioned studies. Marek et al. (15) reported that stretching-induced deficit in the strength of extension at 60˚/s and 300˚/s angular speeds. As reported, two factors have been proposed to explain the stretching-induced strength loses (19); first one is the mechanical factors associated with decreased musculotendinous stiffness that may alter the length–tension relationship and second one is the neural factors caused by decreased muscle activation.

We found that majority of the studies in the literature involved the effects of different stretching exercises on the isokinetic strength of the knee muscles. In this regard, results of the current study also suggested that differences in isokinetic ankle flexion and extension PT after NS, SS, and DS are not significant. Yamaguchi and Ishii (24) reported that the effect of 30-sec static stretching exercises on isokinetic strength was not found significant. Balcı (25) defined SS and DS exercises have no effect on the effect of concentric isokinetic strength. In a study examining the mechanomiographic responses of concentric isokinetic muscle contractions, it was concluded that the reduction-induced reduction in slow and fast angular velocities may not be velocity-specific (16). In addition, some researchers found that stretching exercises had no effect on force, and suggested that this was related to SS time and severity (26).

In previous studies, there are different findings on unknown effects of SS and DS on isokinetic power (27); however, these may be related to the duration and intensity of the SS exercise, the training or competitive experience level of athletes (19, 28) and the isokinetic test rates (29). Ayala et al. (17), the total SS duration per ≤60-90 s isolate muscle group may not have changes in strength and stress due to CON and ECC isokinetic muscle movements. In addition, well-trained athletes who are accustomed to static or dynamic movement actions as in handball may be less susceptible to the stretching-induced strength deficit. These results should only be considered in male handball players who can perform SS and DS prior to training and competition without strength loses (19, 22, 30).

The limitations of this study are: (a) failure to evaluate the effects of SS and DS through EMG. Therefore, the results are interpreted in the literature according to the findings of previous studies. (b) when conducting isokinetic tests, it was observed that players were familiar with low warm-up and tests and rested for a long time between stretching sessions and post-test. For this reason, more specific test protocols should be developed for this type of future research. (c) difficulty of positioning participants in the supine position and aligning the dynamometer axis with the large trochanter of the femur same as in the study of Zapporoli and Liberto (31). (d) small sample group limited results. Therefore, larger working groups may be needed.

Conclusion

According to the our findings and the results of similar studies supporting it, as the causes of changes in isokinetic PT parameters; It can be said that in sports activities, the application of the set numbers and stretching times higher than the normal ratios recommended in the literature and causing neurogenic inhibition in SS exercises and exhaustion in DS exercises. In addition, it can be considered that for a long time, a minimum effect on power performance is observed, but insufficient increase in blood parameters is observed. In sports, where slight positive increases, strength and performance differences affect the competitive outcome, it is recommended that stretching protocols applied in warm-up are performed according to the requirements of the related sports branch. In our study, considering the fact that short-term

maximal loadings are related to force performance in a sport such as handball, where explosive strength and continuity in strength are combined with the flawless playing feature, these are recommended to do that mechanical and neuromuscular factors including the appropriate set numbers and stretching times to reach strong positive increase rates are considered.

References

1. Behm DG, Chaouachi A. A review of the acute effects of static and dynamic stretching on performance. Eur J Appl Physiol. 2011; 111(11): 2633-2651.
2. Chatzopoulos D, Galazoulas C, Patikas D, Kotzamanidis C. Acute effects of static and dynamic stretching on balance, agility, reaction time and movement time. J Sports Sci Med 2014; 13: 403.
3. Leone DC, Pezarat P, Valamatos MJ, Fernandes O, Freitas S, Moraes AC. Upper body force production after a low-volume static and dynamic stretching. Eur J Sport Sci 2014; 14: 69-75.
4. Chaouachi A, Padulo J, Kasmi S, Othmen AB, Chatra M, Behm DG. Unilateral static and dynamic hamstrings stretching increases contralateral hip flexion range of motion. Clin Physiol Funct Imaging 2017; 37: 23-9.
5. Amiri-Khorasani M, Kellis E. Static vs. dynamic acute stretching effect on quadriceps muscle activity during soccer instep kicking. J Hum Kinet 2013; 39: 37-47.
6. Hough PA, Ross EZ, Howatson G. Effects of dynamic and static stretching on vertical jump performance and electromyographic activity. J Strength Cond Res 2009; 23: 507-512.
7. Yamaguchi T, Ishii K, Yamanaka M, Yasuda K. Acute effects of dynamic stretching exercise on power output during concentric dynamic constant external resistance leg extension. J Strength Cond Res 2007; 21: 1238.
8. Robbins DW. Postactivation potentiation and its practical applicability: a brief review. J Strength Cond Res 2005; 19 :453.
9. Haddad M, Dridi A, Chtara M, Chaouachi A, Wong DP, Behm D, Chamari K. Static stretching can impair explosive performance for at least 24 hours. J Strength Cond Res 2014; 28: 140-146.
10. Sevim Y. Antrenman Bilgisi. 3. Baskı, Nobel Yayınevi, Ankara, 2007. (in Turkish)
11. Hedrick A. Dynamic flexibility training. J Strength Cond Res 2000; 22: 33–38.
12. Kim HB, Stebbins CL, Chai JH, Song JK. Taekwondo training and fitness in female adolescents. Balt J Sport Health Sci 2011; 29: 133-138.
13. Miyahara Y, Naito H, Ogura Y, Katamoto S, Aoki J. Effects of proprioceptive neuromuscular facilitation stretching and static stretching on maximal voluntary contraction. J Strength Cond Res 2013; 27: 195-201.
14. Cramer JT, Housh TJ, Weir JP, Johnson GO, Coburn JW, Beck TW. The acute effects of static stretching on peak torque, mean power output, electromyography, and mechanomyography. Eur J Appl Physiol 2005; 93: 530-539.
15. Marek SM, Cramer JT, Fincher AL, Massey LL, Dangelmaier SM, Purkayastha S, et al. Acute effects of static and proprioceptive neuromuscular facilitation stretching on muscle strength and power output. J Athl Train 2005; 40: 94.
16. Evetovich TK, Nauman NJ, Conley DS, Todd JB. Effect of static stretching of the biceps brachii on torque, electromyography, and mechanomyography during concentric isokinetic muscle actions. J Strength Cond Res 2003; 17: 484-488.
17. Ayala F, Croix MD, de Baranda PS, Santonja F. Acute effects of two different stretching techniques on isokinetic strength and power. Rev Andal Med Deporte 2015; 8:93-102.
18. Winke MR, Jones NB, Berger CG, Yates JW. Moderate static stretching and torque production of the knee flexors. J Strength Cond Res 2010; 24: 706-710.
19. Egan AD, Cramer JT, Massey LL, Marek SM. Acute effects of static stretching on peak torque and mean power output in National Collegiate Athletic Association Division I women's basketball players. J Strength Cond Res 2006; 20: 778.
20. Manoel ME, Harris-Love MO, Danoff JV, Miller TA. Acute effects of static, dynamic, and proprioceptive neuromuscular facilitation stretching on muscle power in women. J Strength Cond Res 2008; 22(5): 1528-1534.
21. Kapo S, Smajlovi N, Kajmovi H, ovi N, iri A, utuk M, et al. Effects of different stretching protocols on knee muscles strength and power parameters measured by Biodex dynamometer. TEH VJESN 2016; 23: 273-278.
22. Sekir U, Arabaci R, Akova B. Acute effects of static stretching on peak and end-range hamstring-to-quadriceps functional ratios. World J Orthop 2015; 6: 719.
23. Kim YK, Kim YH, Im SJ. Inter-joint coordination in producing kicking velocity of Taekwondo kicks. J Sports Sci Med 2011; 10: 31.
24. Yamaguchi T, Ishii K. Effects of static stretching for 30 seconds and dynamic stretching on leg extension power. The Journal of Strength & Conditioning Research. 2005; 19(3): 677-683.
25. Balcı A. Statik ve Dinamik Germe Egzersizlerinin Ayak Bile i Kuvveti ve Denge Üzerinde Etkisi. A.Ü., Tıp Fakültesi Spor Hekimli i Anabilim Dalı, Tıpta Uzmanlık Tezi, 2014, Ankara (in Turkish).
26. Bandeira CBU, Mello ML, Pereira MJR. Time-stretching effects on peak torque in the hip joint in dancers. Annals of XXVI Simpósio Internacional de Cie cias do Esporte. 2003; 9-11.
27. Pinto MD, Wilhelm EN, Tricoli V, Pinto RS, Blazevich AJ. Differential effects of 30-vs. 60-second static muscle stretching on vertical jump performance. J Strength Cond

Res 2014; 28: 3440-3446.
28. Amiri-Khorasani M, Sahebozamani M, Tabrizi KG, Yusof AB. Acute effect of different stretching methods on Illinois agility test in soccer players. J Strength Cond Res 2010; 24: 2698-2704.
29. Nelson AG, Allen JD, Cornwell A, Kokkonen J. Inhibition of maximal voluntary isometric torque production by acute stretching is joint-angle specific. Res Q Exerc Sport 2001; 72: 68-70.
30. Unick J, Kieffer HS, Cheesman W, Feeney A. The acute effects of static and ballistic stretching on vertical jump performance in trained women. J Strength Cond Res 2005; 19: 206.
31. Zapparoli FY, Riberto M. Isokinetic evaluation of the hip flexor and extensor muscles: a systematic review. Journal of Sport Rehabilitation, 2017; 26(6): 556-566.

Correspondence:
Mahmut Alp
Süleyman Demirel University, Faculty of Sport Sciences, Department of Physical Education and Sports, Isparta, Turkey - E-mail: mahmut.alp@windowslive.com

Progress in Nutrition 2020; Vol. 22, N. 1: 167-176 DOI: 10.23751/pn.v22i1.9141

Original article

The relationship between subjective well-being levels and physical self-perception and eating attitudes of students receiving sports education

Yasemin Çakmak Yıldızhan, Mehmet Yazıcı

[1]Erzincan Binali Yıldırım University, School of Physical Education and Sports, Department of Sports Management, Erzincan Turkey - Email: ycakmak@erzincan.edu.tr; [2]Erzincan Binali Yıldırım University, School of Physical Education and Sports, Department of Sports Management, Erzincan Turkey

Summary. The study aims to investigate students' physical selves, eating attitudes and subjective well-beings besides the predictive status of their physical self and eating attitude in subjective well-being. Method: The research which has been designed on the basis of relational research method was conducted with 267 participants from physical education and sports schools. The data of the research consist of participants' demographic information (the department that they study, grade level, gender), Physical Self Inventory scores, Eating Attitude Test scores, and Subjective Well-Being Scale scores. Descriptive analysis, Pearson Correlation and regression tests have been used for data analysis. Results: As a result of the analyzes, it is found that subjective well-being levels of the participants are moderate, 26.6% are at risk of eating attitude disorder, that the physical self, general self, physical condition and sport competence perceptions of physical self-perception sub-dimensions are moderate, and that physical fitness and physical strength perceptions were low. In addition, it is observed that there is a weak negative correlation between subjective well-being and eating attitude behavior, a weak positive correlation between subjective well-being and general self, physical self, physical condition and physical attraction perceptions, a moderate negative correlation between subjective well-being and physical strength perception, no significant relationship between subjective well-being and sport competence perception, and that eating attitude and physical self-perception are significant predictors of subjective well-being and explain 42% of the total variance.

Key words: subjective well-being, eating attitude, physical self

Introduction

It is possible for a person in contemporary social life to lead a happy and systematic life, to express a meaning in society and to play their social roles duly via a healthy individual and personality development. Nutrition, which forms the basis of being a healthy individual, is an individual's consumption of nutrients that provide each of the energy and nutritional elements necessary for the growth, development and healthy and productive life of the individual in sufficient amounts and their usage in the body in the most economical way without losing its nutritional value or making it health-impairing (1). Nutrition is an essential requirement for the development of the body, maintenance of life and protection of health. Adequate and necessary intake of all nutrients required for the growth of the body, regeneration and functioning of the tissues and the proper use in the body are defined as adequate and balanced nutrition (2). Although people have enough nutrition, human health is affected negatively since body's needs cannot be adequately met as a result of consuming the same food group frequently or not receiving the right foods, the necessary energy cannot be provided and the body tissues cannot be structured (3). Overeating leading to obesity, refusal to eat, individual's becoming vegetarian or certain eating restrictions due to the psychological causes, eat-

ing things that cannot be categorized as food, disposal of the food digested immediately after being eaten or irresistible behavior of eating at night arise with the situations referred to as imbalanced nutrition or eating disorder (4). It has been observed that there is no single cause of eating disorders. There are approaches that relate the occurrence of eating disorders to problems in the family, the presence of eating disorders in the family, obesity, adolescent problems, acceptance of sexuality, sexual trauma, socio-cultural norms, biological and genetic factors, and low self-esteem (5, 6).

As mentioned above, low self-esteem is one of the factors effective in the formation of eating disorders. Self-worth in eating disorder depends on appearance, body shape and weight (7). When the symptoms of eating disorder (diet, weight loss, and binge eating, vomiting behavior) are evaluated as an effort to increase self-woth, it is very important to consider individuals' eating attitudes in the context of self. Self is the way individuals perceive their own personality (8). There are many definitions about self in the literature. Self is the whole of one's self-evaluations and beliefs about himself (9). In another definition, self is expressed as an object, the totality of the thoughts and feelings that one imposes on themselves. Beane and Lipka (10) defined the concept of self as individuals' perception of various roles and personal characteristics.

Based on the content of the self-concept, as a result of a large number of researches and a consensus on the multi-faceted and hierarchical structure of the self-concept, defining and evaluating individual's thoughts regarding themselves in different living conditions have started to be dealt with (11). Physical self-perception, which is becoming more and more important and starting to be investigating by the researchers more frequently, is a subset of general self-perception. The concept of physical self, general self-esteem, psychological well-being is regarded as a sub-element of health and life. In addition, physical self-perception is the most important element of multifaceted and hierarchical self-perception that is affected by participation in exercise (12-15). Physical self-concept or physical self-perception is important for healthy development, specialization ability and relationship with our physical environment from childhood and is defined to be individual's self-perception and evaluation of the psychomotor dimension (13). It is how the individual perceives and evaluates themselves in motor skills (coordination, sports ability, etc.) and physical fitness parameters (strength, endurance, flexibility, etc.) (16).

Self-esteem is important when studying mental wellbeing because of its close association with emotional stability and adjustment, low self-esteem features in many forms of mental illness and low self-esteem is associated with poor health behaviours. Physical self-esteem has been shown through its associations with measures of emotional adjustment, independent of global self-esteem and socially desirable responding, to have mental wellbeing properties in its own right (17). The influence of physical activity on mental well-being. Like self-perception, physical self-perception is an indicator of psychological health and psychological well-being (18). The concept of psychological well-being expresses positive functionality in one's life. Subjective well-being, expressed as happiness in spoken language, is the evaluation of one's own life. This evaluation is both cognitive (judgments about life satisfaction) and emotional (satisfactory and unsatisfactory emotional responses) (19). Subjective well-being, which is defined as experiencing positive emotions more frequently than negative ones and satisfaction from life (20), is the subjective belief or feeling that life is going well (21). Satisfaction obtained from life expressed in the definition constitutes the cognitive dimension of subjective well-being and includes evaluations of the individual about various living areas (marriage, work, health, success, etc.). The frequency of positive and negative emotions is the affective dimension of subjective well-being. Accordingly, emotions such as joy, enthusiasm, confidence, excitement, interest and gaiety are positive affect; fear, anxiety, guilt, anger, hate and sadness are negative affect (20-23).

When the literature is examined, there are not many studies examining the relationship between these three concepts. Such a study is considered to be more useful in determining some of the factors affecting the subjective well-being levels of the students. In this study, it is aimed to investigate the physical self, eating attitude and subjective well-being levels of students and predicting the subjective well-being levels

of students according to their physical self and eating attitude. In accordance with this purpose, responses to sub-problems below are investigated:

1. What is the level of physical self-perception, subjective well-being and eating attitude of students?
2. Do the students' physical perceptions and eating attitude behaviors predict their subjective well-being statistically significant?

Methods

This research has been designed on the basis of the correlational research method defined as the examination of the correlation between two or more variables without any effect on the variables (24). This method has been preferred in this research because the participants were asked to answer the questions without any effect.

Participants

The research group of the study consists of a total of 267 people studying at the School of Physical Education and Sports. Random sampling method was used to identify the participants. Table 1 shows the distribution of participants by department, grade level and gender.

As can be seen in Table 1, 27.7% (74 people) of the participants are female and 72.3% (193 people) are male. 48.8% (125 people) are in the administration department and 53.2% (147 people) are in the teaching department. 34% (105 people) are 1st grade, 29% (87 people) are 2nd grade, 20% (61 people) are 3rd grade and 17% are 4th grade.

Table 1. Frequency and Percentage Distribution of Demographic Characteristics of Participants

Gender	**n**	**Percentage %**
Female	74	27,7
Male	193	72,3
Total	267	100,0
Department	**n**	**Percentage %**
Administration	125	48,8
Teaching	147	53,2
Grade	**n**	**Percentage %**
Grade 1	105	34
Grade 2	87	29
Grade 3	61	20
Grade 4	52	17

Measures

Four different data have been collected in the study: demographic characteristics (department, class level and gender), physical self-perception, eating attitude habits and subjective well-being levels of the participants. A form was prepared by the researchers to determine the demographic characteristics of the participants.

Physical Self Inventory (PSI)

Data related to the physical self-perception of the participants were collected using the 6-point Likert type Physical Self Inventory (PSI) which was developed by Ninot et al. (2000), adapted to Turkish and whose validity-reliability studies were done by Çağlar et. al. (2017) (25, 26). The physical self-scale consists of 25 items in six sub-dimensions: general self, physical self, physical condition, sport competence, physical strength and physical attraction. In order to determine the reliability of the scale, Cronbach alpha internal consistency coefficients have been calculated in the physical attraction sub-dimension (0.55) = 0.71 in this study; in the general self-concept sub-dimension (0.66) = 0.68 in this study; in the physical condition sub-dimension (0.72) = 0.70; in the physical strength sub-dimension (0.72) = 0.70 in this study; in the physical self-worth sub-dimension (0.80) = 0.71 in this study and in the sports competence sub-dimension (0.89) = 0.69 in this study.

Eating Attitude Test (EAT)

The 6-point Likert-type Eating Attitude Test (EAT), which was developed by Garner and Garfinkel (1979), adapted to Turkish and whose validity-reliability studies were done by Savaşır and Erol (1989), was used to evaluate the disorders in eating behaviors and attitudes of the participants and to measure the symptoms of anorexia nervosa. EAT consists of 40 questions (27, 28). The cut-off point of the scale is 30 points. For items 1, 18, 19, 23, 27, 39 sometimes 1 point, rarely 2 points and never 3 points and other options are evaluated as 0

points. For the other items of the scale, always 3 points, very often 2 points and often 1 point and other options are calculated as 0 points. As a result, the total score of the scale is obtained by adding the scores taken from each item of the scale. In the current study, people who scored ≥30 according to the EAT-40 evaluation scale have been described as "susceptible to eating behavior disorder". The Cronbach's alpha reliability coefficient for the whole inventory was calculated to be (0.83) by Garner and Garfinkel (1979), (0.70) by Savaşır and Erol (1989), and in this research = 0.87.

Subjective Well-Being Scale

Data related to subjective well-being of the participants were developed by Tuzgöl (2004) based on the theories explaining subjective well-being, mainly Diener's (1984) views on subjective well-being (20, 29). The 5-point Likert-type Subjective Well-Being Scale consists of 46 items. The scale includes personal judgments about living spaces and positive and negative emotion expressions. The scores of each item ranged from 5 to 1. 26 of the scale items are negative expressions. Negative expressions are items 2, 4, 6, 10, 13, 15, 17, 19, 21, 24, 26, 28, 30, 32, 35, 37, 38, 40, 43 and 45. Scoring negative expressions is done by reversing. The lowest score that can be obtained from the scale is 46 and the highest score is 230. High score indicates a high level of subjective well-being. Cronbach's alpha reliability coefficient (0.93) of the scale has been found to be = 0.92 in this study.

Ethical Consideration

Ethics committee approval for this study was obtained from Erzincan Binali Yıldırım University Ethical Committee of Scientific Research (Decision number: 2019.05.05-03). We thank all study participants for their willingness to participate.

Statistical Analysis

Data have been analyzed using SPSS version 22. Frequency and percentage values were used to identify the demographic characteristics of the participants (department, grade level, gender).

In the research, firstly, the appropriateness of the data to the normal distribution has been examined when deciding the analysis of the sub-problems. Since Skewness and Kurtosis values are between +2 and -2, it is accepted that the movement distributions are normal (30). In addition, when the Q-Q plot graphs are examined, it can be assumed that the data are normally distributed because all the sub-dimensions and total values are collected on or near the diagonal.

Arithmetic mean and standard deviation techniques have been used to reveal the physical self-perception, eating attitude habits and subjective well-being of the participants. Pearson Correlation Coefficient has been also calculated to determine the level or amount and direction of the correlation between the variables. A simple regression analysis has been used to find out the answer to the question as to whether physical self-perception and eating attitude are a significant predictor of subjective well-being. The significance level of the tests has been taken as .05.

Results

In this section, the findings obtained from the students are given.

Table 2. Descriptive statistics of variables

	n	Min.	Max.	$\bar{x}$	Ss	Skewness	Kurtosis
Subjective Well-being	267	99.00	230.00	171.31	26.08	-.109	-.557
Eating Attitude	267	3.00	85.00	22.88	15.57	1.677	1.986
General Self	267	8.00	30.00	19.03	5.96	.608	-.931
Physical Self	267	5.00	30.00	19.79	4.16	-.343	.252
Physical Condition	267	6.00	49.00	16.97	4.30	1.506	1.406
Sport Competence	267	4.00	24.00	13.52	2.67	.531	1.895
Physical Attractiveness	267	6.00	18.00	10.00	3.18	1.017	.273
Physical Strength	267	3.00	18.00	7.87	3.37	.598	-.380

Table 3. Eating attitude test score percentages of participants

	EAT<30		EAT≥30	
EAT-40	**n**	**%**	**n**	**%**
	204	76,4	63	23,6

As seen in Table 2, subjective well-being levels (=171.31) of the participants have been found to be moderate and eating attitudes (=22.88) are normal eating attitudes according to student perceptions. It has been observed that the physical self (= 19.7general self (= 19.03), physical condition (= 16.97) and sport competence (=13.52) perceptions of physical self-perception sub-dimensions are moderate; physical fitness (=16.97) and physical strength (=7.87) perceptions are low. In addition, as presented in Table 2, when the distribution of the data is examined, it is accepted that the related distributions are normal due to the fact that the sub-dimensions of the inventory and Skewness and Kurtosis values related to the distribution of all data are between +2 and -2 (30). Moreover, when the Q-Q plot graphs are examined, it can be assumed that the data are distributed normally as all the sub-
Dimensions and all the values according to total scores are collected on or near the diagonal.

A score of 30 or higher on the Eating Attitude Test indicates impaired eating behavior. Accordingly, when Table 3 is examined, it is determined that 23.6% of the students have the risk of eating disorder.

As shown in Table 4, it can be observed that there is a weak negative linear correlation between subjective well-being levels and eating attitudes of the participants (r = -. 241, p <0.01); there is a weak positive linear correlation between subjective well-being levels and general self (r = .387, p <0.01), physical self (r = .214, p <0.01), physical condition (r = .242, p <0.01), physical attractiveness (r =. 122, p <0.05); there was a moderate negative linear correlation between subjective well-being levels (r = -600, p <0.01) and physical strength, and there is no significant correlation between subjective well-being levels (r = -103, p <0.01) and perception of sport competence.

In Table 5, when the regression results between eating attitude and physical self-perception as the predictive variables and participants' subjective well-being levels as the predicted variables are examined, it is observed that there is a negative weak correlation between eating attitude and subjective well-being of participants (r = -. 24). And when other variables are controlled, this correlation is calculated as (r = -. 02). It is clear that there is a positive moderate (r =

Table 4. Results of correlation analysis according to the views of the participants

		1	2	3	4	5	6	7	8
1. Subjective Well-being	r	1	-.241**	.387**	.214**	.242**	-.103	.122*	-.600**
	p		.000	.000	.000	.000	.047	.023	.000
2. Eating Attitude	r			.017	.109	.092	.231**	-.006	.449**
	p			.389	.038	.066	.000	.464	.000
3. General Self	r				.370**	.454**	.095	.516**	-.320**
	p				.000	.000	.061	.000	.000
4. Physical Self	r					.411**	.366**	.242**	-.041
	p					.000	.000	.000	.254
5. Physical Condition	r						.159**	.281**	-.135*
	p						.005	.000	.014
6. Sport Competence	r							.031	.284**
	p							309	.000
7. Physical Attractiveness	r								-.151*
	p								.007
8. Physical Strength	r								1
	p								

***p<0.01, *p<0.05*

Table 5: Regression analysis results regarding the predictive status of participants' subjective well-being of eating attitudes and their physical self-perceptions.

Variable	B	Standard Error	β	t	p	Binary r	Partial r
Constant	178.390	8.850		20.157	.000		
Eating Attitude	-.022	.046	-.026	-491	.624	-.241	-.023
General Self	.833	.277	.202	3.189	.002	.387	.150
Physical Self	.806	.353	.129	2.287	.023	.214	.107
Physical Condition	.390	.336	.064	1.162	.246	.242	.055
Sport Competence	-.215	.524	-.022	-.411	.681	-.103	-.019
Physical Attractiveness	-.896	.451	-.109	-1.985	.048	.122	-.093
Physical Strength	-4.024	.454	-.520	-8.855	.000	-.600	-.416
R=.655 R^2=.429; F=27.785 p=.000							

.38) relationship between the general self and subjective well-being of the participants, but when the other variables are controlled, the relationship between the two variables is (r = .15). While the correlation between the physical self and subjective well-being of the participants is positive and low (r = .21), it is seen that, when the other variables are controlled, it is again calculated to be positive and low (r=.10). When the relationship between physical fitness and subjective well-being scores of the participants is examined, it is observed that the calculated correlation is positive and low (r = .24). However, when the other variables are controlled, this relationship has again been found to be low positive (r = .05). It is seen that the relationship between sport competence which is another predictor variable and subjective well-being levels of participants is negative and low level (r = -. 10), but this coefficient changes to become (r = -. 01) when other variables are controlled. While there is a positive and low (r = .12) correlation between the scores of physical attraction and subjective well-being of the participants, it is observed that this value is negative and low (r = .09) when other variables are kept under control. There is a negative and high level (r = -. 60) relationship between physical strength and subjective well-being of the participants, but when the other variables are controlled, the relationship between the two variables turn into a moderate and negative (r = -. 41) relationship.

The established regression model shows that the general self which is called the participants' eating attitudes and physical self-perception, physical self, physical condition, sport competence, physical attractiveness, physical strength, and the subjective well-being of the participants form a moderate and significant relationship (R = .65). R2 = .42, p <.001). All independent variables included in the model explain 42% of the total variance related to subjective well-being levels of the participants that are dependent variables. This signifies that the 48% change in subjective well-being scores can be explained by different variables not included in the regression model. According to the standardized regression coefficients (β), the order of relative significance of the predictive variables on the subjective well-being levels of the participants is as follows: physical strength, general self, physical self, physical attraction, physical condition, eating attitude and sport competence.

When the results of the t-test regarding the significance of regression coefficients are examined, it is seen that the general self-perception (t = 3.189, p <.005) and physical self-perception (t = 2.287, p <.005), which are one of the predictive variables, are important predictors in analyzing the subjective well-being scores of the participants. Accordingly, considering the positive relationship direction, it is possible to claim that the increase in the general self and physical self-perceptions positively affected the increase in the scores of the participants' subjective well-being levels. In addition, according to the t-test results related to the significance of regression coefficients, physical attractiveness (t = -1.985, p <.005) and physical force perceptions (t = -8.855, p <.005), which are the predictive variables, are an important predictive in explaining the scores related to subjective well-being of the par-

ticipants. Accordingly, considering the negative aspect of the relationship, it can be said that the increase in physical attraction and physical strength perceptions adversely affect the increase in subjective well-being scores of the participants.

Discussion

In the current research, physical self-perception, eating attitude behaviors and subjective well-being levels of students, the predictive aspects of physical self-perception and eating attitudes regarding their subjective well-being levels have been investigated. The findings of the research have been discussed and supported by the literature.

The subjective well-being levels of the participants have been found to be moderate. In the literature, subjective well-being levels of students are parallel to this study (31-35).

In this study, it was found that the mean eating attitude of the participants are 22.88 and 26.6% are at risk of eating attitude disorder. In the studies conducted, it has been seen that the mean scores of eating attitude of the students are close to the findings of this study (36-40).

The physical self, general self, physical condition and sport competence perceptions of the physical self-perception sub-dimensions have been found to be moderate, while physical condition and physical strength perceptions are low. Crocker et al. (2000) reported that physical condition and sport competence perceptions of the physical self perception sub dimensions have been found to be high in adolescence. Raustorp et al. found that (2004) physical self-worth, sport competence, body attractiveness, physical strength and physical condition were moderate (41). In the study conducted by Makar (42), it was found that the levels of physical self-perception of the students participating in the study had normal values. In the study of Pehlivan (43), it was specified that teacher candidates' sports ability, physical strength, flexibility and appearance were high. In their study, Kılıçarslan (44) found that physical education teachers have a high level of physical self-perception. It was seen that the students participating in the study had normal values in terms of their physical self-perception levels. It can be said that the differences in the sub-dimensions of physical self-perception between other studies and this one are due to the gender variable, the different physical activity levels of individuals, and the individual's self-perception at the psychomotor level (45-49).

In the study, it has been specified that there is a weak negative relationship between subjective well-being and eating attitudes of the participants, a weak positive relationship between subjective well-being levels and general self, physical self, physical condition and physical attraction perceptions, a moderate negative correlation between subjective well-being level and physical strength perception, and it has been found that there is no significant relationship between subjective well-being level and sport competence perception. In addition, it has been found that eating attitude and physical self (general self, physical self, physical condition, sporting competence, physical attraction, physical strength) explain 42% of total variance in which subjective well-being is a significant predictor.

When the literature is researched, it is seen that there are negative and significant relationships between eating attitude behavior and subjective well-being (35, 50-53). The reason for this is that the high level of subjective well-being of university students can lead to the development of healthy emotion-thought and behavior, and accordingly, the incidence of psychopathological symptoms may decrease. From this point of view, the incidence of problems related to eating attitude can decrease with increasing subjective well-being. In addition, the study result can be supported from a different point. There are also studies in the literature that reveal positively significant relationships between eating attitude disorder and depression (54-57).

Edwars et. al. (58) data analysis revealed moderate positive correlations within and between the two scales (physical self perception and well-being), supporting the conceptualisation that physical self-perception is a subsystem of the more general construct of psychological well-being. Significant relationships were found between psychological well-being and self-acceptance, sport competence, conditioning, conditioning importance, and body importance. The results generally indicated the two scales were moderately positively cor-

related. In addition, there are studies in the literature that reveal significant relationships between physical self-perception / self-perception and subjective well-being / psychological well-being (20,59-67).

In the literature, there are very limited studies investigating the relationship between these three concepts (eating attitude, physical self and subjective well-being). Interviews confirmed that those showing abnormal eating behavior in the questionnaires did indeed show greater eating pathology as well as lower self esteem. In their study, Oktan and Palancı (51) find that age, body image and eating attitude are identified as significant precursors of subjective happiness and it is determined that the model explains 17% of total variance with regard to subjective happiness. In the study that Hwang at all (68) conducted, female students' eating attitudes are negatively associated with parental attachment and self-esteem, but positively with a depressive mood. In their study Button et. al. (69) found that abnormal eating behavior of 15-16 year old girls in the questionnaires did indeed show greater eating pathology as well as lower self esteem and interviews also revealed that those with high levels of eating concern showed greater levels of global self dissatisfaction and higher dissatisfaction with their physical appearance. The results are similar.

Conclusion

In this study, it is revealed that physical self-perceptions and eating attitude habits are effective in individuals having more positive emotions, less negative emotions, and feeling satisfied with their lives. As the subjective well-being of individuals decreased, they are more prone to be at risk of eating behavior disorders, and the better physical condition they feel they have and the higher their body perceptions, the happier they are. It is considered that the physical activity levels and the physical self-perception of the students studying in the sports education department are better than the students studying in another department. In this regard, it is recommended that this study should be conducted for students studying in other departments and for people of middle age and above who have a lower level of physical activity.

References

1. Yücecan S. Changes in food consumption and new trends: Turkish culinary culture research and promotion foundation 1999;23:235-244.
2. Baysal A, General nutrition. Ankara: Hatipoğlu Publisher;1997.
3. Seçken N, Morgil İ. About views about healthy nutrition by using draw and write technique. Hacettepe University Journal of Education 2000;18:123-127.
4. Tam C, Cherry FN, Chak MY. Disordered eating attitudes and behaviours among adolescent in Hong Kong: prevalence and correlates. Journal of Pediatrics Child Health 2007;47:811-817.
5. Bulik CM, Reba L, Siega-Riz AM, Reichborn-Kjennerud T. Anorexia nervosa: Definition, epidemiology, and cycle of risk, Int J Eat Dis 2005;37:2-9.
6. Çelikel FC, Cumurcu BE, Koç M, Etikan I, Yücel B. Psychologic correlates of eating attitudes in Turkish female college students. Compr Psychiatry 2008;49(2):188-94.
7. Fairburn CG, Harrison PJ. Eating disorders. The Lancet 2003; 361:407-416.
8. Cüceoğlu D. Human and behavior. İstanbul: Remzi Publisher;1993.
9. Burn R. Self-concept development and education. New York: Holt, Rinehart and Winston:1982.
10. Beane IA, Lipka RP. Self-concept, self-esteem and the curriculum. Newton, Mass: Allyn and Bacon, Inc.:1984.
11. Aşçı FH. Self-perception and exercise. Hacettepe J. of Sport Sciences 2004;15 (4):233-266.
12. Fox KR. Physical Education and the development of self-esteem in children. In N. Armstrong. (Edit.). New Directions in Physical Education Towards a National Curricuium. (sf. 33-54). Champaign: Human Kinetics,1990.
13. Fox KR. The physical self-perception profile. DeKalb, IL: Office for Health Promotion, Northern Illinois University,1990.
14. Fox KR. The physical self: from motivation to well-being. Champaign, IL: Human Kinetics:1997.
15. Fox KR. Physical activity and mental health promotion: the natural partnership. International Journal of Mental Health Promotion 2000;2: 4-12.
16. Aşçı FH. Comparison of physical self-perception with regard to gender and physical actlvity level. Spor Bilimleri Dergisi 2004;15 (1):39-48.
17. Fox KR. The influence of physical activity on mental well-being, Public Health Nutrition 1999;2(3a):411–418.
18. Edwards DJ, Edwards SD, Basson CJ. Psychological well-being and physical self-esteem in sport and exercise. Int J Ment Health Promot 2004;6:25–32.
19. Diener E, Diener C. Most people are happy. Psychological Science 1996;7:181-185
20. Diener E. Subjective Well-Being. Psychological Bulletin 1984;95 (3):542-575.
21. Diener E. Lucas RE. Personality and subjective well-being across the life span. Temperament and personality develop-

ment across the life span 2000;19:221-244.
22. Argyle M, Martin M, Crossland J. Happiness as a function of personality and social encounters. InJ .P .Forgas, & J .M .Innes (Eds .),Recent advances in social psychology: an international perspective(pp .189–203);1989: North-Holland: Elsevier.
23. Lyubomirsky S. The How of happiness: A scientific approach to getting the life you want. The Penguin Press:NY,2007.
24. Fraenkel JR, Wallen NE. How to design and evaluate research in education. Mc Graw Hill Higher Education. New York:NY,2006.
25. Ninot G, Delignières D, Fortes M. L'évaluation de l'estime de soi dans le domaine corporel [Assess-ment of the physical self]. Sciences et Techniques des Activit es Physiques et Sportives 2000;53:35-48.
26. Çağlar A, Aşçı F, Bilgili N. Psychometric properties of physical self inventory-Turkish version among university students. Anatolian Journal of Psychiatry 2017;18(6):594-601.
27. Garner DM, Garfinkel PE. The eating attitudes test: An index of the symptoms of anorexia nervosa. Psychological Medicine 1979;9:273-279.
28. Savaşır I, Erol N. Eating attitude test: anorexia neurosis symptoms ındex. Turkish Journal of Psychology 1989;7:19-25.
29. Tuzgöl-Dost M. Developing a Subjective well-being scale: validity and reliability studies. Turkish Psychological Counseling and Guidance Journal 2005;23:103-111.
30. DeCarlo LT. On the Meaning and Use of Kurtosis. Psychological Methods 1997;2(3):292-307.
31. Fujita F, Diener E, Sandvik E. Gender differences in negative affect and well-being: the case for emotional intensity. J Pers Soc Psychol. 1991;61(3):427-34.
32. Kermen U. The analysis of the concept of need satisfaction level and subjective well-being level on university students. (Master Thesis). Abant İzzet Baysal University, Bolu, 2013.
33. ahin GN. Comparison of levels pertain to self disclosure, subjective well-being and perceived social support of university students. (Master Thesis). Dokuz Eylül University, İzmir, 2011.
34. Gündoğdu R, Yavuzer Y. Examining subjective well-being and psychological needs of students at the educational faculty according to demographic variables. Mehmet Akif Ersoy University Journal of Education Faculty 2012;12(23):115-131.
35. Buhsi B. Examination of levels in eating attitude anxiety and subjective well-being among university students. (Master Thesis) Beykent University, İstanbul, 2016.
36. Russell S, Ryder S. BRIDGE (Building the relation- ships between body image and disordered eating graph and explanation): A tool for parents and professionals. Eating Disorders: The Journal of Treatment and Prevention 2001;9:1-14.
37. Ünalan D, Öztop DB, Elmalı F, Öztürk A, Konak D, Pırlak B, Güneş D. Eating attitudes and healthy lifestyle behaviors of a group of health school students. İnönü University Journal of Medical Faculty 2009;16:75-81.
38. Kadıoğlu M, Ergün A. The eating attitudes of the university students, self-efficacy and affecting factors. Marmara University Institute of Health Sciences Journal. 2015; 5(2): 96-104.
39. Akdevelioğlu Y, Yörüsün TÖ, Investigation of some factors related to eating attitudes and behaviors of unıversity students. Gazi Journal of Health Sciences 2019;4(1):19-28
40. İlhan MN, Özkan S, Aksakal FN, Aslan S, Durukan E, Maral I. Prevalence of potential eating disorders among the students of a medical school. Psychiatry in Turkey 2006;8(3): 151-155.
41. Crocker PRE, Eklund R, Kowalski KC. Children's physical activity and physical self-perceptions. J Sports Sci. 2000;18:383–394.
42. Makar E. Determine of relationship between social skill, physical self-perceptions and physical activity level of sports education students. (Master Thesis). Bartın University Bartın, 2016.
43. Pehlivan Z. Analysis of physical self-perceptions of physical education teacher candidates and their attitudes toward teaching profession. Education and Science 2010; 35(156):126-141.
44. Kılıçarslan E. The comparison of physical personality perceptions between physical education teachers and the other branches. (Master Thesis). Sakarya University, Sakarya, 2006.
45. Fox KR, Corbin C. The physical self-perception profile: Development and preliminary validation. Journal of Sport & Exercise Psychology 1989;11:408–430.
46. Hayes SD, Crocker PRE, Kowalski KC. Gender differences in physical self-perceptions, global self-esteem and physical activity: Evaluation of the physical self-perception profile model. Journal of Sport Behavior 1999;22: 1–14.
47. Sonstroem RJ, Speliotis ED, Fava JL. Perceived physical competence in adults: An examination of the Physical Self-Perception Profile. Journal of Sport and Exercise Psychology 1992;14:207-221.
48. Whitehead JR. A study of children's physical self-perceptions using an adapted physical self-perception questionnaire. Pediatric Exercise Science 1995;7:133–51.
49. Biddle SJH, Page A, Ashford B, Jennings D, Brooke R, Fox K. Assessment of chil-dren's physical self-perceptions. International Journal of Adolescence and Youth 1993;4:93–109.
50. Hazzouri LE. The relationship between attachment anxiety and avoidance, objective physical attractiveness, maladaptive eating and subjective well-being: a path analysis. Submitted to the Faculty of Marywood University in Partial Fulfillment of the Requirements for the Degree of Doctorate in Clinical Psychology United States Code. (2008).
51. Oktan V, Palancı M. Body image, subjective happiness and eating attitudes among 19 to 63 years old Turkish women. The Journal of Happiness & Well-Being 2018;6(2):114-124.
52. Ouwens MA, Van Strien T, Van Leeuwe JFJ. Possible pathways between depression, emotional and external eating. A

structural equation model. Appetite 2009;53:245-248.
53. Konu AI, Lintonen TP, Rimpela MK. Factors associated withschoolchildren's general subjective well-being,Health Education Research 2002; 17:155–165.
54. Godart NT, Perdereau F, Rein Z, Berthoz S, Wallier J, Jeammet P, et al. Comorbidity studies of eating disorders and mood disorders. Critical review of the literature. J Affect Disord 2007; 97:37-49.
55. Holtkamp K, Muller B, Heussen N, Remschmidt H, Herpetz-Dahlmann B. Depression, anxiety, and obsessionality in long term recovered patients with adolescent-onset anorexia nervosa. Eur Child Adolesc Psychiatry 2005; 14:106-110.
56. Garfinkel PE, Kennedy SH, Kaplan AS. Views on classification and diagnosis of eating disorders. Can J Psychiatry 1995; 40:445-456.
57. McGee BJ, Hewitt PL, Sherry SB, Parkin M. Flett GL. Perfectionist self-presentation body image, and eating disorder symptoms. Body Image 2005; 2:29-40.
58. Edwards SD, Palavar K, Ngcobo HSB, Edwards DJ. Exploring the relationship between physical activity, psychological well-being and physical self-perception in different exercise groups. South African Journal for Research in Sport, Physical Education and Recreation 2005; 27(1):59-74.
59. Abbasi A, Zubair A. Body image, self-compassion and phychological well being among university students. Pakistan Journal of Social and Clinical Psychology 2015;13(1):41-47.
60. Oktan V. The predictive powerof body image and rejection sensitivity on subjective well-being. Educational Sciences and Practice 2012;11(22):119-134.
61. Baumeister RF, Campbell JD, Krueger JI, Vohs KD. Does high self-esteem cause better performance, interpersonal success, happiness, or healthier lifestyles? Psychological Science 2003;4:1–44.
62. Kong F, Zhao J, You X. Self-esteem as mediator and moderator of the relationship between social support and subjective well-being among Chinese university students. Social Indicators Research 2013; 112(1):151-161.
63. Karatzias A, Chouliara Z, Power K, Swanson V. Predicting general well-being from self-esteem and affectivity: an exploratory study with Scottish adolescents.Quality of Life Research 2006;15(7):1143-1151.
64. Padhy M, Rana S, Mishra M. (2011). Self-esteem and subjective wellbeing: Correlates of academic achievement of students. Research Journal of Social Science & Management 2011;1(7):148-156.
65. Fennell M. Low self-esteem: A cognitive perspective. Behavioral and Cognitive Psychotherapy 1997;25:1–25.
66. Karacam A, Pullur A. Academic optimism, psychological well-being and physcal self esteem as a predictor of success perceptions of physical education teachers. Kastamonu Education Journal 2019;27(1):209-218.
67. Stokes R, Frederick-Recascino C. Women's perceived body image: Relations with personal happiness. Journal of Women & Aging 2003;15:17-29.
68. Hwang MJ, Chung, HJ, Lee EA. Relationships parent-child relationships and psychological well-being with eating attitude of female adolescents. Journal of Korean Home Management Association 2008; 26(4):103-117.
69. Button EJ, Loan P, Davies J, Sonuga-Barke EJS. (1997). Self-esteem, eating problems, and psychological well-being in a cohort of schoolgirls aged 15-16: A questionnaire and interview study. International Journal of Eating Disorders 1997;21(1):39–47. doi:10.1002/(sici)1098-108x(199701)21:1<39::aid-eat5>3.0.co;2-4

Correspondence:
Yasemin Çakmak Yıldızhan
Erzincan University, School of Physical Education and Sports,
Erzincan -Turkey
Tel: +90 04462240020
Fax: +90 04462240024
E-mails: ycakmak@erzincan.edu.tr; ycakmakyildizhan@gmai.com

Progress in Nutrition 2020; Vol. 22, N. 1: 177-184 DOI: 10.23751/pn.v22i1.8846

Orignal article

The relationship between serum leptin and VO2max levels in pre-puberty swimmer girls: effect of acute exercise

Esin Güllü[1], Abdullah Güllü[1], Halil Düzova[2], Bilge Özgör[3], Evren Kilinç[4], Faruk Akçinar[5]

[1]Hitit University, Faculty of Sports Sciences, Corum- E-mail: gullu.esn@gmail.com; [2]İnönü University, Faculty of Medicine Department of Physiology, Malatya; [3]İnönü University, Faculty of Medicine Department of Child Health and Diseases, Malatya; [4]Mehmet Ali Aydınlar University, Faculty of Medicine, Department of Biophysics, Istanbul.
[5]İnönü University, Faculty of Sports Sciences, Malatya

Summary. *Objective*: The aim of this study was to determine the relationship between serum leptin (SL) level, which is the sensor of changes in energy intake and consumption, and maximal oxygen consumption (VO_{2max}) level in pre-puberty swimmer girls. *Methods:* Voluntary participants were divided into swimmer group (n: 16) and the control group (n: 15). Bruce protocol was used for acute exercise effect. Body composition, VO_{2max} and SL concentrations of the study group were measured before and after acute exercise. The paired-samples t-test and independent samples t-test were used for intra- and inter-group comparisons. The linear relations between the VO_{2max} and SL levels were determined by Pearson correlation coefficient. The level of significance was used at 0.05. *Results:* There was a significant difference between the SL level and test stage, test duration, HR of test-end, and VO_{2max} variables in both groups ($p<0.05$). There was a high level of negative correlation between VO_{2max} and SL levels in both groups after exercise (SG, $r=-0.63$; $p<0.01$, and CG, $r=-0.60$; $p<0.05$, respectively). *Conclusion:* Acute exercise resulted in decreased SL levels of both groups. It was concluded that regular swimming sports has a positive effect on body composition, VO_{2max}, and SL values of pre-pubertal girls.

Key words: serum leptin, VO_{2max}, body composition, acute exercise, pre-pubertal girl

Introduction

Regular participations in physical activity during adolescence lead to significant changes in hormone concentrations that may affect growth and development. For this reason, having knowledge about specific hormonal and metabolic responses during exercise of children is very important to understand the physiological benefits and potential risks of participation in sports activities on a regular basis (1). In this case, understanding metabolic responses to exercise can help to create better physical activity and nutritional recommendations for children of all ages.

Leptin is one of the hormones consisting of cytokine family and 167 amino acids with various effects on the organism (2). Leptin hormone, which acts as a sensor of changes in energy intake and consumption, secreted by adipocytes may contribute to the long-term control of energy balance and body composition by interaction with receptors in the hypothalamus (3, 4). Also, it plays an important role in the development of fetus, the onset and development of adolescence in children (5) and weight control, energy consumption and nutrient intake (6, 7), in the regulation of neuroendocrine functions (5) has a great effect. Leptin has a short-term and stimulating effect on lipid oxidation in skeletal muscle. Thus, leptin decreases lipid stores in skeletal muscle by increasing fatty acid catabolism (8).

The exercises with sufficient intensity, which may alter the balance of energy consumption, may also lead to changes in leptin levels (8). Because, the exercise is a strong stimulant for the secretion of many hormones and has been suggested to also affect serum leptin (SL) levels (9). Exercise doesn't only increase energy

consumption, but also decrease the fatty mass. Since leptin hormone increases the energy consumption, it is ensured the increasing of energy consumptions during exercise, and so the fat mass also decreases. Several studies that have evaluated the effects of exercise on leptin are based on this fact (10), and so exercise can decrease SL levels (11).

Maximum oxygen consumption (VO_{2max}), which is a criterion of cardio-respiratory development, is the most reliable test to determine the maximum aerobic capacity. If someone can consume high amounts of oxygen over a unit time, this means that this person has a high aerobic capacity. That is, there is a high correlation between the maximum aerobic capacity and the ability to sustain severe effort (12). During the high-intensity exercises, fats are metabolized by hydrolysis, and so provide energy. Therefore, many studies show that fat oxidation increases significantly during exercises that are performed at the level of 85% of VO_{2max} (13, 14).

Findings showing the increasing effects of leptin on food intake and energy metabolism have led to the need to investigate the relationships between leptin levels and exercise (15). But unfortunately, despite the potential importance of pediatric exercise metabolism, a limited number of studies are currently available on this topic (1, 9). On the other hand, there are almost no studies that investigate the effect of acute exercise on SL levels of the pre-puberty girls. In conclusion, the aim of this study was to investigate the relationship between serum leptin concentrations and VO_{2max} levels of acute exercise that applied to pre-puberty girls who regularly practice swimming sports.

Materials and Methods

Ethical Considerations

Prior to the research, this study was approved by the Clinical Research Ethical Committee of the Inonu University Faculty of Medicine. The purpose and possible risks of the study were explained to the parents of the intervention group. The informed voluntary parental consent and acceptance forms prepared according to the Helsinki Declaration were read and signed by each parents. All candidates that agreed voluntarily participate in the study were included in the study after going through the required medical examinations.

The Study Group

The study group consisted of 31 healthy pre-puberty girls. The swimmer group (SG) consisted of 16 licensed swimmers who applied training for 90 minutes a day, 6-day a week at a local swimming club. The mean age of SG was 9.88±1.41 years, the height was 140.38±9.75 cm, the weight was 35.66±9.71 kg and the sport age was 26.31±10.22 months. Also, 15 healthy and sedentary girls participated in this research as control group (CG) that the mean age was 9.73±1.16 years, the height was 142.93±10.33 cm, and the weight was 41.20±10.63 kg. Both of the groups were living in the same city, and they came from similar socioeconomic backgrounds.

Study Procedures

Care was taken to ensure that both groups did not participate in any intensive exercise or activity until 48 hours before the study. In the evening before the study, the last meal and fluid consumption was asked to be terminated at 08.00 pm. Children with fasting were called to the physiology laboratory at 8.00 am with their parents and they were informed about the study. The participants were given a light and standard breakfast of 500 kcal (1.5 cheddar cheese toast, 1 egg, and 1 cup of tea with sugar) prepared earlier (16, 17). Participants were shown a movie until 10.30 am (start time of the study) and after the brief information were made again, the study started.

Concurrent Variables

The anthropometric measurements of the intervention groups were taken as shorts, t-shirts and bare feet. A stadiometer (Harpenden, Holtain Ltd. UK) with a precision of ±1 mm was used for height measurement. Body mass index (BMI), which is considered a better index for evaluating adiposity in children, was calculated using the ($Kg/m^{2.88}$) formula for corrected BMI (18). Body weight, basal metabolic rate (BMR), body fat percentage (BFP) and body fat mass (BFM) were determined by bioelectrical impedance analysis (Tanita BC-418 MA Professional, Japan). The adolescent maturity level of the subjects was determined by

a pediatric specialist through the evaluation of pubic hair according to the Tanner (sexual maturity) scale ((Tanner Stage I/II/III/IV), the swimmer group was 7/4/2/3 (n=16), and control group was 4/6/1/4 (n=15), respectively) (19).

Acute Exercise Test Protocol

The Bruce test protocol, performed in a laboratory environment and on a computer-controlled treadmill (Cosmed T-150, Italy), was considered an acute exercise program. Every subject started the exercise with a 5-minute warm-up jogging at 0% slope, and subsequently the automatic protocol that was loaded in the treadmill was started. The heart rate (HR) was controlled using the (95% x 220 bpm – Age) formula (20), and it was monitored with a portable pulse meter (Polar S800i, Finland). The test was ended when the observed exhaustion against a certain workload (voluntarily fatigue) and according to the Borg scale criteria (21). The test ending stage, test duration, and ending HR values were recorded for each subject. VO_{2max} capacities of the subjects were determined using the (VO_{2max} (mL/kg/min) = 4.38 × Time (min) – 3.9) formula (22).

Collection of Blood Samples

7 ml of forearm venous blood samples were taken from every subject before and 3 minutes after the acute exercise protocol. The collected blood samples were centrifuged for obtaining serum samples, and then they were stored at -80˚C for further study. The SL levels before acute exercise (SL1) and the SL levels after acute exercise (SL2) were determined by Elisa Kit (Boster Biological Technology Co. CA, USA).

Statistical Analysis

The descriptive statistics of the data were calculated and presented in the form of mean and standard deviation (X±SD) in the text. The normality of the variables was tested with the Shapiro-Wilk test and they were found to have parametric distributions ($p>0.05$). Thus, the independent samples t-test was used for the evaluation of the variables between groups. The paired samples t-test was used for the intra-group comparison of the VO_{2max} and the SL values. The relationship between VO_{2max} and SL levels was tested with the Pearson's simple linear correlation (r) test. The statistical analyses were conducted with the IBM SPSS 25.0 package program, and the level of significance was accepted as $p<0.05$.

Results

In this study, the physical, anthropometric, metabolic and physiological responses of the pre-pubertal swimmer girl group (SG; n: 16) and sedentary girl group (CG; n: 15) to acute exercise were examined. The results of obtained data before and after acute exercise of the research group are given below in tabular forms.

As a result of the research, it was observed that both groups were in the weak category when the corrected BMI values for the children were examined, and they were in stage II, according to Tanner's sexual maturity grading. For this reason, the mean of the physical, anthropometric, and sexual maturity variables of both groups were similar, since there was no significant difference between the age, height, weight, BMI, BMR, BFP and LBM values of the research group (Table 1; $p>0.05$).

Discussion

This study is based on the relationship between the acute exercise protocol and leptin VO_{2max} by comparing two groups of healthy pre-puberty girls (swimmers and sedentary). It was observed that there was no significant difference between the pubertal levels of the 31 girls who were taken into the study as they were in stage II of the Tanner (sexual maturity) scale. This can be explained by the fact that there are no hormonal sex-dependent changes in the early ages (23), and that the two groups were physically and anthropometrically similar. It was observed that there were a significant relationship and a direct correlation between the acute exercise (Bruce) protocol ending stages and duration. When the corrected BMI ($Kg/m^{2.88}$) results – which is considered a better index for the evaluation of adiposity of children (18) – it was determined that the both groups were healthy and non-obese.

Table 1: Descriptive statistics and inter-group comparisons of the research group

	Swimmer Group (SG, n:16)		Control Group (CG, n:15)			
Variable	**X**	**SD**	**X**	**SD**	**t**	**p**
Age (year)	9,88	1,41	9,73	1,16	0,304	0,763
Height (cm)	140,38	9,75	142,93	10,33	-0,709	0,484
Body mass (kg)	35,66	9,71	41,20	10,63	-1,517	0,140
BMI (kg/m$^{2.88}$)	13,31	2,56	14,58	2,64	-1,365	0,183
BMR (kcal)	1150	136,13	1222	147,75	-1,412	0,169
BFP (%)	23,36	6,28	26,87	6,48	-1,529	0,137
BFM (kg)	8,78	4,60	11,59	5,15	-1,607	0,119
Test ending stage	4,38	0,89	3,73	0,70	2,224	0,034*
Test duration (min)	12,88	2,67	10,31	2,05	2,977	0,006*
Ending HR (bpm)	203,44	7,47	197,53	4,79	2,636	0,014*
VO_{2max} (ml/kg/min)	52,49	11,71	40,98	9,10	3,040	0,005*
SL1 (ng/mL)	11,67	8,17	14,82	9,86	-0,971	0,339
SL2 (ng/mL)	8,53	5,55	12,20	9,22	-1,354	0,186
Sports age (months)	26,31	10,22				
Tanner stage1/2/3/4	7/4/2/3 (X±SD: 2,06±1,18)		4/6/1/4 (X±SD: 2,33±1,18)			

**p<0.05; X±SD: mean±standard deviation; SL1: SL level before acute exercise; SL2: SL level after acute exercise.*

The VO_{2max} values of the subjects after the acute exercise protocol, together with the other intra-group variables, were found to be strongly correlated with the test ending stage and test duration (p<0.01; Table 3). The HR and VO_{2max} values of the swimmer and control groups were found to be similar with results of other studies (24, 25). The inter-group comparisons revealed that the SG had better HR and $VO2_{max}$ values according to CG (p<0.05; Table 2). The inter-group comparisons revealed that the SG had better HR and VO_{2max} values (p<0.05; Table 2). This result was not a surprise. Because, swimming raises heart rate and respiratory frequency, and normally provides increased blood flow to the skeletal muscles while increasing the blood pressure (26). Thus, the chronic effect of regular training (26.31±10.22 months) is decreased HR and increased heart beat volume (27), which may have led to the significant improvement of the HR values in favor of SG. The increased VO_{2max} can depend on the frequency, intensity and the duration of the training, which can provide 5-30% increase (28, 29). Therefore, it can be said that regular swimming practices positively affect the VO_{2max} levels of the swimmer subjects.

It was observed that there was a highly linear correlation between the SL concentrations and BMI of

Table 2: Intra-group SL1 and SL2 levels of the research group

Group	Variable	X	SD	t	p
SG (n:16)	SL1 – SL2 (ng/mL)	3,14	3,66	3,43	0,004*
CG (n:15)	SL1 – SL2 (ng/mL)	2,62	4,60	2,21	0,045*

**p<0.05; SL1: SL level before acute exercise; SL2: SL level after acute exercise.*

Table 3: Intra-group correlation between VO_{2max} and other variables of the research group

Variable	SG (n:16)	CG (n:15)
Age (year)	0,04	-0,12
Height (cm)	-0,20	-0,07
Body mass (kg)	-0,45	-0,33
BMI (kg/m$^{2.88}$)	-0,42	-0,48
BMR (kcal)	-0,42	-0,274
BFP (%)	-0,43	-0,48
BFM (kg)	-0,48	-0,39
Test ending stage	0,97**	0,83**
Test duration (min)	1,00**	0,99**
Ending HR (bpm)	0,36	-0,06
SL1 (ng/mL)	-0,54*	-0,53*
SL2 (ng/mL)	-0,63**	-0,60*

***p<0.01; *p<0.05; SL1: SL level before acute exercise; SL2: SL level after acute exercise.*

the research group (p<0.01; Table 4). This suggests that the leptin levels were influenced by the adipose tissue that is increasing with age (23), other than the logical differences in BMIs that caused by young ages. While energy consumption increases with exercise, fat mass decreases accordingly. Since leptin increases energy consumption (10), thus, the leptin hormone can also explain the high level of correlation between the SL levels and the BMR, body fat percentage, and body fat mass variables of both groups (p<0.01; Table 4). In addition, it was observed that the negative moderate correlation between the test ending stage and test duration of the acute exercise and the SL levels was in the favor of the SG (p<0.01; p<0.05; Table 4). This may be the result of the CG's shorter exercise time and conclusion of the exercise in easier phases of the treadmill program (30), despite encouragement and psychological support.

The SL1 and SL2 levels were not significantly different between the two groups (p>0.05; Table 1). This may be because SL levels are stable among girls aged 9 to 11 (31). But also, a significant decrease was revealed in intra-group comparisons (p<0.05; Table 2). This may be because of the fact that chronic exercise often decreases leptin levels (32-35). Thus, the energy consumption increases with exercise while the fatty mass decreases, and subsequently, the leptin hormone contributes to the energy metabolism (32-34). This is due to key role of leptin in energy homeostasis and adipocyte secretion (36). The decreased BMI and fatty mass that results from long-term exercise can decrease leptin concentration (32-34). However, while strenuous exercises cause a decrease in leptin levels in men and women (37), a decrease in plasma leptin levels may be observed after acute exercise of 30 minutes (at 50% VO_{2max}) (38). In addition, 41 minutes or shorter exercises, if they are exhausting enough to consume, may change the concentrations of SL due to their effects on fatty acid partitioning in muscle cells (36, 39). These data support the notion that high-intensity exercises are more effective on SL levels. On the other hand, the retrieval period of blood sample after the acute exercise session (3 minutes after the protocol) (40) may have caused this decrease in SL levels. In addition, these decreases in SL levels of both groups can be attributed to circadian rhythm or hemoconcentration (41, 42). These findings suggest that exercise-related reductions in leptin may be due to changes in nutrient availability or changes in the nutrient flow in the production and secretion of leptin, the primary site of adipocytes (39).

Table 4: Intra-group correlation between SL levels and other variables of the research group

Variable	SG (n:16)		CG (n:15)	
	SL1 (ng/mL)	SL2 (ng/mL)	SL1 (ng/mL)	SL2 (ng/mL)
Age (year)	0,02	-0,05	0,32	0,08
Height (cm)	0,21	0,12	0,50	0,25
Body mass (kg)	0,71**	0,69**	0,85**	0,68**
BMI ($kg/m^{2.88}$)	0,77**	0,85**	0,67**	0,72**
BMR (kcal)	0,68**	0,65**	0,83**	0,63*
BFP (%)	0,74**	0,79**	0,73**	0,74**
BFM (kg)	0,77**	0,78**	0,83**	0,75**
Test ending stage	-0,51*	-0,65**	-0,26	-0,24
Test duration (min)	-0,54*	-0,63**	-0,53*	-0,62*
Ending HR (bpm)	-0,36	-0,34	-0,15	-0,22
VO_{2max} (ml/kg/min)	-0,54*	-0,63**	-0,53*	-0,60*
SL1 (ng/mL)	1	0,93**	1	0,89**

***p<0.01; *p<0.05; SL1: SL level before acute exercise; SL2: SL level after acute exercise.*

It was observed that there was a significant correlation between the SL and VO_{2max} measurements in both groups, both before and after acute exercise (p<0.05; Table 3). There was a moderate negative correlation between VO_{2max} and SL1 levels, and a high inverse relationship with SL2 in both groups. As similar studies had found a negative correlation between VO_{2max} and SL levels (2, 43), these results of current study were consistent with the findings of various studies in this field.

The VO_{2max} considered as a determiner for evaluation of cardiovascular fitness (44), and it is a very suitable test to reflect the type, duration and performance of exercise in a given population (45). The VO_{2max} is measured by exercise tests such as treadmill or cycle ergometer (44), and Bruce test is the most widely used of them. In this study, we observed that there were significant differences in test ending stage, test duration, ending HR and VO_{2max} values after acute exercise. This situation can be interpreted for SG and CGs as a sign of different physical performance. Because, compared to the pre-puberty swimmers, pre-pubertal sedentary

girls required more oxygen and energy for an equivalent workload. Therefore, the CG working in the lesser phase of the treadmill program failed to work hard and relatively quickly exhausted despite the adequate encouragement and psychological support (30). For this reason, despite moderate changes in CG's mean leptin values, some individuals showed a large increase or decrease in leptin levels, while there were no any changes for the others (36). Several factors influence the interpretation of these results. For example, exercise training may produce changes in leptin production and/or lack of leptin, at a given time point, that leptin levels cannot be reflected by a single plasma measurement. Furthermore, there are studies indicating that leptin circulates in free form (possibly in bioactive form) or that it is due to leptin binding proteins and that the ratio of these two forms changes even among weak and obese individuals (46). Because leptin is one of the most important regulators of energy balance (37), and given the important role of fat mass in circulating leptin levels, the role of adipose tissue in the secretion of these hormones can be directly correlated with VO_{2max} (47). Thus, it can be interpreted that individuals who regularly exercise can also decrease serum leptin levels as the VO_{2max} (aerobic) levels increase (43). To date, it has been suggested that different results of the effects of exercise on leptin may be responsible for leptin changes in exercise, fluctuations in food intake, intensity, duration of exercise, and circadian rhythm. Therefore, it can be said that even acute exercises with enough intensity to affect energy balance or body fat mass may change leptin secretion (39). So, an increase in VO_{2max} can be expected as long as exercise can be sustained. Correspondingly, regular and prolonged exercises increase fat metabolism and decrease fatty mass, and also suppress SL levels (2). For these reasons, acute exercises with enough intensity to affect the energy balance or the body fat mass may justify our findings that lead to a decrease in serum leptin levels.

Conclusion

The increases in VO_{2max} during acute exercise were found directly related to BMI, BMR, BFP, BFM, and SL levels. Regular and long-term swimming exercises increased the pre-puberty girls' fat metabolism, and thus reduced the amount of body fat and suppressed serum leptin levels, too. Correspondingly, it can be said that long-term and regular swimming exercises (due to tendency to adapt to chronic exercises) cause a decrease in leptin levels. Therefore, it was observed that swimming sport performed regularly in pre-puberty period of girls had a positive effect on BMI, BFP, BFM, HR, VO_{2max} and SL values. For all these reasons, regular swimming exercises may be recommended for healthy development of pre-puberty girls.

Acknowledgement

This work was supported by Academic Research Projects Unit of Inonu University (Project Grants No. 2012/100), and it was presented as a verbal presentation at the 15th International Sports Science Congress, Antalya/Turkey.

References

1. Riddell MC. The endocrine response and substrate utilization during exercise in children and adolescents. J Appl Physiol. 2008; 105:725-733.
2. Unal M, Unal DO, Baltaci AK, Mogulkoc R, Kayserilioglu A. Investigation of serum leptin levels in professional male football players and healthy sedentary males. Neuro Endocrinology Letters. 2005; 26(2):148-151.
3. Dalğın D, Çon M, Çenesiz M, Çenesiz S. The association of leptin and adiponectin with energy and exercise. Journal of Sports and Performance Researches. 2017; 8(2): 166-174.
4. Sinha MK. Human leptin: the hormone of adipose tissue. Eur J Endocrinol. 1997; 272: 562–566.
5. Matthew WH, Joseph AH.Plasma Leptin and Exercise. Sports Med. 2003; 33 (7): 473-482.
6. Arıkan Ş, Serpek B. The effects of endurance training on the relationships body composition plasma ghrelin and leptin levels. Turkish Journal of Sport and Exercise. 2016; 18 (1): 119-126.
7. Rahmouni K, Haynes WG. Leptin signaling pathways in the central nervous system: interactions between neuropeptide Y and melanocortins. Bioassays. 2001; 23: 1095- 1099
8. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature. 1994; 372: 406-407.
9. Weltman A, Pritzlaff CJ, Wideman L and et al. Intensity of acute exercise does not affect serum leptin concentrations in young men. Med Sci Sports Exerc. 2000; 32(9): 1556-1561.
10. Üçok K, Gökbel H. Egzersizin Leptin Düzeylerine Etkileri [The effects of exercise on leptin concentrations]. Genel Tıp Dergisi, 2004; 14(3): 121-124.

11. Gondim OS, de Camargo VT, Gutierrez FA and et al. Benefits of regular exercise on inflammatory and cardiovascular risk markers in normal weight, overweight and obese adults. PloS One. 2015; 10(10): 1-14.
12. Akgün N. Egzersiz Fizyolojisi [Exercise Physiology]. Ege Üniversitesi Basımevi, İzmir: 2. Press; 1994.
13. Wolfe R R. Fat metabolism in exercise. Adir Exp Bial. 1994; 441: 147-56.
14. Smith T, Smith B, Davis M. Predictors of Physical Fitness in a collage sample. Percept Mat Skills. 2000; 1: 1009-10.
15. Ünal M, Ünal DÖ, Salman F, Baltacı AK, Mogulkoç R. The relation between serum leptin levels and MaxVO2 in male patients with type I diabetes and healthy sedentary males. Endocr Res. 2004; 30: 491- 498.
16. Bouassida A, Chatard J, Chamari K and et al. Effect of energy expenditure and training status on leptin response to submaximal cycling. J Sports Sci Med. 2012; 8: 190–6.
17. Nourshahi M, Hidayeti M, Ranjbar K. The correlation between resting serum leptin and serum angiogenic indices at rest and after submaximal exercise. Regulatory Peptides. 2012; 10; 173 (1-3): 6-12.
18. Rosenthal M, Bain SH, Bush A, Warner JO. Weight/height2.88 as a screening test for obesity or thinness in school age children. Eur J Pediatr. 1994; 153: 876–883.
19. Roberts C. Tanner's Puberty Scale: Exploring the historical entanglements of children, scientific photography and sex. Sexualities. 2016; 19(3):328-346.
20. Fredriksen PM, Ingjer F, Nystad W, Thaulow E. Aerobic endurance testing of children and adolescents-a comparison of two treadmill-protocols. Scand J Med Sci Sports. 1998; 8(4): 203-207.
21. Borg GA. (1982): Psychophysical bases of perceived exertion. Med Sci Sports Exerc. 1982; 14(5):377-381.
22. Mackenzie B. 101 Performance Evaluation Test. London. Electric Word Plc. 2005: 96-117.
23. Garcia-Mayor RV, Andrade MA, Rios M, Lage M, Dieguez C, Casanueva FF. Serum leptin levels in normal children: Relationship to age, gender, body mass index, pituitary-gonadal hormones, and pubertal stage. J Clin Endocrinol Metab. 1997; 82(9): 2849-55.
24. Silva OB, Saraiva LCR, Filho DCS. Treadmill Stress Test in Children and Adolescents: Higher Tolerance on Exertion with Ramp Protocol. Arq Bras Cardiol. 2007; 89(6): 354-359.
25. Kotte EM, De Groot JF, Bongers BC, Winkler AM, Takken T. Validity and Reproducibility of a New Treadmill Protocol: The Fitkids Treadmill Test. Med Sci Sports Exerc. 2015; 47(10): 2241-2247.
26. Israel, RG. Influence of Cardiorespiratory Fitness on Measure of Obesity and Fat Distribution in Man. Med. and Science in Sport and Exercise. 1993; 25 (5): 152.
27. Güllü A, Güllü E. Genel Antrenman Bilgisi: sportif performansı geliştirmenin yolları [General Training Theory: ways to improve sports performance], Umut Matbaacılık, 1. Press, İstanbul. 2001.
28. Hickson RC, Rosenkdetter MA. Reduced training frequencies and maintenance of increased aerobic power. Med. and Science in Sports and Exercise. 1981; 13(1): 13-16.
29. Astandrat PO, Rodahl I. Textbook of Work Physiology, McGraw-Hill Company, N.Y. 1997.
30. Marilisa SF, Cardoso SAL, Yasbek PJr., Faintuch J. Aerobic endurance, energy expenditure, and serum leptin response in obese, sedentary, prepubertal children and adolescents participating in a short-term treadmill protocol. Nutrition. 2004; 20: 900–904.
31. Wang T, Morioka I, Gowa Y and et al. Serum leptin levels in healthy adolescents: Effects of gender and growth. Environmental Health and Preventive Medicine. 2004; 9(2): 41–46.
32. Polak J, Klimcakova E, Moro C and et al. Effect of aerobic training on plasma levels and subcutaneous abdominal adipose tissue gene expression of adiponectin, leptin, interleukin 6, and tumor necrosisfactor alpha in obese women. Metabolism. 2006; 55 (10): 1375-1381.
33. Azizi M. The effect of 8-weeks aerobic exercise training on serum leptin in un-trained females. Procedia–Social and Behavioral Sciences. 2011; 15:1630–1634.
34. Koushki MH, Hamedinia MR, Mollanovruzi A. The response of plasma leptin and some selected hormones to one session of progressive running in non-athlete males. Iranian Journal of Health and Physical Activity. 2012; 3 (1): 50-55.
35. Shahram S, Elham Y, Heshmatolah P, Abdolali B. The effect of intermittent aerobic exercise on serum leptin and insulin resistance index in overweight female students. Annals of Biological Research. 2012; 3 (6): 2636-2641.
36. Pe´russe L, Collier G, Gagnon J and et al. Acute and chronic effects of exercise on leptin levels in humans. J. Appl. Physiol. 1997; 83(1): 5–10.
37. Mota GR, Orsatti FL, Delbin MA, Zanesco A. Resistance exercise improves metabolic parameters and changes adipocyte-derived leptin: a comparison between genders in untrained adults. Motriz. 2016; 22 (3): 217-222.
38. Van Aggel-Leijssen DP, Van Baak MA, Tenenbaum R, Campfield LA, Saris WH. Regulation of average 24 h human plasma leptin level: The influence of exercise and physiological changes in energy balance. Int J Obes Relat Metab Disord. 1999; 23 (2):151-158.
39. Hulver M, Houmard J. Plasma leptin and exercise: recent findings. Sports Med. 2003; 33 (7): 473-482.
40. Elias AN, Pandian MR, Wang L, Suarez E, James N, Wilson AF. Leptin and IGF-I levels in unconditioned male volunteers after short-term exercise. Psychoneuroendocrinology. 2000; 25 (5): 453-461.
41. Kraemer RR, Johnson LG, Haltom R and et al. Serum leptin concentrations in response to acute exercise in postmenopausal women with and without hormone replacement therapy. Proc Soc Exp Biol Med. 1999; 221(3):171-177.
42. Kraemer RR, Acevedo EO, Synovitz LB, Hebert EP, Gimpel T, Castracane VD. Leptin and steroid hormone responses to exercise in adolescent female runners over a 7-week season. Eur J Appl Physiol. 2001; 86(1): 85-91.
43. Kecetepen LO, Dursun N. Effects of exercise on plasma leptin concentrations and relation of leptin to respiratory,

cardiovascular parameters. Journal of Health Sciences. 2006; 15 (1): 1-7.
44. Parveen N, Shahid RA, Riaz L, Shahwar, Shaikh AS. Comparison of mean VO2 max in normal weight, overweight and obese students of a local medical college using analysis of variance. Pakistan Journal of Medical Research, 2019; 58(1): 22-25.
45. Sothern MS. Exercise as a modality in the treatment of childhood obesity. Pediatric Clinics of North America. 2001; 48(4): 995-1015.
46. Sinha MK, I Opentanova J, Ohannesian PJ and et al. Evidence of free and bound leptin in human circulation. Studies in lean and obese subjects during short-term fasting. J. Clin. Invest. 1996; 98 (6): 1277-1282.
47. Moradi F. The relationship between circulating levels of IL-18 and leptin, HsCRP, blood pressure and cardiorespiratory function in obese and lean men. Hormozgan Medical Journal. 2016; 20(4): 233-240.

Correspondence:
Esin Güllü
Hitit University, Faculty of Sports Sciences, Corum
E-mail: gullu.esn@gmail.com

Progress in Nutrition 2020; Vol. 22, N. 1: 185-192 DOI: 10.23751/pn.v22i1.7909

Original article

The fatty acid composition in some economic and wild edible mushrooms in Turkey

Aydin Sukru Bengu

Department of Medical Services and Techniques, Vocational School of Health Services, University of Bingol, Turkey - E-mail: abengu@bingol.edu.tr, aydinbengu75@gmail.com

Summary. The aim of this study was to determine the fatty acid composition of different mushroom species collected from the Yozgat and Tokat region in Turkey. Fatty acid composition was determined in the mushroom species (*Morchella elata*, *Macrolepiota procera, Lactarius delicious, Helvella lacunosa, Boletus edulis, Cantharellus cibarius, Bovista plumbea, Agaricus bisporus)*. The fatty acids copmpsition in mushrooms were identified and quantified by gas chromatography. The amounts % of fatty acids in species were different, Fatty acids with 14-24 carbons were occured in mushrooms samples linoleic acid was the major fatty acid detected in all species linoleic acid respectively in species " *Bovista plumbea, Agaricus bisporus, Macrolepiota procera*" were higher than other mushrooms. Percentage of linoleic acid (-6) in species varied from 10 % to 51 %. The other major fatty acids were respectively, palmitic, stearic, oleic acids other abundant fatty acids in the mushrooms. These four fatty acids were present in all of the mushrooms examined. The high level of docosadienoic acid (22:2) detected in *Morchella elata* is remarkable. When fatty acid composition results are compared as saturated and unsaturated, the fatty acid composition of the mushrooms showed that the unsaturated fatty acids are in higher proportions than the saturated fatty acids.

Key words: edible mushroom, fatty acid composition, GC-MS

Introduction

Fatty acids are the basic building block of many lipids. Polyunsaturated fatty acids such as omega-3 and 6 have many biological properties and are biosynthetic precursors of eicosanoids. Fatty acid levels in blood are closely related to many diseases such as cardiovascular diseases, blood pressure and arthritis (1). These fatty acids act as regulators in cellular functions by participating in the structure of membrane phospholipids and other components.

Two fatty acids are dietary essentials in humans because of our inability to synthesize them: linoleic acid and linolenic acid. Linoleic acid is the precursor of ω-6 arachidonic acid, the substrate for prostaglandin synthesis. α-linolenic acid is the precursor of other ω-3 fatty acids important for growth and development. Plants and mushrooms provide us with the essential fatty acids.

Lack of essential fatty acid and effective metabolism of diet plays an important role in the etiology and progression of many diseases (2).

The consumption of wild mushrooms is increasing with the understanding of human nutrition and pharmacological properties (3). Mushrooms have a low in calories and fats, essential fatty acids, contain vegetable proteins are also important food for human beings due to containing valuable vitamins and minerals (4-11).

Many mushrooms have been used for medical purposes until now to prevent diseases such as hy-

pertension, hypercholesterolemia, atherosclerosis and cancer. The reason for this is the height of the essential unsaturated fatty acid levels (12).

Most researchers have examined the fatty acid composition of various mushroom and have clarified the importance of a diet containing mushroom (3,13,14). Many wild mushrooms have been used since ancient times for medicinal purposes due to their beneficial components and their biological activity. But consumers have been consumed fewer mushrooms to eat because of their relative shortages and some difficulties in the supply. For easier access and commercialization of wild mushrooms, many research groups have focused on the development of artificial production methods using a wide variety of materials and conditions (15).

Most of the studies on fatty acids found in mushroom are limited to certain well-known types of mushroom. However, studies have shown that wild edible mushroom, which are accessible and economically important, contain significant amounts of valuable fatty acids. For this reason, studies on fatty acid contents of all edible mushroom should be made.

Matherials and Methods

The following Table 1 contains information on the characteristics of the mushroom specimens; names, habitat, coordinate, edibility and localite. All samples were collected from of Tokat or Yozgat province in Turkey in 2015. Then the mushrooms were identified. Finally, all samples were dried and ground in appropriate conditions in the laboratory. Prepared samples were stored in the refrigerator until the time of analysis.

Lipid Extraction and Analysis of Fatty Acid

The conventional method of total lipid extraction described by Folch et al. (1957) was used for the dried mushroom. Derivatization of the fatty acids to methyl esters was performed by adding 500 µl of HCSM (hexane/chloroform/sodium methoxide, 75/20/5, v/v/v, Sigma, GC grade) solution to the sample vials (16).

Gas chromatography-mass spectrometry (GC-MS) analyses of the methyl derivatives of fatty acids were performed by Agilent 7890 GC /5970 MS Series gas chromatography system (Agilent, Santa Clara, CA, USA) with a FID and MS and a fused (88% - Cyanopropy) aryl-polysiloxane and high polarity capillary column (HP-88, 100 m x 0.25 mm, 0.20 um film (Part no: 112-88A7, Agilent, Santa Clara , CA, USA) was used injector and detector temperatures were 250 and 270 ˚C, respectively, carrier gas was He at a flow rate of 1.0 mL/min; sample volume 1.0 µL; split ratio 20:1. The detector gas was dry air set at 350 mL min^{-1}, and H_2 gas was set at 35 mL min^{-1}. The detector makeup gas was N_2 at 35 mL min^{-1}. The initial oven temperature was held at 120 ˚C for 5 min, then increased up to 250 °C with 5 °C/min increments and held at this temperature for 16 min. Injection system with auto sampler was used. The injector was rinsed with hexane 5 times before and after each injection to prevent contamination. The relative percentages of separated

Table 1. Information on the Chracteristics of the Mushroom Species

Mushroom samples	Habitat	Coordinates	Edibility	Localite
Morchella elata	In conifers woods	39°34'N-35°58'E	Edible	Yozgat
Macrolepiota procera	Under or near deciduous trees, especially beec or buried wood	39°57'N-35°24'E	Edible	Yozgat
Lactarius delicious	In conifers woods	40°07'N-35°19'E	Edible	Yozgat
Helvella lacunosa	In mixed woodland especially on burnt ground	40°21'N-36°38'E	Edible	Tokat
Boletus edulis	In mixed woods	40°30'N-36°40'E	Edible	Tokat
Cantharellus cibarius	In all kinds of woodland	40°33'N-36°36'E	Edible	Tokat
Bovista plumbea	Amongst short grass, on lawns	39°39'N-35°55'E	Edible	Yozgat
Pleurotus ostreatus	Often in large clusters on stumps and usually of deciduous trees	40°38'N-36°59'E	Edible	Tokat
Agaricus bisporus	On manure heaps, garden waste and roadsides	40°32'N-36°37'E	Edible	Tokat

compounds were calculated by using GC data analysis computer program (FID: flame ionization dedector, MS: mass spectrometry). The results were interpreted by the software recorded in the system.

The fatty acid methyl ester(FAME) results were reported in percent. All samples were studied three times and averaged.

Results and Discussion

In the present study, fatty acid composition of nine species of mushroom namely *Morchella elata, Macrolepiota procera, Lactarius delicious, Helvella lacunosa, Boletus edulis, Cantharellus cibarius, Bovista plumbea, Pleurotus ostreatus, Agaricus bisporus* mushrooms were analyzed. The results for fatty acid composition, total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), total unsaturated fatty acids(∑UFA) and total unsaturated fatty acids to total unsaturated fatty acids ratio (∑UFA/SFA) of the studied mushrooms are shown in Table 2. The major fatty acids found in the studied samples were linoleic acid (C18:2 n-6) and oleic acid (C18:1 n-9), followed by palmitic acid (C16:0) and stearic acid (18:0). All of these results are shown in Table 3 and Figures 2 and 3. An example of the GC-MS chromatogram is given in Figure 4.

Table 2. Distribution According to % Fatty Acid Saturations in Mushroom Samples

Name of mushroom	SFA	MUFA	PUFA	∑UFA	∑UFA/ SFA
Morchelle elata	**48.74**	**22.19**	**29.08**	**51.27**	**1.051**
Macrolepiota procera	38.33	12.23	48.55	60.78	1.585
Lactarius delicious	**44.91**	**39.54**	**15.54**	**55.08**	**1.226**
Helvella lacunosa	30.56	46.94	22.49	69.40	2.270
Boletus edulis	**32.17**	**25.90**	**41.99**	**67.89**	**2.110**
Cantharellus cibarius	29.13	32.33	38.54	70.87	2.432
Bovista plumbea	**32.56**	**13.83**	**53.61**	**67.44**	**2.071**
Pleurotus ostreatus	42.77	24.13	40.31	64.44	1.506
Agaricus bisporus	**46.24**	**4.02**	**49.74**	**53.76**	**1.162**

SFA; Saturated Fatty Acid, MUFA; Monounsaturated Fatty Acid, PUFA: Polyunsaturated Fatty Acid, ∑UFA: Total Unsaturated Fatty Acid, ∑UFA/SFA: Total Unsaturated Fatty Acid to Saturated Fatty Acid ratio

∑UFA/SFA ratio was observed the highest in H. lacunosa and the lowest in M. elata in our samples.

Nervonic acid (C24:1) was detected as 7.22% in *Pleurotus ostreatus* only. This result is consistent with the results of the study of Karine 2007 but higher than it.

Lignoceric acid (C24:0) was detected as 0.71% in *Macrolepiota procera* only. Although amount of this fatty acid is very low, it can be an important finding.

In this study, highest SFA is % 46.24 in *Agaricus bisporus*, lowest SFA is %29.13 in *Cantharellus cibarius*, highest MUFA is % 46.94 in *Helvella lacunosa*, lowest MUFA is % 4.02 in *Agaricus bisporus*, highest PUFA is %53.61 in *Bovista plumbea*, lowest PUFA is %22,49 in *Helvella lacunosa*, highest ∑UFA is % 70.87 in *Cantharellus cibarius*, lowest ∑UFA is % 51.27 in *Morchella elata*, highest ∑UFA/SFA is 2.432 in *Cantharellus cibarius*, lowest ∑UFA/SFA is 1.051 in *Morchella elata* were obtained. All of these results are shown in Table 2.

Results reveal that levels of unsaturated fatty acid were higher than saturated ones in nine mushrooms. These results is in agreement with the earlier findings that unsaturated fatty acid content were predominating fatty acid in different species of mushrooms as compared to saturated ones (17).

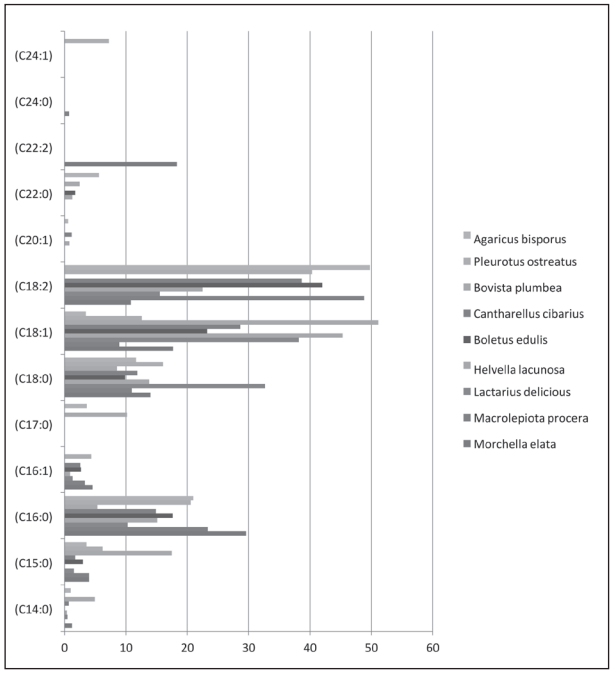


Figure 1: Fatty Acid Distribution in Mushroom Samples

Table 3: % Fatty Acid Distribution in Mushroom Samples

	M. elata	*M. procera*	*L. delicious*	*H. lacunosa*	*B. edulis*	*C. cibarius*	*B. plumbea*	*P. ostreatus*	*A. bisporus*
Myristic acid (C14:0)	1.20	nd	0.45	0.36	nd	0.67	4.93	nd	0.96
Pentadecanoic acid (C15:0)	3.96	3.95	1.53	nd	2.97	1.74	17.46	6.18	3.54
Palmitic acid (C16:0)	29.57	23.32	10.26	15.10	17.61	14.86	5.31	20.54	20.97
Palmitoleic acid (C16:1)	4.54	3.30	1.33	0.88	2.69	2.54	nd	4.34	Nd
Margaric acid (C17:0)	Nd	nd	nd	nd	nd	nd	10.17	nd	3.58
Stearic acid (C18:0)	14.01	10.95	32.67	13.82	9.83	11.86	8.52	16.05	11.61
Oleic acid (C18:1)	17.65	8.93	38.21	45.32	23.21	28.62	51.15	12.57	3.46
Linoleic acid (C18:2)	10.78	48.85	15.54	22.49	41.99	38.64	nd	40.31	49.74
Arachidonic acid (C20:1)	Nd	nd	nd	0.75	nd	1.17	nd	nd	0.56
Behenic acid (C22:0)	Nd	nd	nd	1.28	1.70	nd	2.46	nd	5.58
Docosadienoic acid (C22:2)	18.30	nd	nd	nd	nd	nd	nd	nd	Nd
Lignoceric acid (C24:0)	Nd	0.71	nd	nd	nd	nd	nd	nd	Nd
Nervonic acid (C24:1)	Nd	nd	nd	nd	nd	nd	nd	7.22	Nd

nd: not detected

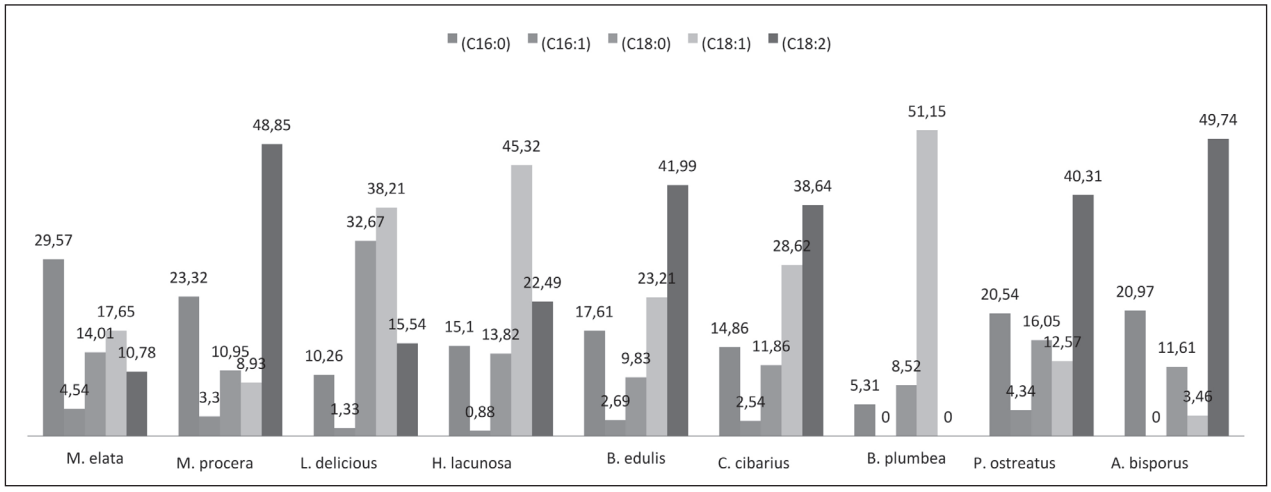


Figure 2: Distribution of the major fatty acids in mushrooms

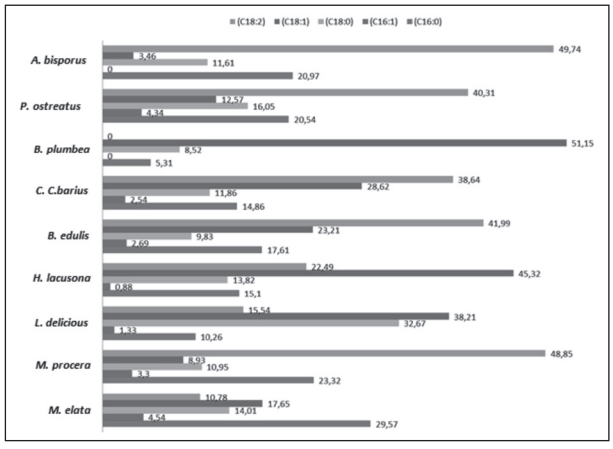


Figure 3: Distribution of the major fatty acids in mushrooms

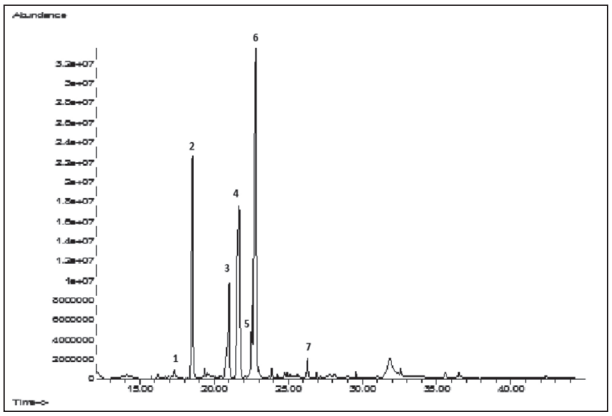


Figure 4: GC-MS chromatogram of *Boletus edulis*: number of 1.C15:0, 2.C16:0, 3.C16:1, 4.C18:0, 5.C18:1, 6.C18:2, 7.C22:0.

Linoleic acid is called an essential fatty acid because it can not be synthesized by the human organism. So it should be derived from diet and a series of ω-6 fatty acids containing γ-linolenic, dihomo-γ-linolenic and arachidonic acids (18). Essential fatty acids are the kind of fatty acids that a living thing needs to take but can not synthesize in their own body (19).

Conclusion

In this study, linoleic acid was the major fatty acid detected in all species of mushroom and it is one of the important essential fatty acid.

The analysis of the obtained profiles showed that linoleic (10.78 to 51.15%..) oleic (3.46-45.32 %), and, to a lesser extent, palmitic (10.26-29.57%) and stearic acids (9.83-32.67%) were the main fatty acids in the studied species in Table 3. This is in agreement with the results reported for other edible mushrooms (20,21). Linoleic amounts varied between 10.78 and 51.15%. The least was in (10.78%), the most in *Bovista plumbea* (51.15%)

Linoleic acid was the preponderant fatty acid in *Macrolepiota procera, Helvella lacunosa, Boletus edulis, Cantharellus cibarius, Bovista plumbea, Pleurotus ostreatus, Agaricus bisporus* in Table 2.

Cis-linoleic acid (18:2) was obtained in high amounts in *Bovista plumbea* (51.15%), *Agaricus bisporus* (49.74%). These results have shown that, in the many of previous reports, the majority of mushroom have higher levels of unsaturated fatty acids, especially linoleic acid (22,23).

Oleic acid is a monounsaturated fatty acid and is found in the ω-9 family. Since people have the enzymes necessary for the synthesis of oleic acid, this fatty acid is not regarded as essential. Under the severe conditions of essential fatty acid deficiency, the mammals elongate and desaturate oleic acid to produce eicosatrienoic acid (C20:3 n-9) (18). It is known that oleic acid which on olive oil has a positive effect human plasma cholesterol levels (24,25).

Other ω-9 fatty acid, such as, nervonic acid was also identified in *Pleurotus ostreatus* in Table 3.

Palmitic acid was found in the highest amounts in *Morchella elata and Macrolepiota procera* species. Stearic acid was found to be the highest amount (32.67%) in *Lactarious delicious*.

Pamitoleic acid was detected in all species except one, as shown in Table 3. As is known, arteriosclerosis can be treated by long-term use of palmitoleic acid.

As seen in Table 2, SFA's were generally found more than MUFA's and PUFA's. But UFA's were found to be higher than SFA's in all mushroom species.

Characterization of saturated, monounsaturated and polyunsaturated fatty acid profiles are shown in Table 2. The total fatty acid content for SFA, MUFA and PUFA was 29.13-48.74%, 4.02-46.94%, 15.54-53.61%.

SFA content was higher in *M. elata* due to palmitic acid (48.74%, 46.24) and in *L. delicious* as result of the high levels of both stearic acids and palmitic (45% of total compounds) in Table 3. The high linoleic acid content contributes to the overall increase in PUFAs, while oleic acid raises MUFAs levels.

PUFAs amount was higher in *B. Plumbea, A. Bisporus, M. Procera* species, in which linoleic acid was the main compound, and MUFA were present in highest levels in *H.lacunosa, L.delicious, C.cibarius* species, due to oleic acid.

These results are consistent with the fact that ratio of unsaturated fatty acids in total fatty acids in mushroom reported in previous studies is higher than saturated fatty acids (26-28).

Increasing the ratio of unsaturated fatty acids in the diet is very important because it leads to an increase in HDL levels, known as good cholesterol, and a decrease in LDL levels and triacylglycerol, also known as bad cholesterol (29).

In addition, it has been proven to be correlated with the increase in atherosclerosis, cardiovascular and other related diseases, with a saturated fat-rich diet (30). Therefore, the consumption of *L. Delicious, H.lacunosa, B.edulis, C.cibarius, B.plumbea, P.ostreatus* species is important for health.

The fatty acid compositions of different mushroom species were different from each other.

Although oleic acid and linoleic acid were in all mushrooms species, linoleic acid was the major compound in *B. plumbea* and *A. bisporus* species. It has been observed that there is a great difference between these compounds of mushroom species. These may be dif-

ferent in the synthesis of the genes responsible for the enzymes involved in the synthesis of fatty acids (31).

Karine et al. examined temperature-related fatty acid composition of *P. ostreatus* collected from Canada in 2007. In this mushrooms of *P. ostreatus* grown at 12, 17, 21 and 27 ^{0}C, changes in SFA, MUFA and PUFA ratios were observed. 18.8-22.9%, 10.1-14.3% and 71-62.8% SFA, MUFA and PUFA respectively were detected. It was determined that SUFA and MUFA ratio gradually increased with temperature and PUFA ratio decreased. This study is significant in terms of explaining the effect of ambient temperature on the changes in the fatty acid composition in this mushroom (32). According to the results of this study, our findings were lower than SFA and MUFA and higher than PUFA. The reason for the difference is thought to be caused by temperature.

Kayode et al. examined the fatty acid composition of *P. ostreatus* collected from Nigeria in 2015. In this study, fatty acid ratios were determined SFA 9.56%, MUFA 49.02% and PUFA 41.43%. PUFA levels are similar to our study. According to our study it was observed that SFA was lower but PUFA was higher than (33). These differences are thought to be caused by climate and environmental factors. On the other hand, Zengin et al. examined the fatty acid levels of *H.lacunosa* collected different from Turkey in 2015. In this study, fatty acid ratios were observed SFA 21.06%, MUFA 41.78 % and PUFA 37.23 % (34). Additionally, Riberio et al. examined the fatty acid profiles of *C.cibarius and B. edulis* collected northeastern of Portugual in 2009 (1). The fatty acid ratios of *C.cibarius, B. edulis* and *H.lacunosa* mushrooms are similar to our same mushrooms results.

Sagia et al. observed the fatty acid composition of *A. bisporus* collected the plains of Punjab, Pakistan in 2008. In this study, unsaturated fatty acid ratio, like our study, was observed to be more dominant in wild edible mushrooms (35).

Abugri et al. observed the fatty acid levels of *Agaricus sp*. And *Lactarius sp.* purchased commercially in the USA in 2016 (36). Oleic acid (C18:1) was no detected in the cultivated species, but in the wild species it detected 20.66 to 37.21 percentage of total fatty acids. This results similar to ours.

The fatty acid levels of the mushroom species in Table 3 can be different from those reported in the literature. These differences can be from different extraction methods, quantification methods, or derivatization of fatty acids.

In previous studies of the same region, it was observed that the fatty acid profile was different in the wild edible mushroom samples (1,2,11,12,20,23,27,28,37).

Rathore 2017, one of his studies, reported that the *Agaricus bisporus* had insulin secretion and anti-aging properties, and *Pleurotus* species also has positive effects on the usage of glucose and HbA_{1C} levels in human body (38).

In another study of the same region, the fatty acid results of the *Lactarius delicious* are similar to ours (39).

Due to its low saturated fatty acid content, fungi are excellent nutrients that can be used in low-calorie diets. For this reason, it can be consumed by humans for low cholesterol levels. Mushrooms are important for human health due to the unsaturated fatty acid variety. Therefore, they can be used as food supplements by the food industry.

Acknowledgements

The author would like to thank all persons of Bingol University Central Research Laboratory.

References

1. Ribeiroa B, Paula G P, Paula B A, Paula B, PatríciaV. Fatty acid composition of wild edible mushrooms species: A comparative study. Microchemical Journal 2009; 93 (1) 29–35.
2. Yilmaz N, Türkekul I, Bulut S, Sahin F, Bayrak, Ö F. Fatty Acid Composition in Ten Mushroom Species Collected from Middle Black Sea Region of Turkey. Asian Journal of Chemical 2013; 25 (3) 1216 – 1220.
3. Barros P B, Correia M, Casal S, Oliveira B, and Ferreira I C F R. Fatty acid and sugar compositions, and nutritional value of five wild edible mushrooms from Northeast Portugal. Food Chemistry 2007; 105 (1) 140–145.
4. Agrahar-Murugkar D and Subbulakshmi G. Nutritional value of edible wild mushrooms collected from the Khasi hills of Meghalaya. Food Chemistry 2005; 89 (4) 599–603.
5. Bobek P, Ginter E, Jurcovicova M, Kunia K. Cholesterol-lowering effect of the mushroom Pleurotus ostreatus in heredi-tary hypercholesterolemic rats. Annals of Nutrition and Metabolism 1991; 35, 191–195.
6. Breene W M. Nutritional and medicinal value of specially

mushrooms. Journal of Food Protection 1990; 53(10) 883–894.
7. Chang S T, Hayes W A. The biology and cultivation of edible mushrooms, New York: Academic Press 1978; 727–793
8. Kurasawa S, Sugahara T, Hayashi J. Studies on dietary fiber of mushrooms and edible wild plants. Nutrition Reports International 1982; 28 (2) 413–421.
9. Manzi P, Gambelli L, Marconi S, Vivanti V, Pizzoferrato L. Nutrients in edible mushrooms: an inter-species comparative study "Nutrients in edible mushrooms: an inter-species comparative study. Food Chemistry 1999; 65(4) 477-482.
10. Manzi P, Aguzzi A, Pizzoferrato L. Nutritional value of mushrooms widely consumed in Italy. Food Chemistry. 2001;73 (3) 321–325.
11. Sanmee R, Dell B, Lumyong P, Izumori K, Lumyong S. Nutritive value of popular wild edible mushrooms from northern Thailand. Food Chemistry 2003; 82 (4) 527–532.
12. Türkekul I, Yılmaz N, ahin F, Bayrak Ö F. Fatty Acid Composition of Six Mushroom Samples of Black Sea Region of Turkey. Asian Journal of Chemistry 2010; 22 (2) 1479-1486.
13. Wang D, Sakoda A, Suzuki M. Biological efficiency and nutritional value of Pleurotus ostreatus cultivated on spent beer grain. Bioresource Technology 2001;78 (3) 293–300.
14. Li C, Li Z, Fan M, Cheng W, Long Y, Ding T, Ming L. The composition of Hirsutella sinensis, anamorph of Cordyceps sinensis. Journal of Food Composition and Analysis 2006;19 (8) 800-805.
15. Zacharia S, Doshi A. Artificial indoor cultivation of Tricholoma crassa (Berk.) Sacc. An ectomycorrhizal mushroom using some local substrates. Journal of Mycology and Plant Pathology 2004; 34 (1) 125–126.
16. Folch J, Lees M and Sloane-Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 1957; 226 (1) 497-509.
17. Senatore Dini A. and A. Marino. Chemical constituents of some Basidiomycetes. Journal of the Science of Food and Agriculture 1988; 45,337–345.
18. Wiley & Sons, Hoboken, N. J. Voet Biochemistry 2004 (3rd ed.)
19. Maga J A, Mushroom flavor. Journal of Agricultural and Food Chemistry 1981;29 (1)1–4.
20. Barros L, Baptista P, Correia D M, Morais J S, Ferreira I C F R. Effects of conservation treatment and cooking on the chemical composition and antioxidant activity of Portuguese wild edible mushrooms. Journal of Agricultural and Food Chemistry 2007;55 (12) 4781-4788.
21. León-Guzmán M F, Silva I, López M G. Proximate chemical composition, free amino acid contents, and free fatty acid contents of some wild edible mushrooms from Querétaro. México Journal of Agricultural and Food Chemistry 1997;45 (11) 4329-4332.
22. Kala P, Chemical composition and nutritional value of European species of wild growing mushrooms: a review. Food Chemistry 2009;113 (1) 9–16.
23. Ruess L, Häggblom M M, García Zapata E J, Dighton J. Fatty acids of fungi and nematodes—possible biomarkers in the soil food chain. Soil Biology and Biochemistry. 2002;34 (6) 745–756.
24. Puiggrós C, Chacón P, Armadans L I, Clapés J, Planas M. Effects of oleic-rich and omega-3-rich diets on serum lipid pattern and lipid oxidation in mildly hypercholesterolemic patients. Clinical Nutrition 2002;21 (1) 79-87.
25. Tomás M, Sentí M, Elosua R,Vila J, Sala J, Masià R, Marrugat J. Interaction between the Gln-Arg 192 variants of the paraoxonase gene and oleic acid intake as a determinant of high-density lipoprotein cholesterol and paraoxonase activity. Eurepean Journal of Pharmacology 2001;432 (2-3) 121-128.
26. Pacheco Y M, López S, Bermúdez B, Abia R, Villar J, Muriana F J G. A meal rich in oleic acid beneficially modulates postprandial sICAM-1 and sVCAM-1 in normotensive and hypertensive hypertriglyceridemic subjects. The Journal of Nutrional Biochemistry 2008;19 (3) 200-205.
27. Barros L, Venturini B A, Baptista P, Estevinho L M, Ferreira I C F R. Chemical composition and biological properties of Portuguese wild mushrooms: a comprehensive study. Journal of Agricultural Food and Chemistry 2008;56 (10) 3856-3862.
28. Kavishree S, Hemavathy J, Lokesh B R, Shashirekha M N, Rajarathnam S. Fat and fatty acids of Indian edible mushrooms. Food Chemistry 2008;106 (2) 597-602.
29. Isanga J, Zhang G N, Biologically active components and nutraceuticals in peanuts and related products: review. Food Reviews International 2007;23, 123-140.
30. Wang L, A Folsom R, Eckfeldt J H. Plasma fatty acid composition and incidence of coronary heart disease in middle aged adults: the Atherosclerosis Risk in Communities (ARIC) Study, Nutrition, Metabolism and Cardiovascular Diseases. 2003;13 (5) 256-266.
31. Qi B, Fraser T, Mugford S, Dobson G, Sayanova O, Butler J, Napier JA, Stobart A K, Lazarus C M. Production of very long chain polyunsaturated omega-3 and omega-6 fatty acids in plants. Nature Biotechnology. 2004; 22 (6) 739-745.
32. Pernedault K, Angers P, Avis T J, Gosselin A, Tweddel R J. Fatty acid profiles of polar and non-polar lipids of Pleurotus ostreatus and P. Cornucopiae var. 'citrino-pileatus' grown at different temperature. Mycolojical Research 2007;111, 1228-1234.
33. Kayode R M O, Olakulehin T F, Adedeji B S, O. Ahmed, Aliyu T H, Badmos A H A. Evaluation of amino acid and fatty acid profiles of commercially cultivated oyster mushroom (Pleurotus sajor-caju) grown on gmelina wood waste. Nigeria Food Journal 2015;33, 18-21.
34. Zengin G, Sarikurcu C, Aktumsek A et al. A Comparative fatty acid compositional analysis of different wild species of mushrooms from Turkey. Emirates Journal of Food and Agriculture 2015; 27(7), 532-536.
35. Sadiq S, Nawaz B H, Asif H M. Studies on chemical composition and nutritive evaluation of wild edible mushrooms, Iran J. Chem. Chem. Eng. 2008;27(3), 151-154.
36. Abugri D A, McElhenney W H, Willian K R. Fatty acid

profiling in selected cultivated edible and wild medicinal mushrooms in southern Unites States. Journal of Experimental Food Chemistry 2016; 2 (1), 1-7.
37. Bengu A S, Some elements and fatty acid profiles of three different wild edible mushrooms from Tokat province in Turkey. Progress in Nutrition 2019; 21, 1: 00-00 DOI: 10.23751/pn.v21i1.7562
38. Himanshi R, Shalinee P, Satyawati S. Mushroom nutraceuticals for improved nutrition and better human health. A review, Pharma Nutrition 2017; 5:35–46.
39. Yılmaz H C, Bengü A S. The investigation of fatty acids and mineral profiles of some edible Lactarius species (L. Delicious, L. Deterrimus, L. Salmonicolor, L. Sanguifluus, L. Semisanguifluus) in the Usak/Turkey province of Aegean Region. Biological Diversity and Conversation 2018;11:1 95-104.

Correspondence:
Aydin Sukru Bengu
Department of Medical Services and Techniques,
Vocational School of Health Services, University of Bingol, 12000 Bingol, Turkey
E-mail: abengu@bingol.edu.tr; aydinbengu75@gmail.com

Progress in Nutrition 2020; Vol. 22, N. 1: 193-198 DOI: 10.23751/pn.v22i1.7949

Original article

Varietal and time dependent differences in juglone and total phenolic contents of the walnut (*Juglans regia* L.) leaves

İsmail Kocaçalışkan[1], Emel Turan[2], Ümran Ertürk[3], Yavuz Demir[4], İrfan Terzi[5]

[1]Molecular Biology and Genetics Department, Yıldız Technical University, İstanbul, Turkey; [2]Institute of Science and Technology, Dumlupınar University, Kütahya, Turkey; [3] Agricultural Faculty, Uludağ University, Bursa, Turkey; [4*] Biology Department, K. K. Education Faculty, Atatürk University, Erzurum, Turkey, E-mail: ydemir@atauni.edu.tr; [5] Education Faculty, Dumlupınar University, Kütahya, Turkey.

Summary. From May to October, time dependent differences with two weeks intervals in juglone (5-Hydroxy-1,4-naphthoquinon) and total phenolic content of the leaves of five walnut (*Juglans regia* L.) cultural varieties grown in Turkey (cv. Şebin, cv. Yalova-2, cv. Yalova-3, cv. Yalova-4 and cv. 1974/7) were investigated. Juglone and phenolic contents were determined by spectrophotometric methods. Correlations between juglone-phenolic content, juglone-climatic factors and phenolic content-climatic factors were also established. In conclusion, there were significant differences between the varieties and the highest values of both juglone and phenolic content were recorded in Yalova-2 cultivar among the varieties. For example, maximum juglone content was determined in Yalova-2 (3,51 mg/g) but minimum in Yalova-4 (2,26 mg/g). Maximum phenolic content was also determined in Yalova-2 (51,8 mg/g), but minimum phenolic level was seen in 1974/7 cultivar (49,3 mg/g), as a mean. On the other hand, both juglone and phenolic content were generally higher from middle of August to middle of September and the lowest levels were seen in May. Further, while a significant positive correlation between juglone and phenolic contents of the leaves was found, there was almost no significant correlation between juglone and climatic factors except a significant negative correlation between phenolic content and wind speed in Yalova-2 cultivar.

Key words: climatic factors, juglone, phenolic content, walnut cultivars, walnut leaf

Introduction

The walnut family (Juglandaceae) contains several species and is rather distributed on the world. The most common species among the species is *Juglans regia* (1). This species is grown commercially in several countries such as China, USA, France, India, Italy, Spain and Turkey for nut production (2). Several parts of the walnut mainly kernels, shells, bark and leaves are using for the pharmacological and cosmetic aims (3). Walnut leaves are easily available in abundant amounts and are considered a source of healthcare compounds and are intensively using in traditional medicine for treatment of several disorders (4).

Juglone (5-hydroxy-1,4-naphthoquinone) is a quinone group aromatic compound has been found in all parts of the walnut tree and it has also been reported that the amounts of juglone were in order of green peel>leaves>bark and it has phytotoxic and allelopathic properties (5). Juglone can be toxic for surrounding plant species, and is of great interest due to its chemical reactivity. Juglone is involved into the walnut pathogenic defense mechanism (6). Walnuts had the highest antioxidant activity among analyzed foods and drinks (7). A balance among competing source and sink mechanisms and rates will ultimately determine whether juglone is capable of attaining sufficient levels to be allelopathic to intercrops in a walnut

tree agroforestry system. Although the allelopathic effect of Juglone on plants is generally toxic (8), in some cases it may also be beneficial (9).

Detrimental impact of Juglone may be associated with suppressing the intensity of a wide range of physiological processes and biochemical reactions occurring in plant tissues. Some researchers reported that Juglone may inhibit the growth of both shoots and roots, photosynthesis, chlorophyll content, respiration, transpiration, stomatal conductance and disrupting root plasma membrane and decreasing of water uptake. Juglone influence the germination, growth, development, reproduction and distribution of a number of plant species (8). In spite of the large number of papers published on the biological activity of Juglone, little is known about the mechanism of its toxic effect on plant cells growth (10). Juglone has been found to have herbicidal effect on some weed species (11) and antioxidant activity on some enzymes (12). Its antiviral and antimicrobial activities have also been detected (1, 13). Recently its antifungal and antibacterial activities have been enhanced using PLGA nanoparticle system, as well (14, 15).

Phenolic compounds are secondary metabolites synthesized in plants by mainly mevalonic or shikimic acid pathways. Their synthesis depends on numerous enzymes involved in different metabolic pathways. They show pharmacological activities such as antioxidant, immune stimulating, antiseptic and spasmolytic so on (16). Phenolics are also involved in physiological processes such as fruit growth and they affect different aspects of fruit development (17). Phenols are active in defense against various types of stresses caused by pathogens or abnormal environmental conditions. Wounding in plants can also cause stress situations affecting the biosynthesis of phenolic compounds (18). Since phenolic compounds have been shown to have antioxidant, free radical-scavenging and metal-chelating activity in addition to their anticarcinogenic properties, they are considered beneficial for human health (19, 20).

Walnut is a rich source of phenolic compounds, including phenolic acids, naphtoquinones and flavonoids. In walnut leaves, naphtoquinones and flavonoids are considered as major phenolic compounds (19). The increasing interest in the powerful biological activities of plant phenolics has outlined the necessity of determining their content (21). There have been reports of seasonal variation in juglone level in leaves and soil beneath of *J. nigra* (22). But no reports regarding the varietal differences and time dependent variation of juglone and phenolic content in the leaves of Turkey's cultivars of *J. regia* have been encountered. For this reason, the present study was conducted to understand time dependent variations in juglone and total phenolic contents in the leaves of five walnut cultural varieties grown in Turkey, and relations between juglone-phenolic content, juglone-climatic factors and phenolic content-climatic factors. This information should allow for a better time dependent use of the leaves of walnut to extract juglone and phenolics, as well as for a better ecological definition of these compounds.

Materials and Methods

Leaf sampling

Walnut leaves were picked up from the five cultivars (cv. Şebin, cv. Yalova-2, cv. Yalova-3, cv. Yalova-4 and cv. 1974/7) of 20 years old walnut trees (*Juglans regia* L.) grown in the walnut orchard of Agricultural Faculty of Uludağ University in Bursa city of Turkey. Three trees per cultivar were chosen randomly and leaf collecting procedure was carried out during two following years with two weeks intervals from May to October. The collected leaves were put into the plastic bags and bring to the laboratory. Then the leaf samples were dried in an oven by keeping for 48 h at 70°C. The dried leaf samples were put into the plastic bags and labelled, and so they were kept in a cold room until to use. On the other hand, the meteorological values of Bursa city were obtained from General Directorate of Meteorology of Turkey (Table 1).

Determination of Juglone Content

Measurement of juglone content of the walnut leaves was carried out by spectrophotometric method (23). 2 g of leaf was homogenized in 50 ml petroleum ether and after filtration the filtrate was centrifuged at 18.000 rpm in refrigerated centrifuge for 15 min. The supernatant was diluted ten-fold with petroleum ether and its absorbance was recorded by spectrophotomet-

Table 1. Meteorological values of Bursa city where the walnut orchard from which the leaves were picked is exist. The values are mean of two following years of the research conducted from May to October.

Meteorological Parameters	May	June	July	August	Septemb.	October
Temperature (°C)	19.4	23.5	25.2	26.2	20.7	16.1
Rainfall (mm)[3]	10.7	55.0	7.7	2.2	47.2	60.5
Humidity (%)	59.0	57.0	53.5	55.5	63.5	74.5
Wind Speed (m/s)	1.9	1.8	2.0	1.7	1.7	1.3

ric measurement at 410 nm. Blank sample was only petroleum ether. In determining of juglone content of the leaves was used standart curve prepared by a series of pure juglone solutions in the range of 0.01, 0.02, 0.03, 0.04 and 0.05 mg juglone contents. The juglone content was expressed as "mg juglone/g dry leaf".

Determination of Total Phenolic Content

The amount of total phenolic compounds in the walnut leaves was determined spectrophotometrically (24). 2 g of leaf was homogenized in 50 ml methanol and after filtration the filtrate was centrifuged at 4.000 rpm in refrigerated centrifuge for 15 min. 10 ml of supernatant was taken and its methanol was evaporated in a rotary evaporator. Then the residue was dissolved in 2 ml of methanol, and 0.1 ml of this solution was mixed with 0.1 ml of Folin reagent, 0.5 ml of Na-carbonate solution (%20) and 9.3 ml of distilled water. After keeping at 25°C for 2 hours, absorbance of the mixture was measured in spectrophotometer at 765 nm. The mixture without 0.1 ml of leaf extract was used as blank sample. In determining of phenolic content of the leaves was used a standart curve prepared by a series of gallic acid standart solutions in the range of 0.02, 0.04, 0.06, 0.08 and 1 mg gallic acid contents. The phenolic content was expressed as "mg phenolic/g dry leaf".

Statistical Analyses

The mean values obtained from three replications were shown on the tables. LSD multiple comparison test was applied for comparing the significant differents among cultural varieties and sampling dates at 0.05 level for both juglone and phenolic content of the walnut leaves. Further, correlation test was applied to show significant correlations between juglone - phenolic content, as well as between juglone - meteorological parameters and phenolic content - meteorological parameters.

Results and Discussion

The increasing interest in the powerful biological activities of plant phenolics has outlined the necessity of determining their content in leaves of different walnut cultivars. In this study, walnut leaves from five walnut cultivars originating from the same orchard and from the same year of production were analyzed for their seasonal variations in juglone and phenolic content from May to October. The walnut cultivars were grown under the same agricultural, geographical and climatic conditions.

In this study, in all the cultivars, the lowest content of juglone in leaves was measured in May which the stage of vigorous development of spring growth unit. In a former study, the lowest content of juglone of walnut in annual shoots was also measured in May (18). It was followed an increase during the resting time between the spring and summer growth. That is, juglone contents increased from the spring growth cycle in May to the summer flush of growth in middle of September. The juglone pattern of seasonal variations was almost similar in all cultivars with some differences. In Yalova-3 and Yalova-4, it increased continuously from May until to October. The other varieties have showed some fluctuations in juglone content (Table 2). On the other hand, maximum juglone content was determined in Yalova-2 (3, 51 mg/g leaf) and minimum in Yalova-4 (2, 26 mg/g leaf), as average of the months. Some researchers proved that the content of juglone in leaves and nuts of pecan (*Carya illinoensis* K.) was low in early season and increased through July. In black walnut (*Juglans nigra* L.) great seasonal fluctuations in the concentrations of juglone were ascertained (25).

Formerly, it has been shown showed that measurements of seasonal change in juglone content among various tissues of pecan walnut revealed that the highest concentrations occurred in leaves in June

Table 2. Time dependent juglone contents of the leaves of the five walnut cultivars. The values in the table are mean of triplicate determinations of two following years. The last row values are mean of juglone contents for each cultivar.

Sampling Date	Şebin	Yalova-2	Yalova-3	Yalova-4	1974/7
May 15	1.03	0.61	0.68	0.21	0.65
June 1	1.08	2.11	1.20	0.72	0.98
June 15	1.71	2.73	2.00	1.75	2.32
July 1	2.92	3.45	2.71	2.27	3.06
July 15	2.92	4.23	2.96	2.31	2.83
Aug 1	2.37	4.86	3.23	2.86	3.70
Aug 15	3.45	5.31	3.03	2.92	4.73
Sept 1	3.57	4.21	3.36	3.18	4.61
Sept 15	3.55	4.46	3.87	3.02	3.81
Oct 1	3.20	3.67	3.53	3.65	3.23
Mean	2.53	3.51	2.66	2.26	3.01

A multiple comparison test LSD (5%) values for sampling dates and the cultivars were 1.07 and 0.81, respectively.

Table 3. Time dependent total phenolic contents of the leaves of the five walnut cultivars. The values in the table are mean of triplicate determinations of two following years. The last row values are mean of total phenolic contents for each cultivar.

Sampling Date	Şebin	Yalova-2	Yalova-3	Yalova-4	1974/7
May 15	18.7	29.3	19.3	25.0	24.3
June 1	20.6	32.5	17.5	15.0	24.3
June 15	23.7	31.8	25.0	19.3	27.5
July 1	34.3	38.7	42.5	25.6	35.6
July 15	43.1	36.5	51.8	59.3	48.7
Aug 1	58.1	63.1	56.8	66.2	56.2
Aug 15	65.0	68.7	66.2	58.7	63.7
Sept. 1	88.1	69.3	80.2	82.5	66.8
Sept. 15	85.0	72.5	71.2	76.8	66.8
Oct. 1	78.1	68.7	68.7	61.2	72.5
Mean	51.2	51.8	50.0	50.0	49.3

A multiple comparison test LSD (5%) values of sampling dates and the cultivars were 4.5 and 2.1, respectively.

and in nuts in September. On the other hand, at the end of vegetation period, low juglone was reported in the walnut *J. regia* (17). Also, regarding the seasonal changes, juglone showed a linear decrease over growing season in the leaves of green husks of *J. regia* **(**26**)**. This opposite result may be derived from different cultivars used or from different climatic conditions along the varied seasons. Other researchers showed that potential juglone abundance estimated in walnut leaves, hulls, and roots ranges from less than 0.1% to as much as 5% dry weight-basis, depending on the growing season and extraction techniques used **(**26**)**.

In our study, the total content of phenolic compounds was the lowest in the stage of vigorous spring growth in May (Table 3). After that, in the mid-time between the spring and summer growth (second sampling date) and at the beginning of the summer growth flush (third sampling date) in middle of September it strongly increased. As average of the months, maximum phenolic content was determined in Yalova-2 variety and minimum phenolic level was seen in 1974/7 variety (49, 3 mg/g leaf). Considering that the light may plays an essential role in phenol biosynthesis, some researchers attributed the increase in phenolics in July to the highest value of solar radiation in July.

Total content of phenols depends on sampling time (3). Our study is also consistent with this result. Differences in terms of total phenols at different sampling times are supposed to be the effect of change in ecological parameters. Biosynthesis of phenolic compounds can be induced by stronger sunlight and length of daytime, therefore the phenols content is increasing until the beginning of August. High temperature stress promotes production of phenolic compounds; the increases observed in phenolic content of walnut leaves collected in July may be attributed to higher values of temperatures. However, no significant correlation between climatic parameters such as temperature, rain, humidity and wind, and phenolic or juglone contents was established (Table 4); variations in total phenols and juglone of walnut leaves reported in the present study (Table 2,3) may be explained by genetically programmed inner conditions of walnut species.

Most of the results show that contents of flavonoids and phenolic acids are lowest at the beginning of vegetative period and increase during summer to achieve the highest amounts at the end of phenological cycle (27). Our results suit to that pattern that cultivar variations in phenolic contents were estimated. The levels of phenols are influenced by environmental factors, soil composition, maturation level, cultivar and harvest year. Some researchers indicated that the average values of phenolic acids, flavonoids and total phenols seem to point out to a decrease until September,

Table 4. Correlations between meteorological parameters and contents of juglon (J) and total phenolic (P). The values in the table are correlation coefficients.

Comparative Parameters	Şebin	Yalova-2	Yalova-3	Yalova-4	1974/7
J-P	0.869**	0.756*	0.922**	0.809**	0.848**
J-Temperature	-0.026	0.380	-0.017	-0.085	0.181
J-Rainfall	-0.046	-0.211	0.044	0.093	-0.125
J-Humidity	0.299	-0.006	0.350	0.443	0.150
J-Wind Speed	-0.331	-0.272	-0.450	-0.585	-0.347
P-Temperature	-0.305	-0.210	-0.152	-0.149	-0.241
P-Rainfall	0.195	0.111	0.018	-0.028	0.079
P-Humidity	0.585	0.530	0.443	0.391	0.550
P-Wind Speed	-0.628	-0.692*	-0.508	-0.438	-0.658

*** (P<0.01), * (P<0.05)*

while others indicated that each phenolic group had its own curve of seasonal fluctuations (3).

On the other hand, in this study, a significant positive correlation between juglone and phenolic contents of the leaves was found (r: 0,893; P<0, 01). While there was no significant correlation between juglone and climatic factors, except a significant negative correlation between phenolic content and wind rate in Yalova-2 cultivar (r:-0,692 ; P<0,05) (Table 4).

The results obtained indicated that walnut leaves may become important in obtaining a noticeable source of the compounds with health protective potential and antimicrobial activity; therefore the walnut leaves should preferentially be collected from the middle of August to the early September, in that time juglone and phenolic contents are higher. It has been shown that cultivars and sampling date is important for the juglone and phenolic contents in the walnut leaves. Juglone was considered as a useful tool for distinguishing different genotypes, and may be a potential genetic marker to study variation. This approach may be more useful for achieving a quick and short-term objective of either increasing juglone content for juglone production or decreasing its content for nut production in horticulture and reducing allelopathy in agroforestry (2).

In conclusion; it may be concluded that there is a significant positive relation between juglone and phenolic content in walnut leaves depending on seasonal variation. Juglone and phenolic contents rather change according to walnut cultivars that the highest juglone content was determined in Yalova-2 cultivar. The results of the present study apparently indicated that the walnut (*J. regia* L.) leaves are constitute a suitable source of juglone and phenols and they could be used as alternative natural antioxidants. As juglone and phenolic contents are higher in that time, walnut leaves should preferentially be collected between late August and early September. The information obtained in the walnut leaves may be useful in planning the collecting time and cultivar selection for producing medicinal extracts.

References

1. Montenegro RC, Araújo AJ, Molina MT, Marinho Filho JD, Rocha DD, Lopéz-Montero E, Goulart MO, Bento ES, Alves AP, Pessoa C, De Moraes MO, Costa-Lotufo LV. Cytotoxic activity of naphthoquinones with special emphasis on juglone and its 5-*O*-methyl derivative. Chemico-Biol Interact 2010; 184: 439–448
2. Thakur A, Cahalan C. Geographical variation of *Juglans regia* L. in juglone content: rapid analysis using micro-plate reader. Current Science 2011; 100(10): 1483-1485
3. Cosmulescu SN, Trandafir I. Variation of phenolics content in walnut (*Juglans regia* L.). South West. J Hort Biol Environ 2011; 2(1): 25-33
4. Cosmulescu SN, Trandafir I, Nour V. Seasonal variation of the main individual phenolics and juglone in walnut (*Juglans regia* L.) leaves. Pharm Biol 2014; 52(5): 575-580
5. Sytykiewicz H. Expression Patterns of Glutathione Transferase Gene (*GstI*) in Maize Seedlings Under Juglone-Induced Oxidative Stress. Int J Mol Sci 2011; 12: 7982-7995
6. El Hadrami A, Kone J, Lepoivre P. Effect of juglone on active oxygen species and antioxidant enzymes in susceptible and partially resistant banana cultivars to Black Leaf Streak Disease. Euro. J Plant Pathol 2005; 113: 241–254
7. Colaric M, Veberic R, Solar A, Hudina M, Stampar F. Phenolic Acids, Syringaldehyde, and Juglone in Fruits of Different Cultivars of *Juglans regia* L. J Agric Food Chem 2005; 53: 6390-6396
8. Achatz M, Morris EK, Muller F., Hilker M, Rillig MC. Soil hypha-mediated movement of allelochemicals: arbuscular mycorrhizae extend the bioactive zone of juglone. Functional Ecol 2014; 28: 1020–1029
9. Kocaçalışkan I, Terzi I. Allelopathic effects of walnut leaf extracts and juglone on seed germination and seedling growth. J Hort Sci Biotech 2001; 76: 436- 440
10. Rudnicka M, Polak M, Karcz W. Cellular responses to naphthoquinones: juglone as a case study. Plant Growth Regul 2014; 72: 239–248
11. Topal S, Kocacalışkan I, Arslan O, Tel AZ. Herbicidal effects of juglone as an allelochemical. Phyton 2007; 46: 259-269

12. Altıkat S, Terzi İ, Kuru H, Kocaçalişkan İ. Allelopathic effect of juglone on growth of cucumber and muskmelon seedlings with respect to antioxidant enzyme activities and lipid peroxidation. J Environ Protect Ecol 2013; 14: 1244-1253
13. Tan DTC, Osman H, Mohamad S, Kamaruddin AH. Synthesis and antibacterial activity of juglone derivatives. J Chem Engin 2012; 6: 84-89
14. Arasoglu T, Derman S, Mansuroglu B, Yelkenci G, Kocyigit B, Gümüş B, Acar T, Kocacaliskan I. Enhancement of antifungal activity of juglone (5-hydroxy-1,4-napphthoquinone) using a poly (D,L-lactic-co-glycolic acid) (PLGA) nanoparticule system. J Agric Food Chem 2016; 64: 7087-7094
15. Arasoglu T, Derman S, Mansuroglu B, Yelkenci G, Kocyigit B, Gümüş B, Acar T, Kocacaliskan I. Synthesis, characterization and antibacterial activity of juglone encapsulated PLGA nanoparticles. J Applied Microb 2017; 123: 1-13
16. Mallavadhani UV, Prasad CV, Shrivastava S, Naidu VG. Synthesis and anticancer activity of some novel 5,6-fused hybrids of juglone based 1,4-naphthoquinones. Europ. J Med Chem 2014; 83: 84-91
17. Solar A, Colaric M, Hudina M, Stampar F. Phenolic content of walnut fruit as affected by cultivar and developmental stage. Acta Hortic 2005; 705: 231-240
18. Solar A, Colaric M, Usenik V, Stampar F. Seasonal variations of selected flavonoids, phenolic acids and quinones in annual shoots of common walnut (*Juglans regia* L.). Plant Sci 2006; 170: 453-461
19. Pereira JA, Oliveira I, Sousa A, Valentão P, Andrade PB, Ferreira IC, Ferreres F, Bento A, Seabra R, Estevinho L. Walnut (*Juglans regia* L.) leaves: Phenolic compounds, antibacterial activity and antioxidant potential of different cultivars. Food and Chem Toxic 2007; 45: 2287–2295
20. Jin R. A study on the radical scavenging activity of juglone and its derivatives. J Mol Structure 2010; 939: 9–13
21. Babula P, Mikelova R, Adam V, Kizek R, Havel L, Sladky Z. Using of liquid chromatography coupled with diode array detector for determination of naphthoquinones in plants and for investigation of influence of pH of cultivation medium on content of plumbagin in *Dionaea muscipula*. J Chromatog *B* 2006; 842: 28–35
22. De Scisciolo B, Leopold DJ, Walton DC. Seasonal patterns of juglone in soil beneath *Juglans nigra* (black walnut) and influence of *J. nigra* on understory vegetation. J Chem Ecol 1990; 16(4): 1111–1130
23. Ponder F, Tadros SH. Juglone concentration in soil beneath black walnut interplanted with nitrogen-fixing species. J Chem Ecol 1985; 11: 937-42
24. Amaral JS, Seabrar M, Andrade PB, Valentao P, Pereira JA, Ferreres F. Phenolic profile in the quality control of walnut (*Juglans regia* L.) leaves. Food Chem 2004; 88: 373-379
25. Coder, KD. Seasonal changes of juglone potential in leaves of black walnut (*Juglans nigra* L.). Journal of Chemical Ecology 1983; 9(8): 1203-1212
26. Cosmulescu SN, Trandafir I, Achim G, Baciu A. Juglone Content in Leaf and Green Husk of Five Walnut (*Juglans regia* L.) Cultivars. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 2011; 39(1): 237-240
27. Skrzypczak-Pietraszek E, Pietraszek J. Chemical profile and seasonal variation of phenolic acid content in bastard balm (*Melittis melissophyllum* L., Lamiaceae). J Pharm Biomed Anal 2012; 66: 154– 161

Correspondence:
Yavuz Demir
Biology Department, K. K. Education Faculty, Atatürk University, Erzurum, Turkey
E-mail: ydemir@atauni.edu.tr

Progress in Nutrition 2020; Vol. 22, N. 1: 199-203 DOI: 10.23751/pn.v22i1.8128

Original article

Evaluation of some commercial dairy rations in terms of chemical composition, methane production, net energy and organic matter digestibility

Mustafa Boga[1], Ozer Kurt[2], Cagri Ozgur Ozkan[3], Ali İhsan Atalay[4], Adem Kamalak[5]

[1]Bor Vocational School, Nigde Ömer Halisdemir University, TR 51700, Nigde, Turkey - E-mail: mboga@ohu.edu.tr; [2]Mus Alparslan Unıversıty Faculty of Applıed Science, Department of Anımal Production and Technology, Mus Turkey; [3]Kahramanmaras Sutcu Imam University, Goksun Vocational School, Organic Farming Department, Kahramanmaras, Turkey; [4]Igdır Unıversıty Faculty of Agrıculture, Department of Animal Science, Igdır, Turkey; [5]Kahramanmaras Sutcu Imam Unıversıty Faculty of Agrıculture, Department of Animal Science, Kahramanmaras, Turkey

Summary. The aim of the present study was to evaluate some commercial dairy rations in terms of chemical composition, methane production, net energy for lactation (NE_L) and organic matter digestibility (OMD). There are considerable variations among total mixed rations (TMRs) used by farms, especially in terms of chemical composition. Crude protein (CP) contents of TMRs ranged from 9.83 to 14.32 %. Acid detergent fiber (ADF) and neutral detergent fiber (NDF) contents of TMRs ranged from 22.79 to 32.32 % and 42.5 to 52.98 % respectively. There are significant differences among TMRs in terms of gas, methane production, NE_L and OMD. Gas production ranged from 82.25 to 97.25 ml. Gas production, NE_L and OMD for TMR 3, 6 and 8 was significantly higher than the others. Methane production (ml) for TMR 8 was significantly higher than that for TMR 7. As a conclusion, TMRs investigated in the current study are not well balanced to meet the nutritional requirements of lactating cows. Therefore, it is not likely that lactating cows fed with these TMR will not explain their genetic potential very well. Especially CP and NE_L of TMRs should be taken into consideration to improve efficiency in milk production of lactating cows in small farms in Turkey.

Key words: chemical composition, digestibility, methane production, net energy lactation, total mixed ration

Introduction

As most parts of the world, diet formulation of lactating cows are based on protein and energy requirements. The chemical compositions and energy contents of feed ingredients or TMRs used in lactating cow diets were not determined due to lack of analytic facilities in most of small dairy farms in Turkey. In addition, most of small dairy farms have no a qualified consulting nutritionist. As a result, in practice, formulating of well-balanced diets is very difficult in the most of small dairy farms. Preliminary investigation clearly showed that diets of lactating cows were not well balanced in Turkey due to lack of analytic facilities and qualified consulting nutritionist in most of small dairy farms. Recently the chemical composition and *in vitro* gas production technique were widely used for evaluation of uninvestigated forages (1-5). The *in vitro* gas production technique not only allows estimation of energy content but also allows methane production of feedstuffs. Recently methane production potentials of some commercial dairy rations and some feedstuffs were evaluated using *in vitro* gas production technique (1, 6). It is well known that methane is one of the most important greenhouse gases. The methane production from ruminant animal has a considerable contribution to the global worming during the fermentation. It was also reported that during the ruminal fermentation 2-12 % of dietary energy intake is lost as methane (7). Therefore, the aim of current experiment was to evaluate the diets of lactating cows in terms of chemical composition, NE_L, OMD and methane production.

Materials and methods

This experiment was conducted in the laboratory of Department of Animal Science, Faculty of Agriculture, University of Kahramanmaras Sutcu Imam, Kahramanmaras in Turkey. Studies performed using *in vitro* experimental model was approved by the Animal Experimentation Ethics Committee of University of Kahramanmaras Sutcu Imam, Faculty of Agriculture (Protocol No: 2018/01).

The ingredients of TMRs were collected from eight different farms in 2017 in Nigde Province of Turkey and mixed in the laboratory as exactly done in farm. The TMRs dried under the shadow at room temperature. Dried TMRs samples were milled to pass 1 mm screen and kept in airtight plastic bags for chemical analysis and *in vitro* gas production. Dry matter (DM), ash, CP and ether extract (EE) contents of TMRs were determined according to AOAC (8). Cell wall (NDF and ADF) contents of TMRs were determined according to method described by Van Soest and Wine (9) and Van Soest (10) respectively. Chemical analysis was carried out in triplicate. The chemical compositions of TMRs are given in Table 1.

The *in vitro* gas and methane production of TMR samples were determined using the *in vitro* gas production technique (11). Three Awassi lambs (approximately 50 kg average weight) were used as inocolum donor's animal for *in vitro* gas production trials. Lambs were fed with diet containing of alfalfa hay (800g) and barley (400g). Equal amount of rumen fluid was transferred into thermo flask before morning feeding and strained through four layered cheesecloths under flushing with CO_2. The rumen fluid and buffer solution were combined in the ratio 1:2 (V/V). 40 ml of buffered rumen fluid were transferred into syringes containing TMR samples (0.5 gram) in quadruplicate. 40 ml of buffered rumen fluid were transferred into four syringes without TMR samples to obtain the blanks. All syringes were incubated for 24 h in water bath maintained at 39 °C. The gas and methane production were detected from the syringes containing TMR samples to determine the net gas production at 24 h incubation. Net gas productions of TMR samples were obtained after correction for blank and hay standard (University of Hohenheim, Germany).

NE_L (MJ/kg DM) and OMD of TMR samples were estimated using equation of Menke and Steingass (11) as follows:

- NE_L (MJ/kg DM) = -0.22 + 0.1062GP+ 0.048CP +0.1329EE
- OMD (%) = 14.88 + 0.8893GP + 0.448CP +0.651 Ash
- Where GP = 24 h net gas production (ml/200 mg); CP = Crude protein (%), EE: Ether extract (%), ash content (%). NE_L was converted into kcal multiplying by 0.239

The methane contents of gas produced after 24 h incubation of TMR samples were determined using an infrared methane analyzer (Sensor Europe GmbH, Erkrath, Germany) (12).

Methane production (ml) = Total gas production for 24 h incubation (ml) X Percentage of Methane (%)

The effect of TMR on gas production, methane production, NE_L and OMD were determined using the one-way analysis of variance (ANOVA). Tukey's multiple range tests was employed to identify the significance between means. Mean differences were considered significant at $P<0.05$.

Results and discussion

There is considerable variation among TMRs in terms of feed ingredients and their levels in TMRs used for dairy cows in Nigde Province, Turkey. TMRs used in the current experiment contained up to 10 ingredients. The concentrate obtained from commercial feed companies used by the all farms, which is common practice in Turkey. In addition to the concentrates, the oat grain was used by one dairy farm. The concentrate level of TMRs ranged from 23.17 to 54.32 %. Wheat straw, corn silage and sugar beet pulp are the mainly used forages for TMRs. Alfalfa hay is used for only three dairy farms.

There is also considerable variation among TMRs in terms of chemical composition. CP contents of TMRs ranged from 9.83 to 14.32 % respectively. It is well known that CP requirements of lactating cows are considerable high due to milk production. NRC (13) suggested that lactating cows with an average 30.9 kg/d of milk production should be fed with a diet av-

eraging 16.1 % of CP of DM with a range from 13.8 to 20.8 % of DM. As can be seen from Table 1, CP contents of TMRs 1, 3, 5, 6 and 7 did not fall into this range suggested by NRC (13). On the other side, CP contents of TMRs 2, 4 and 8 was close to lower and of this range suggested by NRC (13).

As can be seen from Table 1, ADF and NDF contents of TMRs ranged from 22.79 to 32.32 % and 42.5 to 52.98 % respectively. NRC (13) recommends a minimum of 17 to 21% of ADF, 25 to 35 % of NDF for lactating cows. It is unlikely that metabolic disorders such as low milk fat, off feed problems, acidosis and feet sore will occur in the farms involved in current study since ADF and NDF contents of all TMRs studied in the current experiment higher than NRC (13) recommendation. On the other hand, Allen (14) summarized 15 studies and showed a general decline in dry matter intake with increasing NDF concentrations in diets when diets exceeded 25 percent NDF. Therefore high level of NDF contents of TMRs used in the current experiment will results in depression of feed intake, thus reducing milk production.

Estimated NFC (Non Fibrous Carbohydrates) contents of TMRs ranged from 23.9to 34.1 %. Hoover and Stokes (15) regressed data from Nocek and Russell (16) and found that when dietary NFC was greater than 45 to 50 % or less than 25 to 30 %, milk production was decreased. As can be seen from Table 1, NFC contents of TMRs 1, 2, 5 and 7 were very close to lower end of this recommended range. The others except for TMR 3 was lower than the upper end of recommended end.

In vitro gas production, methane production, NE_L and OMD of TMRs are given in Table 2. There are significant differences among TMRs in *in vitro* gas, methane production, NE_L and OMD. Gas production ranged from 82.25 to 97.25 ml. Methane productions of most of TMRs studied in the current experiment was similar whereas methane production (ml) for TMR 8 was only significantly higher than that for

Table 1. Ingredient composition (%) of TMR fed lactating cows on selected dairy farms in Turkey, (as dry matter)

	TMRs							
Ingredients	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Wheat straw	33.44	33.14	-	20.37	40.3	15.49	19.31	21.73
Corn silage	14.85	10.09	10.4	18.26	17.58	15.54	4.25	-
Sugar beet pulp	11.57	6.91	-	8.39	8.87	15.18	3.09	18.52
Concentrate	40.13	42.61	51.49	44.82	33.25	41.3	23.17	54.32
Patato	-	7.24	7.21	-	-	-	-	-
Bean straw	-	-	30.9	-	-	-	-	-
Oat grain	-	-	-	8.15	-	-	-	-
Alfala hay	-	-	-	-	-	12.49	11.58	5.43
Barley straw	-	-	-	-	-	-	19.31	-
Corn stover	-	-	-	-	-	-	19.31	-
Total	100	100	100	100	100	100	100	100
Composition (% DM)								
DM	91.00	91.47	91.12	91.49	92.57	91.92	92.43	91.66
Ash	10.94	9.17	13.17	9.60	10.14	10.25	8.70	13.17
CP	10.06	14.32	12.41	14.08	9.83	12.78	10.38	13.95
ADF	29.5	28.11	22.79	23.78	30.4	26.09	32.32	23.82
NDF	51.56	50.17	38.06	43.10	52.98	48.46	55.27	42.5
EE	2.88	2.46	2.3	3.33	2.69	2.28	1.65	2.15
NFC	24.0	23.9	34.1	29.9	24.4	26.2	24.0	28.2

DM: Dry matter (% as feed), CP: Crude protein, ADF: Acid detergent fiber, NDF: Neutral detergent fiber, EE: Ether extract, NFC= 100-(NDF+CP+EE+CA)

Table 2. Gas, methane production, net energy for lactation and organic matter digestibility of TMR fed lactating cows on selected dairy farms in Turkey

	TMRs									
Parameters	1	2	3	4	5	6	7	8	SEM	Sig.
GP(ml)	84.50^{b}	84.50^{b}	97.25^{a}	85.75^{b}	84.00^{b}	92.25^{a}	82.25^{b}	92.75^{a}	1.625	***
CH_4(ml)	12.71^{ab}	13.26^{ab}	13.39^{ab}	13.33^{ab}	12.43^{ab}	13.04^{ab}	11.88^{b}	13.74^{a}	0.473	***
CH_4(%)	15.04^{abc}	15.69^{a}	13.77^{c}	15.54^{ab}	14.80^{abc}	14.14^{bc}	14.46^{abc}	14.81^{abc}	0.440	***
NE_L	1012.0^{d}	1047.5^{cd}	1149^{a}	1084.7^{bc}	997.7^{de}	1102.7^{ab}	953.5^{e}	1117.0^{ab}	16.53	***
OMD	56.56^{cd}	57.33^{cd}	63.61^{a}	57.94^{c}	55.77^{de}	60.09^{b}	54.46^{e}	61.88^{ab}	0.578	***

*$^{a\,b\,cd}$ Row means with common superscripts do not differ (P<0.05); S.E.M. – standard error mean; Sig. – significance level; GP: Gas production (ml), CH_4 – Methane emission(ml or %), NE_L: Net energy for lactation (MJ /kg DM), OMD: Organic matter digestibility (%), , ***P<0.001.*

TMR 7. The gas and methane production of TMRs at 24 h incubation were considerably lower than those reported by Getachew *et al.* (1) who measured gas and methane production of seven TMR from selected dairies. The low gas production of TMRs in the current experiment might be associated with low NFC and high level of NDF and ADF, which ranged from 23.9 to 34.1%, 42.5 to 55.27 % and 22.79 to 32.32% respectively. NFC, NDF and ADF contents TMRs selected by Getachew *et al.* (1) ranged from 34.9 to 46.3, 25.0 to 31.5% and 18.3 to 24.6% respectively.

TMRs used in the current experiment were collected from small dairy farms without qualified consulting nutritionist. Therefore, it was expected that there would be considerable variations in chemical composition of TMRs, which was the case and the variation in chemical compositions of TMRs would affect the methane production. However, the type of TMR has a significant effect on methane emission but not great as much as expected. The methane production of TMRs ranged from 11.88 to 13.74 ml per 0.5 g incubated DM. Methane production is been affected by forage species and quality.

Gas production, NE_L and OMD for TMRs 3, 6 and 8 was significantly higher than the others. As can be seen from Table 2, NE_L of TMRs ranged from 953.5 to 1149 kcal/kg DM. The recommendation of NRC (13) for NE_L ranged 1234 to 1640 kcal /kg DM for lactating cows with 10-30 kg milk production. Therefore, TMRs studied in the current study is not likely to meet the energy requirement of lactating cows since the NE_L of TMRs offered were lower than those suggested by NRC (13).

The cell wall contents of feedstuff are very important factors affecting the nutritive value of feedstuffs. As can be seen from Table 1, an increase in NDF and ADF of TMRS at the expense of NFC decreased the gas production, digestibility and NE_L value of TMRs since cell wall contents of TMRs are less fermentable than NFC contents of TMRs. As can be seen from Table 1, NFC contents of TMRs increased with increased level of concentrate. Concentrates are rich in NFC than that for forages. Therefore, cell wall structural elements contents are negatively correlated with nutritive value parameters such as gas production, digestibility and energy value of feedstuffs. As can be seen from Table 3, *in vitro* gas and methane production of TMRs were negatively correlated with NDF or ADF contents of TMRs.

It is well know that concentrate contains more fermentable substrate than forages. Therefore, the concentrate produces more gas and methane when fermented by rumen micro-organisms.

Table 3. Correlation coefficient (r) of relationship of chemical composition with gas, methane production and estimated parameters

	GP	CH_4	NE_L	OMD
Ash	0.828*	0.624^{NS}	0.714*	0.848**
CP	0.399^{NS}	0.828*	0.688^{NS}	0.538^{NS}
ADF	-0.808*	-0.906**	-0.965**	-0.890**
NDF	-0.830*	-0,840**	0.929**	0.904**
EE	-0.177^{NS}	0.302^{NS}	0.143^{NS}	-0.072^{NS}
NFC	0.791*	0.609^{NS}	0.812*	0.814*

*Ash (% of DM), CP – Crude protein (% of DM), ADF – Acid detergent fiber (% of DM), NDF – Neutral detergent fiber(% of DM), EE: Ether extract (% of DM), CT – Condensed tannin (% of DM), GP: Gas production (ml), CH_4 – Methane emission (ml), NE_L: Net energy for lactation (kcal /kg DM), OMD: Organic matter digestibility (%) NS: Not significant, ** P<0.01, *P<0.05*

As can be seen from Table 3, NE_L and OMD decreased with increasing NDF or ADF contents of TMRs whereas NE_L and OMD increased with increasing NFC content. Therefore, TMRs studied in the current study should be supplemented with concentrate to increase NE_L contents.

Conclusion

All theTMRs investigated in the current study are not well balanced to meet the nutritional requirements of lactating cows. Therefore, it is not likely that lactating cows fed with these TMRs will not explain their genetic potential very well. Especially CP and NE_L of TMRs should be taken into consideration to improve efficiency in milk production of lactating cows in small farms in the Turkey and worldwide.

References

1. Gatechew G, Robinson P, DePeters EJ, Taylor SJ, Gisi DD, Higginbotham GE, Riordan TJ. Methane production from commercial dairy rations estimated using an *in vitro* gas production technique. Animal Feed Science and Technology 2005; 123-124:391-402.
2. Kamalak A. Determination of potential nutritive value of Polygonum aviculare hay harvested at three maturity stages. Journal of Applied Animal Research 2010;38: 69-71.
3. Nijdda AA, Nasiru A. *In vitro* gas production and dry matter digestibility of tannin containing forages of semi –arid region of north-eastern Nigeria. Pakistan Journal of Nutrition 2010; 9:60-66.
4. Purcell, P.J., O'Brien, M., Boland, T.M. and O'Kiely, P. In vitro rumen methane output of perennial ryegrass samples prepared by freeze drying or thermal drying (40°C). Animal Feed Science Technology 2011; 166:175-182.
5. Kaya E, Kamalak A, Potential nutritive value and condensed tannin contents of acorns from different oak species. Journal of Veterinary Faculty, Kafkas University 2012; 18(6):1061-1066.
6. Prirondini M, Malagutti L, Colombini S, Amodeo P, Crovetto GM. Methane yield from dry and lactating cows diets in the Po Plain (Italy) using an *in vitro* gas production technique. Italian Journal of Animal Science 2012;11:330-335.
7. Johnson KA, Johnson DE. Methane emissions from cattle. Journal of Animal Science 1995; 73:2483-2492, 1995.
8. AOAC, 1990. Official method of analysis. 15th ed., pp.66-88. Association of official analytical chemists, Washington, DC, USA.
9. Van Soest PJ, Wine RH. The use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell wall constituents. Journal of the Association of Official Analytical Chemist 1967; 50: 50–55.
10. Van Soest PJ. The use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fiber and lignin. Journal of the Association of Official Analytical Chemist 1963; 46: 829–835.
11. Menke KH, Steingass H. Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. Animal Research Development 1988; 28:7-55.
12. Goel G, Makkar HPS, Becker K. Effect of *Sesbania sesban* and *Carduus pycnocephalus* leaves and Fenugreek (*Trigonella foenum-graecum* L) seeds and their extract on partitioning of nutrients from roughage and concentrate-based feeds to methane. Animal Feed Science and Technology 2008;147 (1-3): 72-89.
13. NRC, 2001. Nutrient requirements of dairy cattle. 7.ed. Washington, D.C. National Academy Press.
14. Allen MS. Effects of diet on short-term regulation of feed intake by lactating dairy cows. Journal of Dairy Science 2000;83:1598– 1624.
15. Hoover WH, Stokes SR. Balancing carbohydrates and proteins for optimum rumen microbial yield. Journal of Dairy Science 1991;74:3630– 3644.
16. Nocek JE, Russell JB. Protein and energy as an integrated system. Relationship of ruminal protein and carbohydrate availability to microbial synthesis and milk production. Journal of Dairy Science 1988;71:2070– 2107.

Correspondence:
Mustafa Boga
Bor Vocational School
Nigde Omer Halisdemir University, TR 51700, Nigde, Turkey,
Tel: (90) 5308356251
Fax: (90) 388 311 84 37
E-mail: mboga@ohu.edu.tr

Progress in Nutrition 2020; Vol. 22, N. 1: 204-213 DOI: 10.23751/pn.v22i1.8037

Original article

The relationship between nutritional status, serum folic acid and homocyteine levels in hemodialysis and peritoneal dialysis patients

Handan Akalın[1], *Neslişah Rakıcıoğlu*[2]

[1]Atatürk University, Faculty of Health Sciences, Department of Nutrition and Dietetics, Erzurum,Turkey; [2]Hacettepe University, Faculty of Health Sciences, Department of Nutrition and Dietetics, Ankara,Turkey - E-mail: neslisah@hacettepe.edu.tr

Summary. *Background.* Nutritional deficiencies and imbalances may be encountered in hemodialysis (HD) and peritoneal dialysis (PD) patients. The aim of this study is to investigate the relationship between nutritional status, and serum folic acid and homocysteine levels in hemodialysis and peritoneal dialysis patients. *Methods.* Information about demographics and eating habits were recorded using a questionnaire in 30 hemodialysis and 30 peritoneal dialysis patients. The subjective global assessment was used to assess the nutritional status of the patients. Individual food consumption records were taken in three consecutive days. Some routine blood parameters were recorded from the patient files. Folic acid, vitamin B_{12} and homocysteine analysis were also performed from the serum samples from the remaining blood. *Results.* All of the dialysis patients were found malnourished. Body weight, body mass index, waist and hip circumferences values was lower HD patients than PD patients ($p<0.05$). Serum homocysteine levels of PD patients were higher than HD patients ($p<0.05$). A positive correlation was identified between serum homocysteine and BUN levels, whereas there was an inverse relationship between homocysteine and vitamin B_{12} level, total protein, albumin level and malnutrition score ($p<0.05$). In general, no significant difference was found between the nutrients intake of HD and PD patients. *Conclusion.* Nutritional status of patients of HD was found better than PD patients. Assessment of nutritional status periodically is important for the prevention of malnutrition and early intervention in dialysis patients. Dietary folic acid, vitamin B_{12}, vitamin B_6 and protein intake may be improved by foods or supplementation for prevention of hyperhomocysteinemia in dialysis patients.

Key words: nutritional status, dialysis, malnutrition, folic acid, vitamin B_{12}, homocysteine

Background

Renal cachexia, malnutrition and inflammation are the most important risk factors for medical complications, cardiovascular deaths and all-cause morbidity and mortality in dialysis patients (1, 2). The control of blood pressure (3), excessive fluid accumulation (4), pulmonary hypertension (5), fatigue (6), anemia (7), the management of hyperphosphatemia (8, 9) and the effects of inflammation, to prevent and fix the weakening and sarcopenia, reduce costs of medication (1), improve quality of life and survival (2). Course these all are affected by the nutritional status of patients with dialysis.

Assessment and monitoring of nutritional status in dialysis patients is important to determine malnutrition and to make timely nutritional therapy. In this context, to know effect of dialysis types on blood homocysteine, folic acid, vitamin B_{12} levels and association of these molecule levels with nutritional status will affect the medical approach and the prognosis of patients. In other words, to protect dialysis patients from cardiovascular diseases; how much folic acid and vitamin B_{12} supplementation should be given, and what type of food supplements may be used can be deter-

mined by examining the relationship between nutrient intake, folic acid, vitamin B_{12}, and homocysteine levels. The nutritional interventions to be applied without evaluation will lead to nutritional deficiencies and over dosage in the vitamins, or unnecessary costs. As far as we detect in literature, studies on dialysis patients have been based on generally clinical data but limited malnutrition measures (2, 10). Sarcopenic obesity, coagulability, risk of mortality and morbidity from infection and death risk in elderly is higher for patients receiving PD than in those receiving HD (11, 12). Insufficient home-based nutrition therapy, nutritional self-care or nutritional education facilities, consulting with a dietitian may have an additional effect on nutrition status in PD compared with HD. In this study, we evaluated the influence of hemodialysis (HD) and peritoneal dialysis (PD) on nutritional status and patient's plasma levels of homocysteine, folate, and vitamin B_{12}. Also, malnutrition has been evaluated as versatile through food consumption records, clinical examination, anthropometric measurements and biochemical parameters as a different aspect. The hypothesis of this study was hemodialysis is superior in nutritional aspects and B vitamins levels (folate, vitamin B_{12}) and, decreases the plasma level of homocysteine of dialysis patients than peritoneal dialysis.

The Ethics Committee of Medicine Faculty at Atatürk University, Erzurum, Turkey approved the study protocols with the number of 15 on 09.02.2007 and informed consent was obtained from all participants. Study procedures followed were in accordance with the Helsinki Declaration.

Material and methods

Subjects

The study recruited patients from Atatürk University Faculty of Medicine, Yakutiye Research Hospital, and Dialysis Unit in Erzurum province, Turkey. The study design was descriptive, cross-sectional, and single-centered. All dialysis patients who are suitable for the inclusion criteria and are eager to participate in the study were included in the study. Thirty (16 male, 14 female) HD patients and 30 (14 male, 16 female) PD patients were enrolled. Patients with dialysis have osteoporosis (12.5%), rheumatism (4.2%), and hepatitis C (4.2%) diseases. Inclusion criteria included being on maintenance HD and PD patients for >3 months whose age was >18 years, voluntary individuals who were literate. Exclusion criteria included patients exposed to nitric oxide in the last 3 months, life expectancy shorter than 6 months (metastatic cancer, terminal HIV), who have diseases such as stroke, thrombosis, myocardial infarction within the last 3 months, have received vitamin therapy in the last 4 weeks, who use anti-folate and anti-epileptic medication, alcohol, who support enteral and parenteral nutrition, who have kidney transplantation, who have blood transfusion within 30 days. All HD patients were dialyzed for 4 hours and 3 times per week with bicarbonate-buffered dialysate. Patients were dialyzed with the flow rate of blood at 350 mL/min and the flow rate of dialysate at 500mL/min. Polysulfone membranes whose surface area was 1.5-1.8 m^2 with low flow and low heparinization were used in all cases. PD patients perform four exchanges per day. PD was performed with dialysis fluids containing 1.36%, 2.27%, and 3.86% glucose. Bicarbonate was used as a buffer for PD patients.
Sociodemographic characteristics and eating habits of patients were obtained by questionnaire. Subjective Global Assessment Form was used for assessing the malnutrition status of patients (13, 14).

Anthropometric measurements

Anthropometric measurements (body weight, height, mid-arm circumference, waist circumference, and hip circumference) were performed after exiting dialysis session of HD patients and the day when they came to the doctor's control of PD patients. Dry weight was used as the body weight at the end of dialysis in HD patients. For PD patients, weighing was done with the empty abdominal cavity. The evaluation of fluid status is made by clinical observation of body weight change, edema, and blood pressure and checking of biomarkers. Participants were weighed in kilograms using a NAN brand digital weight scale with scale sensitive to 0.1kg. Standing height was measured with a tape measure. All measurements were obtained as described previously (15). Body mass index and mid-arm circumference were compared to clinical references proposed by HEMO study for dialysis patients (16).

Modified Subjective Global Assessment (MSGA)

A screening test used in the evaluation of protein-energy malnutrition. MSGA consists of 2 section and 7 variables. MSGA examines weight change in the preceding 6 months. The history focused on 7 variables, namely: weight change in preceding 6 months, presence of GI symptoms (anorexia, nausea, vomiting, and diarrhea), change in dietary intake and functional capacity, subcutaneous loss of fat, muscle wasting and edema. Variables scores are graded from 1 to 5 such as 1 = Never, 2 = Mild, 3 = Moderate, 4 = Severe 5 = Very seriously. MSGA total score ranges from 7 (normal) to 35 (severe malnutrition). MSGA score; between (7-10) is considered as well-nourished, between 11-22 is considered as having mild to moderate malnutrition, and between 23-35 is considered as severely malnourished in patients on dialysis. Application of MSGA form to dialysis patients is explained in detail in previous studies (13, 14).

Dietary intake

Food consumption record was taken in three consecutive days of all dialysis patients by the researcher. The days for the hemodialysis patients were selected as one day on the weekday, one day on the dialysis day and one day at the weekend; and it was determined as two days on the weekday, and one day on the weekend for the PD patients (17). Dietary energy and other nutrients intake were analyzed by the Food Information System (BEBİS) computer program (18). For PD patients, dialysate calorie added to total calorie intake considering dextrose concentration of dialysate, dialysate volume and dialysis frequency. The mean percentages of meeting energy and other nutrient requirements according to the RDA were determined of all dialysis patients (19-21).

Biochemical Data

Biochemical tests were made following an overnight fast, prior to the midweek dialysis session in HD patients and without interruption of the dialysis schedule in PD patients for some routine analysis With the serum samples from the remaining blood, folic acid, vitamin B_{12} and homocysteine analysis were performed. The blood was placed in EDTA tubes and iced. The plasma was separated in 5000 rpm at 5 minutes within 30 minutes. Plasma samples were stored in Eppendorf tubes at −80˚C until analyzed. The iced plasma was thawed at +4 ˚C after seven days. Homocysteine values were analyzed using the Immuchrom ready commercial kit by means of the Hewlett Packard (HP) 1100 HPLC (high-pressure liquid chromatography) system. The reference value for adult male and female is 5-15 μmol/dL (22). Folic acid and vitamin B_{12} were studied by immunochemistry without electrochemiluminescence by means of Roche Modular Analytical E 170 system. The normal range for adult male and female is 3-17 ng/mL for folic acid and 193-982 pg/mL for B_{12} vitamins (23). All other biochemical tests were taken from patient files.

Statistical Evaluation of Data

Statistical analysis was performed using SPSS 20.0 (SPSS, Inc., IBM, Illinois, USA). Continuous variables were expressed as the mean ± standard deviation and minimum-maximum ranges. Descriptive statistics are used according to the feature of data. Group differences were assessed using the unpaired *t*-test taking into account showing the normal distribution in table. Chi-square test of independent groups (Fisher Chi square or Pearson square) was used for frequency tables. Linear correlation between two variables was assessed by the Pearson test. The coefficient of correlation *r* is given. $P<0.05$ is considered a statistically significant difference (24).

Results

The mean age of HD and PD patients were 48.07±14.62 and 47.70±13.77 years old, respectively. The duration of dialysis was 52.00±44.12 months in HD patients and 27.67±29.31 months in PD patients. Mean duration of education was 6.07±4.78 years of HD patients, and 5.53±4.32 years of PD patients. Malnutrition score according to MSGA was found 17±4.14 and 14.87±4.44 in HD and PD patients, respectively ($p>0.05$). The anthropometric measurements of the patients according to dialysis were given in Table 1. There was a statistically significant difference between the weight, body mass index (BMI), waist circumference, waist/hip

Table 1. Anthropometric measurements of patients according to type of dialysis

	Type of Dialysis					
	Hemodialysis		Peritoneal dialysis			
Anthropometric measurements	**Male (n:16) $\bar{X}$±SD**	**Female (n:14) $\bar{X}$±SD**	**Male (n:14) $\bar{X}$±SD**	**Female (n:16) $\bar{X}$±SD**	**P1**	**P 2**
Body weight (kg)	66.42±7.96	55.99±12.51	73.45±8.35	68.35±19.07	**0.03***	**0.05***
Height (m)	1.69±.06	1.54±0.08	1.70±0.04	1.57±0.06	0.61	0.26
Body mass index (kg/m^2)	23.18±2.55	23.38±4.47	25.32±2.85	27.53±7.27	**0.04***	0.08
Mid arm circumference (cm)	27.12±1.82	27.14±3.82	28.10±2.41	29.81±5.55	0.22	0.14
Waist circumference (cm)	89.68±7.96	92.57±16.56	97.92±7.91	103.87±19.39	**0.02***	0.10
Hip circumference (cm)	99.43±5.25	96.35±11.94	100.57±6.83	108.5±18.39	0.61	**0.04***
Waist/hip ratio	0.90±0.07	0.95±0.10	0.97±0.07	0.95±0.60	**0.01***	0.95

**p<0.05, P1=male; P2=female*

ratio of male patients undergoing peritoneal dialysis and hemodialysis (p<0.05). These measurements of the patients with PD were found higher than HD patients.

Daily intake of energy and nutrients according to dialysis types is given in Table 2. No significant difference was found between the nutrient consumption of patients who underwent HD and PD. The percentages of receiving insufficient energy those are the normal weight were found 53.3% and 80% in HD and PD patients, respectively. 76.7% of patients with hemodialysis and 90% of patients with peritoneal dialysis consume inadequate protein. The daily intake of thiamine, vitamin B_6, folic acid, vitamin C, vitamin D, vitamin E, iron and fiber were found to have lower levels in both dialysis patient groups. Vitamin B_{12} intake was lower in females with PD than the HD patients (p>0.05). Vitamin B_{12} intake is less than RDA levels for female patients with HD and PD. Vitamin A intake is lower than recommended levels in both genders with HD whereas it is normal in PD patients.

Biochemical measurements of patients according to dialysis type are given in Table 3. In general blood parameters for health condition were found higher in PD patients than HD patients (p<0.05). Only, HDL-cholesterol, albumin, calcium, vitamin B_{12}, ferritin, and parathyroid hormone (PTH) levels were found lower in PD patients than HD patients (p<0.05). Serum folic acid level was found to be normal in 63.3% of HD patients and 73.4% of PD patients. There was no low folic acid level in HD patients but, 3.3% of PD patients had low serum folic acid level. The percentages of normal serum B_{12} levels were found 60% of hemodialysis patients and 83.3% of peritoneal dialysis patients. Although no patients with HD lower serum B_{12} levels, 3.3% of patients with PD had lower serum B_{12} levels. Also, serum homocysteine level was found a normal level in 73.3%, mild high level in 26.7% of HD patients. The percentages of mild, moderate, severe high homocysteine levels were found 13.3%, 80%, 6.7% of patients with PD, respectively.

The correlation between some blood nutritional parameters and homocysteine levels in dialysis patients are given in Table 5. A positive correlation was identified between serum homocysteine and BUN levels, whereas there was an inverse relationship between homocysteine and vitamin B_{12} level, total protein, albumin level and malnutrition score (p<0.05).

Discussion

The aim of this study was to evaluation of modality in dialysis (HD versus PD) on nutritional status and patient's plasma level of homocysteine, folate, and vitamin B_{12}. In this study, all of the hemodialysis (HD) and peritoneal dialysis (PD) patients were found to be malnourished by using the MSGA. Anthropometric measurements of PD patients were found higher than HD patients. Both male and female body weights and body mass index, waist/hip ratio were found higher in male PD patients than male HD patients (p<0.05) (Table 1). Therefore, PD patients are more prone to

Table 2 Daily energy and nutrients intake according to types of dialysis

	Type of Dialysis					
	Hemodialysis (n:30)		Peritoneal dialysis (n:30)			
Energy and Nutrients	**Male (n:16) $\bar{X}$±SD**	**Female (n:14) $\bar{X}$±SD**	**Male (n:14) $\bar{X}$±SD**	**Female (n:16) $\bar{X}$±SD**	**P1**	**P 2**
Energy (kcal)	1594.09±860.48	1200.73±504.08	1655.82±1376.71	1059.99±426.33	0.88	0.41
Protein (g/kg)	1.03±0 .65	0.88±0.47	1.02±0.76	0.60±0.25	0.56	0.25
Lipid (g)	56.47±31.57	52.16±27.56	77.83±84.58	48.67±24.74	0.36	0.72
Cholesterol (mg)	214.41±137.62	172.02±107.38	307.18±307.70	192.43±165.90	0.29	0.70
Saturated fat (g)	23.96±15.35	22.66±14.07	34.47±41.84	21.42±11.49	0.36	0.79
MUFA (g)	18.84±9.93	18.18±9.69	27.49±30.58	17.08±8.53	0.29	0.74
PUFA (g)	9.28±5.94	7.70±3.52	10.29±7.35	7.05±3.52	0.68	0.62
Carbohydrates (g)	201.07±120.89	133.78±63.81	159.41±102.90	113.84±48.78	0.32	0.34
Fibre (g)	17.38±8.94	11.93±5.44	13.75±4.59	10.16±4.39	0.18	0.33
Vitamin A (µg)	643.41±318.18	567.80±327.51	976.72±592.87	694.30±397.81	0.06	0.35
Vitamin B_1 (mg)	0.67±0.36	0.43±0.17	0.62±0.36	0.42±0.13	0.67	0.84
Vitamin B_2 (mg)	1.14±0.62	0.76±0.31	1.20±0.83	0.84±0.24	0.82	0.46
Vitamin B_6 (mg)	1.03±0.58	0.66±0.29	0.98±0.58	0.62±.22	0.83	0.70
Vitamin B_{12} (µg)	3.54±3.88	2.29±1.47	4.01±3.87	1.94±1.58	0.74	0.53
Total Folic Acids (µg)	243.98±138.01	167.73±73.28	213.96±125.31	156.18±61.87	0.54	0.64
Vitamin C (g)	62.61±37.36	42.62±31.86	54.16±31.00	42.47±20.37	0.51	0.97
Sodium (mg)**	3168.72±1570.42	2538.92±1022.87	3474.82±2311.59	2849.77±1503.61	0.67	0.52
Potassium (mg)	1789.11±1017	1101.18±434.30	1680±763.57	1138.02±321.53	0.75	0.79
Calcium (mg)	565.35±356.09	371.65±202.23	590.57±583.20	451.99±129.42	0.89	0.20
Magnesium (mg)	178.80±91.53	124.61±46.88	190.33±112.34	121.79±31.48	0.76	0.85
Phosphorus (mg)	959.95±539.99	654.53±270.99	1036.90±846.97	648.59±192.08	0.77	0.95
Iron (mg)	8.68±4.64	6.33±2.63	9.78±5.49	5.35±2.01	0.56	0.26
Zinc (mg)	8.68±4.77	6.27±2.53	10.38±8.79	5.64±2.45	0.51	0.49

*t-test; *p<0.05, P1=male; P2=female ; ** Sodium from salt wasn't included in calculation; MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids*

chronic diseases. High CRP and blood lipids in PD patients also support this finding. There is a close relationship between body size and composition with physical functions and life quality (25). Mid-Upper Arm Circumference (MUAC) is one of the most important markers that represent the nutritional status and inflammation, also independently increases the survival time in HD patients (4, 26). In this study, when the arm circumference measurements of dialysis patients are compared to standard values, it was shown that male (49%) and female (47%) hemodialysis patients were in the 5^{th}-10^{th} and 10^{th}-25^{th} percentile, and male (47%) and female (49%) PD patients were in the 10^{th}-25^{th} and 25^{th}-50^{th} percentile, respectively. It shows that the protein mass of skeletal muscle of dialysis patients is low. Liman et al. (27) reported that 51% patients with hemodialysis were malnourished according to MUAC. İsoyama et al. (28) found that 20% of dialysis patients had sarcopenia, 24% of them had low muscle mass, and 15% of them had low muscle strength. Also, obese sarcopenia was found higher in the PD group than in the HD group (12). Decreased protein intake, cardiovascular insufficiency, proinflammatory cytokines and changes in DNA methylation decrease the protein synthesis in muscle and increase the muscle destruction. Decreased muscle mass and strength were increased the risk of heart failure, fractures, infections, insulin resistance, weakness, and deaths (29).

Table 3. Biochemical measurements of patients according to type of dialysis

	Type of Dialysis §		
Nutritional blood parameters	**Hemodialysis (n:30) $\bar{X}$±SD**	**Peritoneal dialysis (n:30) $\bar{X}$±SD**	**P values**
High-density lipoprotein cholesterol (mg/dl)	46.86±9.59	40.46±7.48	**0.006***
Low-density lipoprotein cholesterol (mg/dL)	121.06±37.20	146.96±45.17	**0.018***
Triglyceride (mg/dL)	169.26±98.43	213.96±138.28	0.155
Total cholesterol (mg/dL)	181.36±45.31	207.60±54.17	**0.046***
Blood urea nitrogen (mg/dL)	19.90±11.87	45.30±15.57	**0.000***
Creatinine (mg/dL)	2.95±1.40	10.85±15.51	**0.007***
Albumin (g/dL)	4.21±0.74	3.24±0.58	**0.000***
Sodium (mmol/L)	140.16±3.72	138.30±4.44	0.083
Potassium (mmol/L)	3.36±0.55	4.30±0.82	**0.000***
Calcium (mg/dL)	10.13±0.88	8.92±1.29	**0.000***
Phosphorus (mg/dL)	2.49±0.67	4.57±1.18	**0.000***
C-reactive protein (mg/dL)	0.58±0.47	2.84±4.42	**0.007***
Hemoglobin (g/dL)	11.30±1.79	10.22±3.22	0.112
Hematocrit (%)	35.13±6.09	30.32±9.58	**0.024***
Folic Acid (ng/ mL)	12.63±6.27	13.74±22.13	0.792
Homocysteine (μmol/L)	13.53±4.03	53.44±38.05	**0.000***
Vitamin B_{12} (pmol/L)	899.64±412.16	592.13±354.91	**0.014***
Alkaline phosphatase (U/L)	197.68±152.23	120.46±67.45	0.490
Lactate dehydrogenase (U/L)	248.30±95.83	265.53±96.25	**0.000***
Total Protein (g/dL)	7.90±1.19	6.41±1.01	0.340
Glucose (mg/dL)	100.93±31.80	111.33±49.91	**0.019***
Ferritin (ng/mL)	585.96±335.22	384.92±307.02	**0.000***
Parathyroid hormone (pg/mL)	723.76±627.12	256.41±226.32	**0.006***

*t-test, *p<0.05; § Dialysis output values were taken into account for HD and PD patients*

It has been found that %52-85.4 of HD patients (10, 30-32) and %40.6-74.8 of PD patients (33) were malnourished in these previous studies. It has been indicated that malnutrition increases the risk of infection in dialysis patients (34), triggers arterial calcification in HD patients, and has an important role in cardiovascular deaths (2). In this study, it was found that albumin level of PD patients was significantly lower than the HD patients (p<0.05). Similarly, Mathew et al. specified that the albumin level of PD patients was lower than the HD patients. Low albumin levels can be explained with loss of high-molecular protein during the PD and inflammation in PD patients. Low albumin levels in dialysis patients cause impairment of the Ca, P, cholesterol, and triglyceride transportation (10). Also, low levels of albumin led to impairment in total cholesterol and LDL cholesterol transport in PD patients. In this study, C-reactive protein, which is a marker of inflammation, total and LDL-cholesterol levels were found higher of PD patients than HD patients (p>0.05) (Table 3).

Various studies have shown that serum and red blood cell folic acid levels of PD patients are higher than HD patients (35, 36). However the difference was not statistically significant, it was also found that HD patients had lower serum folic acid levels than PD patients, in this study (Table 3). Presence of low folic acid level might be explained by folic acid is a small molecule and it loses during the HD process and it can also interact with other medications in HD patients (37, 38). Also, vitamin B_{12} level was found higher in HD patients than PD patients (p>0.05). Similar re-

Table 4 Correlation between some nutritional blood parameters and homocysteine levels in dialysis patients

Nutritional blood parameters	Homocysteine levels	
	Pearson Correlation	p
Triglyceride (mg/dL)	0.023	0.860
Cholesterol (mg/dL)	0.113	0.391
Blood urea nitrogen (mg/dL)	0.549	**0.000****
Creatinine (mg/dL)	0.108	0.413
Albumin (g/dL)	-0.369	**0.004****
C-reactive protein (mg/dL)	0.04	0.761
Hemoglobin (g/dL)	-0.019	0.883
Folic acid (ng/mL)	-0.147	0.262
Vitamin B_{12} (pmol/L)	-0.316	**0.014***
Lactate dehydrogenase (U/L)	-0.011	0.936
Total protein (g/dL)	-0.315	**0.014***
Body mass index (kg/ m^2)	0.163	0.212
Malnutrition score	-0.389	**0.020***
Calcium (mg/dL)	-0.315	**0.014***
Phosphorus (mg/dL)	0.380	**0.003****
Parathyroid hormone (pg/mL)	-0.256	**0.048***

**p<0.05, **p<0.01*

sults have shown in the literature (39). Vitamin B_{12} is a large molecule and its binds to haptocorrin (big non-glycoprotein) at 80-90%, thus the loss of vitamin B_{12} is less due to hemodialysis (40). Homocysteine and methylmalonic acid (MMA) are novel markers which are used to determine the functional status of vitamin B_{12} and folate in tissues. High levels of homocysteine and normal levels of MMA show the folate deficiency. On the other hand, high levels of homocysteine and MMA show vitamin B_{12} deficiency (41). Vitamin B_{12} serves as a vehicle molecule in the transfer of the methyl groups from 5-methyltetrahydrofolate to homocysteine. Therefore, even though folic acid levels are normal, vitamin B_{12} deficiency cannot ensure the methionine remethylation from homocysteine. Furthermore, adenosyl-cobalamin, as one form of vitamin B_{12}, ensures the transformation of methylmalonic acid to succinyl CoA. This reaction has an important role in the metabolism of fatty acids and aliphatic amino acids. Vitamin B_{12} deficiency causes abnormal lipid accumulation (42, 43). In this study, abnormal lipid levels of PD patients could be correlated with the functional vitamin B_{12} deficiency. Normal blood vitamin B_{12} level is associated with the late emptying of vitamin B_{12} stores in the body. Vitamin B_{12} is stored in the body for 3-5 years, folic acid is stored in the body for 1-1.5 years, and vitamin B_6 is stored in the body for 3-4 months (40). Also, it has been found that 80% of the PD patients have moderate levels of homocysteine and 73.3% of the HD patients have normal levels of homocysteine in this study. It is shown that homocysteine levels were higher than the cutoff value (13.5μmol/L) in both dialysis modality (HD and PD) (44). Decrease of cystathionine synthase activity occurs due to PEM, decreases the synthesis of cytosine and cysteine from methionine and it leads to hyperhomocysteinemia (45). In this study, dietary protein intake of PD patients was found lower than HD patients ($p>0.05$) (Table 2). Furthermore, there is a positive relationship between serum homocysteine and BUN levels and there is an inverse relationship between vitamin B_{12} levels, total protein levels, albumin levels, and malnutrition scores in this study ($p<0.05$) (Table 4). Despite normal vitamin B_{12} and folate levels, high homocysteine levels may show the decrease in the function of vitamin B_{12} receptors dependent on uremia. The decrease in transcobalamin II level may lead to the reduction of vitamin B_{12} intake from peripheral tissues in inflammation prone HD patients. This mechanism may occur in order to remove the vitamin B_{12} from pathogenic microorganisms which lead to inflammation and infection in peripheral tissues. High serum vitamin B_{12} concentrations in case of inflammatory conditions such as HD, lead to functional vitamin B_{12} deficiency in peripheral tissues and thus hyperhomocysteinemia occurs (37). Saifan et al, there was a 58% prevalence of vitamin B_{12} deficiency as defined by high MMA level in HD patients (46). This hypothesis is supported by vitamin B_{12} level and the relationship between CRP and ferritin levels. Hyperhomocysteinemia could be impaired vitamin B_{12} metabolism (38). Despite normal vitamin B_{12} and folate levels, one reason for high levels of homocysteine can be explained vitamin B6 deficiency. Even though vitamin B_{12} and folate levels are normal in hemodialysis patients, it was previously shown in other studies that dialysis patients had a vitamin B_6 deficiency (41). Vitamin B_6 deficiency is observed in 24-56% of hemodialysis patients. Also, observing more

common compared to other vitamin B deficiencies because the molecular size of vitamin B_6 (MW 245) is smaller than the molecular sizes of folate (MW 441) and vitamin B_{12} (MW 1355). Therefore, the loss of vitamin B_6 is more during dialysis. Furthermore, vitamin B_6 stores in the body are limited and this also increases the deficiency risk (40). Thus, hyperhomocysteinemia can occur because of malnutrition, vitamin B_{12} functional deficiency, vitamin B_6 deficiency and uremia. In present study; the dietary consumption of vitamin B_6 is found 1.03 ± 0.58 mg and 0.66 ± 0.29 mg in male and female patients with hemodialysis, respectively. Vitamin B_6 intakes were 0.98 ± 0.58 mg and 0.62 ± 0.22 mg in the male and female patients with peritoneal dialysis patients, respectively. Although pyridoxine is recommended 1.3-1.7 mg in diet of healthy adults (47) , this daily intake of pyridoxine does not meet requirements of hemodialysis and peritoneal dialysis patients. It is proposed that dialysis patient should consume more vitamin B_6 than RDA (20, 48).

Strengths of the study are as follows; measurements were performed in only one laboratory and subjective global assessments and anthropometric measurements were performed by one dietitian. These strengths ensure the minimum measurement bias. In this study, there were an equal number of patients from each group, age characteristics of the two groups are similar, and both genders were well represented. These strengths decreased the error dependent on confounding factors. This study is a single-centered study. Vitamin B_6 levels were not measured, functional indexes/biomarkers were not used in the determination of the vitamin B_{12} and folate levels, and only static indexes were used. These are the important limitations of this study. MMA level was not measured whereas only homocysteine levels were measured. This prevented to determine whether or not hyperhomocysteinemia occurs due to the functional deficiency of vitamin B_{12} or folate.

Conclusion

This study was conducted to compare nutritional status, serum folic acid and homocysteine levels of hemodialysis and peritoneal dialysis patients. Malnutrition is identified in both dialysis modalities. Body mass indexes (BMI), waist circumference, waist/hip ratio of male PD patients were found higher than male HD patients. PD patients had a higher level of homocysteinemia than HD patients. Hyperhomocysteinemia can be affected by malnutrition, functional deficiency of vitamin B_{12}, vitamin B_6 deficiency and uremia. These findings suggest that when anthropometric measurements are assessed in patients on dialysis, a health professional should be considered to use with body composition methods. While making a decision about dialysis modality in renal units, it should be also considered that the types of dialysis which could effect on nutritional status and homocysteine, folic acid, vitamin B_{12} levels of patients. Further analysis of data after longer follow-up is needed to suggest a nutritional status benefit for a particular dialysis modality. Also, further studies are required for the determination of well and malnutrition nutritional status at the beginning of dialysis in patients. In addition, change of nutritional status with dialysis modality in the long term should be detected.

Supplementary Data

None declared.

Acknowledgements

We thank Prof. Dr. Ramazan Çetinkaya from Atatürk University for supporting clinical applications.

Conflict of interest statement

None declared.

References

1. Kalantar-Zadeh K, Ikizler TA. Let them eat during dialysis: an overlooked opportunity to improve outcomes in maintenance hemodialysis patients. J Ren Nutr 2013;23(3):157-63.
2. Nakagawa N, Matsuki M, Yao N, Hirayama T, Ishida H, Kikuchi K, et al. Impact of metabolic disturbances and malnutrition-inflammation on 6-year mortality in Japanese patients undergoing hemodialysis. Ther Apher Dial 2015;19(1):30-9.

3. Harper J, Nicholas J, Webbc L, Casula A, Williams AJ. UK Renal Registry 12th Annual Report (December 2009): chapter 11: blood pressure profile of prevalent patients receiving dialysis in the UK in 2008: national and centre-specific analyses. Nephron Clin Pract. 2010;115 Suppl 1:c239-60.
4. Mathew S, Abraham G, Vijayan M, Thandavan T, Mathew M, Veerappan I, et al. Body composition monitoring and nutrition in maintenance hemodialysis and CAPD patients—a multicenter longitudinal study. Ren Fail 2015;37(1):66-72.
5. Genctoy G, Arikan S, Eldem O. Pulmonary hypertension associates with malnutrition and body composition hemodialysis patients. Ren Fail 2015;37(2):273-9.
6. Nowak Z, Laudanski K. The perception of the illness with subsequent outcome measure in more favorable in continuos peritoneal dialysis vs hemodialysis in the framework of appraisal model of stress. Int J Med Sci 2014;11(3):291-7.
7. Frazao CM, de Sa JD, Medeiros AB, Fernandes MI, Lira AL, Lopes MV. The adaptation problems of patients undergoing hemodialysis: socio-economic and clinical aspects. Rev Lat Am Enfermagem 2014;22(6):966-72.
8. Cupisti A, Kalantar-Zadeh K. Management of Natural and Added Dietary Phosphorus Burden in Kidney Disease. Semin Nephrol 2013;33(2):180-90.
9. Jiang N, Fang W, Yang X, Zhang L, Yuan J, Lin A, et al. Dietary phosphorus intake and distribution in Chinese peritoneal dialysis patients with and without hyperphosphatemia. Clin Exp Nephrol 2015;19(4):694-700.
10. Zhang K, Cheng G, Cai X, Chen J, Jiang Y, Wang T, et al. Malnutrition, a new inducer for arterial calcification in hemodialysis patients? J Transl Med 2013;11(1):1.
11. Han SS, Park JY, Kang S, Kim KH, Ryu D-R, Kim H, et al. Dialysis modality and mortality in the elderly: a meta-analysis. Clin J Am Soc Nephrol 2015;10(6):983-93.
12. Koefoed M, Kromann CB, Juliussen SR, Hvidtfeldt D, Ekelund B, Frandsen NE, et al. Nutritional status of maintenance dialysis patients: low lean body mass index and obesity are common, protein-energy wasting is uncommon. PloS one. 2016;11(2):e0150012.
13. Janardhan V, Soundararajan P, Rani NV, Kannan G, Thennarasu P, Chacko RA, et al. Prediction of malnutrition using modified subjective global assessment-dialysis malnutrition score in patients on hemodialysis. Indian J Pharm Sci 2011;73(1):38.
14. Mohammed FA, Farhood HF, AtheemWtwt M. Prediction of malnutrition using modified subjective global assessment-dialysis malnutrition score in patients on chronic hemodialysis. J Community Med Health Educ 2014;4(3):291.
15. Control CfD, Prevention. National Health and Nutrition Examination Survey (NHANES). Anthropometry Procedures Manual. Atlanta, GA: Centers for Disease Control and Prevention; 2007. National Center for Health Statistics, editor. 2016.
16. Chumlea WC, Dwyer J, Bergen C, Burkart J, Paranandi L, Frydrych A, et al. Nutritional status assessed from anthropometric measures in the HEMO study. J Ren Nutr 2003;13(1):31-8.
17. Ortega RM, Pérez-Rodrigo C, López-Sobaler AM. Dietary assessment methods: dietary records. Nutr Hosp 2015;31(3):38-45.
18. Bebis (Beslenme Bilgi Sistemi) Nutrition Data Base Software [Internet]. Data base: The German Food Code and Nutrient Data Base (BLS II.3, 1999) with additions from USDA-sr and other sources. 2004.
19. Kopple JD. National kidney foundation K/DOQI clinical practice guidelines for nutrition in chronic renal failure. Am J Kidney Dis 2001;37(1):S66-S70.
20. Kosmadakis G, Da Costa Correia E, Carceles O, Somda F, Aguilera D. Vitamins in dialysis: who, when and how much? Ren Fail 2014;36(4):638-50.
21. Rucker D, Thadhani R, Tonelli M, editors. Trace element status in hemodialysis patients. Semin Dial 2010: Wiley Online Library.
22. Ueland PM, Refsum H, Stabler SP, Malinow MR, Andersson A, Allen RH. Total homocysteine in plasma or serum: methods and clinical applications. Clin Chem 1993;39(9):1764-79.
23. Gutcho S, Mansbach L. Simultaneous radioassay of serum vitamin B12 and folic acid. Clin Chem 1977;23(9):1609-14.
24. Aktürk Z., Acemoğlu H. Sağlık çalışanları için araştırma ve pratik istatistik (Research and practical statistics for health workers). Istanbul: Anadolu Matbaası; 2010
25. Stenvinkel P, Carrero JJ, von Walden F, Ikizler TA, Nader GA. Muscle wasting in end-stage renal disease promulgates premature death: established, emerging and potential novel treatment strategies. Nephrol Dial Transplant 2016;31(7):1070-7.
26. Chen J, Peng H, Zhang K, Xiao L, Yuan Z, Chen J, et al. The insufficiency intake of dietary micronutrients associated with malnutrition-inflammation score in hemodialysis population. PLoS One. 2013;8(6):e66841.
27. Liman H, Anteyi E, Oviasu E. Prevalence of malnutrition in chronic kidney disease: A study of patients in a tertiary Hospital in Nigeria. Sahel Medical Journal. 2015;18(5):8.
28. Isoyama N, Qureshi AR, Avesani CM, Lindholm B, Bàràny P, Heimbürger O, et al. Comparative associations of muscle mass and muscle strength with mortality in dialysis patients. Clin J Am Soc Nephrol 2014;9(10):1720-8.
29. Stenvinkel P, Carrero JJ, Von Walden F, Ikizler TA, Nader GA. Muscle wasting in end-stage renal disease promulgates premature death: established, emerging and potential novel treatment strategies. Nephrol Dial Transplant 2016;31(7):1070-7.
30. Tan SK, Loh YH, Choong LHL, Suhail SM. Subjective global assessment for nutritional assessment of hospitalized patients requiring haemodialysis–a prospective cohort study. Nephrology 2015.
31. Sedhain A, Hada R, Agrawal RK, Bhattarai GR, Baral A. Assessment of Nutritional Status of Nepalese Hemodialysis Patients by Anthropometric Examinations and Modified Quantitative Subjective Global Assessment. Nutr Metab Insights 2015;8:21.

32. Rani VN, Kavimani S, Soundararajan P, Chamundeeswari D, Kannan G. Correlation between anthropometry, biochemical markers and subjective global assessment-dialysis malnutrition score as predictors of nutritional status of the maintenance hemodialysis patients. Int J Med Res Health Sci 2015;4(4):852-6.
33. Prasad N, Sinha A. Impact of Malnutrition And Comorbities On Survival Of Peritoneal Dialysis Patients. Indian Journal of Peritoneal dialysis. 2013;24(1):18-30.
34. van Diepen AT, Infections in peritoneal dialysis patients: Incidence, determinants and the association with peritoneal transport. 2015
35. Shaban H, Ubaid Ullah M, Berns JS, editors. Measuring vitamin, mineral, and trace element levels in dialysis patients. Semin Dial 2014: Wiley Online Library.
36. Lee E-Y, Kim J-S, Lee H-J, Yoon D-S, Han B-G, Shim Y-H, et al. Do dialysis patients need extra folate supplementation? Adv Perit Dial 1999;15:247-52.
37. Soohoo M, Ahmadi S-F, Qader H, Streja E, Obi Y, Moradi H, et al. Association of serum vitamin B12 and folate with mortality in incident hemodialysis patients. Nephrol Dial Transplant 2017;32(6):1024-32.
38. Yeh E-L, Huang Y-C, Tsai S-F, Yu T-M, Wu M-J, Chen C-H. Relationship between plasma levels of homocysteine and the related B vitamins in patients with hemodialysis adequacy or inadequacy. Nutrition 2018;53:103-8.
39. Perna AF, Lanza D, Sepe I, Conzo G, Altucci L, Ingrosso D. Altered folate receptor 2 expression in uraemic patients on haemodialysis: implications for folate resistance. Nephrol Dial Transplant 2013;28(5):1214-24.
40. Corken M, Porter J. Is vitamin B(6) deficiency an underrecognized risk in patients receiving haemodialysis? A systematic review: 2000-2010. Nephrology (Carlton). 2011; 16(7): 619-25.
41. Sarvari GR, Naseri M, Esmaeeli M, Azarfar A. Serum Folate and Vitamin B12 Levels in Hemodialysis Patients: Is There any Correlation with Plasma Homocysteine Levels? J Ped Nephrology 2014;2(4):140-6.
42. Capelli I, Cianciolo G, Gasperoni L, Zappulo F, Tondolo F, Cappuccilli M, et al. Folic acid and vitamin B12 administration in CKD, why not? Nutrients 2019;11(2):383.
43. Şen S, Durat G, Atasoy I. Vitamin B 12 ve Folik Asit Eksikliğinin Psikiyatrik ve Nörolojik Bozukluklarla İlişkisi. Türk Klinik Biyokimya Derg 2009;7(1):31-6.
44. De Vecchi A, Bamonti-Catena F, Finazzi S, Campolo J, Taioli E, Novembrino C, et al. Homocysteine, vitamin B12, and serum and erythrocyte folate in peritoneal dialysis and hemodialysis patients. Perit Dial Int 2000;20(2):169-73.
45. McCully KS. Chemical pathology of homocysteine. V. Thioretinamide, thioretinaco, and cystathionine synthase function in degenerative diseases. Ann Clin Lab Sci 2011; 41(4): 301-14.
46. Saifan C, Samarneh M, Shtaynberg N, Nasr R, El-Charabaty E, El-Sayegh S. Treatment of confirmed B12 deficiency in hemodialysis patients improves Epogen® requirements. Int J Nephrol Renovasc Dis 2013;6:89.
47. Chan M, Kelly J, Tapsell L. Dietary modeling of foods for advanced CKD based on General Healthy Eating Guidelines: what should be on the plate? Am J Kidney Dis 2017; 69(3): 436-50.
48. Ross EA, Shah GM, Reynolds RD, Sabo A, Pichon M. Vitamin B6 requirements of patients on chronic peritoneal dialysis. Kidney Int 1989;36(4):702-6.

Correspondence:
Neslişah Rakıcıoğlu 2
Hacettepe University, Faculty of Health Sciences, Department of Nutrition and Dietetics, Ankara,Turkey
E-mail: neslisah@hacettepe.edu.tr

Progress in Nutrition 2020; Vol. 22, N. 1: 214-223 DOI: 10.23751/pn.v22i1.7810

Original article

Investigation of aflatoxin levels in chips by HPLC using post-column UV derivatization system

Jale Çatak, Mustafa Yaman, Halime Uğur

Department of Nutrition and Dietetics, Faculty of Health Sciences, İstanbul Sabahattin Zaim University, Istanbul, Turkey - E-mail: jalecatak@gmail.com

Summary. This study was designed to investigate the aflatoxin B1 (AFB1), B2 (AFB2), G1(AFG1), G2(AFG2) and total aflatoxin levels in 27 samples of several packaged chips randomly obtained from various markets in Istanbul using immunoaffinity chromatography and post-column UV derivatization system by HPLC. Seventeen of the samples contained some aflatoxins at detectable levels, whereas 10 did not. The levels of aflatoxin ranged between 1 and 39 μg/kg. AFB1 and AFB2 were detected in 63% and 41% in analyzing chip samples respectively. The levels of AFB1 varied from 1 to 24 μg/kg in chip samples and the levels of AFB2 varied from 1 to 16 μg/kg in chip samples. Low levels of AFG2 ranging from 0 to 3 μg/kg were found in chips. Groundnut chips showed high aflatoxin concentrations and recorded relatively higher AFB1, AFB2, AFG2 and Total aflatoxin levels ranging from 1 to 39 μg/kg, indicating that groundnut is potentially causing serious health risks to consumers of these products than other chips. The aim of this study is to determine the presence and amount of aflatoxin B1, B2, G1, G2 in various chips samples consumed in Istanbul.

Key words: aflatoxin, chips, HPLC, mycotoxin, post-column UV derivatization system

Introduction

Chips are nutrients with high energy and low nutritive value. Chips products have a huge potential as a snack for children and young people and depending on this its market is increasing and also export of these products is growing day by day in the World and Turkey (1). Aflatoxins are mycotoxins, which are considered to be toxic metabolites, produced mainly in foods by some fungi such as *Aspergillus flavus* and *Aspergillus parasiticus* (2, 3). In the United States daily calorie intake for children between the ages of 2 and 5 increased by 30% with the contribution of snack foods in between 1977 and 1996. It is also reported in several publications that there is an association between the increase in consumption of these snack foods and the increase in obesity and other chronic diseases related to obesity in children and adolescents (4).

Worldwide obesity has shown a threefold increase compared to 1975 and it has been appointed that there are 1.9 billion adults over the age of 18 being overweight and 650 million of them are obese in 2016. In other words, while 39% of adults over 18 years old were overweight, 13% of them determined to be obese (5). Obesity is increasingly widespread among children and has increased 10-fold over the past 40 years. 41 million children between 0-4 years of age and 340 million children aged 5-19 years were found to be overweight or obese in 2016 (5, 6). If the current situation continues, the number of overweight or obese children aged 0-5 years is expected to increase globally to 70 million by 2025 (7).

In 2016 Turkey Health Interview Survey conducted by the Turkish Statistical Institute (Turkstat), Body Mass Index (BMI) was calculated using height and weight values. It has been determined in this study

that the proportion of obese individuals aged 15 years and over has declined from 19.9% in 2014 to 19.6% in 2016 (8). It has been stated in 'Turkey School-age Children (Age 6-10) Growth Monitoring Project' in 2009 that being obese and overweight status among the children of this age was found 14.3% and 6.5%, respectively (9). Additionally, according to the another study of the same age group which was the COSI-TUR research, 14.9% of the children between this age group has been identified as overweight and 9.9% as obese in 2016 (6).

Over the last few years, the growth in the sales of salty snacks in the US continues to be steady. Sales in 2015 reached 22 billion dollars with an increase of 3.5%. The best salty snack was potato chips, which remained unchanged at 2015 and had a share of 7.5 billion dollars. Prospectively, an annual growth rate of 4% is predicted in the salted snack market between 2015 and 2020 (10). Considering the number of people consuming potato chips in the US based on years, the number of people in 2011 was 257 million and it reached to 276 million in 2017. Consumption of 16 packets or more per year, which is the highest amount of these in annual consumption, covered 19.9 million people in 2011 and rose to 32.8 million people in 2017 (11, 12).

When considering the chip market development over the years in Turkey, the market, which has a size of approximately 233 million dollars in 2004, reached a turnover of 1.1 billion dollars in 2012 and about 1.3 billion dollars in 2013. The sales volume of the market in 2012 reached 90 thousand tons with a growth of 300% has reached 105 thousand tons of sales volume in 2013. With the increased production, the consumption of chips, which was 400 g per capita in 2004, has increased to about 1 kg by 2012 (13). In 'Turkey Childhood Obesity Research' (COSI-TUR 2013) research, the nutritional behaviours of children in Turkey are determined according to the declarations of families in 2013 for the first time. When classified according to the frequency of consumption, it was found that chips and popcorn were consumed in 1-3 days a week with the highest rate of 59.6% (14). In repetitive work in 2016, three years later, salted nut consumption was included and salted snacks consumption of children within the frequency of 1-3 days in a week was found 29.6%. They consumed less than once in a week with the highest rate (34.6%) (6).

Mycotoxins are found in various foods which were stored in hot, humid and unhealthy conditions such as cereals, especially maize and rice, some animal source foods such as milk and cheese, hard-shelled fruits such as walnuts, hazelnuts and peanuts, oilseeds such as sunflower and soybean, dried fruits and spices (red pepper, black pepper, turmeric, ginger, coriander) which are highly toxic compounds (15-17). It is estimated that mycotoxins contaminate about 25% of the world's nutrients each year and that approximately 4.5 billion people are chronically exposed to mycotoxins, according to the US Centers for Disease Control and Prevention. Among the different type of mycotoxins; aflatoxins are widespread in major food crops such as groundnuts, maize, dried fruits and spices as well as milk and meat products (17-20).

Aflatoxins are the most dangerous ones in between known 400 toxins. Aflatoxin B_1, aflatoxin B_2, aflatoxin G_1 and aflatoxin G_2 are the main ones produced naturally between 20 types of aflatoxins (21). Among these aflatoxin B_1 (AFB1) is the most predominant and toxic mycotoxin that seriously threatens the human health. Many experimental, clinical and epidemiological studies indicate that the aflatoxins have been found to be carcinogenic, genotoxic, mutagenic, teratogenic, immunotoxic, hepatotoxic, nephrotoxic and they also inhibit several metabolic systems of humans and animals (15, 16, 22-24). Prolonged exposure to aflatoxin contamination even at low levels in the crops, affecting the main functions of the organism, can lead to immune system disorders as well as liver damage or can cause cancer in various organs, especially liver and kidney (25). Aflatoxins, along with other mycotoxins, are thought to play a role in the pathogenesis of malnutrition and kwashiorkor in children, as well as in the development of edema in malnourished people (26, 27). High doses of aflatoxin exposure can result in vomiting, abdominal pain and even death (28).

Regarding these negative effects, many countries and some international organizations have made important regulations on "Acceptable health risk" to control aflatoxin contamination in foods and to ban the trading of contaminated products. These regulations generally depend on the level of economic develop-

ment of a country, the rate of consumption of high-risk products and the susceptibility of crops to contamination (25, 29). The safe limits of aflatoxin for human consumption was determined as 4-30 μg/kg. The EU has indicated that no direct human consuming product should be present at levels greater than 2 μg/kg for AFB1 and greater than 4 μg/kg for total aflatoxin (30, 31). Therefore, controls of these risky nutrients, especially risky in the context of aflatoxin (such as corn, peanut, oilseeds, dried fruits and spices) and like chips, which can be contain aflatoxin have a high importance in terms of food safety, protecting consumer health especially children and economics (15, 23, 32). In the literature, there are studies based on the aflatoxin content of foods that focused on cereals, particularly maize, oilseeds like groundnut and spices (33-42) but studies on the aflatoxin content of chips are limited (43-48) and more important these limited number studies are based on traditional foods. This study was conducted in order to determine the presence and levels of aflatoxins, which have been proven to have adverse effects on health and the residues of aflatoxin B_1 (AFB1), B_2 (AFB2), G_1 (AFG1), G_2 (AFG2) and total aflatoxin in 27 chips which are purchased from various markets in the province of Istanbul, Turkey.

Materials and methods

This study has focused on the aflatoxin levels of chips. Immunoaffinity chromatography and post-column UV derivatization system by HPLC were used to determine the presence and levels of AFB1, AFB2, AFG1 and AFG2 in the 27 chips samples.

The following chemicals; methanol (MeOH), acetonitrile (ACN), sodium chloride (NaCI), potassium dihydrogen phosphate (KH_2PO_4), sodium hydroxide (NaOH) and aflatoxin mix 4 solutions (B_1+B_2+G_1+G_2) were obtained from Sigma (St. Louis, MO, U.S.A). Immunoaffinity column (AFLAPREP, Product Code: P07) was obtained from R-Biopharm (Glasgow, UK) for the purification of aflatoxins. Teflon tube "tubing" (length: 20 m diameter: 0.25 mm) was purchased from Supelco Analytical. UVA lamp (20W, 60cm) was supplied by Sylvania. In this study, all other chemicals were used in high purity.

Sampling and sample preparation

27 different kinds of chips were sampled which are obtained from different markets in Istanbul according to the sampling protocol of Turkish Food Composition Table (49) The samples were homogenized by grinding. Then, 50 g of each sample was weighed and placed in a 250 mL plastic beaker. Then, 5 g of sodium chloride and 100 mL of distilled water were added and thoroughly mixed in a high speed mixer for 1 min. After that, 150 mL of methanol was added to the prepared mixture and mixed again in the high-speed mixer for 2 minutes. The mixture was filtered through a filter paper and centrifuged at 4000 rpm for 10 minutes and then adjusted to pH 7.4 with a 2M NaOH solution. After purification 5 mL of this obtained liquid was taken and 5 mL of buffer solution was added onto this (50)

Immunoaffinity chromatography

The extract obtained in the preparation of the sample was passed through the immunoaffinity column in a volume of 2 mL per minute with the prepared pump system. After the sample loading was completed, the column was rinsed with 20 mL of buffer solution to remove residual impurities. The toxins were eluted with 1 mL of methanol at a flow rate of approximately 5 mL per minute and then filtered through a 0.22 μm cellulose-based filter and injected into the HPLC.

HPLC conditions

The content of aflatoxins was determined by HPLC, consisting of Shimadzu Nexera-İ LC-2040C 3D pump with a Shimadzu RF-20A fluorescence detector (Shimadzu Corporation, Kyoto, Japan) according to the procedure described by (51) with some modifications. The Mobile phase consisted of a mixture of water/acetonitrile/methanol (60/15/30 //v/v/v). Excitation and emission wavelengths 365 nm and 460 nm for aflatoxins, respectively. The separation was performed with a Luna (5μm, 250x4.6 mm), C18 100 Å analytical column (Phenomenex, USA) and the flow rate was 1.2 mL/ min. The column oven temperature was maintained at 35°C, the analysis time was 30 min and the injection volume was 50 mL.

Derivatization System

As an alternative to the post-column derivatization system of Kobra Cell, the photochemical derivatization system was established in a laboratory environment. The derivatization system was formed by wrapping a 60 cm long UV-A lamp with a length of 20 m and a 0.5 mm diameter Teflon tube.

Results and Discussion

In this study, 27 packaged chip samples were analyzed for aflatoxin B_1 (AFB1), B_2 (AFB2), G_1 (AFG1), G_2 (AFG2) and total aflatoxin. They were obtained from various markets in Istanbul. The presence and concentration range of AFB1, AFB2, AFG1 and AFG2 in the samples were investigated by HPLC using immunoaffinity chromatography and post-column UV derivatization system. Figure 1 shows the HPLC chromatogram of aflatoxins mix standard (B_1, B_2, G_1, G_2) and Figure 2 shows the HPLC chromatogram of aflatoxins in chips.

The limit value for aflatoxin B_1, which can be found in human food according to the legal limits of aflatoxins in the European Union (EU) member countries, is accepted at 2-4 μg/kg (30, 31) Table 1 shows the legal limits for aflatoxins of foods like chips in Turkey, European Union member states and the United States.

The maximum acceptable value is determined as 5 μg/kg for aflatoxin B_1 and 10 μg/kg for Total aflatoxin ($B_1 + B_2 + G_1 + G_2$) content for processing snacks containing peanut, other oilseeds and spices in the Turkish Food Codex Legislation (52, 53)

Table 2 lists the results of determining aflatoxin values in chips. According to this, AFB1 was detected in 63% of analyzing chip samples. AFB1 has been detected in 17 chip samples and 5 of them were found above the maximum limits. In other words, 19% of the chips products are above the maximum limits considering AFB1. It has been known for many years that aflatoxin B_1 is the most carcinogenic form of aflatoxin that occurs naturally and has toxigenic properties in living organisms (54) The levels of aflatoxin B_1 varied from 1 to 24 μg/kg in chip samples. According to the results of the analysis, the amount of 24 μg/kg of AFB1 is close to about five times to legal tolerance limits applied in Turkey at 5 μg/kg.

However, AFB2 was detected in 41% of chip samples. AFB2 was found in 11 chips. AFB2 was detected in 41% in chips. The levels of aflatoxin B_2 varied from 1 to 16 μg/kg in chip samples.

AFG1 was not detected at all. However, according to the Table 2, the results of the analysis of 27 samples in total indicate that aflatoxin G_2 was found in only 1 of the samples. Low levels of AFG2 ranging from 0 to 3 μg/kg were found in chips.

With regards to Total aflatoxin, it detected in 63% of the samples. When the amount of Total aflatoxin ($B_1 + B_2 + G_1 + G_2$) in chips products is considered the results of the analysis obtained as 39 μg/kg is approximately twice as much as the tolerable level of 20 μg/kg (55) as determined by the FDA which is an international organization as well as it is approximately quadrupled of 10 μg/kg which is the legal tolerance limits applied in Turkey. In addition, 2 samples with 33 μg/kg and 39 μg/kg were well above the FDA limits whose tolerable level is 20 μg/kg. And more importantly, 39 μg/kg total aflatoxin level is almost 10 times higher than the limit values determined by the EC. However, according to the Table 2, Total aflatoxin in 41% of the analyzed samples were above the limit values determined by the EC.

With regard to Turkish Food Codex; when the amounts of aflatoxin found in the analysis of chip samples compared with the maximum acceptable val-

Table 1. Legal limits for aflatoxins of groundnut-chips in Turkey, European Union member states and United States.

Aflatoxins	Maximum acceptable levels in Turkey (μg/kg) (TFC)	Maximum acceptable levels in EU Member States (μg/kg) (EC)	Maximum acceptable levels in United States (μg/kg) (FDA)
AFB1	5	2	-
Total aflatoxin (AFB1+AFB2+AFG1+AFG2)	10	4	20

Table 2. Aflatoxin levels in chips

Chips samples	B_1 (µg/kg)	B_2 (µg/kg)	G_1 (µg/kg)	G_2 (µg/kg)	Total (µg/kg)
1*	14	16	-	3	33
2	2	1	-	-	3
3*	-	-	-	-	-
4	-	-	-	-	-
5	-	-	-	-	-
6	-	-	-	-	-
7	-	-	-	-	-
8	-	-	-	-	1
9*	5	6	-	-	11
10*	24	15	-	-	39
11	2	1	-	-	3
12*	1	-	-	-	1
13*	4	2	-	-	6
14	-	-	-	-	-
15	1	-	-	-	1
16	2	1	-	-	3
17	12	6	-	-	18
18	-	-	-	-	-
19	-	-	-	-	-
20	1	-	-	-	1
21	3	1	-	-	4
22	-	-	-	-	-
23	2	1	-	-	3
24	-	-	-	-	-
25	5	2	-	-	7
26	1	-	-	-	1
27*	1	-	-	-	1

*-: not detected; *Groundnut containing chips*

ues for aflatoxin in Turkey; it can be seen that the 15% of the chips products is above the maximum limits in terms of Total aflatoxin.

However, Total aflatoxin was detected in 17 chips. 17 of the samples contained some aflatoxins at detectable levels, whereas 10 did not. The levels of aflatoxin ranged between 1 and 39 µg/kg. Total aflatoxin levels were found above the maximum limits in 3 chips, according to the Turkish Food Codex and more importantly all of them were groundnut-containing chips.

Remarkably, 75% of the Total aflatoxin detected chips were groundnut-chips.

When we assess the groundnut-containing chips;

- Aflatoxin was detected in 86% of them and could not be detected in only 1 groundnut containing chips.
- AFB1 levels were found above the limits in 3 of the groundnut chips. In other words, 43% of the groundnut chips were found above limits considering AFB1 level.
- AFB2 was detected in 57% of the groundnut chips. Table 2 provides the AFB2 levels that there are 3 groundnut-chips in the range of 6-16 µg /kg.
- Total aflatoxin levels were detected in 3 groundnut-chips above the maximum limits as 11 µg/kg, 33 µg/kg and 39 µg/kg as can be seen in Table 2.
- 10 numbered samples (groundnut-chips) was almost 2 times the limit of 20 µg/ kg that the FDA has determined with the 39 µg/kg.
- Groundnut-chips showed high aflatoxin concentrations, with means ranging from 5 to 39 µg/kg potentially causing serious health risks to consumers of these products.
- Groundnut chips recorded relatively higher AFB1, AFB2, AFG2 and Total aflatoxin levels ranging from 1 to 39 µg/kg, indicating that groundnut is potentially a more serious risk to consumer health than other constituents.

Groundnut (Arachis hypogaea L.) is an important crop for domestic markets as well as for foreign trade in several developing and developed countries. It is also one of the most valuable crop for snack foods like chips. Inadequate hygiene conditions during drying, transport and storage stages in the production of groundnut could cause microbiological and Mycological growth which could result in the formation of mycotoxins. However, groundnut can easily be damaged by fungi, especially by *Aspergillus* species, which cause quantitative losses and produce highly toxic and carcinogenic chemical substances known as aflatoxins (15, 32)

Aflatoxin contamination is one of the main problems about dried foods and groundnuts are also suitable for aflatoxin contamination. Therefore, controlling of aflatoxins in groundnuts and groundnut-contained products such as chips has a great importance for protecting consumers.

Njumbe et al., reported in their study done in Cameroon that 74% of the maize samples were be-

ing contaminated with one or more toxins while 62% of the groundnut samples were contaminated. In the same study, aflatoxin B1 was found to be one of the most common contaminants in maize (6-645 μg/kg). Aflatoxin B1 (6-125 μg/kg) was detected as one of the main contaminants in groundnut samples (56)

Tosun and Arslan, showed that the AFB1 levels of 41 organic spice samples were found above the EU regulation limit (5 μg/kg). In a study, 93 pieces of organic spices were selected randomly from organic markets and organic shops in Turkey. In 58 organic spices AFB1 was detected. The maximum value was determined by cinnamon sample between organic spice samples (53 μg/kg). AFB1 was not detected in thyme samples. A recent study has shown that stricter measures must be taken in order to prevent mold contamination in the production of organic spices (57)

The present study evaluated the aflatoxin presence in chips obtained from markets in İstanbul. Number of samples was 27. Number of positive samples was 17. Number of samples exceeding EU limits was 11. More importantly, the results of this study also indicate that a person can easily exceed these limits. When a person consumes 1 package from the sample which numbered 1 (product pack 130 g), the EC daily intake limit for aflatoxin is excessively exceed. However, when a person consumes 2 packages from the sample which numbered 10 (product pack 55 g), the EC daily intake limit for aflatoxin is also exceeded. If we take into account that aflatoxin intake will occur also from other foods, we may have a better understanding of the seriousness of this risky situation.

In 2013, Republic of Turkey Ministry of Health Turkish Public Health Institution, Department of Obesity, Diabetes and Metabolic Diseases performed 'Obesity Surveillance Initiative' for the first time as part of WHO European Childhood Obesity Surveillance Initiative – COSI (14) In this study, the nutritional behaviours of children (Ages 7-8) in Turkey are determined according to the declarations of families.

Table 3 provides the nutritional behaviours of children for snacks consumption, such as chips and popcorn based on localities. According to the table 3, the frequency of consumption for chips and popcorn between 1-3 in a week with the highest percentage (59.6%), 60.5% in urban areas and 54.8% in rural areas. It has been reported that the frequency of consumption of foods such as chips and popcorn between 4 and more in a week more in rural areas than in urban areas with 27.7% and 21.0%, respectively. Besides, it has been stated that nearly twenty percent of children, never consume such foods (18.3%). While approximately three out of every four schools can be reached the wafers and chocolates, foods such as chips and snacks can be reached in one out of every seven schools. Accessibility is much higher in schools in the urban areas. This shows that the possibility of reaching unhealthy foods at schools is high. The data of this study were compared with the findings of the TOÇBİ (Research Report of the School-age Children (Age 6-10) Growth Monitoring Project in Turkey) research (9) While the chips and popcorn consumption frequency every day was 8.7% in this study, it was 19.0% in TOÇBİ Research. It has been suggested in a recent review that establishing of reliable and effective low-cost testing methods to monitor aflatoxin contamination levels in rural areas is necessary (25)

Table 4 provides the distribution of the salty snacks (potato chips, corn chips, snack) consumption frequency of children in Turkey and in Istanbul (6) According to the results of 'Turkey Childhood Obesity Research (2016)' 7.6% of children consume salty snacks (potato chips, corn chips, cookies) every day, 13.7% frequently (4-6 days a week), 29.6% rarely (1-3 days a week) and 34.6% consume less than once a week. However, 14.5% of children, never consume. It

Table 3. The nutritional behaviours of children in Turkey, according to the declarations of families (%), Turkey, 2013 (Republic of Turkey Ministry of Health Turkish Public Health Institution, Department of Obesity, Diabetes and Metabolic Diseases. Turkey Childhood (Ages 7-8) Obesity Surveillance Initiative) (COSI-TUR, 2013).

Nutrition	Locality	Every day	4-6 times a week	1-3 times a week	Never	Total
Chips, popcorn	Urban	8.1	12.9	60.5	18.5	3795
	Rural	11.5	16.2	54.8	17.4	788
	Total	8.7	13.4	59.6	18.3	4583

Table 4. Distribution of the salty snacks (potato chips, corn chips, snack) consumption frequency of children in Turkey and in Istanbul (COSI-TUR, 2017).

Consumption frequency	Turkey (%)	Istanbul (%)
Never	14.5	17.7
Less than once a week	34.6	38.3
1-3 day	29.6	26.4
4-6 day	13.7	11.7
Every day	7.6	5.9
Total	100.0	100.0

can be seen from the table that the levels of consumption frequency of chips and snacks in Istanbul, one of the most populous cities in Turkey.

Aflatoxins are a critical problem for food safety in many developing countries. Groundnuts are one of the most important oilseed crops and snack foods in the agro-processing sector in the industrialised world trade market. Aflatoxin contamination in groundnut is both a pre-harvest and postharvest problem (58) Preserving foods by drying is an effective and ancient method, but inefficient drying and inappropriate storage conditions, may cause aflatoxin production in dried foods. Aflatoxins are not only a problem during cropping, but also during storage, transport, processing, and handling steps due to their high stability. Aflatoxin contamination of groundnuts is one of the most important factors determining the quality of groundnuts and has caused significant financial losses for producing and exporting countries. In addition, aflatoxins pose serious public health issues in many developing countries, since the occurrence of these toxins can be considerably common and even extreme. In the literature studies have shown that raw material selection and drying process parameters are the key elements in food drying. It is known that mycotoxin formation which have negative effects on human health can be prevented by providing adequate and appropriate drying conditions (59) The best way to control aflatoxin contamination of groundnuts is to get under control and prohibit it in the first place (60) Recent studies have shown that aflatoxin levels during drying and pre-storage were significantly higher than those during harvest and post-harvest. In other words the drying and pre-storage terms are the most critical periods for aflatoxin contamination (59) It is evident that, alternative technologies necessary at pre- and post-harvest levels, aiming to minimize contamination of commercial foods and food commodities, at least to ensure that aflatoxin levels remain below safe limits (61) and providing the required control systems which create an effective regulatory environment for ensuring domestic food safety in rural and urban areas.

The occurrences of mycotoxins as food contaminants in different localities, especially in developing countries and the inevitable exposure of populations and particularly children to these toxins with probable adverse outcomes need be scientifically assessed.

This study was undertaken to determine the presence and levels of aflatoxin B_1, B_2, G_1, G_2 and total aflatoxin in chips consumed in the province of Istanbul, Turkey. High levels of aflatoxin in the finished product show that there is inadequate control for aflatoxin in chips.

As a result, the aflatoxin levels were high regarding the tolerance level in food for human consumption in chips, which are snack products with high energy and low nutritional value, may have some potential risks to human and causes negative impacts on human life. It is clear that high aflatoxin levels caused human health risks and created an obstacle to expanding trade both internally and internationally. In order to reduce aflatoxin contamination, it is necessary for monitoring contamination levels in different snack products like chips and raising the awareness of public health impacts associated with aflatoxin contamination.

Conclusions

In this study, aflatoxin B_1, B_2, G_1 and G_2 were analyzed in 27 chips with an effective analytical method for the safe determination of aflatoxins in food samples. Results regarding aflatoxin levels in chips show that high levels of aflatoxin in the finished product show that there is inadequate control for aflatoxin in chips, remarkably for groundnut-chips. Aflatoxin levels of the groundnut-chips and the difficulty of meeting tolerance limits by importers and food processors must lead to the rejection of the groundnut for chip production and the reduction in market demand of the chips. Groundnuts shall be subjected to sorting or

other physical treatment to reduce aflatoxin contamination before human consumption or use as an ingredient in chips. Therefore, new methods of detoxification are necessary to prevent health risks and economic losses that result from aflatoxin contamination. It was concluded that the widespread presence of aflatoxins in chip samples were considered to be possible hazards to public health especially, children. On account of this, chips products have to be controlled continuously for the presence of aflatoxin contamination by the Turkish public health authorities. Moreover, multidisciplinary and comprehensive research is required to effectively control and minimizing aflatoxin contamination maintaining healthy living and economic development.

Raising public health awareness is crucial regarding aflatoxin to;

- improved health conditions of human and animals
- increase food safety and security
- enhance the quality of foods
- conserve natural resources
- increase economic benefits and reduce related costs.

Acknowledgment

This study was carried out in the laboratory of the Halal Food R&D center of Istanbul Sabahattin Zaim University.

References

1. USDA-FAS. Global Agricultural Information Network (GAIN). Report TR6057. Washington, DC: USDA Foreign Agricultural Service, 2016.
2. Steyn, P. The biosynthesis of mycotoxins. Rev Med Vet. 1998, 149(6), 469-478.
3. Rai, M.; Jogee, PS.; Ingle, AP. Emerging nanotechnology for detection of mycotoxins in food and feed. Int J Food Scı Nutr. 2015, 66(4), 363-370.
4. Jackson, P.; Romo, MM.; Castillo, MA.; Castillo-Durán, C. Junk food consumption and child nutrition. Nutritional anthropological analysis. Rev Med Chil. 2004, 132(10), 1235-1242.
5. World Health Organisation, Obesity and overweight, Geneva, Switzerland, 2018.
6. Republic of Turkey Ministry of Health Turkish Public Health Institution, Department of Obesity, Diabetes and Metabolic Diseases Turkey Childhood (Age 8) Obesity Research-COSI-TUR 2016, Turkish Public Health Institution, Ministry of National Education, World Health Organization Regional Office for Europe, Ministry of Health Publication number: 1080, Ankara 2017. Available at: https://hsgm.saglik.gov.tr/depo/haberler/turkiye-cocukluk-cagi-sismanlik/COSI-TUR-2016-Kitap.pdf (accessed Aug 2018).
7. World Health Organisation, Facts and Figures on Childhood Obesity, Geneva, Switzerland, 2017.
8. Turkish Statistical Institute (TURKSTAT). Turkey Health Research 2016, Ankara, Turkey.
9. Irmak, H.; Kesici, C.; Kahraman, N. Turkey School Age Children (6-10 age group) Growth Monitoring (TOÇBİ) Project Research Report. 2011. T.C. Ministry of Health General Directorate of Primary Health Care. 2011.
10. Institute of Food Technologists (IFT), Potato chips drive $22 billion U.S. salty snacks market, Chicago, IL, USA, 2016.
11. Statista, A. The statistics portal. U.S. population: Consumption of potato chips from 2011 to 2020. Web site: https://www.statista.com/statistics/285913/consumption-of-potato-chips-in-the-us-trend/ (accessed Aug 2018).
12. Statista, A. The statistics portal. U.S. population: Amount of potato chips consumed from 2011 to 2017. Web site: https://www.statista.com/statistics/285926/amount-of-potato-chips-consumed-in-the-us-trend/ (accessed Aug 2018)
13. Özdemir, P.; Malayoğlu, HB. Potato Processing Industry By-products and Their Evaluation in Animal Nutrition. TURJAF. 2017, 5(1), 93-97.
14. Republic of Turkey Ministry of Health Turkish Public Health Institution, Department of Obesity, Diabetes and Metabolic Diseases, Turkey Childhood (Ages 7-8) Obesity Surveillance Initiative (COSI-TUR) 2013. Ministry of Health, Hacettepe University. Ministry of Health Publication number: 920. Ankara, 2014. Available at: http://www.halksagligiens.hacettepe.edu.tr/cosi_tr-2014.pdf (accessed Aug 2018).
15. Kensler, TW.; Roebuck, BD.; Wogan, GN.; Groopman, JD. Aflatoxin: a 50-year odyssey of mechanistic and translational toxicology. Toxicol Sci. 2011, 120(suppl. 1), 28S-48S.
16. Chen, Y.; Kong, Q.; Chi, C.; Shan, S.; Guan, B. Biotransformation of aflatoxin B 1 and aflatoxin G 1 in peanut meal by anaerobic solid fermentation of Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus. Int J Food Microbiol. 2015, 211, 1-5.
17. Bhatnagar-Mathur, P.; Sunkara, S.; Bhatnagar-Panwar, M.; Waliyar, F.; Sharma, KK. Biotechnological advances for combating Aspergillus flavus and aflatoxin contamination in crops. Plant Sci. 2015, 234, 119-132.
18. Emmott, A. Market-led aflatoxin Interventions: Smallholder groundnut value chains in Malawi. In 2020 Focus brief, 20(16) Washington, DC: International Food Policy Research Institute, 2013.
19. Monson, MS.; Settlage, RE.; McMahon, KW.; Mendoza, KM.; Rawal, S.; El-Nezami, HS.; Coulombe, RA.; Reed, KM. Response of the hepatic transcriptome to aflatoxin B1 in domestic turkey (Meleagris gallopavo). PLoS One. 2014,

9(6), e100930.
20. Yentür, G.; Er, B. The evaluation of the aflatoxin presence in foods. Turkish Bulletin of Hygiene and Experimental Biol. 2012, 69(1), 41-52.
21. Gürhayta, O.; Çağındı, Ö. Evaluation of the Occurrence and Health Implications of Aflatoxin and Ochratoxin A in Dried Fruits. CBUJOS. 2016, 12(2), 327-338.
22. Binder, EM.; Tan, LM.; Chin, LJ.; Handl, J.; Richard, J. Worldwide occurrence of mycotoxins in commodities, feeds and feed ingredients. Anim Feed Sci Technol. 2007, 137(3-4), 265-282.
23. Sherif, SO.; Salama, EE.; Abdel-Wahhab, MA. Mycotoxins and child health: The need for health risk assessment. Int J Hyg Environ Health. 2009, 212, 347-368.
24. Barberis CL.; Carranza, CS.; Chiacchiera SM.; Magnoli, CE. Influence of herbicide glyphosate on growth and aflatoxin B1 production by Aspergillus section Flavi strains isolated from soil on in vitro assay. J Environ Sci Health B. 2013, 48(12), 1070-1079.
25. Udomkun, P.; Wiredu, AN.; Nagle, M.; Müller, J.; Vanlauwe, B.; Bandyopadhyay, R. Innovative technologies to manage aflatoxins in foods and feeds and the profitability of application–A review. Food Control. 2017, 76, 127-138.
26. Coulter, JBS.; Hendrickse, RG.; Lamplugh, SM.; Macfarlane, SBJ.; Moody, JB.; Omar, MIA.; Suliman, GI.; Williams, TE. Aflatoxins and kwashiorkor: Clinical studies in sudanese children. Trans R Soc Trop Med Hyg. 1986, 80(6), 945-951.
27. Hendrickse, RG. Malnutrition and mycotoxins (editorial). J Trop Pediatr. 1982, 2, 99-100.
28. Etzel, RA. Mycotoxins. JAMA. 2002, 287, 425-427.
29. Kendra, DF.; Dyer, RB. Opportunities for biotechnology and policy regarding mycotoxin issues in international trade. Int J Food Microbiol. 2007, 119(1-2), 147-151.
30. European Commission-EC. No 118/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union. 2006; 5–24.
31. European Commission-EC. Commission regulation (EU) no 165/2010 of 26 February 2010, amending regulation (EC) no 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards aflatoxin. Official Journal of the European Union, 8-12. L 50.2010.
32. Mutegi, CK.; Ngugi, HK.; Hendriks, SL.; Jones, RB. Prevalence and factors associated with aflatoxin contamination of peanuts from Western Kenya. Int J Food Microbiol. 2009, 130, 27-34.
33. Tabata, S.; Kamimura, H.; Ibe, A.; Hashimoto, H.; Iida, M.; Tamura, Y.; Nishima, T. Aflatoxin contamination in foods and foodstuffs in Tokyo: 1986-1990. J AOAC Int. 1993, 76(1), 32-35.
34. Qian, GS.; Ross, RK.; Yu, MC.; Yuan, JM.; Gao, YT.; Henderson, BE.; Groopman, JD. A follow-up study of urinary markers of aflatoxin exposure and liver cancer risk in Shanghai, People's Republic of China. Cancer Epidemiol Biomarkers Prev. 1994, 3(1), 3-10.
35. Rustom, IY. Aflatoxin in food and feed: occurrence, legislation and inactivation by physical methods. Food Chem. 1997, 59(1), 57-67.
36. Henry, SH.; Bosch, FX.; Troxell, TC.; Bolger, PM. Reducing liver cancer--global control of aflatoxin. Science. 1999, 286(5449), 2453-2454.
37. Aycicek, H.; Aksoy, A.; Saygi, S. Determination of aflatoxin levels in some dairy and food products which consumed in Ankara, Turkey. Food Control. 2005, 16(3), 263-266.
38. Ulca, P.; Evcimen, MK.; Senyuva, HZ. Surveys of aflatoxin B1 contamination of retail Turkish foods and of products intended for export between 2007 and 2009. Food Addit Contam Part B Surveill. 2010, 3(2), 120-125.
39. Roy, M.; Harris, J.; Afreen, S.; Deak, E.; Gade, L.; Balajee, SA.; Luby, S. Aflatoxin contamination in food commodities in Bangladesh. Food Addit Contam Part B Surveill. 2013, 6(1), 17-23.
40. Hepsag, F.; Golge, O.; Kabak, B. Quantitation of aflatoxins in pistachios and groundnuts using HPLC-FLD method. Food Control. 2014, 38, 75-81.
41. Battilani, P.; Toscano, P.; Van der Fels-Klerx, HJ.; Moretti, A.; Leggieri, MC.; Brera, C.; Robinson, T. Aflatoxin B 1 contamination in maize in Europe increases due to climate change. Sci Rep. 2016, 6, srep24328.
42. Eivazzadeh-Keihan, R.; Pashazadeh, P.; Hejazi, M.; de la Guardia, M.; Mokhtarzadeh, A. Recent advances in Nanomaterial-mediated Bio and immune sensors for detection of aflatoxin in food products. Trends Analyt Chem. 2017, 87, 112-128.
43. Bankole, SA.; Mabekoje, OO. Mycoflora and occurrence of aflatoxin B 1 in dried yam chips from markets in Ogun and Oyo States, Nigeria. Mycopathologia. 2004, 157(1), 111-115.
44. Mestres, C.; Bassa, S.; Fagbohoun, E.; Nago, M.; Hell, K.; Vernier, P.; Cardwell, KF. Yam chip food sub-sector: hazardous practices and presence of aflatoxins in Benin. J Stored Prod Res. 2004, 40(5), 575-585.
45. Gnonlonfin, GJB.; Hell, K.; Fandohan, P.; Siame, AB. Mycoflora and natural occurrence of aflatoxins and fumonisin B1 in cassava and yam chips from Benin, West Africa. Int J Food Microbiol. 2008, 122(1-2), 140-147.
46. Kaaya, AN.; Eboku, D. Mould and aflatoxin contamination of dried cassava chips in Eastern Uganda: association with traditional processing and storage practices. J. Biol. Sci. 2010, 10(8), 718-729.
47. Gnonlonfin, GJB.; Adjovi, CSY.; Katerere, DR.; Shephard, GS.; Sanni, A.; Brimer, L. Mycoflora and absence of aflatoxin contamination of commercialized cassava chips in Benin, West Africa. Food Control. 2012, 23(2), 333-337.
48. Jonathan, SG.; Abdul-Lateef, MB.; Olawuyi, OJ.; Oyelakin AO. Studies on bio-deterioration, aflatoxin contamination and food values of fermented, dried and stored Ipomoea batatas chips. Nat Sci. 2012, 10(11), 123-128.
49. Löker, GB.; Yaman, M.; Çevikkalp, SA.; Başaran, N.; Özalp, Ç.; Ünlü, ŞG.; Türker, I. Food composition of fruits in Turkey: research outputs from the new Turkish Food Composi-

tion Database, TürKomp. Fruits. 2016, 71(6), 419-431.
50. Aflaprep IFU P07.v14, 16 April 2003, R-Biopharm Rhone Ltd, Glasgow, UK.
51. Papadopoulou-Bouraoui, A.; Stroka, J.; Anklam E. J AOAC Int. 2002, 85, 411-416.
52. Turkish Food Codex (TFC). Regulation on Contaminants, Maximum levels for contaminants in foodstuffs. Official Journal, 29 December 2011, Vol. 28157. Ankara, Turkey.
53. Turkish Food Codex (TFC). Regulation on Contaminants. Official Journal, 19 December 2012, Vol. 28502. Ankara, Turkey.
54. Scheidegger, KA.; Payne, GA. Unlocking the secrets of aspergillus flavus from pathogenicity to functional genomics. J Toxicol Toxin Rev. 2003, 22(2-3), 423-459.
55. Food and Drug Administration (FDA). Guidance for Industry: Action Levels for Poisonous or Deleterious Substances in Human Food and Animal Feed. Aflatoxin in Peanuts and Peanut Products. 2017, Sec. 570.375 https://www.fda.gov/food/guidanceregulation/ucm077969.htm#afla (accessed Aug 2018).
56. Njumbe Ediage, E.; Hell, K.; De Saeger, S. A comprehensive study to explore differences in mycotoxin patterns from agro-ecological regions through maize, peanut, and cassava products: a case study, Cameroon. J Agric Food Chem. 2014, 62(20), 4789-4797.
57. Tosun, H.; Arslan, R. Determination of aflatoxin B1 levels in organic spices and herbs. ScientificWorldJournal. 2013.
58. Guchi, E. Aflatoxin contamination in groundnut (Arachis hypogaea L.) caused by Aspergillus species in Ethiopia. J Appl Environ Microbiol. 2015, 3(1), 11-19.
59. Lavkor, I.; Bicici, M. Aflatoxin occurrence in peanuts grown in Osmaniye at harvest, post-harvest, drying and pre-storage periods. J Agric Sci. 2015, 21(3), 394-405.
60. Dorner, JW. Management and prevention of mycotoxins in peanuts. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2008, 25(2): 203-208.
61. Prietto, L.; Moraes, PS.; Kraus, RB.; Meneghetti, V.; Fagundes, CAA.; Furlong, EB. Post-harvest operations and aflatoxin levels in rice (Oryza sativa). Crop Prot. 2015, 78, 172-177.

Correspondence:
Dr. Jale Çatak
Department of Nutrition and Dietetics, Faculty of Health Sciences, İstanbul Sabahattin Zaim University,
34303, Küçükçekmece, Halkalı, Istanbul, Turkey
E-mail: jalecatak@gmail.com

Progress in Nutrition 2020; Vol. 22, N. 1: 224-235 DOI: 10.23751/pn.v22i1.7701

Original article

Antioxidant activity and phenolic content of commonly consumed fruits and vegetables in Algeria

Habiba Djenidi, Seddik Khennouf, Amel Bouaziz

Laboratory of Phytotherapy Applied to Chronic Diseases, Department of Biology and Animal Physiology, Faculty of Nature and Life Sciences, University Setif 1, Algeria - E-mail: habibadjenidi@yahoo.fr

Summary. The purpose of this study was to evaluate the phenolic content and the antioxidant activity of some commonly foods consumed in Algeria. 22 vegetables and 14 fruits extracts were evaluated for their polyphenolic content and antioxidant potential using different methods. Results showed that beans, cauliflower and courgette were rich in total polyphenols. However, Jew's mallow was the richest in flavonoids and the highest content of tannins was noticed in the pomegranate. The antioxidant activity of fruits and vegetables extracts using -carotene bleaching assay showed that lettuce, courgette, cauliflower, artichoke, mallow, bean, green bean, green pea, peach and apricot were the most effective with antioxidant activity percentage greater than 70%. However, eggplant, lettuce, courgette, artichoke, mallow, chard, green bean, green pea, black grapes and pomegranate showed the highest antioxidant activity against DPPH radical with $IC_{50} \leq 0.8$ mg/ml. Also, potato, lettuce, carrot, courgette, pumpkin, turnip, cucumber, fennel, cauliflower, cabbage, artichoke (flower), Jew's mallow, chard, green bean, bean and green pea showed the highest chelating activity with $IC_{50} \leq 0.8$mg / ml and onion, potato, courgette, Jew's mallow, chard, green bean, bean and green pea showed the highest reducing power ($IC_{50} \leq 5$ mg / ml). Finally, these selected consumed fruits and vegetables are natural source of polyphenols and have an important antioxidant activity and their consumption may reduce the risk of pathologies induced by oxidative stress.

Key words: antioxidant activity, phenolic content, fruits, vegetables, DPPH, chelating activity, reducing power, β-carotene bleaching

Introduction

Oxidative stress is an imbalance between the production of reactive oxygen species (ROS) and antioxidant defense mechanisms, leading to damage to lipids, proteins and nucleic acids (1), which are involved in several diseases such as cancer, aging skin, inflammatory, cardiovascular and neurodegenerative diseases (2).

Antioxidants which can neutralize free radicals are of great importance in preventing the development of these diseases (3). Thus, to avoid the toxic effect of synthetic antioxidants, many studies have investigated new antioxidants of natural origin as polyphenols (4).

Polyphenols are bioactive compounds usually found in fruits, vegetables, legumes, grains, chocolate, and beverages such as fruit juices, tea, coffee and red wine (5). These secondary metabolites can act as scavengers of free radicals which are responsible for the initiation of oxidation, as well as chain breaking antioxidants, singlet oxygen deactivators, reducing agents, metal chelating agents and inhibitors of specific oxidative enzymes (6).

Many studies showed that diets rich in fruits and vegetables are good antioxidants and can reduce the risk of development of many diseases associated with oxidative stress as cancers, atherosclerosis, aging, inflammatory, cardiovascular and neurodegenerative diseases (7, 8).

The health effects of polyphenols and their properties, especially when these compounds are present in

large quantities in food, are important to consumers, which requires the evaluation of their antioxidant activity. Thus, the objective of this study was to evaluate the total polyphenols content and *in vitro* antioxidant activity of some consumed vegetables and fruits in Algeria.

Material and methods

Plants materials

All fresh vegetables and fruits used in this study (Table 1) were bought from the local market in Biskra (south eastern of Algeria) and Setif (north eastern of Algeria) regions at the time of their most frequent consumption during 2012-2013. At least 1 kg of the good quality produces without bruises and damage were purchased.

Extraction of phenolics from food samples

The extraction was carried out according to the method described by Hossain et al (9). 1 kg of consumed part of fresh fruits and vegetables previously cleaned and washed with distilled water, except for Jew's mallow which was used in dry form were cut into small pieces and crushed. Then 100 g of each crushed material were macerated in 625 ml of methanol / water mixture (80/20: V / V) for 3 days at 4 °C. The macerate was filtered and the filtrate was subjected to a rotary evaporation under reduced pressure at 45 °C. The extract obtained was dried and stored at 4 °C until use.

Determination of total phenolic content

Total polyphenol content was assayed by Folin-Ciocalteu reagent described by Li et al (10). 100 µl of each extract were added to 500 µl of Folin-Ciocalteu reagent (10 times diluted in distilled water). After 4 min of incubation, 400µl of 7.5% sodium carbonate were added and the solution mixtures were kept in the dark for 1 h and 30 min at room temperature. Then, the absorbance of each solution was read at 765 nm against a blank by a spectrophotometer.

The concentration of the total polyphenols was calculated from the regression equation of the calibration curve of gallic acid at different concentrations (12.5 to 100 µg/ml) and expressed in micrograms of gallic acid equivalent per milligram of dry extract (µg of GAE / mg of extract).

Determination of total flavonoids content

The flavonoids evaluation was assayed by the method of Quettier-Deleu et al (11) using aluminum trichloride ($AlCl_3$). 500µl of each extract was added to an equal volume of a solution of $AlCl_3$ (2% in methanol). The mixture was vigorously stirred and after 10 minutes of incubation, the absorbance was read at 430 nm by a spectrophotometer. The quantification of flavonoids was evaluated from the calibration curve of quercetin at different concentrations (1.25 to 40 µg /ml). The results were expressed in micrograms of quercetin equivalent per milligram of dry extract (µg of QE / mg of extract).

Determination of total tannins content

Tannins were assayed by the method described by Bate-Smith (12). A volume of 500 µl of fresh bovine blood (which had an absorbance equal to 1.6 at a wavelength of 576 nm) was added to 500 µl of the extract. After stirring and centrifugation for 10 min at 4000 rpm, the absorbance of the supernatant was read at 576 nm. The quantification of tannins was carried out using the calibration curve of tannic acid at different concentrations (200 to 600 µg/ml).

The results were expressed in micrograms of equivalent tannic acid per milligram of dry extract (µg of TAE / mg of extract).

In vitro antioxidant activity

The diversity of nature and the strucure of plant compounds require the development of many methods to evaluate their antioxidant activity. Thus, different methods are used to measure the antioxidant activity of the extracts. Each method uses or generates a different radical that is involved in the oxidation process. Only one method is insufficient to represent the total antioxidant capacity of the extracts, and for this purpose four different tests were used to evaluate the antioxidant activities of the extracts which are DPPH radical scavenging assay, ferrous ion chelating, ferric reducing power and β-carotene/ linoleic acid bleaching assay.

Table 1. Common name, region of purchase, scientific name and used part of fruits and vegetables

	Common name	Region of purchase	Scientific name	Used part
Vegetables	Artichoke (flower)	Setif	*Cynara cardunculus* L.var. *scolymus*	Flower
	Artichoke (stem)	Setif	*Cynara cardunculus* L.var. *scolymus*	Stem
	Bean	Setif	*Vicia faba* L.	Seed
	Beetroot	Biskra	*Beta vulgaris* L.var. *rapacea Koch*	Root
	Cabbage	Setif	*Brassica oleracea* L.	Leaves
	Carrot	Biskra	*Daucus carota* ssp. *sativus*	Tuber
	Cauli flower	Biskra	*Brassica oleracea* L.	Flower
	Chard	Setif	*Beta vulgaris* L.var. *cicla Pers*	Leaves
	Courgette	Biskra	*Cucurbita pepo* L.	Fruit
	Cucumber	Biskra	*Cucumis sativus* L.	Fruit
	Eggplant	Biskra	*Solanum melongena* L.	Fruit
	Fennel	Biskra	*Foeniculum dulce* Mill.	Leaves
	Green bean	Setif	*Phaseolus vulgaris* L.	Fruit (clove)
	Green pea	Setif	*Pisum sativum*	Seed
	Jew's mallow	Biskra	*Corchorus olitorius* L.	Leaves
	Lettuce	Setif	*Lactuca sativa* L.	Leaves
	Onion	Biskra	*Allium cepa* L.	Bulb
	Pepper	Biskra	*Capsicum annum* L.	Fruit
	Potato	Biskra	*Solanum tuberosum* L.	Tuber
	Pumpkin	Setif	*Cucurbita maxima* Duch.	Fruit
	Tomato	Biskra	*Solanum lycopersicum* L.	Fruit
	Turnip	Biskra	*Brassica napus* L.	Root
Fruits	Apple	Biskra	*Malus communis* Poir.	Fruit
	Apricot	Setif	*Prunus armentiaca* L.	Fruit
	Banana	Setif	*Musa sapientum* L.	Fruit
	Dates " Deglat-Nour"	Biskra	*Phoenix dactylifera* L.	Fruit
	Dates "Ghars"	Biskra	*Phoenix dactylifera* L.	Fruit
	Dates "Mech-Degla"	Biskra	*Phoenix dactylifera* L.	Fruit
	Grapes (black)	Biskra	*Vitis venifera* L.	Fruit
	Grapes (white)	Biskra	*Vitis venifera* L.	Fruit
	Mandarin	Setif	*Citrus reticulata*	Fruit
	Medlar of Japan	Setif	*Eryobotrya japonica* Lindl.	Fruit
	Orange	Setif	*Citrus sinensis* Osb.	Fruit
	Peach	Setif	*Prunus persica* L.	Fruit
	Pear	Biskra	*Pyrus communis* L.	Fruit
	Pomegranate	Setif	*Punica granatum* L.	Fruit

DPPH radical scavenging assay

The DPPH radical scavenging method is a spectrophotometric procedure used to determine the antioxidant capacity of the components. It is based on the ability of the DPPH radical to discolor from purple to yellow color in the presence of antioxidants by accepting an electron or hydrogen atom given by an antioxidant compound (13).

The antiradical activity of the extracts in this study was measured by the 2,2'-diphenyl-1-picrylhydrazyl

(DPPH) test according to the method of Brand-Williams et al (14) with slight modification. A range of extract concentrations and quercetin as antioxidant reference were prepared. A volume of 50µL of each extract solution was mixed with 1.25 ml of DPPH (0.04 mg / ml) prepared in methanol. After stirring, the mixture was incubated for 30 minutes in the darkness at room temperature and the absorbance was read at 517 nm against a blank. The inhibition of free radical activity was calculated according to the following equation:

The antiradical activity (%) = [(Abs $_{control}$ - Abs $_{sample}$) / Abs $_{control}$] x 100.

The IC_{50}, which is the concentration of extract or quercetin responsible for 50% of inhibition of DPPH radical was determined from the plot of inhibition percentage against extract or quercetin concentration.

Ferrous ions chelating activity

Transition metal ions such as copper and iron are important for the generation of highly reactive hydroxyl radicals via the Fenton reaction in *in vivo* and *in vitro* systems. Compounds that bind to metal ions can alter the redox potential of these ions, making them catalytically silent. Therefore, compounds that can act as effective chelators for the sequestration of copper and iron ions are considered antioxidants by intercepting and / or suppressing radicals (15).

The chelating activity of the extracts was measured following the inhibition of the formation of the Fe (II) -Ferrozine complex after incubation of the samples with divalent iron according to the method described by Le et al (16). The sample solutions (250 µl) were initially mixed with 50 µl $FeCl_2$ (0.6 mM in distilled water) and 450 µl of methanol. After 5 min, 50 µl of ferrozine (5 mM in methanol) were added to the reaction medium and the mixture was stirred well and then left to react for 10 min at room temperature. The red chromophore (Fe (II) -Ferrozine) had maximum absorption at 562 nm and the chelation activity was calculated according to the following equation:

The chelating activity (%) = [(Abs $_{control}$-Abs $_{sample}$) / Abs $_{control}$] x 100

Where IC_{50} is the concentration of extract responsible for chelate 50 % of iron ions.

Ferric reduction power

The reducing power of the extracts can provide a significant indication of the potential antioxidant activity of the plant. The presence of antioxidants in the extracts would result in the reduction of Fe^{3+} to Fe^{2+} by giving an electron. The amount of Fe^{2+} complex can be monitored by measuring the absorbance at 700 nm (13).

The reducing capacity of the various vegetables and fruits extracts was evaluated using the method of Ozsoy et al (17). 100µl of each extract was mixed with 100 µl of phosphate buffer solution (0.2 M, pH 6.6) and 100 ml of 1% potassium hexacyanoferrate solution. The mixture was incubated for 20 minutes at 50 ° C in a water bath. After that, 250 µl of 1% trichloroacetic acid was added and the mixture was centrifuged for 10 minutes. Then, 250 µl of the supernatant were taken and mixed with 250 µl of distilled water and 500 µl of 0.1% aqueous solution of $FeCl_3$. The absorbance was read at 700 nm. A calibration curve was plotted from the line obtained with the BHT used as reference at different concentrations. The IC_{50} value was defined as the effective concentration of the extract or standard which had the absorbance of 0.5.

β-carotene/ linoleic acid bleaching assay

The β-carotene bleaching test measures the ability of antioxidants to retard the β-carotene decolorization induced by conjugated diene hydroperoxides resulting from the oxidative degradation of linoleic acid (13).

In this test, the antioxidant capacity of the extracts is determined according to the method of Kartal et al (18). 25 µl of linoleic acid and 200 mg of tween 40 were added to 0.5 mg of β-carotene dissolved in 1 ml of chloroform. After evaporation of chloroform by a rotavapor, 100 ml of distilled water saturated with oxygen were added with vigorous stirring. 2.5 ml of this emulsion were transferred into tubes and 350 µl of each extract or BHT as reference antioxidant with a concentration of 2 mg / ml were added. The kinetics of discoloration of the emulsion in the presence and the absence of antioxidant (negative control in which the sample was replaced by 350 µl of methanol) was monitored at 490 nm at regular intervals of time for 24 hours. The relative antioxidant activity of the extracts was calculated according to the following equation:

Antioxidant Activity (%) = (Abs $_{sample}$ / Abs $_{BHT}$) x 100

Statistical analysis

Statistical analysis was performed using the Graph Pad Prism software (version 5.01 for Windows). The results were presented as mean ± standard deviation (SD) and were analyzed by the one way analysis of variance (ANOVA) followed by Dunnet's test. For the comparison of the results, the $P \leq 0.05$ values were considered statistically significant.

Results

Total phenolic content

Phenolic compounds are widely distributed in the plant kingdom and have significant antioxidant activity because of their ability to yield hydrogen and to form stable intermediate radicals. The total polyphenols, flavonoids and tannins contents of vegetables and fruits was presented in Table 2 and 3.

Table 2. Polyphenols, flavonoids, tannins content in vegetables

Common name	Polyphenols content in extract (µg GAE/ mg)	Flavonoids content in extract (µg QE /mg)	Tannins content in extract (µg TAE /mg)
Artichoke (flower)	108 ± 5.34	0.89 ± 0.02	2.74 ± 0.17
Artichoke (stem)	34.51 ± 4.35	1.46 ± 0.03	3.47 ± 0.07
Bean	360.2 ± 8.20	11.07 ± 0.56	7.89 ± 0.04
Beetroot	87.11 ± 3.79	2.37 ± 0.06	17.91 ± 0.74
Cabbage	62.22 ± 1.35	0.73 ± 0.09	4.48 ± 0.03
Carrot	43.88 ± 1.72	1.43 ± 0.05	7.35 ± 0.04
Cauliflower	340.22 ± 4.66	2.78 ± 0.28	5.26 ± 0.01
Chard	255.88 ± 4.87	32.13 ± 0.99	19.59 ± 0.12
Courgette	305.85 ± 3.79	19.93 ± 0.35	21.59 ± 0.12
Cucumber	69.11 ± 2.78	0.34 ± 0.00	5.53 ± 0.02
Eggplant	292.96 ± 4.29	4.50 ± 0.16	8.68 ± 0.18
Fennel	43.77 ± 9.23	0.53 ± 0.01	2.70 ± 0.21
Green bean	91.92 ± 9.00	2.53 ± 0.06	8.47 ± 0.52
Green pea	259.2 ± 3.95	10.07 ± 0.80	9.06 ± 0.06
Jew'smallow	276.37 ± 5.30	34.8 ± 0.80	20.91 ± 1.24
Lettuce	300.66 ± 5.97	12.28 ± 0.25	8.81 ± 0.25
Onion	245.88 ± 7.07	4.38 ± 0.07	6.54 ± 0.91
Pepper	294.07 ± 5.62	2.49 ± 0.20	6.51 ± 0.01
Potato	167.22 ± 3.61	2.72 ± 0.23	5.88 ± 0.24
Pumpkin	80 ± 3.14	2.90 ± 0.00	2.98 ± 0.51
Tomato	197.40 ± 9.06	2.08 ± 0.04	8.04 ± 0.54

Results were expressed as mean ±SD, n=3

Total phenolic content

As seen in Table 2, the total polyphenols content in vegetables ranged from 34.51 to 360.2 µg of GAE / mg of extract. Bean, cauliflower, courgette and lettuce showed the highest total polyphenol contents with values of 360.2 ± 8.20, 340.22 ± 4.66, 305.85 ± 3.79 and 300.66 ± 5.97 µg of GAE / mg of extract, respectively, followed by pepper (294.07 ± 5.62 µg GAE / mg extract), eggplant (292.96 ± 4.29 µg of GAE / mg of extract) and Jew's mallow (276.37 ± 5.30 µg of GAE / mg of extract).

However, artichoke stems (34.51 ± 4.35 µg of GAE / mg of extract), fennel (43.77 ± 9.23 µg of GAE / mg of extract), cabbage (62.22 ± 1.35 µg of GAE / mg extract) and cucumber (69.11 ± 2.78 µg GAE / mg extract) contained low phenolics content.

Concerning the quantification of phenolics in fruits, it was found that the pomegranate contained the highest total polyphenols content (200.51 ± 1.26 µg of GAE / mg of extract) followed by pear (151.55 ± 1.25 µg of GAE / mg of extract), apple (115.77 ± 0.00 µg of

Table 3. Polyphenols, flavonoids, tannins content in fruits

Common name	Polyphenols content in extract (µg GAE/ mg)	Flavonoids content in extract (µg QE /mg)	Tannins content in extract (µg TAE /mg)
Apple	115.77 ± 0.00	1.19 ± 0.01	5.33 ± 0.09
Apricot	48.3 ± 4.76	0.66 ± 0.13	2.26 ± 0.01
Banana	41.55 ± 2.19	0.04 ± 0.00	3.65 ± 0.02
Dates " Deglat-Nour" cultivar	84.15 ± 3.14	0.22 ± 0.01	1.86 ± 0.00
Dates "Ghars" cultivar	56.77 ± 1.72	0.20 ± 0.06	2.25 ± 0.10
Dates "Mech-Degla" cultivar	29.48 ± 7.18	2.94 ± 0.05	1.77 ± 0.06
Grapes (black)	91 ± 2.98	0.63 ± 0.02	4.99 ± 0.07
Grapes (white)	92.11 ± 3.45	0.41 ± 0.05	5.17 ± 0.01
Mandarine orange	115.25 ± 0.12	0.08 ± 0.00	10.91 ± 0.29
Medlar of Japan	80.36 ± 1.40	0.83 ± 0.09	1.84 ± 0.02
Orange	65.22 ± 1.72	1.14 ± 0.00	7.50 ± 0.19
Peach	46.86 ± 8.30	1.05 ± 0.11	2.27 ± 0.01
Pear	151.55 ± 1.25	1.03 ± 0.03	5.15 ± 0.10
Pomegranate	200.51 ± 1.26	0.86 ± 0.16	39.44 ± 0.83

Results were expressed as mean ±SD, n=3

GAE / mg of extract) and mandarin (115.25 ± 0.12µg of GAE / mg of extract) (Table 3).

Total flavonoids content

As shown in Table 2, flavonoids content in vegetables ranged from 0.34±0.00 to 34.8±0.80 µg of QE / mg of extract. Cucumber, turnip and fennel contained the lowest content, while Jew's mallow, chard, courgette and lettuce contained the highest flavonoids content with values of 34.8±0.80, 32.13±0.99, 19.93 ± 0.35 and 12.28±0.25 µg of QE / mg of extract, respectively. However, the dates "Mech-Degla", apple, orange, contained the highest content of flavonoids (2.94±0.05, 1.19±0.01, 1.14±0.00 µg of QE / mg of extract respectively) (Table 3).

Total tannins content

As shown in Table 2, tannins content in the vegetables was found to be ranged from 2.70 ±0.21 to 21.59 ±1.24 µg of TAE /mg of extract. Courgette contained the highest content (21.59±0.12µg of TAE / mg of extract) followed by Jew's mallow (20.91±1.24 µg of TAE/ mg of extract), chard (19.59±0.12 µg of TAE / mg of extract) and beetroot (17.91±0.74 µg of TAE / mg of extract). Whereas, the pomegranate was the richest fruit in tannins with a content of 39.44±0.83 µg of TAE / mg of extract (Table 3).

In vitro antioxidant activity

The antioxidant activity of 22 vegetables and 14 fruits extracts assessed by DPPH radical scavenging assay, ferrous ion chelating assay, reducing power and β-carotene/linoleic acid bleaching assay were presented in Table 4 and 5.

DPPH radical scavenging activity

In this study (Table 4 and 5), green bean, Jew's mallow, lettuce, eggplant, artichoke (flower), courgette, green pea, chard, pomegranate and black grapes showed high antioxidant activity against DPPH radical with IC_{50} ≤0.8 mg/ml. Pepper, tomato, onion, potato, beetroot, pumpkin, cauliflower, cabbage, bean, white grapes, apple, peach, medlar of Japan and apricot had IC_{50} value ranged between 0.8 and 2 mg / ml. Whereas, carrot, turnip, cucumber, fennel, artichoke (stem), pear, banana, mandarin, orange and dates had low antiradical activity with IC_{50} ≥ 2 mg / ml.

Ferrous ion chelating activity

As shown in Table 4 and 5, vegetables and fruits can be classified into four groups according to their chelating capacity (high, medium, low chelating capacity).

The chelating potential of the extracts was inversely proportional to the IC_{50} value. Vegetables and fruits with high chelating activity included potato, lettuce, carrot, courgette, pumpkin, turnip, cucumber, fennel, cauliflower, cabbage, artichoke (flower), Jew's mallow, chard, green bean, bean and green pea with IC_{50} ≤0.8mg / ml. Pepper, onion, eggplant, white grapes and dates "Ghars" showed IC_{50} between 0.8 and 2 mg /ml. Tomato, beetroot, artichoke (stem), pear, black grapes, apple, pomegranate, banana, mandarin , orange, peach, medlar of Japan, apricot, dates "Mech-Degla" and "Deglat-Nour" had low chelating activity (IC_{50} ≥ 2 mg / ml).

Ferric reducing power

In the same manner, vegetables and fruits have been classified into four groups according to their reducing capacity (high, medium, low reducing capacity).

Table 4 and 5 showed that extracts with high antioxidant activity (IC_{50} ≤ 5 mg / ml) included onion, potato, courgette, Jew's mallow, chard, green bean, bean and green pea. Tomato, eggplant, pumpkin, turnip, cabbage, artichoke (flower), artichoke (stem), and pomegranate had the medium activity with IC_{50} ranged between 5 and 10 mg / ml. However, extracts with low reducing power (IC_{50} ≥10 mg / ml) included pepper, lettuce, carrot, beetroot, cucumber, fennel, cauliflower, pear, black grapes, white grapes, apple, banana, mandarin, orange, peach, apricot, medlar of Japan and dates.

β-carotene/ linoleic acid bleaching assay

According to our results, the tested fruits and vegetables can be classified also into four groups according to their antioxidant activity which is ranging from 13 % to 92% (high, medium, low and very low).

From the 22 vegetable and 14 fruit extracts which were tested for their inhibition of linoleic acid oxidation, 7 vegetables and 2 fruits were found in the group that had high antioxidant activity (> 70%) including lettuce, courgette, artichoke (flower), artichoke (stem),

Jew's mallow, green bean, bean, green peas, peach and apricot. The group having a medium activity (50-70%) was represented by pepper, beetroot, cabbage, chard, mandarin, orange, medlar of Japan. However, tomato, onion, eggplant, potato, cucumber, fennel, black grapes, pomegranate and banana represented a group with a low antioxidant activity (<50%). Carrot, pumpkin, turnip, pear, white grapes, apple and dates showed a very low antioxidant activity percent (<40%) (Table 4 and 5).

Discussion

In the present study, the total phenolic, flavonoids and tannins contents of 14 fruits and 22 vegetables consumed commonly in Algeria were evaluated and their antioxidant activity using four different antioxidant assays were also assessed.

Phenolic compounds such as flavonoids and tannins are widely distributed in fruits and vegetables and have gained much attention due to their antioxidant

Table 4: Antioxidant activities of vegetables extracts

Common name	DPPH radical scavenging assay (IC_{50} mg/ml)	Ferrous ion chelating activity (IC_{50} mg/ml)	Ferric reducing power (EC_{50} mg/ml)	β-carotene / linoleic acid assay bleaching assay (% of inhibition after 24 h of incubation)
Artichoke (flower)	0.36±0.02$^{(d)}$	0.04±0.01$^{(a)}$	7.73±0.076$^{(d)}$	72.12±2.53$^{(d)}$
Artichoke (stem)	4.02±0.28$^{(d)}$	2.21±0.16$^{(d)}$	7.68±0.57$^{(d)}$	78.73±1.43$^{(d)}$
Bean	1.58±0.04$^{(d)}$	0.09±0.00$^{(a)}$	4.08±0.20$^{(d)}$	88.42±2.97$^{(a)}$
Beetroot	1.76±0.01$^{(d)}$	5.23±0.17$^{(d)}$	37.30±1.27$^{(d)}$	51.11±5.37$^{(d)}$
Cabbage	1.79±0.06$^{(d)}$	0.22±0.01$^{(d)}$	9.07±0.18$^{(d)}$	66.06±3.64$^{(d)}$
Carrot	3.77±0.06$^{(d)}$	0.32±0.01$^{(d)}$	76.25±1.90$^{(d)}$	35.45±5.38$^{(d)}$
Cauliflower	1.67±0.03$^{(d)}$	0.72±0.02$^{(d)}$	16.75±0.66$^{(d)}$	72.07±1.55$^{(d)}$
Chard	0.77±0.01$^{(d)}$	0.07±0.01$^{(a)}$	4.95±0.18$^{(d)}$	64.31±7.69$^{(d)}$
Courgette	0.48±0.02$^{(d)}$	0.18±0.01$^{(d)}$	3.32±0.01$^{(d)}$	84.89±2.66 $^{(c)}$
Cucumber	7.21±0.24$^{(d)}$	0.17±0.00$^{(d)}$	60.91±3.46$^{(d)}$	47.17±9.22$^{(d)}$
Eggplant	0.36±0.00$^{(d)}$	1.27±0.06$^{(d)}$	7.13±0.09$^{(d)}$	49.14±5.28$^{(d)}$
Fennel	3.29±0.07$^{(d)}$	0.29±0.02$^{(d)}$	53.28±2.28$^{(d)}$	47.72±1.20$^{(d)}$
Green bean	0.04±0.00 $^{(b)}$	0.23±0.00$^{(d)}$	3.44±0.31$^{(d)}$	91.70±3.37$^{(a)}$
Green pea	0.65±0.01$^{(d)}$	0.69±0.01$^{(d)}$	3.40±0.11$^{(d)}$	92.31±7.64$^{(a)}$
Jew's mallow	0.06±0.00$^{(d)}$	0.35±0.03$^{(d)}$	2.30±0.03$^{(d)}$	76.92±2.93$^{(d)}$
Lettuce	0.22±0.00$^{(d)}$	0.19±0.00$^{(d)}$	12.19±0.48$^{(d)}$	77.17±5.38$^{(d)}$
Onion	1.14±0.16$^{(d)}$	0.97±0.08$^{(d)}$	4.02±0.18$^{(d)}$	41.36±5.47$^{(d)}$
Pepper	1.71±0.01$^{(d)}$	1.63±0.01$^{(d)}$	26.02±0.57$^{(d)}$	53.78±11.54$^{(d)}$
Potato	1.02±0.11$^{(d)}$	0.32±0.02$^{(d)}$	4.32±0.05$^{(d)}$	48.93±0.46$^{(d)}$
Pumpkin	1.69±0.03$^{(d)}$	0.16±0.00$^{(c)}$	6.59±0.54$^{(d)}$	21.13±2.92$^{(d)}$
Tomato	0.96±0.01$^{(d)}$	10.17±0.83$^{(d)}$	5.22±0.60$^{(d)}$	44.39±8.18$^{(d)}$
Turnip	2.15±0.03$^{(d)}$	0.18±0.01$^{(d)}$	5.35±0.44$^{(d)}$	20.25±3.81$^{(d)}$
Rutin	0.0072±0.00			
EDTA		0.0064±0.00		
BHT			0.32±0.00	94.94±3.69
H_2O				6,41±0.38
MeOH				9.59±0.74

*$^{(a)}$: No significant difference, $^{(b)}$: * (P<0.05), $^{(c)}$:** (P<0.01), $^{(d)}$: *** (P<0.001) compared to standards*
Results were expressed as mean ±SD, n=3

Table 5: Antioxidant activities of fruits extracts

Common name	DPPH radical scavenging assay (IC_{50} mg/ml)	Ferrous ion chelating activity (IC_{50} mg/ml)	Ferric reducing power (EC_{50} mg/ml)	β-carotene bleaching / linoleic acid assay (% of inhibition after 24 h of incubation)
Apple	1.65±0.04$^{(d)}$	9.18±0.11$^{(d)}$	19.68±0.48$^{(d)}$	13.38±0.31$^{(d)}$
Apricot	1.67±0.03$^{(d)}$	7.94±1.33$^{(d)}$	30.00±1.47$^{(d)}$	75.76±2.00$^{(d)}$
Banana	9.20±0.87$^{(d)}$	5.48±0.38$^{(d)}$	52.30±3.01$^{(d)}$	45.15±5.21$^{(d)}$
Dates " Deglat-Nour" cultivar	3.72±0.08$^{(d)}$	2.04±0.04$^{(d)}$	108.53±15.98$^{(d)}$	15.93±2.19$^{(d)}$
Dates "Ghars" cultivar	4.15±0.13$^{(d)}$	1.60±0.07$^{(d)}$	31.73±0.54$^{(d)}$	13.58±0.08$^{(d)}$
Dates "Mech-Degla" cultivar	4.59±0.10$^{(d)}$	2.97±0.04$^{(d)}$	195.25±10.92$^{(d)}$	34.69±16.11$^{(d)}$
Grapes (black)	0.74±0.00$^{(d)}$	3.29±0.03$^{(d)}$	16.08±0.74$^{(c)}$	46.76±0.71$^{(d)}$
Grapes (white)	1.40±0.11$^{(d)}$	1.07±0.08$^{(d)}$	16.12±2.54$^{(c)}$	22.32±2.59$^{(d)}$
Mandarin	4.92±0.09$^{(d)}$	23.41±0.60$^{(d)}$	52.07±0.02$^{(d)}$	58.68±3.84$^{(d)}$
Medlar of Japan	0.95±0.02$^{(d)}$	18.81±0.06$^{(d)}$	18.43±0.34$^{(d)}$	61.01±0.80$^{(d)}$
Orange	2.48±0.04$^{(d)}$	6.66±0.18$^{(d)}$	23.64±2.18$^{(d)}$	53.18±5.50$^{(d)}$
Peach	0.98±0.02$^{(d)}$	21.47±1.44$^{(d)}$	14.62±0.66 $^{(c)}$	80.97±1.30$^{(d)}$
Pear	3.16±0.04$^{(d)}$	11.08±0.89$^{(d)}$	28.28±0.30$^{(d)}$	17.17±1.00$^{(d)}$
Pomegranate	0.32±0.01$^{(d)}$	2.78±0.36$^{(d)}$	9.58±0.22$^{(d)}$	42.02±4.97$^{(d)}$
Rutin	0.0072±0.00$^{(d)}$			
EDTA		0.0064±0.00		
BHT			0.32±0.00	94.94±3.69
H_2O				6.41±0.38
MeOH				9.59±0.74

*$^{(a)}$: No significant difference, $^{(b)}$: * (P<0.05), $^{(c)}$:** (P<0.01), $^{(d)}$: *** (P<0.001) compared to standards.*
Results were expressed as mean ±SD, n=3

activities and free radical scavenging abilities, which potentially have benefit for human health. Thus, many reports had evaluated the phenolic content of fruits and vegetables (7).

Results obtained in the present study revealed that the level of these phenolic compounds in beans, cauliflower and courgette extract were considerable. In comparison with other studies, the total polyphenols content in tomato , onion, courgette, white grapes, orange, bean, lettuce, eggplant and pepper have been found in this study were higher than those of Cie lik et al (19), Liu et al (20), Baginsky et al (21) and Mokhtar et al (22). However, phenolic content in cauliflower, carrot and pea were lower than those of Dos Reis et al (23) and Kähkönen et al (24). The total phenolic content estimated in pomegranate (200.51 ± 1.26 µg of GAE / mg of extract) was higher than that of Derakhshan et al (25) which was equal to 23.8 ± 6.74 µg GAE/mg of juice extract.

Flavonoids are plant polyphenols found frequently in fruits, vegetables, and grains and are divided into several subclasses including anthocyanins, flavanols (catechins), flavones, flavanones, and flavonols (26).

The flavonoids content in this study was higher in Jew's mallow and chard which were higher than those of Oboh (27) and Sacan and Yanardag (28). However, the flavonoids content in three varieties of dates and courgette were higher than those of other authors (29, 30).

The orange, red, and blue or violet coloration in vegetables, fruits, flowers, and plant storage tissue are due to water-soluble anthocyanins, which are natural pigments reduced from the yellow flavonoids due to loss of oxygen (26). Thus, the anthocyanins detected in eggplant, onion, apple, black grapes and pomegranate may be considered as responsible for the high phenolic content in this study.

Tannins are a group of polyphenols present in various concentrations in many fruits and vegetables

consumed by human. Studies revealed that the phytoconstitutents belonging to tannins class possess potent antioxidant activity; some exhibit radical scavenging activity as well (31).

The obtained results showed that the highest level of tannins was detected in the pomegranate which was higher than that of Orak et al (32) who estimated the tannin content in Turkish pomegranate (16.38 ± 0.35 µg TAE/mg of juice extract) . Several classes of pomegranate tannins include ellagitannin such as ellagic acid, punicalagin and punicalin that are found in pomegranate juice and peel showed a great antioxidant activity as reported by Zarfeshany et al (33). Also, many vegetables and fruits have shown to be a rich source of polyphenols, flavonoids and tannins as ferulic, chlorogenic, coumaric and syringic acids, luteolin, quercetin, kaempferol, and catechins which showed marked antioxidant activities (34, 35). However, the comparison the presented results with those of bibliography remains difficult because each study uses a different extraction method.

In this study, the extraction of the polyphenols was carried out by maceration in a hydro-methanol mixture (80% methanol) which is frequently used for the extraction of phenolic compounds, where the solubility of the phenolic compounds was influenced by the degrees of polarity of solvent, the degree of polymerization of the phenolic compounds, and the interaction of the phenolic compounds with other food constituents and the formation of insoluble complexes. Thus, there is no uniform or completely satisfactory procedure suitable for the extraction of all phenols or a specific class of phenolic compounds in plant materials. (36). As mentioned by Pérez-Jiménez et al (37), a procedure for the extraction of antioxidants from plant foods should combine at least two extraction cycles performed with aqueous-organic solvents with different polarities in order to extract antioxidant compounds with different chemical structures. Also, several factors may influence the phenolic content of food plants such as the geographic region where they were cultivated (38, 39), altitude, environmental factors as soil, irrigation, temperature range, light quality , exposure to diseases and pests, the harvest season, , industrial processing, the way of drying , storage and method of extraction and quantification (34). Furthermore, many studies showed that the highest polyphenol content and antioxidant activity has been reported for fruits and vegetables grown in arid zones which is explained by the fact that fruits and vegetables increase their phytochemicals to adapt with abiotic stress (38, 39).

The total antioxidant properties of plants cannot be evaluated by single method because of complex nature of their phytochemicals which may act through different mechanisms. Therefore, two or more methods should always be employed in order to evaluate the total anti-oxidative effects of fruits and vegetables extracts (40). Of these, DPPH scavenging, ferrous ion chelating, reducing power test and β- carotene bleaching assay are used for the evaluation of the antioxidant activities of the extracts.

The radical scavenging activity of extracts of fruits and vegetables was evaluated using DPPH assay which is a commonly used for its rapidity and effectiveness (41). In this assay, the lowest IC_{50} value indicated the more potent antioxidant activity of the extract in terms of hydrogen atom or electron donating capacity.

Results showed that fruits and vegetables extracts exhibited a good DPPH radical scavenging effect that may be related to their higher polyphenols contents. Hence, polyphenol-rich foods found in vegetables and fruits can serve as free radical scavenger. Furthermore, the obtained results corroborate with findings of Jiang et al (42), Lui et al (20), Oboh (27) and Marathe et al (43) who studied the correlation between the antioxidant activity and the polyphenolic content of different varieties of vegetables and fruits. Pincemail et al (44) reported also that fruits and vegetables rich in anthocyanins generally have a greater total antioxidant capacity than those rich in flavanones and flavonols and this may be explain the high antioxidant activity of eggplant, pomegranate and black grapes.

The metal chelating assay is based on the ability of extract to chelate transition metals by binding them to ferrous (Fe^{2+}) ion catalyzing oxidation and disrupting the formation of Fe^{2+}- ferrozine complex (intense red purplish in color). This chelating capacity is important, since it reduces concentration of the catalyzing transition metal in lipid peroxidation through the inhibition of lipid peroxides to peroxyl and alkoxyl radicals via the Fenton reaction (13).

The obtained results showed that the vegetables and fruits extracts exhibited appreciable chelating ef-

fect with the highest antioxidant capacity noticed by potato, lettuce, carrot, courgette, pumpkin, turnip, cucumber, fennel, cauliflower, cabbage, artichoke (flower), Jew's mallow, chard, green bean, bean and green pea. This iron chelating activity of extracts shown in this study could be related to their amount of total phenolic and flavonoids contents. Similar correlations between polyphenols and iron-chelating ability were also noted by Nathan and Brumaghim (45) whose reported that the strong iron-binding properties of polyphenols, whether the iron chelating ability of catechol or gallol containing polyphenols are actually plays a key role in their antioxidant activity and anti-lipid peroxidation by blocking the Fenton reaction. Gebhardt and Fausel (46) mentioned also that artichoke extract have a marked chelating potential which can be due, at least, to some ubiquitous and artichoke-specific polyphenolic and flavonoid compounds.

Reducing power assay is also widely used in evaluating antioxidant activity of plant polyphenols. The samples with higher reducing power show higher absorbance. The presence of reductants like antioxidants in the fruits and vegetables extracts causes the reduction of the ferric to the ferrous form, indicating that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation process (41).

Onion, potato, courgette, Jew's mallow, chard, green bean, bean and green pea showed the a considerable reducing power indicating that they can act as electron donors and could react with free radicals to convert them into more stable products and then terminate the free radical chain reactions. These obtained results agree with other reports on reducing power of plants food who reported that the reducing power of polyphenolics is probably due to the presence of hydroxyl group, which might act as electron donors (47, 48).

β-carotene bleaching test is based on the oxidation of linoleic acid generates peroxide radicals, following the abstraction of hydrogen atoms from diallyl methylene groups of linoleic acid. These free radicals will subsequently oxidize the highly unsaturated β-carotene, thus causing the disappearance of its red color. However, in the presence of an antioxidant compound, this degradation process is prevented. It also reflects the ability to inhibit the lipid peroxidation *in vitro* (13).

In this study, the vegetables and fruits extracts found to hinder the extent of β-carotene bleaching by neutralizing the linoleate free radical and other free radicals formed in the system which is in agreement with results of many studies (49) who studied the lipid peroxidation of food rich vegetables and fruits. The antioxidant activity percentage of peroxidation inhibition of the presented vegetables and fruits extracts was similar to that reported by Ismail et al (50). Also, Karadeniz et al (51) found close results for pears, grapes and apples. But, pomegranate showed a lower antioxidant activity (42.02%) than that found in the study of Singh et al (52).

The statistical analysis indicated that legumes (green bean, bean and green pea) and courgette had significantly high antioxidant activity (91.70%, 88.42%, 92.31%, 84.89%) compared with BHT as a reference antioxidant and the same results were reported by Amarowicz and Pegg (53). Also, previous studies indicated the good correlation between the antioxidant capacity of the fruits and vegetables and their phenolic content (54). However, many studies have found no correlation between total polyphenols content and antioxidant activity of plant extracts (50, 55) and this may be explained by the fact that the molecular antioxidant response of the phenolic compounds varies considerably according to their chemical structure (56). Thus, the antioxidant activity of fruits and vegetables depends on not only to its content of phenolic compounds but also on the type of phenolics and their relative distribution (57) and the interactions between antioxidants (58).

Conclusion

This study showed that consumed fruits and vegetables in Algeria contain polyphenols, flavonoids and tannins which are affected mainly by the geographical region and harvesting time and showed a good antioxidant activity in relation to their phenolic content and their consumption may deliver greater health benefits thought the supply of natural antioxidant. So, the use of a balanced diet containing enough fruits and vegetables as a source of natural antioxidants could be much more effective and economical than artificial supplementation with antioxidants such as ascorbic acid

or -tocopherol for body protection against oxidative stress. Also, this work highlighted that it is important to use different free radicals and oxidation systems to evaluate the antioxidant activity of fruit and vegetable extracts because the extracts didn't present the same results in all methodologies and this could be due to difference in chemical composition of extracts and different mediums and principals of technics.

References

1. Sies H. Oxidative stress: oxidants and antioxidants. Exp Physiol 1997; 82: 291-295.
2. Pham-Huy L A, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. Int J Biomed Sci 2008; 4: 89-96.
3. Li S, Chen G, Zhang C, Wu M, Wu S, Liu Q. Research progress of natural antioxidants in foods for the treatment of diseases. Food Sci Human Wellness 2014; 3: 110-116.
4. Xiu-Qin L, Chao J, Yan-Yan , Min-Li Y, Xiao-Gang C. Analysis of synthetic antioxidants and preservatives in edible vegetable oil by HPLC/TOF-MS. Food Chem 2009; 113 : 692-700.
5. Manach C, Williamson G, Morand C, Scalbert A, Rémésy C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. Am J Clin Nutr 2005; 81: 230S-242S.
6. Rice-Evans C, Miller N, Paganga G. Antioxidant properties of phenolic compounds. Trends Plant Sci 1997; 2: 152-159.
7. Gunathilake K D P P, Ranaweera K K D S. Antioxidative properties of 34 green leafy vegetables. J Funct Foods 2016; 26: 176-186.
8. Z et al. Profiling of Phenolic Compounds and Antioxidant Activity of 12 Cruciferous Vegetables. Molecules 2018; 23: 1139.
9. Hossain M B, Barry-Ryan C, Martin-Diana A B, Brunton N P. Effect of drying method on the antioxidant capacity of six Lamiaceae herbs. Food Chem 2010; 123: 85-91.
10. Li H B, Cheng K W, Wong C C, Fan K W, Chen F, Jiang Y. Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. Food Chem 2007; 102: 771-776.
11. Quettier-Deleu C et al. Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour. J Ethnopharmacol 2000; 72: 35-42.
12. Bate-Smith E C. Haemanalysis of tannins: the concept of relative astringency. Phytochemistry 1973; 12: 907-912.
13. Bouaziz A, Abdalla S, Baghiani A, Charef N. Phytochemical analysis, hypotensive effect and antioxidant properties of *Myrtus communis* L. growing in Algeria. Asian Pac J Trop Biomed 2015; 5: 19-28.
14. Brand-Williams W, Cuvelier M E, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT - Food Sci Technol 1995; 28: 25-30.
15. Bilto Y Y, Alabdallat N G, Salim M. Antioxidant properties of twelve selected medicinal plants commonly used in Jordan. Br J Pharm Res 2015; 6: 121-130.
16. Le K, Chiu F, Ng K. Identification and quantification of antioxidants in *Fructus lycii*. Food Chem 2007; 105: 353-363.
17. Ozsoy N, Can A, Yanardag R. Antioxidant activity of *Smilax excelsa* L. leaf extracts. Food Chem 2008; 110: 571-583.
18. Kartal N, Sokmen M, Tepe B, Daferera D, Polissiou M, Sokmen A. Investigation of the antioxidant properties of *Ferula orientalis* L. using a suitable extraction procedure. Food Chem 2007 ; 100: 584-589.
19. Cie lik E, Gr da A, Adamus W. Contents of polyphenols in fruit and vegetables. Food Chem 2006; 94: 135-142.
20. Liu X et al. Total phenolic content and DPPH radical scavenging activity of lettuce (*Lactuca sativa* L.) grown in Colorado. LWT-Food Sci Technol 2007; 40: 552-557.
21. Baginsky et al. Phenolic compound composition in immature seeds of fava bean (*Vicia faba* L.) varieties cultivated in Chile. J Food Compost Anal 2013; 31: 1-6.
22. Mokhtar M et al. Determination of the polyphenolic content of a *Capsicum annuum* L. extract by liquid chromatography coupled to photodiode array and mass spectrometry detection and evaluation of its biological activity. J Sep Sci 2015; 38: 171-178.
23. Dos Reis L C R, de Oliveira V R, Hagen M E K, Jablonski A, Flôres S H, de Oliveira Rios A. Carotenoids, flavonoids, chlorophylls, phenolic compounds and antioxidant activity in fresh and cooked broccoli (*Brassica oleracea* var. *Avenger*) and cauliflower (*Brassica oleracea var. Alphina* F1). LWT-Food Sci Technol 2015; 63: 177-183.
24. Kähkönen M P et al. Antioxidant activity of plant extracts containing phenolic compounds. J Agric Food Chem 1999; 47: 3954-3962.
25. Derakhshan Z et al. Antioxidant activity and total phenolic content of ethanolic extract of pomegranate peels, juice and seeds. Food Chem Toxicol 2018; 114 : 108-111.
26. Merken H M, Beecher G R. Measurement of food flavonoids by high-performance liquid chromatography: a review. J Agric Food Chem 2000; 48: 577-599.
27. Oboh G, Raddatz H, Henle T. Characterization of the antioxidant properties of hydrophilic and lipophilic extracts of Jute (*Corchoru solitorius*) leaf. Int J Food Sci Nutr 2009; 60 : 124-134.
28. Sacan O, Yanardag R. Antioxidant and antiacetylcholinesterase activities of chard (*Beta vulgaris* L. *var. cicla*). Food Chem Toxicol 2010; 48: 1275-1280.
29. Zineb G, Boukouada M, Djeridane A, Saidi M, Yousfi M. Screening of antioxidant activity and phenolic compounds of various date palm (*Phoenix dactylifera*) fruits from Algeria. Med J Nutr Metab 2012; 5: 119-126.
30. Baljeet S Y, Roshanlal Y, Ritika B Y. Effect of cooking methods and extraction solvents on the antioxidant activity of summer squash (*Cucurbita pepo*) vegetable extracts. Int

Food Res J 2016; 23: 1531-1540.
31. Ghosh, D. Tannins from foods to combat diseases. Int J Pharm Res Rev 2015; 4: 40-44.
32. Orak H H, Yagar H, Isbilir S S. Comparison of antioxidant activities of juice, peel, and seed of pomegranate (*Punica granatum* L.) and inter-relationships with total phenolic, tannin, anthocyanin, and flavonoid contents. Food Sci Biotechnol 2012; 21: 373-387.
33. Zarfeshany A, Asgary S, Javanmard S H. Potent health effects of pomegranate. Adv Biomed Res 2014; 3:1-8.
34. Manach C, Scalbert A, Morand C, Rémésy C, Jiménez L. Polyphenols: food sources and bioavailability. Am J Clin Nutr 2004; 79: 727-747.
35. Stafussa A P et al. Bioactive compounds of 44 traditional and exotic Brazilian fruit pulps: phenolic compounds and antioxidant activity. Int J Food Prop 2018; 21: 106-118.
36. Naczk M, Shahidi F. Extraction and analysis of phenolics in food. J Chromatogr A 2004; 1054 : 95-111.
37. Pérez-Jiménez J, Neveu V, Vos F, Scalbert A. Identification of the 100 richest dietary sources of polyphenols: an application of the Phenol-Explorer database. Eur J Clin Nutr, 2010; 64: S112-S120.
38. Kumari D, Madhujith T, Chandrasekara A. Comparison of phenolic content and antioxidant activities of millet varieties grown in different locations in Sri Lanka. Food Sci Nutr 2017; 5: 474-485.
39. Taghizadeh S F, Davarynejad G, Asili J, Nemati S H, Karimi G. Assessment of phenolic profile and antioxidant power of five pistachio (*Pistacia vera*) cultivars collected from four geographical regions of Iran. Avicenna J Phytomed 2018; 8: 33-42.
40. Chu Y H, Chang C L, Hsu H F. Flavonoid content of several vegetables and their antioxidant activity. J Sci Food Agric 2000; 80: 561-566.
41. Amarowicz R, Pegg R B, Rahimi-Moghaddam P, Barl B, Weil J A. Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. Food Chem 2004; 84: 551-562.
42. Jiang H, Ji B, Liang J, Zhou F, Yang Z, Zhang H. Comparison on the antioxidant capacity of selected fruits and vegetables and their separations. Chem Nat Compd 2006; 42: 410-414.
43. Marathe S A, Rajalakshmi V, Jamdar S N, Sharma A. Comparative study on antioxidant activity of different varieties of commonly consumed legumes in India. Food Chem Toxicol 2011; 49 : 2005-2012.
44. Pincemail J, Degrune F, Voussure S, Malherbe C, Paquot N, Defraigne J O. Effet d'une alimentation riche en fruits et légumes sur les taux plasmatiques en antioxydants et des marqueurs des dommages oxydatifs. Nutr Clin Metab 2007; 21: 66-75.
45. Perron N R, Brumaghim J L. A review of the antioxidant mechanisms of polyphenol compounds related to iron binding. Cell Biochemi Biophys 2009; 53: 75-100.
46. Gebhardt R, Fausel M. Antioxidant and hepatoprotective effects of artichoke extracts and constituents in cultured rat hepatocytes. Toxicol In Vitro 1997; 11: 669-672**.**
47. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J Agric Food Chem 1992; 40: 945-948.
48. Pellegrini N et al. Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different *in vitro* assays. J Nutr 2003; 133: 2812-2819.
49. Kaur C, Kapoor H C. Anti-oxidant activity and total phenolic content of some Asian vegetables. Int J Food Sci Technol 2002; 37: 153-161.
50. Ismail A, Marjan Z M, Foong C W. Total antioxidant activity and phenolic content in selected vegetables. Food Chem 2004; 87: 581-586.
51. Karadeniz F, Burdurlu H S, Koca N, Soyer Y. Antioxidant activity of selected fruits and vegetables grown in Turkey. Turk J Agric For 2005; 29: 297-303.
52. Singh R P, Chidambara Murthy K N, Jayaprakasha G K. Studies on the antioxidant activity of pomegranate (*Punica granatum*) peel and seed extracts using *in vitro* models. J Agric Food Chem 2002; 50: 81-86.
53. Amarowicz R, Pegg R B. Legumes as a source of natural antioxidants. Eur J Lipid Sci Technol 2008; 110 : 865-878.
54. Xiao Z P et al. Flavonoids health benefits and their molecular mechanism. Mini Rev Med Chem 2011; 11: 169-177.
55. Jaiswal S G, Patel M, Saxena D K, Naik S. Comparison of measurements of antioxidant activity in the selected leafy vegetables depending on extraction solvent. J Hortic For 2017; 25: 75-80.
56. Satué-Gracia M T, Heinonen M, Frankel E N. Anthocyanins as antioxidants on human low-density lipoprotein and lecithin–liposome systems. J Agric Food Chem 1997; 45: 3362-3367.
57. Heinonen I M, Lehtonen P J, Hopia A I. Antioxidant activity of berry and fruit wines and liquors. J Agric Food Chem 1998; 46: 25-31.
58. Freeman B L, Eggett D L, Parker T L. Synergistic and antagonistic interactions of phenolic compounds found in navel oranges. J Food Sci 2010; 75: C570-C576.

Correspondence:
Habiba Djenidi
Laboratory of Phytotherapy Applied to Chronic Diseases, Department of Biology and Animal Physiology, Faculty of Nature and Life Sciences
University Setif 1, Setif
19000, Algeria
E-mail: habibadjenidi@yahoo.fr

Progress in Nutrition 2020; Vol. 22, N. 1: 236-252 DOI: 10.23751/pn.v22i1.8011

Original article

Phytochemical screening and *in vivo* and *in vitro* evaluation antioxidant capacity of *Fargaria ananassa*, *Prunus armeniaca* and *Prunus persica* fruits growing in Algeria

Saoudi Soulef[1], Khennouf Seddik[1], Mayouf Nozha[2], Amira Smain[1], Dehamna Saliha[1], Karim Hosni[3]

[1]Laboratory of Phytotherapy Applied to Chronic Diseases, Faculty of Nature and Life Sciences, Univ Setif 1, 19000, Algeria - E-mail: Soulef2016bio@gmail.com; [2]Laboratory of Applied Biochemistry, Faculty of Nature and Life Sciences, Univ Setif 1, 19000, Algeria; [3]Laboratoire des Substances Naturelles, Institut National de Recherche et d'Analyse Physico-chimique (INRAP), Sidi Thabet 2020, Tunisia.

Summary. *Purpose:* Fruits are important source of phytochemicals such as polyphenols, flavonoïds, tannins, carteneoides and vitamins which poss antioxidant activity. The aim of this study is the screening of polyphenols and the evaluation of antioxidant activity *in vitro* and *in vivo* of *Fargaria ananassa*, *Prunus armeniaca* and *Prunus persica* (Rosacea family) fruits extracts. *Methods:* The antioxidant activity of hydromethanolic extracts were estimated by methods of the scavenging activity against DPPH, ABTS, hydroxyl radicals (HO.), β-carotene/linoleic acid model system, lipid peroxidation, reducing power and chelating activity. Total phenolics and flavonoids content in these extracts were determined using Folin–Ciocalteu's reagent and Aluminum chloride colorimetric methods respectively. In vivo antioxidant activity was conducted by biological study using male wistar rats. *Result:* The results showed that *Fargaria ananassa* contains high amount of polyphenols and flavonoids (310 ± 0.003µg gallic acid /mg extract and 14.78 ± 0.001µg quercetin equivalent/mg extract respectively), followed by *Prunus armeniaca* and *Prunus persica,* but the highest levels of tannin were found in *Prunus armeniaca* (127.9±0.003 µg tannic acid equivalent/ mg extract). *Fargaria* extract have a high antiradical effect towards DPPH radical, however, *Prunus persica* have a good effect in the inhibition of lipid peroxidation. The administrations of *Fargaria* extract (200 mg/kg and 600 mg/kg) elevates the plasma antioxidant activity and reduced the level of MDA with 85.07 ± 2.06% and 39.54 ± 1.11% respectively and increased the levels of GSH in the liver of rats. The UPLC analysis of extracts demonstrated the presence of various phenolic acids (gallic acid, cinnamic acid and hydrocinnamic acid) and flavonoids (rutin and flavons3-ols) in all extracts. *Conclusion:* These results support the idea that the consumption of fruits can reduce the risk of disease related to free radicals such as cancer, cardiovascular diseases, hypertension, diabetes and stroke. This effect can be attributed at least in part to the antioxidant properties of polyphenols present in the extracts.

Key words: *Fargaria ananassa*, *Prunus armeniaca*, *Prunus persica,* polyphenols, flavonoids, tannin, antioxidant activity.

Introduction

The oxidative stress is the imbalance between antioxidants and the free radicals (1). Free radicals are defined as the molecules with an unpaired electron (2). These molecules are highly reactive and have an important role in cell physiology, such as life cycle regulation, development, migration, induction of signaling pathways, activation of second messengers, and triggering of antioxidant responses (3). The oxidative stress is associated with various diseases such as hypertension, cardiovascular disease, atherosclerosis, diabetes, cancer and arthritis (3, 4). Fruits and vegetables are important source of antioxidants such as phenolic acid, fla-

vonoids, cartonoids, vitamin C. These compounds are important functional food. Fruits can be considered as natural materials to prevent human from various pathologies as they may help to reduce the risk of many age related degenerative diseases (5). Polyphenols can maintain the health by several mechanisms including the elimination of free radicals. The protection and regeneration of other dietary antioxidant and the chelation of pro-oxidant metals (6-8). In this study fruits of *Fargaria ananassa* (Strawberry), *Prunus armeniaca* (apricot) and *Prunus persica* (peach) were used. These fruits belong to the same family *Rosaceae* growing in Algeria and are widely consumed by the Algerian population. Apricot, strawberry and peach are considered as rich sources of phytomolecules such as vitamins, polyphenols, flavonoids, carotenoids, fatty acids and proteins (9, 10). The aim of the present study was to examine the *in vitro* and *in vivo* antioxidant properties and the polyphenols content of these fruits in order to establish a relation between the consumption of these fruits and prevention from pathologies where oxidation stress is implicated.

Materials and methods

Plant material

In this study, fruits of *Fargaria ananassa and Prunus persica* were purchased from commercial market in Amoucha, Setif (Algeria) on April and July 2016. *Purnus armeniaca* fruits were harvested from Tizi Nbachar in Setif region on May 2016. Fresh Fruits were used for the present study.

Animals

Male Wister rats (150-200g) were purchased from Pasteur institute, Algiers. They were kept in cages at room temperature for one week to familiarize with the environment and have free excess to commercial diet and tap water. Ethics committee of the faculty of nature and life sciences, University Ferhat Abbas, Sétif 1 approved the experimental protocol.

Preparation of extract

The extraction of phenolic compounds was carried out according to method used by Markham (11). Hundred gram of the consumed parts of the fruits were washed with water, homogenized and mixed with 1 liter of methanol (85:15 v/v for the first extraction and then in 50:50 v/v for the second step) and kept at room temperature for 5 days to allow maximum extraction of bioactive molecules. The resulting solution was then filtered and the supernatant was evaporated using vacuum rotary evaporator at 40° C to obtain crude methanol extract. The crude extract was dried and stored at 4° C until use.

Determination of total polyphenols

The amount of total phenolic content in fruit samples was estimated using the Folin–Ciocalteu reagent as described by Li (12) with slight modification. In brief 200µl of samples solution were mixed with 1000µl of Folin –Ciocalteu reagent (1:10diluted with distilled water). The mixture was allowed to stand for 5 min, and then 800µL of sodium carbonate (7.5%) was added to the mixture. The mixture was incubated at room temperature in the dark for 90 min. the absorbance was measured against a blank at 760 nm using a UV-Visible spectrophotometer. The standard curve was prepared using 0-160 µg/ml solution of gallic acid. The amount of total phenolic was expressed as mg equivalent gallic acid /g dry extract.

Determination of flavonoids content

Aluminum chloride colorimetric method adapted from Bahromun (13) was used for the determination of total flavonoids. One ml of 2% $AlCl_3$ solution was added to an equal volume of extract. After mixing, the mixture was incubated for 10 min at ambient temperature in the dark. The absorbance was determined against the same mixture without the extract as a blank at 430 nm. The results are expressed in milligram of quercetin equivalent per gram dried extract.

Determination of tannin content

The amount of tannins was determined using the method described by Gharzouli (14). This method is based on the capacity of the tannin to precipitate hemoglobin. Briefly, a volume of samples mixed with an equal volume of hemolysed bovine blood (absorbance = 1.6). After 20 min of incubation at room temperature, the mixture was centrifuged at 4000 rpm for

10 min, and the absorbance of the supernatant was read at 576nm. Results were expressed as mg equivalent tannic acid per gram dried weight (mg TAE/g DW).

Determination of protein in the extracts

The amount of proteins in the extracts was estimated by Commasie bleu method described by Bardford (15). The blue of Commassie 0.004 % was dissolved in 4% ethanol (96 %) and 10% phosphoric acid (85%). SDS 0.1% was added to the mixture.100 µl of extract was added to 2 ml of reagent. Tubes were mixed with vortex, and then the absorbance was measured against a blank at 595 nm. The standard was prepared using 0.1-2 mg/ml solution of BSA in water. The results were expressed as mg BSA equivalent per g extract.

Total soluble sugars content

Total soluble sugars were determined using the method described by Dubois (16). In brief, 1 ml of samples was treated with 1 ml of 5% phenol and 5 ml concentrated sulphuric acid. The absorbance was recorded at 490 nm in UV/VIS spectrophotometer, against a blank (without sample). D-Glucose was used as standard and the amount of sugar was expressed in mg/g dried weight.

Identification of phenolic compounds by UPLC-DAD

The phenolic compounds in samples were analyzed by UPLC-DAD system (Perkin Elmer series 275), a model LC-200 micro pump High pressure Binary with series 200 autosmpler, a model Hypersil Gold reversed phase column(1.9µm* 3nm* 50mm) and a model diode array detector. The flow rate was kept constant throughout the analysis at 0.6 ml/min and the injection volume was 20 µl. The operating condition were a follows: mobile phase water (A) and acetonitrile (B): gradiant 5% B from 0 to 1 min, 5%-21% B from 1 to 5 min, 21%-50% B from 5 to 7 min, 50%-100% B from 7 to 10min, 5% B from 10 to 13 min. The column was maintained at 30° and UV detection was recorded in the range 165 nm- 365 nm. Phenolic compounds were identified by comparing retention time and spectrograms of samples with standards.

Determination of the in vitro Antioxidant activity of extracts

Phosphomolybdate assay (Total Antioxidant Capacity)

Total antioxidant capacity assay is a method used for the quantitative determination of antioxidant capacity, through the formation of phosphomolybdenum complex; this method was described by Prieto (17). An aliquot of 0.1 ml of sample solution was mixed with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance was measured at 695 nm against a blank. A typical blank contained 1 ml of the reagent solution and the appropriate volume of the solvent and incubated under the same conditions. The total antioxidant capacities were expressed as µg ascorbic acid equivalent per mg dry extract.

DPPH radical scavenging assay

Free radical scavenging activity of extracts against stable DPPH (2-diphenyl-2-picrylhydrazyl hydrate) was determined using the method described by Yardpiroon (18). 1 mL of the extract was added to 2.0 mL of 0.1 mM DPPH solution. The mixture was strongly shaken and left to stand at room temperature for 30 min. The changes in color (from deep-violet to light-yellow) and the Absorbance of samples were measured at 517 nm. The percentage of radical scavenging activity was calculated using the following equation:
Radical scavenging activity (%) = $[A_{control} - A_{sample}] / A_{control} \times 100$.

Where $A_{control}$ is the absorbance of the control reaction (containing all reagents except the sample). A_{sample} is the absorbance of the extract. A curve of percent inhibition or percent scavenging effect against samples concentrations was plotted and the concentration sample required for 50% inhibition was determined. The value for each test sample was presented as the inhibition curve at 50% or IC_{50}.

Free radical scavenging ability by ABTS

The free-radical-scavenging activity was determined by ABTS radical cation decolorization assay Re (19). Briefly, $ABTS^{+}$ radical cation was generated by a reaction of 7 mM ABTS with 2.45 mM potas-

sium persulfate. The reaction mixture was allowed to stand in the dark for 16 h at room temperature. The solution was then diluted by mixing ABTS solution with methanol to obtain an absorbance of 0.70 ± 0.02 units at 734 nm. Then, 50 µl of sample was mixed with 1ml of $ABTS^{+}$ solution and kept for 30 min at room temperature. The absorbance of reaction mixture was measured at 734 nm. The ABTS scavenging capacity of the extract was compared with that of VIT C and the percentage inhibition was calculated as ABTS radical scavenging activity (%) = [($Abs_{control}$ - Abs_{sample}) / (Abscontrol)] x 100 where $Abs_{control}$ is the absorbance of ABTS radical + methanol; Abs_{sample} is the absorbance of ABTS radical + sample extract /standard.

Reducing power of extracts

The reducing power of the fruit extract was estimated according to the method described by Ebrahimzadeh (20) .In brief, 100 µl of the extract with various concentration were mixed with an equal volume of 0.2 M phosphate buffer (PH= 6.6) and 1% of potassium ferricyanide [K3Fe (CN_6)]. The reaction was incubated at 50°C in a water bath for 20 min and the reaction was terminated by the addition of 250 µl of 10% trichloroacetic acid followed by centrifugation for 10 min at 3000 rpm.250 µl of the upper layer of solution was mixed with 250µl of distilled water and 500µl of $FeCl_3$ and the absorbance was measured at 700 nm against a blank. Higher absorbance indicates higher reducing power.BHT was used as positive control.

Ferrous ion chelating activity of the extracts

The method described by Decker and Welch (21) was used to investigate the ferrous ion chelating ability of different extracts. This activity ferrous ion chelating ability was monitored by the absorbance of the ferrous iron ferrozine complexe at 562 nm. The mixture contained 500 µl sample or EDTA, 100 µl $FeCl_2$ (0.6 mM in water) and 900µl methanol. Same mixture without the extract or EDTA was considered as a control. The mixture was shaken well and allowed to react at room temperature for 5 min; 100 µL of ferrozine (5 mM in methanol) was then added. The chelating effect was calculated as a percentage, using the same equation as that described for the DPPH assay.

β-Carotene bleaching assay

In this test, the antioxidant capacity of the extracts is determined by measuring the inhibition of the oxidative decomposition of β-carotene (discoloration) by the products of oxidation of the linoleic acid according to the method described by Gursoy (22). The emulsion of β-carotene/ linoleic acid is prepared by solubilization of 0,5mg β-carotene in 1ml of chloroform, 25µl of the linoleic acid and 200mg of Tween 40 are added, after that 100ml of distilled water saturated with oxygen was then added to the reaction. 350µl of extracts or BHT solubilized in methanol (2mg/ml) was mixed with 2,5ml emulsion. The same procedure was repeated with MeOH and H_2O as negative control. The absorbance was measured at 490 nm after: 1heure, 2h, 3h, 4h, 6h and 24h of incubation at room temperature in the dark .The percentage of inhibition of β-carotene decomposition by the extracts antioxidant was measured as follows:

AA% = ABS_{test} / ABS_{BHT} ×100

AA%: Percentage of the antioxidant activity.

ABS_{test}: Absorbance in the presence of the extract (test).

ABS_{BHT}: Absorbance in the presence of positive control BHT.

Ferric thiocyanate (FTC) method

The antioxidant capacity of fruits extracts towards the peroxidation of linoleic acid was tested by the thiocyanate method described by Yen (23). In this test, the concentration of peroxide decreases as the antioxidant activity increases. The mixture contained 0.5 ml of samples, 2.5 ml of 0.02M linoleic acid emulsion at pH 7.0 and 2 ml of 0.2 M phosphate buffer at pH 7.0. The emulsion was prepared by mixing 0.2804 g of linoleic acid, 0.2804 g of Tween 20, and 50 ml of phosphate buffer. The reaction mixture was incubated for 5 days at 37 °C. 0.1 ml of the reaction mixture is transferred to a test tube and 75% EtOH (4.7 ml), 30% ammonium thiocyanate (0.1 ml), 0.02 M ferrous chloride in 3.5% HCl (0.1 ml) were added to tubes each 24 h intervals. Three minutes after the addition of ferrous chloride to the reaction mixture, the absorbance of the resulting mixture (red color) is measured at 500 nm every 24 h until the absorbance of the control reached its

maximum. BHT and vitamin C were used as positive controls and the mixture without the sample is used as the negative control. % Inhibition of lipid peroxidation is calculated by the folowing equation: Inhibition (%) = [Ac – As / Ac × 100].

Where, A_s is the absorbance of the sample on the day when the absorbance of the control is maximum and Ac is the absorbance of the control on the day when the absorbance of the control is maximum.

Thiobarbituric Acid (TBA) assay

According the method of Kikuzaki and Nakatani (24), the TBA was measured on the final day of FTC assay. This method based on the determination of the in levels of malonaldehyde (MDA) formed during lipid peroxidation. The sample contained the same elements used in the lipid peroxidation. 1 ml of sample solution was mixed with 2 ml of trichloroacetic acid (20%) and 2 ml of thiobarbituric acid solution. The mixture was then placed in a boiling water bath for 10 minutes, after cooling tubes were centrifuged at 3000 rpm for 20 min. The absorbance of the supernatant was measured at 532 nm and recorded after it has reached its maximum.

Hydroxyl radical scavenging assay

Hydroxyl radical is one of the potent reactive oxygen species in the biological system that reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids and causes damage to cell. Hydroxyl radical scavenging activity was measured by the ability of the different fruit extracts to scavenge the hydroxyl radicals according the method described by Smirnoff and Cumbes (25) with slight modifications. The reaction mixture consists of100 µL of varying concentration of samples or standard antioxidants, 1 ml of $FeSO_4$ (1.5 mM), 0.7 ml of H_2O_2 (6 mM), 0.3 ml of sodium salicylate (20 mM). This mixture was incubated at 37 C° for 1 h, after which the absorbance of the hydroxylated salicylate complex was measured at 562 nm. The percentage scavenging effect was calculated as flows:

Scavenging rate = $[A_{control} - A_{sample}] / A_{control} \times 100$.

Where $A_{control}$ was the absorbance of the control (without sample) and A_{sample} was the absorbance in the presence of the sample.

Determination of the in vivo Antioxidant activity of fruits extracts

Experimental design

Rats were divided into 08 groups having 6rats in each group: Group 01: received 0.9% of NaCl. Group 02: received vitamin C (200 mg/kg); Group 03: received the dose 200mg /kg of *Fargaria ananassa* extract. Group 04: received the dose 600mg /kg of *Fargaria ananassa* extract. Group 05: received the dose 200mg / kg of *Purnus armeniaca* extract. Group 06: received the dose 600mg /kg of *Purnus armeniaca* extract. Group 07: received the dose 200mg /kg of *Purnus persica* extract. Group 08: received the dose 600mg /kg of *Purnus persica* extract.

At the end of the experimental period (15 days), rats were sacrificed. Blood was collected in heparinized tubes and centrifuged at 3000 rpm for15 min. Plasma was kept in the freezer until use. Tissues are also kept in freezer until use.

In vivo Antioxidant activity of plant extracts

Effect of extracts on plasma antioxidant capacity using DPPH radical

In this assay, the ability of plasma to scavenging the DPPH radical was measured by the method of Burists and Bucars (26) with slight modifications. Briefly, 50 µl of plasma was mixed with DPPH solution (0.004%). the mixture was incubated for 30 min, then tubes were centrifuged at 3000 rpm for 15 min. The absorbance was measured at 517 nm, and the plasmatic antioxidant capacity was calculated as follows:

% scavenging activity = $[(Abs_{control}-Abs_{sample})/Abs_{control}]$ X 100

A control: is the absorbance of the blank solution

A sample: is the absorbance in the presence of plasma.

Effect of extracts on plasma reducing power

According the method of Chung (27) the reducing power was evaluated. 0.1 ml of plasma was mixed with 0.1 ml of sodium phosphate buffer (0.2 M, pH 6.6) and 0.1 ml of potassium ferricyanide (1 %). The mixture was incubated for 20 min at 50°C. After that, 0.250 ml of trichloroacetic (1%) was added. Then, the mixture was centrifuged for 10 min at 3000 rpm. An

aliquot (0.250 ml) of the upper layer was mixed with 0.250 ml of distilled water and 0.5 ml of ferric chloride (0.1%), and the absorbance at 700 nm was measured. Higher absorbance indicates a higher reducing power.

Preparation of liver homogenate

After scarifying the animals, the homogenate of liver were prepared by homogenizing 0.5g of liver tissues in 4.5 ml cold KCl solution (1.15%) using homogenizer on ice. The homogenate was centrifuged at 4000 rpm for 15 min at 4C and the supernatant was used for the determination of (GSH) activity and lipid peroxidation (MDA).

Assessment of reduced glutathione concentration

GSH was measured using a previously described procedure by Ellman (28). GSH can react with 5, 50-dithio-bis (2-nitrobenzoic acid) (DTNB) and formed yellow color. In brief, 50 µl of tissue homogenate was diluted in 10 ml phosphate buffer (0.1 M, PH 8). 3 ml of this mixture was mixed with 20 µl of DTNB. The developed yellow color was then measured immediately after 5 min at 412 nm against a blank (without tissue homogenate). GSH concentrations were calculated using the standard curve of GSH. It was expressed as µmol/ g tissue.

Assessment of lipid peroxidation

According the method of Okhawa (29) lipid peroxidation rate was determined by malondialdehyde level (MDA). This assay was based on the reaction between TBA and MDA. In brief, to 0.5 ml of tissue homogenate, 1 ml of TBA (0.67 %) was added. The mixture was incubated for 15 min in boiling water bath. 4 ml of n-butanol was added to the mixture after cooling, tubes were then centrifuged at 3000 rpm for 15 min. The amount of TBARS formed in each sample was assessed by measuring the optical density of the supernatant at 535 nm against a blank. The concentration of MDA was determined from a standard curve of 1, 1, 3, 3 tetraethoxypropane in the same conditions and it was expressed as nmol/ g tissue.

Statistical analysis

Statistical analysis was performed using Graph Pad Prism (version 5.01 for Windows). In vitro results were expressed as mean ± standard deviation (SD) and were analyzed by one way analysis of variance (ANOVA) followed by Dunnet's test. The pharmacological results were presented as mean ± standard error of mean (S.E.M.) of six experiments. In all cases, The P-values less than 0.05 were considered statistically significant.

Result

Total polyphenols, flavonoids and tannins contents in the extracts

Fruits are major source of biologically active components. Most of these compounds have antioxidants effects such as polyphenols, flavonoids, protein and vitamins. These substances have an important role in the prevention of various diseases (30, 31). Total phenolic, flavonoids and tannins contents in different extracts are shown in Table 1. Total phenolic compounds ranged between 112.5 and 310 µg GAE /mg DW, and the results showed that *Fargaia* is rich in polyphenols followed by *Prunus armeniaca* and *Prunus persica.* Total flavonoids contents were expressed as mg quercetin equivalents per gram of dry weight (µg QE/mg). *Fargaria* extract exhibited the highest flavonoids content. The quantification of tannins contents showed that *Prunus armeniaca* extract contained the highest tannins concentration with the value of 127.9 ± 0.0003 µg TAE /mg extract. The lowest tannins content was noticed for *Prunus persica* extract with a value of 62.83 ± 0.03 µg TAE /mg extract.

Table 1. Total polyphenols, flavonoids and tannins contents in fruits extracts

Extract	Total phenolic content (µg GAE/mg)	Total flavonoids (µg QE/mg)	Total tannins (µg TAE/mg)
Fargaria ananassa	310±0.003	14.78±0.001	81.5±0.01
Prunus armeniaca	232.5±0.02	5.68±0.002	127.9±0.003
Prunus persica.	112.5±0.02	6.02±0.003	62.83±0.03

GAE : Gallic Acid Equivalent QE : Quercetin Equivalent TAE : Tannic Acid Equivalent. Results expressed as means ± SD

Protein and Sugar content in the extracts

Fruits are important source of bioactive molecules such as protein, carbohydrate, amino acid, phenolic compounds and minerals (32). As shown in table 2, *Fargaria* contains the highest amount of sugars with a value of (958± 0.06 mg D-glucose/ g) the lowest amount was observed in *Prunus armeniaca* (285 ±0.06 mg D-glucose/g). Total protein content was high in *Fargaria* (2.73 ±0.04 g/l) and low in *Prunus persica* (1.55±0.01mg/g).

Identification of different phenolic acids, flavonoids and ascorbic acid in fruits extracts

Phenolic compounds are very important fruits constituents because of their scavenging ability due to their hydroxyl groups (33). The UPLC chromatogram of *Fargaria ananassa, Prunus persica* and *Prunus armeniaca* fruits extracts revealed the presence of various phenolic acid and flavonoids such as gallic acid, protocachuic acid, caffeic acid, flavon-3-ols. *Fargaria* extract showed the presence of some compounds which are absent in the *Prunus persica* and *Prunus armeniaca* such as hydroxynammic acid, p-coumaric, cinamic acid as presented in the Fig1. Catechin was detectable in the *Fargaria ananassa, Prunus persica. Prunus persica* and *armeniaca* contained ferulic acid but only rutin and vanillic acid and chlorogenic acid was found in *Prunus armeniaca* (Fig.2).

In vitro antioxidant activity of fruit extracts

Total Antioxidant Capacity (TAC) of fruit extracts

The total antioxidant was estimated using phosphomolybdate assay. It is based on the reduction of molybdenum (VI) to molybdentum (V) in the presence of antioxidant, and this reduction produced green phosphate/ Mo (V) complex in acid pH, which can measured at 695 nm (34, 35). The phosphomolybdate model evaluates both water soluble and fat soluble antioxidant capacity. It increases with the increase in the concentration of extract. The result was expressed as µg Ascorbic acid equivalent per g of extract. The result of total antioxidant capacity showed that *Fargaria* had the highest antioxidant capacity (99.6 ± 0.007) flowed by *Prunus persica* and *Prunus armeniaca* (81.33 ± 0.04 and 81.66 ± 0.008), respectively.

Table 2. Total sugars and proteins in fruits extracts

Extract	Total sugar (mg d-glucose E/g)	Total protein (mg/g)
Fargaria ananassa	958 ±0.06	2.73 ±0.04
Prunus persica	610 ±0.008	1.55 ±0.01
Prunus armeniaca	285 ±0.06	2.12 ±0.02

Results are expressed as Mean ± SD (n=3)

DPPH radical scavenging activity of fruits extracts

DPPH is widely used to assess the radical scavenging activity of antioxidant compounds (20, 36, 37). The 2,2diphényl-1-picrylhydrazyle is the first free radical used to study the relation between the structure

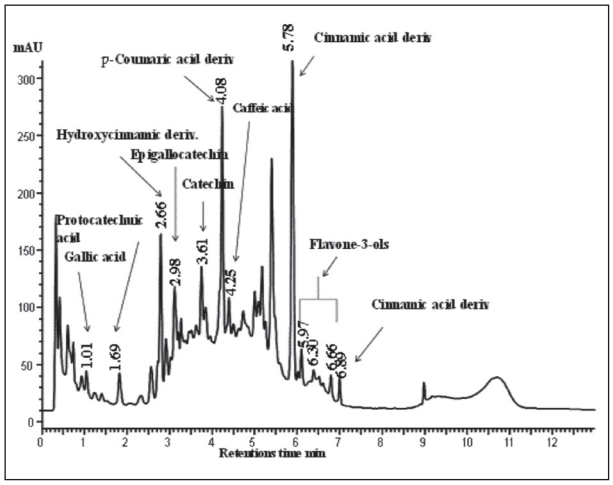


Fig 1. UPLC chromatogram of *Fargaria ananassa* fruit methanol extract

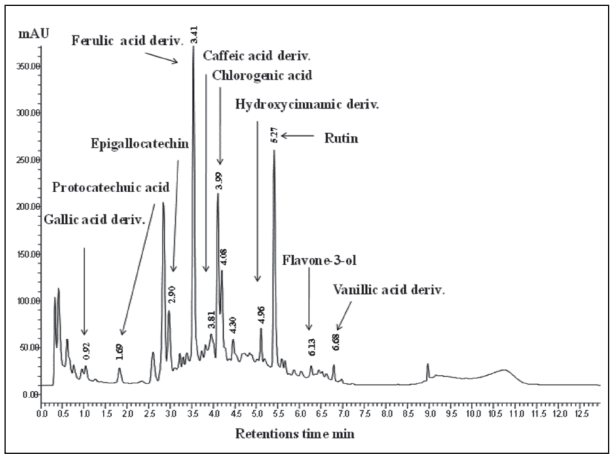


Fig 2. UPLC chromatogram of *Prunus armeniaca* fruit methanol extract

and antioxidant activity of phenolic compounds (38). It was found that the radical- scavenging activity of extract increased with increasing concentration (39). The degree of discoloration indicates the scavenging potential of the antioxidants .The antioxidant activities obtained by the DPPH method for the fruits extracts are presented in Fig.4. This activity was compared with that of BHT as a synthetic antioxidant. The results revealed that methanol extract of *Fargaria* is more effective scavenger than the *Prunus armeniaca* and *Prunus persica* with IC_{50}values 0.142 ± 0.0004, 0.488 ±0.012, 0.673 ±0.02 mg/ml for the three extracts respectively.

Free radical scavenging ability using ABTS

The ABTS radical is one of various radical used for measuring the antioxidant activity in foods (40). ABTS is a stable organic radical that has gained hydrogen; this method determines the antioxidant activity of hydrogen donating antioxidants in fruit crude extract (41, 40). *Fargaria* extract has high scavenging ability of the ABTS radical with an IC_{50} value of 0.040 ± 0.003 mg/ml. *Prunus persica* and *Prunus armeniaca* have good scavenging activity with IC_{50} values of 0.173±0.003 and 0.323± 0.007 respectively.

Reducing power of fruits extracts

Various studies have revealed that the electron donation ability reflects the reducing power of the bioactive compound. The amount of the Fe^{+2} complex was determined by measuring the formation of perls Prussian blue at 700 nm. Results are shows in fig 6 and7. Fig.6 showed a relationship between the increase in the absorbance, the concentration of extract and the reducing power. At 0.5mg/ml the absorbance of fruit extracts were in the flowing order *Fargaria* (1.142) >*Prunus armeniaca* (0.744) >*Prunus persica* (0.592). Figure7 shows the reducing power of fruit extracts. All extracts exhibited low activity compared to BHT with RC_{50} values of: BHT (0.008mg/ml) >*Fargaria* (0.251 mg/ml) >*Prunus armeniaca* (0.329 mg/ml) >*Prunus persica* (0.779 mg/ml).

Metal Chelating activity of extracts

The Ferrosine-Fe^{+2} complex produced a red chromophore which can be measured at 562 nm (42). In this study, the chelating activity of fruits extracts is

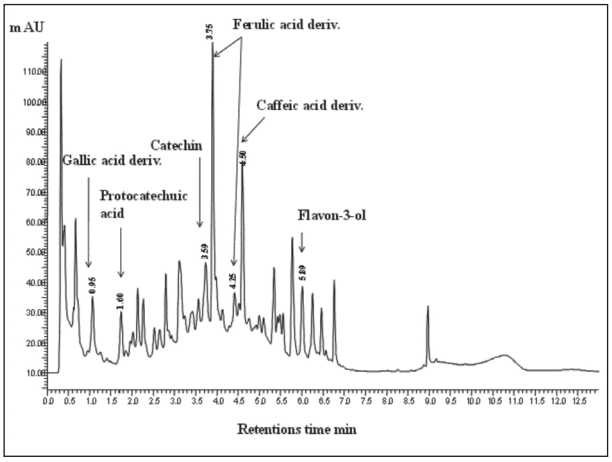


Fig 3. UPLC chromatogram of *Prunus persica* fruit methanol extract.

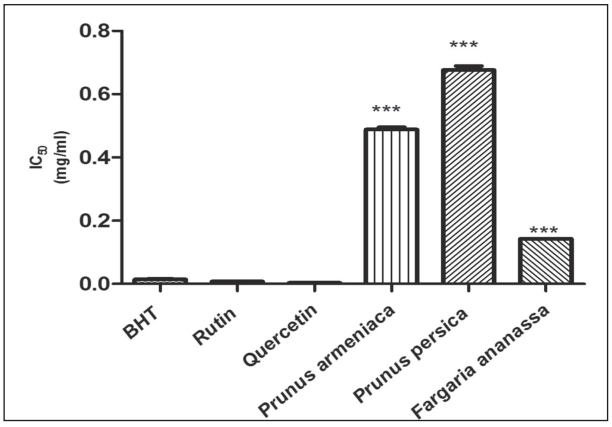


Fig. 4. DPPH free radical scavenging activity of different fruit extracts. Data were presented as IC_{50} means± SD (n = 3). *** ($p < 0.001$) compared to BHT as standard.

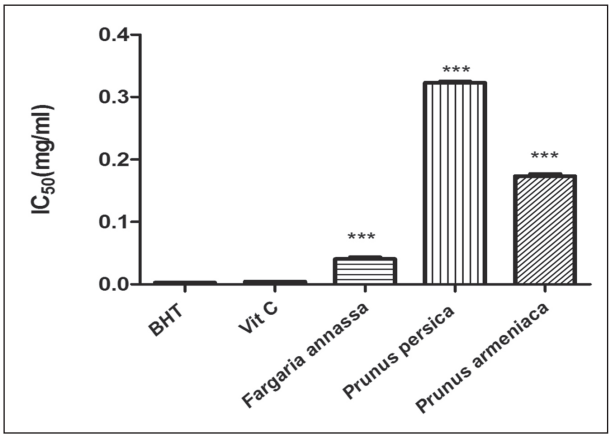


Fig.5. ABTS radical scavenging activity of different fruits extracts. Data were presented as IC_{50} means ± SD (n = 3). (*** $p<0.001$) compared to BHT and vit C as standards

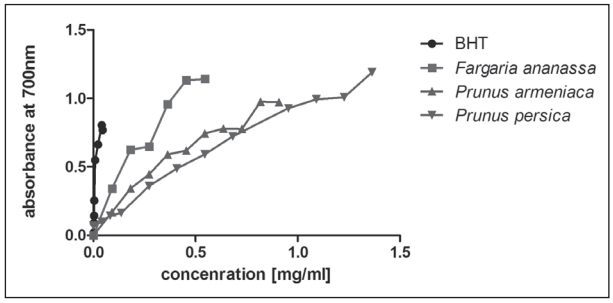


Fig 6. Antioxidant activity of fruits extracts expressed as reducing power. Values are means ± SD (n = 3).

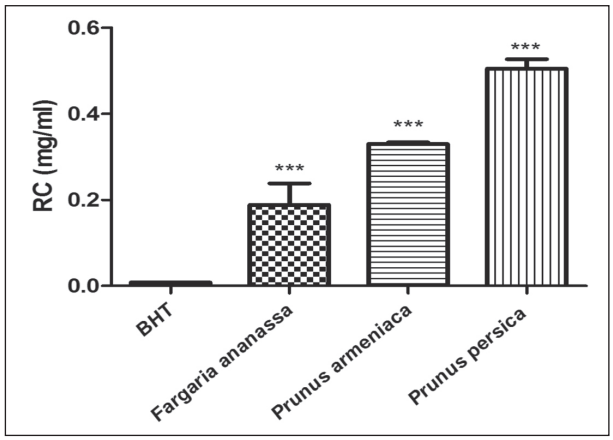


Fig 7. Reducing power of different fruits extract. Data were presented as RC_{50} means ±SD (n=3). (*** P < 0.001) compared to BHT as a standard

presented in Fig 5. *Prunus aremeniaca* had the highest ferrous ion chelating activity compared to *Fargaria*, and both extract had low activity than EDTA. EDTA is a strong metal chelator, hence, it is used as a standard metal chelator agent in this study. The low metal chelating activity was found for *Prunus persica* with EC_{50} value of 9.01 mg/ml.

Antioxidant activities of extracts using β-carotene-linoleate model system

The antioxidant activity of fruits extracts was also estimated by β-carotene bleaching assay. In β-carotene/linoleic acid model system, linoleic acid during incubation forms hydroperoxides free radicals (43). The antioxidant activity is high when the color of β-carotene does not change during the incubation period of 24 h. In the present study, the percentage inhibition of β-carotene bleaching by fruits extracts ranged from 55.22 to 80.72 %. The highest activity was found for

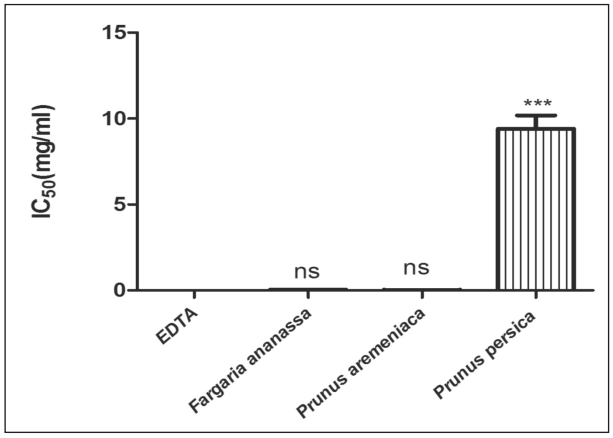


Fig 8. Metal chelating activity of different fruits extracts. Data were expressed as EC50 means ± SD (n = 3).(ns: no significant difference, *** p<0.001) compared to EDTA as a standard chelating agent.

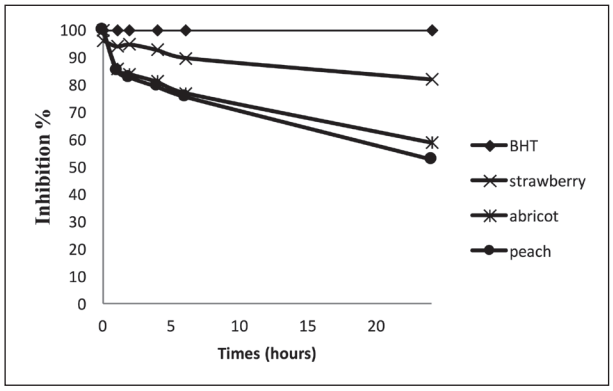


Fig 9. Changes in the percentage of the inhibition ratios of linoleic acid oxidation of different fruit extracts (2mg/ml) using β-carotene bleaching method, compared to BHT as a positive control during 24 h.

Fargaria (80.72 ± 4.57%) and the lowest activity was noticed for *Prunus* (55.22 ± 4.90 %). All extracts had lower activity compared to BHT.

Antioxidant activity of fruit extracts on linoleic acid peroxidation

The ferric thiocyanate method is used to measure the rate of peroxide formation in the first stages of lipid peroxidation (44). During linoleic-acid oxidation, the peroxide formed reacts and oxidize Fe^{2+} into Fe^{3+} to give red color (45). High absorbance demonstrated high concentration of peroxide during the incubation; which has a maximum absorbance at 500 nm. Low absorbance value indicates high level of antioxidant

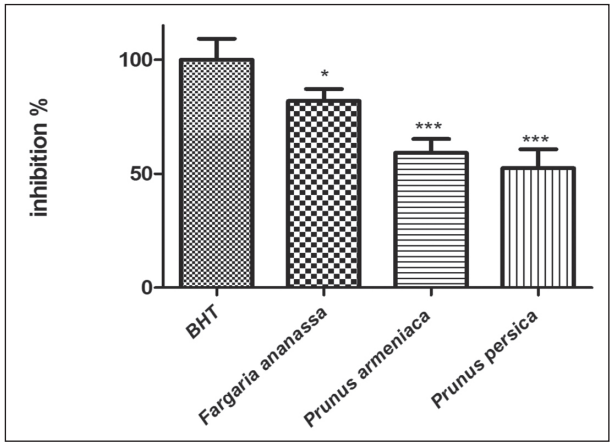


Fig.10. Antioxidant activities of different fruits extracts measured by β-carotene bleaching method (2 mg/ml at 24 h of incubation). BHT was used as standards antioxidant. Values are means ± SD (n = 3). (*p < 0.05, *** p < 0.001) compared to BHT as standard.

activity. Fig.11 shows the results of thiocyanate method. All fruit extracts have varying percentages of inhibition in the formation of peroxides compared with vit C, which is used as positive control. *Fargaria* and *Prunus* exhibited good peroxidation inhibiting activity, with 56.67 ± 3.34 % and 55.22 ± 2.86% respectively. While *Prunus armeniaca* presents the lowest ability to inhibit the formation of peroxide during 5 days with a value of 37.55 ± 4.055 %.

Thiobarbituric Acid (TBA) assay

FTC and TBA are the important methods used to measure the amount of peroxide radicals. TBA is used to indicate the amount of peroxide radicals in the second stage. Fig.11 showed the antioxidant activity of fruits extracts in TBA method. Results showed that *Fargaria* had high antioxidant effect than other fruits extracts. The inhibitions of the formation of Malonaldehyde were in the following order: vit C (62.19 ± 0.342%) >*Fargaria ananassa* (46.20± 3.89%) >*Prunus persica* (42.95 ±2.42%) > *Prunus armeniaca* (38.30 ± 2.22%) Fig 11.

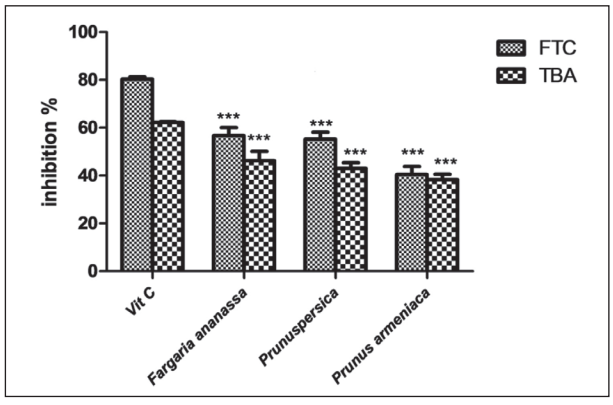


Fig 11. A comparison between total antioxidant activities of fruits extracts using the FTC and TBA methods. Vit C: Positive standards. Values are means ± SD (n=3)

Hydroxyl radical scavenging activity of fruit extracts

Hydroxyl radicals are the major active oxygen species in the biological systems; it reacts with fatty acids of cell membrane phospholipids (46, 47). The high concentration of hydroxyl induced damage to DNA, lipid, protein and produced carcinogenesis, mutagenesis and cytotoxicity (48, 49). The scavenging of hydroxyl is important to prevent cells from oxidative damage. The reaction of Fenton is the first way to produce $OH^{\cdot}$, in this reaction the transition of metal can degrade the hydrogen peroxide and generate hydroxyl radical. The hydroxyl scavenging ability of various fruit extract was measured using a system containing $FeSO_4$ and H_2O_2, this chemicals produced $OH^{\cdot}$, which hydroxylate salicylate . The Hydroxyl radical scavenging activity of methanol fruits extracts were determined and compared to vit C. The results of fruit extract scavenging activity were show in Fig.12. All extract can reduce the formation of hydroxyl radical with different IC_{50} values. *Fargaria* and *Prunus* present strong activity with IC_{50} values of (0.079 ± 0.031 and 0,089 ± 0.003 mg/ml) respectively,

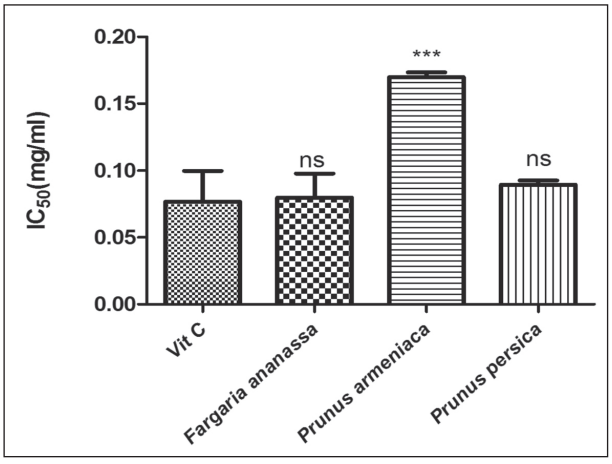


Fig 12. Hydroxyl radical scavenging activity of different fruits extracts. Data are presented as IC_{50} means ± SD (n = 3).(ns: no significant difference, *** p<0.001) compared to vitamin C as standard

but were significantly ($p< 0.05$) lower than vitamin C (IC_{50} = 0,076 ±0.007 mg/ml). *Prunus armeniaca* had an IC_{50} value of (0.16 ± 0.008 mg/ml) and possess good scavenging activity against $OH^{\cdot}$ radicals.

In vivo antioxidant activity of fruit extracts

Plasma antioxidant capacity using DPPH radical scavenging activity

DPPH free radical was used to evaluate the antioxidant activity after animal treatment. This method is based on hydrogen or electron donation and the ability of the extract to reduce the color of DPPH to yellow color. Fig.13 shows that fruit extracts can scavenge the DPPH radical. After the oral administration of 200 mg/kg of fruit extracts to rats , plasma antioxidant activity was lower than that of reference group (vit c) with the following order vit C 26.05 ± 1.28% >*Prunus armenaica* 22.91 ±1.99% >*Fargaria* 15.84 ± 0.94% >*Prunus persica* 10.78 ± 0.72%. For the dose of 600 mg/kg, *Prunus armeniaca* demonstrated high effect than Vit c with an inhibition value of 34.38 ± 1.82%.

Effect of fruits extracts on plasma reducing power in rats

Figure 14 shows the effect of extracts on plasma reducing activity. The results indicate that all extracts have good reducing power. At 200 mg/kg *Fargaria ananassa* and *Prunus armeniaca* have reducing capacity of 0.619 ± 0.14 and 0.625 ± 0.13 respectively approximate to the value of reference group (0.6 ± 0.10). *Prunus persica* show high activity with value of 0.865 ± 0.08. The oral administration of 600 mg/kg of fruit extract resulted in a high reducing capacity in the plasma, the high value was shown for *Prunus armeniaca* (1.044 ± 0.082).

MDA levels

In the present study, TBARS method was used to evaluate the levels of lipid peroxidation during the oxidative stress, Thiobarbituric acid reactive substances were produced and induced lipid peroxidation (50). In lipid peroxidation, MDA is one of the major aldehydes produced. Thus, it is considered as a good biomarker of oxidative damage. MDA reacts with proteins, phospholipids, nucleic acid and induced cell damage (51) and this damage induces various diseases associated with oxidative stress (52). Fig.15 show the capacity of fruit extracts to inhibit lipid peroxidation in the liver of rats and minimize the formation of MDA. The results were compared with reference group, which received Vit C (200 mg/kg). In this assay, the relation between

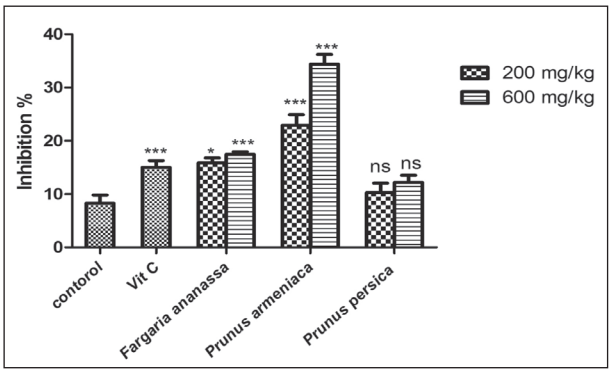


Fig.13. Effect of fruit extracts and vitamin C. on DPPH scavenging activity in plasma of rats. Values are given as means ± SEM (n=6). (ns: no significant difference; * $p < 0.05$; *** $p < 0.001$) compared to control group

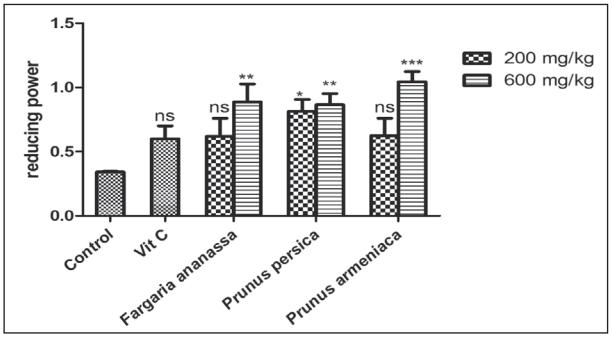


Fig.14. Effect of fruit extracts and vitamin C. on reducing activity in plasma of rats. Values are given as means ± SEM (n=6). (ns: no significant difference; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) compared to control group.

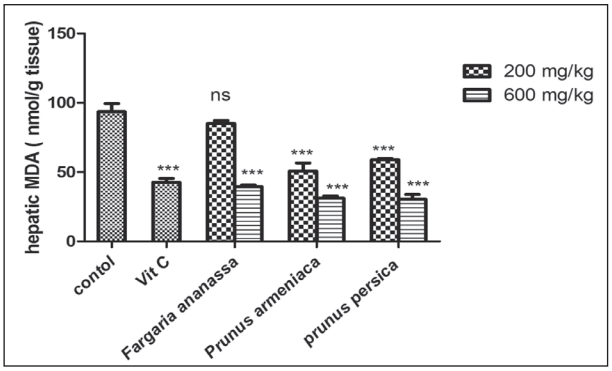


Fig 15. Effect of fruit extracts and vitamin C on MDA level in liver of rats. Values are given as means ± SEM (n=6). (ns: no significant difference; *** $p < 0.001$) compared to control group

the rate of MDA and the concentration of extract was noticed. Extracts of *Fargaria, Prunus and Prunus* at 200 mg/kg had a good effect with values of 85.07±2.06, 50±5.70 and 58.93±0.91 nnomol/g tissue respectively. However, the dose of (600 mg/kg) decreased the MDA levels and this decrease was statistically significant when compared to control group. Aydemir (53) reported that Vit C has antioxidant activity and protect cell membrane against damage. Vit C has strong activity with value of 42.51 ± 2.96 nmol/g tissue.

Effects of fruits extracts on GSH levels in liver homogenate

Reduced glutathione (GSH) is a linear tripeptide of L-glutamine, L-cysteine, and glycine. GSH is an extremely important cell protectant agent. GSH acts as reducing agent and is a vital substance in detoxification. It provides antioxidant protection in the aqueous phase of cellular systems. The central role of GSH in antioxidative defense is because it can regenerate another water-soluble antioxidant, ascorbic acid, via the ascorbate–glutathione cycle (54). Hence, depletion of intracellular GSH is usually regarded a measure of oxidative stress. In this test, the GSH reacts with DTNB in the dark and forms yellow complex. Fig.16 show GSH levels in the liver of rats treated different extracts. *Prunus persica and Prunus armeniaca* at 200 mg/kg and 600 mg/ kg did not change the levels of GSH in the liver. *Fargaria* treatment caused an increase in the levels of GSH at dose of 600 mg/kg with a value of 36.01 ± 2.51 µmol/g tissue.

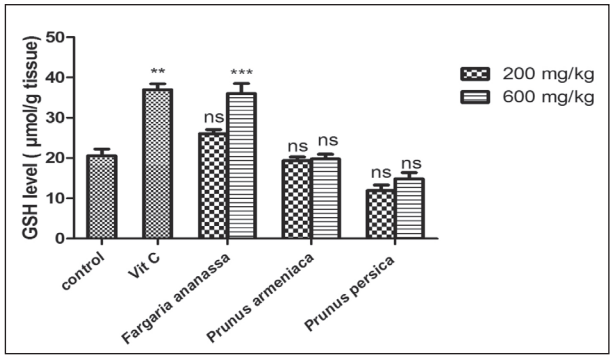


Fig 16. Effect of fruits extract and vitamin C on GSH level in the liver of rats. Values are given as means ± SEM (n=6). (ns: no significant difference; ** p < 0.01; *** p < 0.001) compared to control group.

Discussion

Fruits and vegetables contain phytochemicals with antioxidant activity. These antioxidants have multifunction. Their activity and mode of action in a particular test system may depend on the oxidation conditions, which may in turn affect both the kinetics of oxidation and the composition of the system .Therefore, a multi-dimensional assay protocol would be an advantage by reducing these limitations (55). The chemistry behind the antioxidant capacity assays has been reviewed by Huang (42). Polyphenolic compounds such as flavonoids, phenolic acids and tannins are considered the major contributors to the antioxidant activity of fruits and vegetables (36). The antioxidant activities of polyphenols were attributed to their redox properties, which allow them to act as reducing agents, hydrogen donators and free radicals quenchers, as well as their metal chelating abilities (36). Therefore, multiple methods were used in this study to evaluate the antioxidant properties of fruit extracts.

Various factors can change the fruit phenolic contents such as the variety, the stage of maturation, the area, the harvesting time and the part of the fruit as well as the types and quantity of phytochemicals (56, 57). During the second stage of maturation, the fruit have a high phenolic content, which might be associated with an amplified polyphenol oxidase activity (58). In addition, both genetic and agronomic or environmental factors play a role on the phytochemical composition and nutritional quality of the crops (59). Climate has an important role on quality, including the nutritional value of fruit and vegetables. Light intensity, temperature and water availability affect the antioxidant activity in different fruit and vegetables, and the deficit irrigation influences their polyphenol content (60, 61). Flavonoids have high antioxidant effect, which depends on the environment condition. Various factors may change the action of flavonoids and product alteration in their efficacy as antioxidant (62). In this study, fruits were originated from a semi-arid region of Sétif, Algeria with changing climate (wet and cold in winter and dry and hot in summer).

Tannins bind to and precipitate proteins and various other organic compounds including amino acids and alkaloids. This tannin protein complex can provide persistent antioxidant activity. The amount of tannin

can be depending in their chemical nature, the solvent used and the experimental condition (63).

Sugars are important food constituents and instant source of energy for the body. A high sugar level of a fruit also serves as an index of maturity. An overall view of the obtained data showed variations in sugar levels that might be due to genetic factors, responsible for differences in composition among different varieties (64). The sugars and protein have antioxidant effect (65).

Polyphenols and phenolic acids are also powerful antioxidants and demonstrated various health benefits by exhibiting antibacterial, antiviral, anticarcinogenic, anti-inflammatory and vasodilator actions (66). Several studies suggest that Catechin and rutin have good effects such as antioxidant, anti-ageing and may prevent cardiovascular complications. Their beneficial effects are attributed to their ability to reduce oxidative stress, lipid peroxidation, free radical generation and low density lipoprotein (LDL) cholesterol-oxidation (67, 68). Moreover, other phenolic compounds found in the extracts such as gallic acid also possess beneficial effects on human health and deceases oxidative stress (69). Various natural product containing phenolics and flavonoids have the ability to reduce molybdenum (35, 70, 71).

In the present study, DPPH scavenging activity of extracts is correlated with tannins, flavonoids and various phenolic compounds (37). Generally, the extract with high total phenolic contents had higher scaving-ing activity (18). It was reported that the extract of strawberries (Fargaria) had the highest total antioxidant activity compared with extracts of plums, orange, red grapes, kiwi fruit, pink grape fruit, white grapes, banana, apple, tomato and pears (8).

The scavenging effect of the ABTS+ radical by the extracts was found to be much higher than that of DPPH radical. Factors like stereo selectivity of the radicals or the solubility of the extract in different testing systems have been reported to affect the capacity of extracts to react and quench different radicals (72). Compounds which have ABTS+ scavenging activity did not show DPPH scavenging activity. This is not the case in this study.

In the reducing power, the transformation of Fe+3 to Fe+2 and changes of yellow color to green and blue of test solution depend on the concentration and the presence of reductants in the samples (73, 74, 75). In this study this activity was also related to the amount of polyphenols and flavonoids in the samples.

Ferrous ion has an important role in food systems (76). It is well known as an effective pro-oxidant. Transition metals, like ion can stimulate lipid peroxidation, the formation of free radical such as hydroxyl radical and accelerates lipid proxidation into alkoxyl radical (77). Chelating agent can reduce lipid peroxidation and inhibit the formation of free radicals by stabilizing the transition metals (72). The metal ion chelating activity was measured by the ability of some phenolic compounds to disrupt the formation of Ferrosine-Fe+2 complex (78, 79).

The absences of antioxidant induce discoloration of β-carotene because it will couple with linoleic acid and generates free radicals. The rates of β-carotene blanching can be slow down in the presence of antioxidant (80).Various studies demonstrated that the β-carotene bleaching activity is in relation to flavonoids and polyphenols compounds which can inhibit oxidation of linoleic acid and the formation of hydroperoxides (81, 82).

The inhibition of Self-oxygenation of unsaturated fatty acids is one of the mechanisms of antioxidant activity. Initiation of a peroxidation sequence in a cell membrane or polyunsaturated fatty acid is due to the abstraction of a hydrogen atom from the double bond in the fatty acid molecule. The free radical tends to be stabilized by a molecular rearrangement to produce a conjugated diene which then easily reacts with an oxygen molecule to give a peroxyl radical (83). Peroxyl radicals can abstract hydrogen from another molecule or they can abstract hydrogen to give lipid hydoperoxide R-OOH (84). These results indicate the relation between the amount of polyphenols and flavonoids and lipid peroxidation. The flavonoids can reduce or stop lipid peroxidation by the scavenging the peroxyl free radical. According to several studies, this activity is related to the number of hydroxyl groups present in the molecules of phenolics in the extracts.

When compared the FTC method and TBA the result of the present study indicate that the product of peroxide in the first stage is high compared to the second stage. In this second stage, the Malonaldehyde is a free radical product with high amount but is not stable for a period. Malonaldehyde has low molecular weight, it reacts with proteins, phospholipids, nucleic acid and induced cell damage (51).

Husain (85) reported that flavonoids such as myrcetin, quercetin and rhamnetin were OH scavengers. They also noted that the effectiveness of such compounds increases with increasing the number of hydroxyl groups attached to the aromatic B ring. As is the case for many other free radicals, OH can be neutralized if it is provided with a hydrogen atom. The phenolic compounds present in the crude extract had the ability to donate a hydrogen atom to OH. Strawberry extracts exhibited high enzymatic activity for oxygen detoxification and a high level of antioxidant capacity against free radical species including peroxyl radicals, superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen (86, 87).

Various studies reported the use of several assays for the determination of the antioxidant activity of fruit and vegetable extracts in human plasma after diet. These methods included ABTS, DPPH, reducing power and ORAC. These methods are based on hydrogen donation and other electron (42, 88). Other parameters can be used to determine the effect of crude fruit extracts against oxidative stress in vivo using animal models.

These results demonstrated that fruits have good plasma antioxidant activity using DPPH, reducing power. The plasma contains albumin, bilirubin, reduced glutathione and uric acid endogenous antioxidant and may be take exogenous antioxidants from food. Which can work complementary and synergistic with endogenous antioxidant to protect human health against ROS (89).

These results suggest that fruits extract exhibit free radical scavenging activity, which could exert a beneficial action against pathophysiological alterations, caused by the presence of superoxide and hydroxide radicals indicating the regeneration of damaged liver cells (90). This effect can be attributed to the antioxidant properties of polyphenols present in the extracts (91), because of their strong ability to scavenge free radicals and break the reaction chain of these radicals *in vitro* and *in vivo* (92). Several works with extracts of various plants have reported a reduction in the oxidative stress due to the presence of high antioxidants amount such as polyphenols. Vijayakumar (93), found these effects for black pepper and Gladine (94) reported these effects for rosemary, grape, citrus, and calendula; whereas, Papandreou (95) reported the same results for blue berries (Vaccinium angustifolium).Various studies suggest that polyphenols stimulate the gene expression of SOD, and GPx (96).

The present study showed that *Fargaria ananassa*, *Prunus armeniaca and Prunus persica* consumed by the population and produced in Sétif region, Algeria contain high amounts of polyphenols and flavonoids. The extracts of these fruits exert a good *in vitro* and *in vivo* antioxidant activity; bioactive compounds such as polyphenols are important contributors to the antioxidant activity of fruits. The treatment of animals with these extracts resulted in a reduction in the production of MDA in liver tissue of rats. It is concluded that *Fargaria ananassa*, *Prunus armeniaca and Prunus persica* are important source of phenolic acid and flavonoids and their consumption can reduce the risk of several diseases associated with oxidative stress such as cancer, diabetes, aging and cardiovascular diseases.

Acknowledgements

I acknowledge the University Ferhat Abass, Setif 1 for the financial support. This work was performed in the Laboratory of Phytotherapy Applied to Chronic Diseases, Department of Biology and Animal Physiology,, Faculty of Nature and Life Sciences, University Ferhat Abass Setif 1, Algeria.

Refreces

1. Meda NTR, Bangou MJ, Bakasso S, Millogo-Rasolodimby J, Nacoulma OG. Antioxidant activity of phenolic and flavonoid fractions of Cleome gynandra and Maerua angolensis of Burkina Faso. Journal of Applied Pharmaceutical Science, 2013; 3: 36-42.
2. Das Sarma A, Rahaman Mallick AR, Ghosh AK. Free Radicals and Their Role in Different Clinical Conditions: An Overview. International Journal of Pharma Sciences and Research (IJPSR), 2010; 1:185-192.
3. Duran-Bedolla J, Rodriguez MH, Navor VS, Arancibia SR, Cerbon M, Rodriguez MC. Oxidative stress: production in several processes and organelles during Plasmodium sp development. Oxidants and Antioxidants in Medical Science, 2013; 2: 93-100.
4. Ahmed D, Munim F, Saeed S. Phenolic and flavonoid contents and anti-oxidative potential of epicarp and mesocarp of Lagenaria sicerariafruit: a comparative study. Asian Pac. J. Trop. Med, 2014; 7: 249-255.
5. Singh JP, Kaur A, Singh N, Nim L, Shevkani K, Kaur H, Arora DS. In vitro antioxidant and antimicrobial properties of jambolan (Syzygium cumini) fruit polyphenols. LWT-Food Science and Technology, 2016; 65: 1025-1030.

6. Amin I,Tan, SH. Antioxidant activity of selected commercial seaweeds. Mal J Nutr, 2002; 8: 167–177.
7. Lee WY, Emmy Hainid KI, Abbe Maleyki MJ, Amin I. Antioxidantcapacity and phenolic content of selected commercially available cruciferous vegetables. Mal J Nutr, 2007; 13: 71– 80.
8. Laura A, Alvarez-Parrilla E, Gustavo A, Alez-Aguilar G. Fruit and Vegetable Phytochemicals. Chemistry, nutritional value and stability, 2010; Pp 3-36.
9. Campbell OE, Padilla-Zakour OI. Phenolic and carotenoid composition of canned peaches (Prunus persica) and apricots (Prunus armeniaca) as affected by variety and peeling, Food Research International, 2013; 54: 448–455.
10. Garrigaa M, Munoz CA, Caligari PDS, Retamales JB. Effect of salt stress on genotypes of commercial (Fragaria x ananassa) and Chilean strawberry (F. chiloensis). Scientia Horticulturae, 2015; 195: 37-47.
11. Markham KR. Techniques of Flavonoid Identification (Chap.1 and 2). London: Academic Press, 1982; Pp.1- 113.
12. Li HB, Cheng KWC, Fan KWF, Jiang Y. Evaluation of antioxidant capacity and total phenolic content of different fraction of selected microalgae.Food chem,2007; 102: 771-776.
13. Bahorun T, Gressier B, Trotin F, Brunete C, Dine T, Vasseur J, Luyckx M, Cazin M, Pinkas M. Oxygen species scavenging activity of phenolic extract from Hawthorn fresh plant organs and pharmaceutical preparations .Arzneim Forsch/Drug Res, 1996; 46: 1086-1089.
14. Gharzouli K, Khennouf S, Amira S, Gharzouli A. Effects of aqueous extracts from Quercus ilex L. root bark, Punica granatum L. fruit peel and Artemisia herba-alba Asso leaves on ethanol-induced gastric damage in rats. Phytother Res, 1999; 13: 42-45.
15. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem, 1976; 7: 248-54.
16. Dubois M, Gilis K, Hamilton J, Rebers P, Smith F. Colorimetric method for determination of sugars and related substances. Anal chem, 1956; 28: 350-356.
17. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal Biochem, 1999; 269: 337-341.
18. Yardpiroon B, Aphidech S, Prasong S. Phytochemical and Biological Activities of the Wild Grape Fruit Extracts Using Different Solvents. British J of Pharmaceutical Res, 2014; 4: 23-36.
19. Re R. Antioxidant activity applying an improved ABTS radical cation decolorization assays. Free Radic Biol Med 1999; 26: 1231–1237.
20. Ebrahimzadeh MA, Nabavi SF, Nabavi SM, Eslami B, Asgarirad H. In vitro antioxidant and free radical scavenging activity of Leonurus cardiaca subsp. Persicus, Grammosciadium platycarpum and Onosma demawendicum. African J Biotechnol, 2010; 9: 8865-8871.
21. Decker EA, Welch B. Role of ferritin as lipid oxidation catalyst in muscle food. J Agr Food Chem, 1990; 36: 674-677.
22. Gursoy N, Tepe B, Akpulat AH. Chemical Composition and Antioxidant Activity of the Essential Oils of Salvia palaestina (Bentham) and S. ceratophylla (L). J Records of Natural Product, 2012; 6: 278-287.
23. Yen GC, Chang YC, Su SW. Antioxidant activity and active compounds of rice koji fermented with Aspergillus Candidus. Food Chem, 2003; 83: 49–54.
24. Kikuzaki H, Nakatani N. Antioxidant effects of some ginger constituents. J Food Sci, 1993; 58: 1407–1410.
25. Smirnoff N, Cumbes QJ. Hydroxyl radical scavenging activity of compatible solutes. J Phytochemrstry, 1989; 28: 1057 -1060.
26. Burits M, Bucar F. Antioxidant activity of Nigella sativa essential oil. Phytother Res, 2000; 14: 323–328.
27. Chung YC, Chen SJ, Hsu CK, Chang CT, Chou ST. Studies on the antioxidative activity of Graptopetalum paraguayense. Food Chem, 2005; 91: 419–424.
28. Ellman G. Tissue sulfhydryl groups. Arch biochem biophis, 1959; 82: 70-77.
29. Okhawa H, Ohishi N, Yagi K. Assay of lipid peroxides in animal tissues by thiobarbituric acid. Anal Chem 1979; 95: 351–358.
30. Oomah BD, Mazza G. Functional Foods. In: The Wiley Encyclopedia of Science & Technology, 2nd edition, V2 (edited by F.J. Francis). 2000; Pp. 1176- 1182. NewYork, NY: Wiley
31. Kaur Ch, Kapoor HC. Antioxidants in fruits and vegetables - the millennium's health. J food sci and technology, 2001; 36: 703- 725.
32. Kumar S, Pandey AK. Chemistry and Biological Activities of Flavonoids: An Overview. Scientific World Journal, 2013: 1-16.
33. Uddin R, Saha MR, Subhan N, Hossain H, Jahan I A, Akter R, Alam A. HPLC-analysis of polyphenolic compounds in gardenia jasminoidesand determination of antioxidant activity by using free radical scavenging assays. Advanced Pharmaceutical Bulletin, 2014; 4: 273-281.
34. Aliyu AB, Ibrahim MA, Ibrahim H, Musa AM, Lawal AY, Oshanimi JA, Usman M, Abdulkadir IE, Oyewale AO, Amupitan JO. Free radical scavenging and total antioxidant capacity of methanol extract of Ethulia Conyzoides growing in Nigeria. Romanian Biotechnological Letters, 2012; 17: 7458-7465.
35. Ravisankar N, Sivaraj CH, Seeni S, Joseph J, Raaman N. Antioxidant activites and phytochemical analysis ofmethanol extract of leaves of hypericum hookerianum. Int J of Pharmacy and Pharmaceutical Sci, 2014; 6: 0975-1491.
36. Vladimir-Kneževi S, Biljana Blažekovi B, Štefan MB, Alegro A, K szegi, T, Petrik J. Antioxidant Activities and Polyphenolic Contents of Three selected Micromeria Species from Croatia. J molecules, 2011; 16: 1454-1470.
37. Garg D, Muley A, Khare N, Marar Th. Comparative Analysis of Phytochemical Profile and Antioxidant Activity of Some Indian Culinary Herbs. Res J of Pharmaceutical, Biological and Chem Sci, 2012; 3: 845-854.
38. Popovici C, Saykova I, Tylkowski B. Evaluation de l'activité antioxydant des composés phénoliques par la réactivité avec le radical libre DPPH. Génie Industriel, 2009; 4: 25-39.
39. Ebrahimzadeh MA, Nabavi SF, and Nabavi SM. Antihemolytic and antioxydant activity of Hibiscus esculentusleaves.

Pharmacologyonline, 2009; 2: 1097-1105.
40. Sivakumar ChV, Meera I. Antioxidant and Biological Activities of Three Morphotypes of Murraya koenigii L.from Uttarakhand. Food Processing & Technology, 2013; 4: 2-7.
41. Le K, Chiu F, Ken NG. Identification and quantification of antioxidants in Fructus lycii. Food chem, 2007; 105: 353-363.
42. Huang D, Ou B, Prior RL. The chemistry behind antioxidant capacity assays. J of Agricultural Food Chem, 2005; 53: 1841-1856.
43. Goze I, Alim A, Tepe AS, Sokmen M, Sevgi K Tepe B. Screening of the antioxidant activity of essential oil and various extracts of Origanum rotundifolium Boiss. From Turkey. Journal of Medicinal Plants Research, 2009; 3: 246-254.
44. Ting HCh, Hsu WY, Tsai ChF, Lu FJ, Ming-Chih MCh, Chen WK. Thein vitro andin vivo antioxidant properties of seabuckthorn (Hippophae rhamnoides L.) seed oil. Food chem, 2001; 125: 652-659.
45. Yu L, Zhao M, Wang JSh, Cui Ch, Yang B, Jiang Y, Zhao Q. Antioxidant, immunomodulatory and anti-breast cancer activities of phenolic extract from pine (Pinus massoniana Lamb) bark. Innovative Food Science and Emerging Technologies, 2008; 9: 122–128.
46. Padmanabhan P, Jangle SN. In-vitro antioxidant potential of an herbal preparation containing four selected medicinal plants. Journal of Krishna Institute of Medical Sciences University, 2012; 1: 2231-4261.
47. Archana CM, Showmya J, Harini K, Pradeepa M, Geetha N. Radical scavenging and antioxidant activity of methanolic leaf extract of Chromolaena odorata (l) king and robinson by in vitro assays. International Journal of Pharmaceutical Sciences and Business Management, 2015; 3: 1-12.
48. Saeed N, Khan MR, Shabbir M. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Torilis leptophylla L. BMC Complementary and Alternative Medicine, 2012; 12: 2-12.
49. Rahman MdM, Badrul Islam MdB, Biswas M, Khurshid Alam AHM. In vitro antioxidant and free radical scavenging activity of different parts of Tabebuia pallidagrowing in Bangladesh. BMC Res Notes, 2015; 8: 2-9.
50. Behuliak M, Palfy R, Gardlik R, Hodosy J, Halcak L, Celec P. Variability of thiobarbituric acid reacting substances in saliva. Disease Markers, 2009; 26: 49-53.
51. Pandey KB, Rizvi SI. Markers of oxidative stress in erythrocytes and plasma during aging in humans. Oxidative Medicine and Cellular Longevity, 2010; 3: 2-12.
52. Mateos R, Lecumberri E, Ramos S, Goya L, Bravo L. Determination of malondialdehyde (MDA) by high-performance liquid chromatography in serum and liver as a biomarker for oxidative stress: Application to a rat model for hypercholesterolemia and evaluation of the effect of diets rich in phenolic antioxidants from fruits. Journal of Chromatography, 2005; 827: 76-82.
53. Aydemir T, Ozturk R, Bozkaya LA, Tarhan L. Effects of antioxidant vitamins A, C, E and trace elements Cu, Se on Cu Zn SOD, GSH-Px, CAT and LPO levels in chicken erythrocytes. Cell Biochemistry and Clinical Function, 2000; 18: 109-115.
54. Valko M, Leibfritz D Moncol J, Cronin MTD, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell Biol, 2007; 39: 44–84.
55. Lako J, Trenerry VC Rochfort S (2008) Routine analytical methods for use in South Pacific regional laboratories for determining naturally occurring antioxidants in food. International Food Research Journal 15: 313-323
56. Hind AB, Abu-Bakr A, Abu G. Compositional changes during guava fruit ripening. Food Chemistry, 2003; 80: 557-563.
57. Liu H, Cao J, Jiang W. Evaluation and comparison of vitamin C, phenolic compounds, antioxidant properties and metal chelating activity of pulp and peel from selected peach cultivars. J Food Science and Technology, 2015; 1-7.
58. Parr AJ, Bolwell GP. Phenols in the plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. Journal of the Science of Food and Agriculture, 2000; 80: 985–1012.
59. Chuang Ch, Mcintosh M. Potential Mechanisms by Which Polyphenol Rich Grapes Prevent Obesity-Mediated Inflammation and Metabolic Diseases. Annu Rev Nutr, 2011; 31: 155–176.
60. Dragovic-Uzelac V, Levaj B, Mrkic V, Bursac D, Boras M. The content of polyphenols and cartenoids in three apricot cultivars depending on stage of maturity and geographical. Food chem, 2007; 102: 966-975.
61. Pék Z, Szuvandzseiv P, Daood H, Neményi A, Helyes L. Effect of irrigation on yield parameters and antioxidant profiles of processing cherry tomato.Cent Eur J Biol, 2014; 9: 383-395.
62. Chen HY, Yen GCh. Antioxidant activity and free radical-scavenging capacity of extracts from guava (Psidium guajava L) leaves. Food Chem, 2007; 10: 686-694.
63. Mahmoudi S, Khali M, Mahmoudi N. Etude de l'extraction des composés phénoliques de différentes parties de la fleur d'artichaut (Cynara scolymus L). Nature and Technologie, 2012; 09: 35 -40.
64. Ali S, Masud T, Abbasi KS. Physico-chemical characteristics of apricot (Prunus armeniacaL.) grown in Northern Areas of Pakistan. Scientia Horticulturae, 2011; 130: 386-392.
65. Wong SP, Leong LP, William Koh JH. Antioxidant activities of aqueous extracts of selected plants. Food Chem, 2006; 99: 775-783.
66. Duthie GG, Duthie SJ, Kyle JAM. plant polyphenols in cancer and heart disease: implications as nutritional antioxidants. Nutr Res Rev, 2000; 13: 79-106.
67. Augustyniak A, Bartosz G, Cipak A, Duburs G, Horakova L, Luczaj W, Majekova M, Odysseos AD, Rackova L, Skrzydlewska E, Stefek M, Strosova M, Tirzitis G, Venskutonis PR, Viskupicoca J, Vraka PS, Zarkovic N. Natural and synthetic antioxidants: an updated overview. Free Radic Res, 2010; 44: 1216-62.
68. Ghasemzadeh A, Jaafar HZE Rahmat A. Antioxidant activities, total phenolics and flavonoidscontent in two varieties of Malaysia young ginger (Zingiber officinale Roscoe). Molecules 2010; 15: 4324-4333.
69. Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants

in human health and disease. Oxid Med Cell Longev, 2009; 2: 270-8.
70. Ahmed D, Khan MM, Saeed R. Comparative analysis of phenolics, flavonoids, and antioxidant and antibacterial potential of methanolic, hexanic and aqueous extracts from Adiantum caudatum leaves. Antioxidant, 2015; 4: 394-409.
71. Gupta M, Karmakar N, Sasmal S. in vitro antioxidant activity of aqueous and alcoholic extracts of polyherbal formulation onsisting of Ficus glomerata Roxb and Symplocos racemosa Roxb. Stem Bark Assessed in Free Radical Scavenging Assays. Int J of Pharmacognosy and Phytochemical Res, 2017; 9: 181-189.
72. Yu L, Haley S, Perret J, Harris M, Wilson J, Qian M (2002) Free radical scavenging properties of wheat extracts. J. Agric. Food Chem, 2002; 50: 1619–1624.
73. Chung,YC, Chang, CT, Chao WW, Lin CF, Chou ST. Antioxidative activity and safety of the 50% ethanolic extract from red bean fermented by Bacillus subtilis IMR–NK1. J Agr Food Chem, 2002; 50: 2454–2458.
74. Gülçin I, Oktay M, Kireçci E, Küfrevioglu ÖI. Screening of antioxidant and antimicrobial activities of anise (Pimpinella anisum L.) seed extracts. Food Chem, 2003; 83: 371–382.
75. Gülçin Ï. Antioxidant and antiradical activities of L-carnitine. Life Sci, 2006; 78: 803–81.
76. Al-Hashimi AG. Antioxidant and antibacterial activities of Hibiscus sabdariffa L. extracts. African J of Food Sci, 2012; 6: 506-511.
77. Özkan A, Yumrutaş Ö, Saygideğer SD, Kulak M. (2011) Evaluation of Antioxidant Activities and Phenolic Contents of Some Edible and Medicinal Plants from Turkey's Flora. Advances in Environmental Biology, 2011; 5: 231-236.
78. Norshazila S, Syed Zahir I, Suleiman KM, Aisyah MR, Kamarul RK. Antioxidant Levels and Activities of Selected Seeds of Malaysian Tropical Fruits. Mal J Nutr, 2010; 16: 149-159.
79. Yumrutas O, Saygideger SSD. Determination of antioxidant and antimutagenic activities ofPhlomis armeniacaandMentha pulegium. J of Applied Pharmaceutical Science, 2012; 02: 36-40.
80. Kulisic T, Radoni A, Katalinic V, Milos M. Use of different methods for testing antioxidative activity of oregano essential oil. Food Chem, 2004; 85: 633–640
81. Duh PD, Yen GC. Antioxidative activity of three herbal water extracts. Food Chem, 1997; 60: 639–45.
82. Sun T, Ho CT. Antioxidant activities of buckwheat extracts. Food Chemistry, 2005; 90:743-9.
83. Gorden MH. The mechanism of antioxidant action in vitro. Food Antioxidant, 1990; Pp1-8.
84. Anusuya N, Anusaya S, Manian R, Siddhuraju P, Manian S. Antioxidant and free radical scavenging activity of certain dietary and medicinal plant extracts. Food global science books, 2009; 3: 47-52.
85. Husain SR, Cillard J, Cillard P. Hydroxyl radical scavenging activity of flavonoids. Phytochemistry, 1987; 26: 2489-2491.
86. Wang SY, Lin KS. Antioxidant activity in fruits and leaves of blackberry, raspberry and strawberry varies with cultivar and developmental stage. Journal of agricultural and food chemistry, 2000; 48: 140-146.
87. Wang SY, Jiao H. Scavenging capacity of berry crops on superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen. J of Agricultural and Food Chem, 2000; 48: 5677–5684.
88. Katalinic V, Modun D, Music TI, Boban M. Gender differences in antioxidant capacity of rat tissues determined by 2,2V-azinobis (3-ethylbenzothiazoline 6-sulfonate; ABTS) and ferric reducing antioxidant power (FRAP) assays. Comp Biochem Physiol, 2005; 140: 47-52.
89. Meziti A, Meziti H, Boudiaf K, Benboubetra M, Bouriche H. Polyphenolic Profile and antioxidant Activities of Nigella Sativa Seed Extracts in vitro and in vivo. World Academy of Science, Engineering and Technology, 2012; 6: 04-24.
90. Celep E, Ahmet Aydin A, Kirmızıbekmez H, Yesilada E. Appraisal ofin vitroandin vivo antioxidant activity potential of Cornelian cherry leaves. Food and Chemical Toxicology, 2013; 62: 448–455.
91. Vinson J A Teufel K, Wu N. Red wine, dealcoholized red wine, and especially grape juice, inhibit atherosclerosis in a hamster model. Atherosclerosis, 2001;1567-72.
92. Liu Q, Yao H. Antioxidant activities of barley seeds extracts. Food Chemistry, 2007; 102: 732-737.
93. Vijayakumar RS, Surya D. Nalini N. Antioxidant efficacy of black pepper (Piper nigrum L.) and piperine in rats with high fat diet induced oxidative stress. Redox Report, 2004; 9: 105-110.
94. Gladine C, Morand Ch, Rock E, Guffat D, Bouchart D, Durand D. The antioxidative effect of plant extracts rich in polyphenols differs between liver and muscle tissues in rats fed n-3 PUFA rich diets. Animal Feed Science and Technology, 2007; 139: 257-272.
95. Papandreou MA, Dimakopoulou A, Linardaki ZI Cordopatis P Klimis-Zacas D Margarity M, Lamari FN. Effect of a polyphenol-rich wild blueberry extract on cognitive performance of mice, brain antioxidant markers and acetylcholinesterase activity. Behavioural Brain Research, 2009;198: 352-358.
96. Sánchez-Reus MI, Del Rio MAG, Iglesias I, Elorza M, Slowing K, Benedí J. Standardized Hypericum perforatum reduces oxidative stress and increases gene expression of antioxidant enzymes on rotenone-exposed rats. Neuropharmacol, 2007; 52: 606-516.

Correspondence:
Saoudi Soulef
Laboratory of Phytotherapy Applied to Chronic Diseases,
Faculty of Nature and Life Sciences,
Univ Setif 1, 19000, Algeria
E-mail: Soulef2016bio@gmail.com

Progress in Nutrition 2020; Vol. 22, N. 1: 253-258 DOI: 10.23751/pn.v22i1.7758

Essential oil and fatty acid composition of *Melissa officinalis* L.

Mouna Souihi[1,2], *Ismail Amri*[3], *Amir Souissi*[1], *Karim Hosni*[4], *Nadia Ben Brahim*[1], *Mohamed Annabi*[1]

[1]National Institute for Agronomic Research in Tunisia (INRAT), Carthage University, Agronomic Sciences and Technics Laboratory (LR16INRAT05), Hedi Karray Street, 2049, Ariana, Tunisia - E-mail:mannabi@gmail.com; [2]Faculty of Mathematical, Physical and Natural Sciences of Tunis, [3]National Centre for Nuclear Science and Technology (CNSTN), Sidi Thabet Technopark, 2020 Ariana, Tunisia; [4]National Institute for Research and Physicochemical Analysis, Sidi Thabet, 2020 Ariana, Tunisia

Summary. The leaf material of lemon balm (*Melissa officinalis L.*) from different origins was evaluated for their chemical composition under the semi arid conditions of Tunisia. Qualitative and quantitative variations of the chemical composition of essential oils according to the origin were shown. The main compounds of Tunisian samples were germacrene-D (29.17-24.6%) and caryophyllene (14.91-13.44%) in Tabarka and Nefza, the samples were characterized by the absence of citral and citronellal. Fatty acids from cultivated *M. officinalis* leaves were analyzed by GC–MS. The major fatty acids of all studied samples were polyunsaturated fatty acids, linoleic acid (73.93-66.74%), versus (16.25-13.32%) saturated ones, oleic acid and (6.29-4.26%) of monounsaturated fatty acid palmitic acid.

Key words: *Melissa officinalis* l., essential oil, fatty acids, tunisia

Introduction

Melissa officinalis L. (Lamiaceae) is a medicinal plant native to Southern Europe and Northern Africa (1). Due to several applications in pharmaceutical, food and hygiene industries, *M. officinalis* has been one of the most important commercial plants in recent decades (2). The plant germinate naturally in sandy and scrubby areas (3) but has also been reported to grow on damp wastelands, at heights ranging from sea level to the mountains (4).

There are three subspecies of *M. officinalis*: subsp. *officinalis* L, subsp. *inodora* (Bornm) and subsp. *altissima* (Sibth. & Sm.) Arcangeli or *M. romana* Miller (5). However, only subsp. *officinalis* has commercial value and the characteristic lemony odour of lemon balm (6).

M. officinalis has been credited with many medicinal attributes such as tonic, antispasmodic carminative, diaphoretic, antidepressant, antispasmodic, antibacterial, hypoallergenic (7, 8).

Essential oil yield ranged from 0.03 to 0.47% (9). This is quite low compared to other members of the Lamiaceae family and is considered to be the reason for the high production cost and price of essential oil in the market. Moradkhani, Sargsyan (10); Usai, Atzei (5) reported that the main constituents of the essential oil are citral (geranial and neral), citronellal, β-pinene, α-pinene, β-caryophyllene, totalizing 96% of the oil ingredients.

The essential oil of *M. officinalis* is a prominent antimicrobial agent against food-borne pathogens and food spoilage bacteria (11). It is currently used in medicine and pharmacology (anti-tumor, anti-bacterial, antimicrobial, antihistaminic, antispasmodic and antioxidant, by means of its antiviral effect curing of herpes (12, 13). It was also reported that *M. officinalis* contains substances that inhibit protein biosynthesis in cancer cells (14, 15) antiulcerogenic, moderate Alzheimer's disease, modulation of mood and cognitive performance, and stimulation of the immune system (against anti-HIV-1) (16). Unsaturated fatty acids (UFA) function

as major nutrients, constituents of cell membranes and precursors of various signal molecules, and they are important in both the medical and, as they are involved in the human inflammatory response, blood-pressure regulation, cholesterol metabolism and brain development (17, 18). Epidemiologic prospective cohort studies have suggested that replacing saturated fatty acids with carbohydrates is modestly associated with a higher risk of ischemic heart disease, whereas replacing SFAs with polyunsaturated fatty acids is associated with a lower risk of ischemic heart disease (19).

Due to the continental and geographical conditions, Tunisia is a suitable location for the growth of many medicinal plants, which are genetically valuable resources in fundamental and applied research in plant breeding. However *M. officinalis* has been cultivated in some European and Balkan countries, but not in Tunisia. Its oil is sometimes adulterated with *Cymbopogon* spp.

The Tunisian *M. officinalis* has received little attention, and previous studies have been focused mainly on other species from Lamiaceae family, *M. officinalis* vanished from many parts of Tunisia (20), but some still preserved in two locations in the North West of the country: Tabarka and Nefza (21). Therefore, an investigation of this genetic resource was needed for the assessment of its biochemical composition. The objective of the present study is to determine the chemical composition of the essential oil and fatty acid of *M officinalis* cultivated in Tunisia.

Material and methods

Plant Material

The plant material was botanically characterized by Dr Ben Brahim N. (Laboratory of science and agricultural techniques, National Agricultural Research Institute of Tunisia (INRAT) according to the morphological descriptions in the Tunisian Flora (20). Tunisian seeds were harvested from sites found in northern Tunisia (Tabarka and Nefza). Seeds of French *M. officinalis* were provided by National Institute for Agricultural Research, and the German seeds were provided by the Institute for Food and Resource Economics (ILR) University of Bonn.

Isolation of essential oil

The leaves were air-dried (for 30 days) at room temperature in a shadowy place, protected from direct light. Each sample was powdered and mixed to ensure sample uniformity. The essential oils were obtained from 100 g (dry weight) of plant material using a Clevenger-type Apparatus for 3 h. The hydrodistillation was performed in three replicates, and the oils were stored at 4°C until analysis by GC/MS. The average oil yields were estimated on the basis of the dry weight of the plant material.

GC/MS analysis

The oils were analyzed with a Hewlett-Packard 6890N/5975B inert GC-MSD system (Agilent, USA) equipped with two cap. Columns, a HP-INNOWAX (30 m×0.25 mm i.d., film thickness 0.25 μm) and an HP-5MS (30 m×0.25 mm i.d., film thickness 0.25 μm) column (J&W Scientific, USA). The oven temperature was programmed isothermal at 50°C for 1 min, then rising from 50 to 250 °C at 28/min, and finally held isothermal at 250°C for 15 min; injector temperature, 250 °C; ion source temperature, 230 °C; carrier gas, He (high purity 99.99%; 1.2 ml min^{-1}); injection volume, 1 μl; split ratio, 100:1. The electron impact ionization mode was used with an ionization voltage of 70 eV. Total ion chromatograms were obtained over the scan range of 30-800 a.m.u in the full-scan acquisition mode, and the compounds were identified using the NIST05 and Wiley 7 databases with a resemblance percentage above 85%. Semi-quantitative data were calculated from the GC peak areas without using correction factors and were expressed as a relative percentage (peak area %) of the total volatile constituents identified. Retention indices (RI) were determined for all the detected compounds based on the retention times (tr) of a homologous series of n- alkanes (C8-C30) (22).

Fatty acid methyl ester preparation

Triplicate samples of 1 g of *M. officinalis* leaves were subjected to lipid extraction using a modified version of the Bligh and Dyer (23) method. Thus, leaf samples were kept in boiling water for 5 minutes then ground manually using a mortar and pestle; chloroform/methanol mixture (2:1 v/v) was used for lipid extraction. After washing by fixation water, the organic layer containing lipids was recovered and dried under a nitrogen stream. Total fatty acids (TFAs) of total lipids were

transmethylated using sodium methoxide solution (3% in methanol) (24). Methyl heptadecanoate (C17:0) was used as an internal standard. The fatty acids methyl esters (FAMEs) obtained were subjected to GC analyses.

Results

Essential oil

The *M. officinalis* samples cultivated under the climatic conditions of the INRAT yielded a small amount of essential oil. The oils were analyzed by GC/MS. Forty-two compounds were identified, representing about (86.11%, 83.1%, 96.72% and 71.83%) of the total oils obtained from Nefza, Tabarka, Germany and France respectively. In addition to the differences in the essential oil yield, the GC/MS analyses revealed qualitative and quantitative differences in the composition between the oils of the four origins (Table1).

GC/MS analysis showed that the oils of the German population were characterized by the presence of a significant aldehyde fraction (39.31; 27.71%) with geranial and neral being the dominant components, together with the sesquiterpene (12.23%) β-caryophyllene. The sesquiterpene caryophyllene oxide (27.06%) was found to have the highest value in the French population, which exhibited lower levels (7.12-4.29% respectively) geranial and neral. Germacrene-D (32.08-27.06%) was the highest in the Tunisian samples Tabarka and Nefza, together with the sesquiterpene caryophyllene (16.4- 14.7% respectively).

Fatty acids

The total fatty acids (TFAs) extracted from the French, German, Tabarka and Nefza populations account for 95.38, 98.91, 94.65, 88.02 mg/g DW, respectively (Table 2).

Leaves of the German, Tabarka and Nefza populations have the same FA composition. Linoleic acid is the major compound reaching over (74.08, 70.75, and 66.74% respectively) of TFA followed by palmitic acid (15.77, 15.82, and 13.32% resp), C18:1n-9 (oleic acid) (6.29, 5.89 and 4.26 % resp) and C20:0 and (arachidic acid) (1.06, 1.19 and 1.31% resp). The main FA of the French samples was C18:2n-6 linoleic acid (73.93%), C16:0 (palmitic acid) (16.25%), C18:1n-9 (oleic acid) (4.62%) C20:0 (arachidic acid) (1.60%); and the C16:1 (palmitoleic acid) was not detected. *Melissa officinalis* leaves were characterized by a high proportion of polyunsaturated fatty acid (PUFA) linoleic acid (73.93-66.74%) versus (16.25-13.32%) of saturated ones (SFA) oleic acid and (6.29-4.26%) of monounsaturated (MUFA) palmitic acid (Table 2). To the best of our knowledge, the foliar fatty acid composition of *M. officinalis* is reported herein for the first time. The proportion of the fatty acids did not show any differences according to the origin of samples.

Discussion

Essential oil

All the sampled populations of *Melissa officinalis* yielded a small content of essential oil. These results were similar to those found with Iranian *M. officinalis* which produced 0.06-0.16% (2). However, this yield was lower for *M. officinalis* grown in other countries such as Turkey, for example, the total essential oil content ranged between 0.27-0.36%, (25). In Spain, the yield was 0.5% as reported (26).

In previously investigated oils from cultivated *M. officinalis*, the major components are aldehydes such as citronellal, neral and geranial, and the sesquiterpenes such as (*E*)-caryophyllene and caryophyllene oxide were also important compounds (27). The Iranian oils presented the citral (geranial and neral), citronellal and geraniol as the main components of *M. officinalis* (10). Algerian populations showed that the most dominant constituents obtained were citral, citronellal and caryophyllene oxide (28). The main components of oil from Turkey, were citronellal (39%), citral (33%), citronellol, linalool and geraniol (29). Those results are similar to our findings for the German and the French samples. Essential oil content shows a strong dependence genetic constitution of the different origins (30).

As it is known in the literature, the essential oil of *M. officinalis* subsp. *officinalis* contains significant amounts of citral and/or citronellal, whereas for the *M. officinalis* subsp. *altissima* a strong belong to chemotype germacrene D.(30)

It is noteworthy that the main components of the leaf oils of cultivated *M. officinalis* subsp. *altissima* from Greek as α-caryophyllene (7.27-12.66%), ger-

Table 1. Comparison of the essential oils isolated from different *M. officinalis*.

N°	Components		Content (%)			
		RI	Nefza	Tabarka	Germany	France
1	Camphene	954	-	-	-	1.29
2	£-3-carene	1011	0.32	-	-	-
3	(*Z*)-α-Ocimene	1026	-	0.5	-	-
4	Citronellol	1229	-	-	1.88	-
5	Neral	1240	-	-	27.71	4.29
6	GeraniaL	1267	-	-	39.31	7.12
7	Thymol	1290	-	-	0.4	-
8	α-ylangene	1372	0.42	-	-	-
9	α-Copaene	1376	0.72	0.54	-	-
10	Geranyl acetate	1381	-	-	1.42	-
11	Dehydro-ar-ionene	1389	0.84	-	-	-
12	*(E)*-α-Bergamotene	1412	0.55	-	-	1.24
13	(*E*)- Caryophyllene	1419	1.36	1.25	-	1.06
14	β-Caryophyllene	1420	14.7	16.4	12.23	8.92
15	α-cedrene	1432	0.52	0.27	-	-
16	Alloaromadendrene	1439	-	0.59	-	-
18	Aromadendrene	1441	0.3	-	-	-
19	α-Cubebene	1475	1.45	1.34	1.23	0.42
20	Germacrene D	1468	27.06	32.08	1.67	2.0
21	Bicyclogermacrene	1495	0.18	-	-	-
22	*Cis*-Calamenene	1521	0.75	-	-	-
23	β-sesquiphellandrene	1522	2.75	0.4	-	0.97
24	delta-Cadinene	1523	0.73	-	-	-
25	α -Cadinene	1524	0.34	-	-	-
26	gamma-Cadinene	1538	4.96	-	0.76	1.77
27	α-Calacorene	1542	0.71	1.23	-	0.76
28	Nerolidol	1559	0.9	-	-	-
29	Globulol	1568	0.42	-	-	-
30	Caryophyllenol	1572	0.91	1.47	0.5	2.23
31	Germacrene D-4-ol	1574	0.72	-	-	-
32	Caryophyllene oxide	1576	9.54	16.61	8.76	27.06
33	Spathulenol	1578	0.49	-	-	-
34	Humulene oxide II	1606	0.56	1.01	0.26	1.29
35	α-Cadinol	1654	4.61	3.24	-	5.64
36	t-Muurolol	1627	-	-	0.59	-
37	*iso* Aromadendren epoxide	1641	0.77	-	-	0.46
38	Farnesol	1743	-	0.49	-	-
39	(β-*Z*)Curcumen-12-ol	1756	0.53	-	-	-
40	Phytol	1949	6.96	5.68	-	3.64
41	*epi* manoyl oxide	1993	0.22	-	-	-
42	(*E-E*)-Geranyl linalool	2027	0.82	-	-	1.59
Total compound			86.11	83.1	96.72	71.88
Monoterpene hydrocarbons			0.32	0.5	-	1.29
Oxygenated monoterpenes			-	-	70.72	11.41
Sesquiterpene hydrocarbons			57.79	54.1	15.89	15.98
Oxygenated sesquiterpenes			20 .82	22.82	10.11	39.52
Oxygenated diterpenes			7.18	5.68	-	3.64
Yield (%(w/w))			0.038	0.032	0.164	0.140

Table 2. Fatty acid percentages content of *M. officinalis* leaves

Fatty acid	France	Germany	Tabarka	Nefza
C16:0 (palmitic acid)	16.25	15.77	15.82	13.32
C16 :1 (palmitoleic acid)	-	1.71	1.00	1.47
C18 :1n-9 (oleic acid)	4.62	6.29	5 ,89	4.26
C18 :2n-6 (linoleic acid)	73.93	74.08	70.75	66.74
C20:0 (arachidic acid)	1.60	1.06	1.19	1.31
Total (%)	95.38	98.91	94.65	88.02

macrene-D (34.79-51.50%), sabinene (0.91-14.68%) and α-pinene (0.53-8.03%) (31). These compounds have also been detected as the main ones in the studied Melissa oils of Greek origin from natural populations, whereas no citral or citronellal was detected. The subspecies *M. officinalis* subsp. *altissima* exhibits a different chemical profile and the slight odoUr of lemon perceptible during a short period of flowering is not attributable to the presence of citral (32).

The presented results suggest the existence of two different chemotypes in the species M. officinalis. We declare the three chemotypes ct. caryophyllene oxide, ct. citral and ct. germacrene D.

The present work provides for the first time data about the chemical composition, qualitative and quantitative patterns of essential oils extracted from the Tunisian *Melissa officinalis*. The analysis implies that the samples from Tunisia, Tabarka and Nefza can belong to the *M. officinalis* subsp. *altissima* , according to the previous studies (31-30).

Fatty acids

To the best of our knowledge, the foliar fatty acid composition of *M. officinalis* is reported herein for the first time. The proportion of the fatty acids did not show differences according to the origin of samples. The comparison between different Melissa evidenced a similarity, at least with reference to the presence of the main fatty acid constituents. It is noteworthy that previous findings showed that the genus *Satureja*, *Origanum* and *Thymus* of the Lamiaceae family had some minor variations in fatty acid composition, which are dominated by the chemotypes of linoleic acid, palmitic acid and linolenic acid.(33)

In conclusion, the Tunisian lemon balm has not been studied before, it is founded in small fragmented habitats. Quantitative and qualitative differences between the essential oils of Tunisian and introduced *M. officinalis* have been revealed and three chemotypes have been detected Interestingly, in terms of the dominant compounds, not even trace amounts of neral, geranial, or of citronellal, were detected in Tunisian samples Tabarka and Nefza, which were dominated by germacrene-D. No significant difference was identified with the composition of FA from the four populations which are dominated by the unsaturated fatty acids linoleic acid.

Acknowledgement

The authors would like to extend their sincere appreciation to the Laboratory Agronomic Sciences & Technics of the National Institute for Agronomic Research in Tunisia for their financial support.

Notes on contributors

Mouna SOUIHI: Collected Melissa officinalis specimens from Tabarka and Nefza (north of Tunisia), cultivated the materials and followed their growth and life cycle, interpreted the results and wrote the draft manuscript.

Ismail AMRI: Interpretation of the chemical composition.

Amir SOUISSI: Performed the statistical analyzes.

Karim HOSNI: Performed the chemical analyses of essential oil and fatty acid.

Nadia BEN BRAHIM: Article revision.

Mohamed ANNABI: Data analysis and article revision

References

1. Zargari, A., Herbal medicines. Publication of Tehran University, Tehran, 1990. 83.
2. Aharizad, S., et al., Assessment of relationship between effective traits on yield and citral content of lemon balm (Melissa officinalis L.) populations using path analysis. Indian Journal of Science and Technology, 2013. 6(5): p. 4447-4452.
3. Tucker, A.O. and T. DeBaggio, big book of herbs. 2000: Interweave Press.
4. Brickell, C. and J. Zuk, AZ Encyclopedia of Garden Plants. The American Horticultural Society. DK. Publishing. New York. I. Trinajstic'et al.: Drvenasta lucema—Medicago arborea L. u hrvatskoj flori, 1997.
5. Usai, M., A.D. Atzei, and M. Marchetti, A Comparative Study on Essential Oil Intraspecific and Seasonal Variations: Melissa romana Mill. and Melissa officinalis L. from Sardinia. Chemistry & biodiversity, 2016. 13(8): p. 1076-1087.
6. Craker, L.E. and J.E. Simon, Herbs, spices, and medicinal plants: recent advances in botany, horticulture, and pharma-

cology. Vol. 1. 1992: Psychology Press.
7. Blumental, M., A. Goldberg, and J. Brinckmann, Herbal medicine. Expanded Commission Monographs. Austin, TX: American Botanical Council, 2000.
8. Sepand, M.R., et al., Comparison of neuroprotective effects of Melissa officinalis total extract and its acidic and non-acidic fractions against a β-induced toxicity. Iranian journal of pharmaceutical research: IJPR, 2013. 12(2): p. 415.
9. Ozguven, M., W. Koller, and P. Range, Yield and quality traits of wild balm collections from the South East of Turkey. Zeitschrift fur Arznei-& Gewurzpflanzen, 1999. 4(1): p. 39-43.
10. Moradkhani, H., et al., Melissa officinalis L., a valuable medicine plant: A review. Journal of Medicinal Plants Research, 2010. 4(25): p. 2753-2759.
11. Gutierrez, J., C. Barry-Ryan, and P. Bourke, The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients. International journal of food microbiology, 2008. 124(1): p. 91-97.
12. Allahverdiyev, A., et al., Antiviral activity of the volatile oils of Melissa officinalis L. against Herpes simplex virus type-2. Phytomedicine, 2004. 11(7-8): p. 657-661.
13. Ehsani, A., et al., Evaluation of Thymus vulgaris plant extract as an eco-friendly corrosion inhibitor for stainless steel 304 in acidic solution by means of electrochemical impedance spectroscopy, electrochemical noise analysis and density functional theory. Journal of colloid and interface science, 2017. 490: p. 444-451.
14. Adorjan, B. and G. Buchbauer, Biological properties of essential oils: an updated review. Flavour and Fragrance Journal, 2010. 25(6): p. 407-426.
15. Sousa, A.C., et al., Melissa officinalis L. essential oil: antitumoral and antioxidant activities. Journal of pharmacy and pharmacology, 2004. 56(5): p. 677-681.
16. Scuteri, D., et al., Aromatherapy and Aromatic Plants for the Treatment of Behavioural and Psychological Symptoms of Dementia in Patients with Alzheimer's Disease: Clinical Evidence and Possible Mechanisms. Evidence-Based Complementary and Alternative Medicine, 2017. 2017.
17. Sakuradani, E., et al., Identification of 12 fatty acid desaturase from arachidonic acid producing Mortierella fungus by heterologous expression in the yeast Saccharomyces cerevisiae and the fungus Aspergillus oryzae. European journal of biochemistry, 1999. 261(3): p. 812-820.
18. Kökten, K., et al., Tannin, protein contents and fatty acid compositions of the seeds of several Vicia L. species from Turkey. 2010.
19. Jakobsen, M.U., et al., Major types of dietary fat and risk of coronary heart disease: a pooled analysis of 11 cohort studies–. The American journal of clinical nutrition, 2009. 89(5): p. 1425-1432.
20. Pottier Alapetite, G., Flore de la Tunisie. Angiopermes-Dicotyledones. Gamopetales. Tunis): Ministere de l'Enseignement Superieur et de las Recherche Scientifique et de la Ministere de l'Agriculture, 1981: p. 655-1190.
21. El-Mekki-Ben, B., et al. Rare plants of northern and central Tunisia. in Annales de l'INRAT. 2014. Institut National de la Recherche Agronomique de Tunisie (INRAT).
22. Hosni, K., K. Msaada, and B. Marzouk, Comparative study on Hypericum triquetrifolium Turra. fatty acids. Asian J. Plant Sci, 2007. 6: p. 384-8.
23. Bligh, E.G. and W.J. Dyer, A rapid method of total lipid extraction and purification. Canadian journal of biochemistry and physiology, 1959. 37(8): p. 911-917.
24. Cecchi, G., S. Biasini, and J. Castano, Méthanolyse rapide des huiles en solvant: note de laboratoire. Revue française des corps gras, 1985. 32(4): p. 163-164.
25. Avci, A.B. and R.R.A. Giachino, Harvest stage effects on some yield and quality characteristics of lemon balm (Melissa officinalis L.). Industrial Crops and Products, 2016. 88: p. 23-27.
26. Adzet, T., et al., Content and Composition of M. officinalis Oil in Relation to Leaf Position and Harvest Time1. Planta medica, 1992. 58(06): p. 562-564.
27. Basta, A., O. Tzakou, and M. Couladis, Composition of the leaves essential oil of Melissa officinalis sl from Greece. Flavour and fragrance journal, 2005. 20(6): p. 642-644.
28. Abdellatif, F., et al., Chemical composition and antimicrobial activity of the essential oil from leaves of Algerian Melissa officinalis L. EXCLI journal, 2014. 13: p. 772.
29. Bağdat, R.B. and B. Coşge, The essential oil of lemon balm (Melissa officinalis L.), its components and using fields. J. Fac. Agric. OMU, 2006. 21(1): p. 116-121.
30. Kittler, J., et al., Evaluation of 28 balm and lemon balm (Melissa officinalis) accessions for content and composition of essential oil and content of rosmarinic acid. Genetic resources and crop evolution, 2018. 65(3): p. 745-757.
31. Dawson, B.S., R.A. Franich, and R. Meder, Essential oil of Melissa officinalis L. subsp. altissima (Sibthr. et Smith) Arcang. Flavour and fragrance journal, 1988. 3(4): p. 167-170.
32. Božović, M., et al., Melissa officinalis L. subsp. altissima (Sibth. & Sm.) Arcang. essential oil: Chemical composition and preliminary antimicrobial investigation of samples obtained at different harvesting periods and by fractionated extractions. Industrial Crops and Products, 2018. 117: p. 317-321.
33. Cacan, E., K. Kokten, and O. Kilic, Leaf fatty acid composition of some Lamiaceae taxa from Turkey. Progress in Nutrition, 2018. 20(1-S): p. 231-236.

Correspondence:
Dr. Mohamed Annabi
National Institute for Agronomic Research in Tunisia (INRAT), Carthage University, Agronomic Sciences and Technics Laboratory, Hedi Karray Street, 2049, Ariana, Tunisia
E-mail: mannabi@gmail.com

Progress in Nutrition 2020; Vol. 22, N. 1: 259-264 DOI: 10.23751/pn.v22i1.8627

Original article

Clinoptilolite induces cell death in THP-1 cells in oxidative stress conditions

Gunnur Demircan[1], Ata Onur Kepenek[2], Sule Beyhan Ozdas[3], Demet Akın[4]

[1]Medical Biology and Genetics / Demiroglu Bilim University, Turkey; [2] Bahcesehir University, Medical Faculty, Turkey - E-mail: atakepenek@hotmail.com, [3] Medical Biology and Genetics, Demiroglu paeBilim University, Turkey; [4] Pharmacology, Bahcesehir University, Medical Faculty, Department of Pharmacology, Turkey

Summary. *Aim:* Zeolites are tectosilicates which appear as minerals in nature and can also be synthesized in the laboratory conditions. Clinoptilolite is a natural zeolite which has ion exchanging and adsorbent characteristics. We have aimed to display the effect of clinoptilolite to apoptotic, autophagic and antioxidant characteristics of cancer cell lines under oxidative stress conditions. *Material and Methods:* The cytotoxicity of clinoptilolite was evaluated using the MTT assay. The total antioxidant status was measured by the total antioxidant status (TAS) detection kit. The western blotting analysis was performed in order to assess the protein expression levels of the apoptosis and to autophagy markers like Beclin1, Bcl-2 and LC3B. *Results:* 24 hours incubation of cancer cells with clinoptilolite reduced cell proliferation in a dose dependent manner. Clinoptilolite lowered the autophagy in cancer cells, in particular, directing the cells towards apoptosis and leading to a fall in the TAS levels. Clinoptilolite to cause a decrease in antioxidant defence. *Discussion:* Parallel to these findings, it has been seen that there was an increase in apoptosis and a decrease in autophagy in cells induced with hydrogen peroxide effect. In this study, clinoptilolite was shown to cause a decrease in antioxidant defense.

Key words: clinoptilolite, cancer, autophagy, apoptosis, antioxidant

Introduction

Zeolites are tectosilicates which have been hydrated, and which appear as minerals in nature and can also be synthesized as artificial materials, in the laboratory (1-2). It is known that there are more than 40 zeolite structures in nature, while there are a further 229 types of zeolites which have been synthesized (3). Zeolitization is the process of zeolite formation from feldspathic rocks (4). This zeolitic transformation cause their chemical stability in solutions at different pH values which is essential for human applications (5).

Zeolites have a big potential for biomedical applications. It has been shown that microporous Faujasite Zeolite could be used as a drug delivery system to facilitate the oral delivery of poorly water soluble compound (6). Current needs for the synthesis and characterization of novel mesoporous and microporous materials, which would be better suited for biomedical applications (7).

Zeolites have been used as hemostatic components, gastro-protective drugs and antioxidative agents. Clinoptilolite, is a natural zeolite, which has ion exchanging and adsorbent characteristics and is not toxic. It has been shown in previous studies that clinoptilolite can be used as an auxiliary product in the treatment of cancer (8,9). Pavelic et all. Also shown that antiproliferative and proapoptotic effects of zeolites can be used for tumor treatment (7).

Zeolites can be used as antibacterial agents, especially when Ag is incorporated into these materials by ion exchange. (10,11). Zeolites, are used for as biosensor in some applications (12). Intestinal cell mediated antiinflammatuar effects of clinoplitolite has been shown in some studies (13, 14) Recent studies have estabileshed a possible link "gut brain axis" between intestinal microbiome and neurological disorders (15).

Based on the antioxidant effects of zeolites, clinoptilolite's antioxidant effects on hepatocytes, following partial hepatectomy in rats has been shown. It is found that, the levels of malondialdehyde, has decreased which is an indicator of oxidative stress on the liver tissue, following the oral application of clinoptilolite (16).

Programmed cell death is defined as regulated cell death mediated by an intracellular program (17). Defects in the system of apoptosis play a very important role in cancer. New cancer treatment strategies target to reduce apoptotic mechanisms. There are pro-anti apoptotic proteins like Bcl-2 in the process of apoptosis in the cell (18). Studies have shown that clinoptilolite reduces cell viability, DNA synthesis and increases apoptosis (16).

Autophagy is a cellular mechanism by which cellular materials are delivered to the lysosome for degradation. With this mechanism, cellular components are recycled and cellular energy and precursors of macromolecules are obtained (19). Some studies reveal that autophagy is associated with oncogenes and tumor suppressor genes. The mechanism of the autophagic process is controlled by a series proteins such as Beclin-1, ATG5, LC3B, ULK (20)

Extensive research over the last two decades has shown that ongoing oxidative stress also can cause chronic inflammation, which can mediate cancer (21). Thus extanuate oxidative stress is a potential strategy for therapeutic prevention of cancer. In this study we evaluated the effect of clinoptilolite on cell death mechanisms on the leukemia of the human peripheral blood monocyte cell line (THP-1) under oxidative stress conditions. High levels of oxidative stress exhibit cytotoxicity, inhibiting cell proliferation and leading to apoptotic/necrotic cell death.

Material and Methods

Clinoptilolite (MEGADETOX® TMAZ® Tribomechanisch Mikronisierter Aktivierter clinoptilolite-Zeolith % 100), and the human peripheral blood monocyte cell line (THP-1, ATCC®-TIB202TM-THP-1 Manassas, VA, USA) were used in this study. Cells were cultured with the Roswell Park Memorial Institute1640 medium (RPMI 1640), containing 10 % FBS, 0.2 mM glutamine, 100 µg/ml streptomycin, 100 IU/ml penicillin at 37°C, under 5 % CO2 and 1 atm pressure. THP-1 cell line without clinoptilolite (control) was used as negative control. Only 200 mM H_2O_2 treated group without clinoptilolite administration was used as positive control. Cells were incubated with increase concentrations of clinoptilolite ($5x10^{-5}$, 10^{-5}, $5x10^{-4}$, 10^{-4}, and 10^{-3} M) for 24 hour. After incubation with clinoptilolite, H_2O_2 added each treated groups and only H_2O_2 treated group for 1hour.

The Measurement of Cytotoxicity

Clinoptilolite's effect on cell viability was evaluated using the MTT [3–(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay kit (Promega-The CellTiter 96® Non-radioactive Cell Proliferation Assay) This is a colorimetric assay for measuring cell metabolic activity. It is based on the ability of nicotinamide adenine dinucleotide phosphate (NADPH)-dependent cellular oxidoreductase enzymes to reduce the tetrazolium dye MTT to its insoluble formazan, which has a purple color.

THP-1 cells placed on 96-well plates with 2.5 × 10^4 cells in each well and incubated for 24 h, with serum-medium. Then, the medium was replaced with serum-free medium containing the clinoplitolite at increasing concentrations ($5x10^{-5}$, 10^{-5}, $5x10^{-4}$, 10^{-4}, and 10^{-3} M) concentrations, based on MTT assay, followed by incubation for 24 h. The absorbance was recorded at 570 nm using 96-well plate reader.

Total Antioxidant Status (TAS)

After incubation with serum-free medium containing clinoptilolite for 24 h and H_2O_2 for 1 hour, cell lysates were prepared with lysis buffer. (Thermo Fisher Scientific, Cat No: FNN0021). The total antioxidant status (TAS) was measured using the TAS detection kit

(Rel Assay Diagnostic) in cell lysates. The absorbance was recorded at 660 nm using 96-well plate reader.

Western blot analysis

After incubation with clinoplitolite cells were washed with PBS and centrifugated, the supernatant fluid was discarded and the cellular pellet was lysate in 1 ml RIPA buffer (Mybiosource, Cat No: MBS169028) containing protease inhibitor cocktail. After 20 min incubation with RIPA, the cell lysate was centrifuged at 16.250 g, 20 min, 4°C. The supernatant was collected and the amount of protein was calculated using the Bradford reagent (Sigma Aldrich, USA). Western blotting was performed with the following antibodies Rabbit anti-beclin-1 (Santa Cruz, USA) and mouse anti-Bcl-2 and anti-LCB (Santa Cruz, USA) were used to determine autophagy and apoptosis related protein expression levels.

Signals were detected with an imaging system (Bio-Rad ChemiDoc MP Imaging System, Singapore). The density was analyzed using Image J software (W. Ras Band, Research Service Brunch, NIMH, NIH, Bethesta, MD) and normalized with the signal of actin for equal protein loading control of each sample and each experiment (20)

Statistical Analysis

Data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using non-parametric Mann–Whitney U test. P value < 0.05 (*) and < 0.01(**) was considered statistically significant. Statistical analyses were performed using GraphPad Prism 5 software.

Results

Cell Viability

Cell viability is a parameter for the proliferation index of the cells.

24 hours incubation with clinoptilolite reduced cell proliferation in a dose-dependent manner, in the THP-1 cells. The non-toxic concentrations of $5x10^{-5}$,10^{-5}, $5x10^{-4}$, 10^{-4}, and 10^{-3} M clinoptilolite were determined according to the MTT results (Fig. 1)

The Total Antioxidant Status in the THP-1 Cells Treated with Clinoptilolite

A statistically significant decrease was observed in positive control (H_2O_2 treated) group in TAS level, (*p<0.05). TAS levels were intent to increase with clinoptilolite +H_2O_2 treated groups but these increases were not significantly important (Fig. 2)

The Autophagy Markers

The protein expressions of Beclin-1 and LC3-B, the early and late markers of autophagy respectively

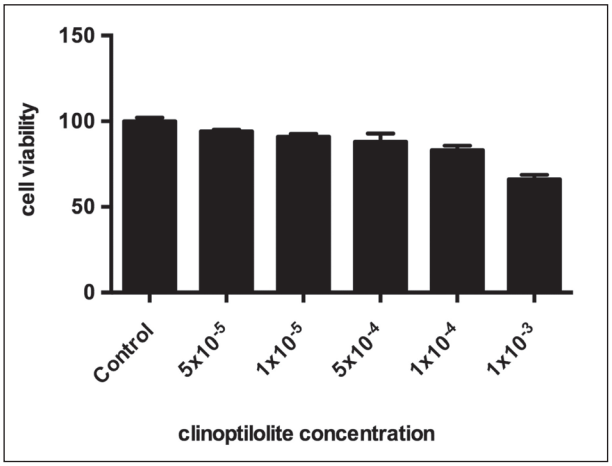


Figure 1: Effect of different clinoptilolite doses on the viability of THP-1cells measured by the MTT assay.

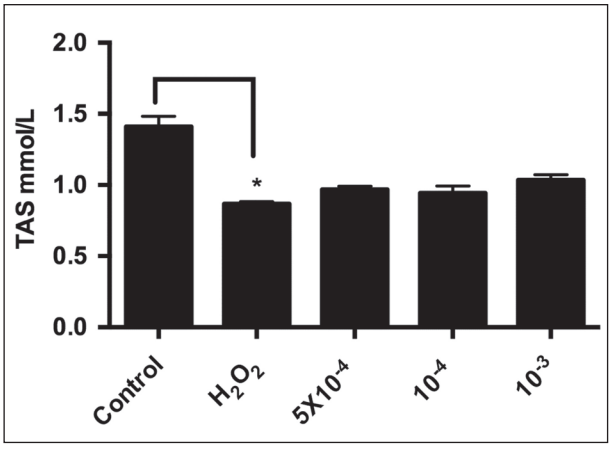


Figure 2: Graphical comparison of Total Antioxidant Status (TAS) mmol/L values for the control, H_2O_2, and different doses of clinoptilolite in the THP-1cells. Mean±SD, *p<0.05 for control group vs H_2O_2.

and the expressions in the THP-1 cells incubated with clinoptilolite for 24 hours and H_2O_2 for 1 hour were determined using the western blot method.

Beclin-1 protein levels were decreased in H_2O_2 and clinoptilolite incubated groups in a statistically significant manner (*p<0.05, **p<0.001 respectively (Fig 3).

LC-3B protein levels were also decreased in H_2O_2 and clinoptilolite incubated groups in a statistically significant manner (*p<0.05, **p<0.005 respectively) There was no significant differences only in $5X10^{-4}$ concentration of clinoplitolite incubated group (Fig 4).

The Apoptosis Markers

BCL-2 is an anti-apoptotic marker. It is known that some tumor containing cells inhibit apoptosis to escape death.

We found significantly increased BCL2 protein level in H_2O_2 treated group compared with control. Clinoplitolite was decreased this protein levels in all concentrations. These decreases were statistically different from H_2O_2 treated group.

Discussion

In the last twenty years, the routes followed in the formation and treatment of cancer have changed to a great extent, at molecular levels. Thus, today, targets have been revealed at numerous molecular levels for the development of alternative cancer treatments, which have started to be clinically implemented (22). Strategically targeted alternative cancer treatments focus on fundamental signal mechanisms, along the lines of cell growth and cell death (23).

Apoptosis, which is also known as programmed cell death, is a physiological cellular process, which is seen in organisms during their normal development (24). Many anti-cancer drugs impact the DNA synthesis and separation of cells in the tumor cell, causing apoptosis inductions (25).

Autophagic cell death is characterized by the appearance of cytoplasmic organelles, such as mitochondria and endoplasmic reticulum, or vesicles with two or multiple membranes, which swallow the cytoplasm mass. Autophagy can be a protective mechanism against apoptosis. Damage to the autophagy process contributes to the development of cancer (26).

At the same time, autophagy may also be a tumor suppressant in the early stages of tumor formation. Reduced autophagy is found in tumor cells and may be associated with malignant transformation. Under these circumstances, the induction of autophagy is seen to be beneficial for the prevention of cancer. Together with this, autophagy could also support the

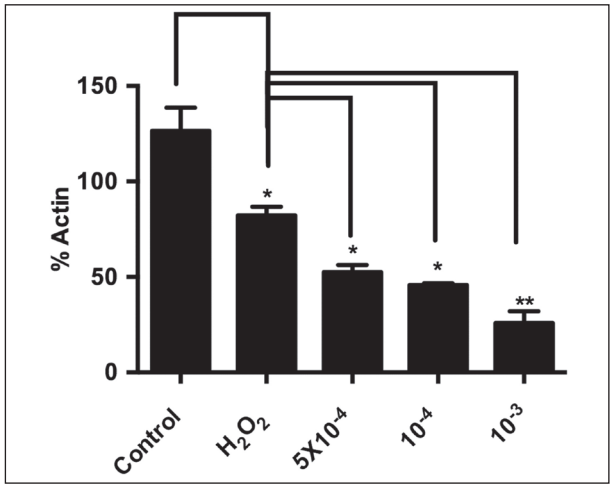


Figure 3: Graphical comparison of protein levels of Beclin-1 using Western Blot for the control, H_2O_2, and drug groups at different doses of clinoptilolite in the THP-1 cells. Mean±SD, *p<0.05 for control group vs H_2O_2 group, H_2O_2 group vs $5X10^{-4}$ M and 10^{-4} M clinoptilolite. **p<0.01 for H_2O_2 vs 10^{-3} M clinoptilolite.

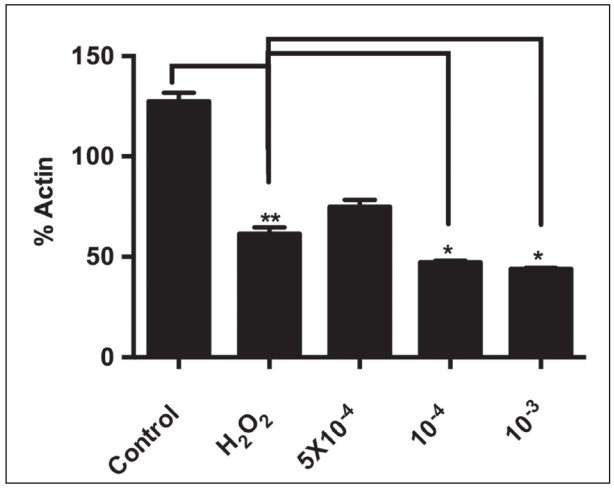


Figure 4: Graphical comparison of protein levels of LC-3B using Western Blot for the control, H_2O_2, and drug groups at different doses clinoptilolite in the THP-1 cells. Mean±SD, **p<0.01 for control group vs H_2O_2 group, H_2O_2 group vs 10^{-4} M and 10^{-3}M clinoptilolite **p<0.01

tumor, when the presence of a tumor has been shown (that is to say, it is a tumor inhibiting mechanism) and cancer cells can use developed autophagy in order to survive under metabolic and therapeutic stress (27).

In many studies have been shown that reactive oxygen species (ROS) could be effective in the formation of autophagosomes, as well as having regulatory effects on autophagy for cell death or survival. At the same time, high levels of ROS within the cell, promotes apoptosis (28).

Autophagy is an alternative mechanism, which is used especially by tumor cells to survive (29). According to our findings, it was seen that clinoptilolite suppresses autophagy in THP-1 cells (Fig 3 and 4). Thus, there was a negative effect on the survival rate of this cancer cells. The inhibition of autophagy will result in an increase in the inflammation within the cell, and this will induce the cell towards apoptosis. In one study, stated that inflammation in the cells would increase during the process of getting away from autophagy (30), authors also stated that apoptosis increases during the process of inflammation (31). Similarly, in our study, it was observed that autophagy had been inhibited as a result of the application of clinoplilolite, and that, in parallel with the information contained in the literature, this inhibition had increased the level of apoptosis, but also led to a relative fall in the TAS level.

The effects of clinoptilolite on Bcl-2, which is an anti-apoptotic marker, were also examined. The Bcl-2 protein levels only showed an increase, when compared to the control group, in the cells incubated with H_2O_2. Accordingly, incubation with H_2O_2 has directed THP-1 cell lines away from apoptosis, in order to protect them from oxidative stress. In one study it has been observed that hydrogen peroxide induced cytotoxicity increased the levels of the Bcl-2 protein (32).

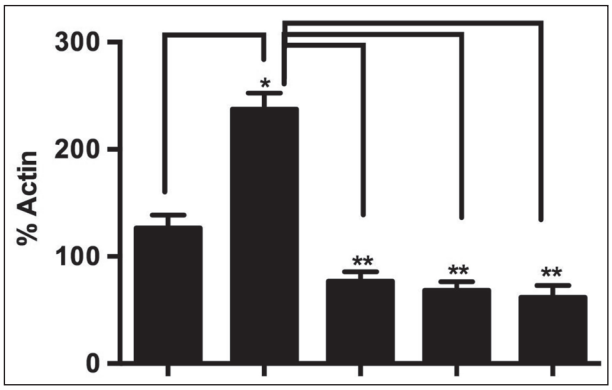


Figure 5: Graphical comparison of protein levels of Bcl-2 using Western Blot for the control, H_2O_2, and drug groups at different doses clinoptilolite in the THP-1 cells. Mean±SD, *p<0.05 for control group vs H_2O_2 group, H_2O_2 group vs $5X10^{-4}$ M, 10^{-4} M and 10^{-3} clinoptilolite, **p<0.01 for H_2O_2 vs 10^{-3} M clinoptilolite.

On the other hand, clinoptilolite incubation, was significantly decreased the Bcl-2 levels, when compared to the H_2O_2 group (Fig. 5). Based on these findings, it can be said that clinoptilolite suppresses the anti-apoptotic routes and directs the cells towards apoptosis, in cells which have suffered oxidative damage.

Even if the previous studies in the literature have yet to fully explain the relationship between clinoptilolite and apoptosis, in their study using macrophages derived from human peripheral blood monocytes and we can also say that clinoptilolite was increased apoptosis in dose dependent manner in THP-1 cells (Fig. 5).

As a summary, in concordance with the literature, there was an increase in apoptosis and a decrease in autophagy in cells induced with hydrogen peroxide effect. In this study, clinoptilolite was shown to cause a decrease in antioxidant defense. Parallel to these findings, it was seen that while clinoptilolite inhibits autophagy, it directs cells to apoptosis. This apoptotic effects of clinoptilolite may related to the suppression of oxidative stress. There is still a need for more extensive and more descriptive research to promote the clinical use of clinoptilolite in cancer. Further human studies are necessary to understand clinoptilolite contributions on anticancer therapy.

References

1. Barrer RM. Zeolites and clay minerals as sorbents and molecular sieve. Academic Press 1978; London, New York.
2. Pekov IV, Grigorieva AA, Turchkova AG, Lovskaya EV. Natural ion exchange in microporous minerals: Different aspects and implications. Miner. Adv. Mater. I 2008; 7–15.
3. Laurino C, Palmieri B. Zeolite: "The magic stone"; Main nutritional, environmental, experimental and clinical fields of application. Nutr. Hosp. 2015; 32(2): 573-81.
4. Monzón JD, Pereyra AM, Conconi MS, Basaldella EI. Phase transformations during the zeolitization of fly ashes. J. Environ. Chem. Eng. 2017; 5, 1548–1553.

5. Hall A. Zeolitization of volcaniclastic sediments; the role of temperature and pH. J. Sediment. Res. 1998; 68, 739–745.
6. Kontogiannidoua E, Karavasilia C, Kouskoura M G, Filippousic M, Tendeloo G V, I.Andreadis I I. Eleftheriadisa G K, Kontopoulou I, Catherine K.Markopoulou K M, Bouropoulos N, Fatouros D G. In vitro and ex vivo assessment of microporous Faujasite zeolite (NaX-FAU) as a carrier for the oral delivery of danazol. Journal of Drug Delivery Science and Technology 2019; 51: 177-184.
7. Pavelic K, Subotic B, Colic M. Biomedical application of zeolites. Studies in Surface Science and Catalysis 2001; 135: 170.
8. Bacakova L, Vandrovcova M, Kopova I, Jirka I. Applications of zeolites in biotechnology and medicine. Biomaterials Science 2018; 6: 974-989.
9. Margeta K, Zabukovec N, Siljeg M, Farkas A. Natural Zeolites in Water Treatment—How Effective is Their Use. In Water Treatment; Elshorbagy, W., Chowdhury, R., Eds.; IntechOpen: London, UK, 2013; Volume 5, pp. 81–112.
10. Hotta M, Nakayima H, Yamamoto K, Aono M. Antibacterial temporary filling materials: the effect of adding various ratios of Ag-Zn-Zeolite. J. of Oral Rehab. 1998; 25: 485
11. Dyer A, Morgan S, Wells P, Williams C. The use of zeolites as slow release anthelmintic carriers. Journal of Helminthology 2000; 74 (2): 137-141
12. Liu B, Yan F, Kong J, Deng J. A reagentless amperometric biosensor based on the coimmobilization of horseradish peroxidase and methylene green in a modified zeolite matrixAnal. Chim. Acta 1999; 386: 31
13. McGhee JR, Fujihashi K. Inside the Mucosal Immune System. PLoS Biol. 2012; 10, e1001397.
14. Lamprecht M, Bogner S, Steinbauer K, Schuetz B, Greilberger JF, Leber B, Wagner B, Zinser E, Petek T, Wallner-Liebmann S, et al. Effects of zeolite supplementation on parameters of intestinal barrier integrity, inflammation, redoxbiology and performance in aerobically trained subjects. J. Int. Soc. Sports Nutr. 2015; 12, 40.
15. Kaelberer MM, Buchanan KL, Klein ME, Barth BB, Montoya MM, Shen X, Bohórquez DV. A gut-brain neural circuit for nutrient sensory transduction. Science 2018; 361, eaat5236.
16. Katic M, Bosnjak B, Gall-Troselj K, Dikic I, Pavelic K. A clinoptilolite effect on cell media and the consequent effects on tumor cells in vitro. Front Biosci. 2006; 11: 1722-32
17. Su Z, Yang Z, Xu Y, Chen Y and Su QY et al. Apoptosis, autophagy, necroptosis, and cancer metastasis. Molecular Cancer 2015; 14:48
18. Wong RSY. Apoptosis in cancer: from pathogenesis to treatment. J Exp Clin Cancer Res. 2011; 30(1): 87
19. Levy JMM, Towers CG, Thorburn A. Targeting autophagy in cancer. Nat Rev Cancer. 2017; 17(9): 528-542
20. Yun CW, and Lee SH. The Roles of Autophagy in Cancer. Int J Mol Sci. 2018; 19(11): 3466.
21. Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: How are they linked? Free Radic Biol Med. 2010; 49(11): 1603–1616
22. Ricci MS, Zong WX. Chemotherapeutic Approaches for Targeting Cell Death Pathways. Oncologist 2006; 11(4): 342-57
23. Alles A, Alley K, Barrett JC. Buttyan R, Columbano A, et al Apoptosis: a general comment. FASEB J. 1991; 5: 2127-8
24. Herrmann M, Kalden JR. Apoptosis and autoimmunity. Wiley-Vch Verlag GmbH & Co. KGaA, University of Erlangen-Nuremberg, Institute for Clinical Immunology. 2003; Weinheim, Germany
25. Gozuacik D, Kimchi A. Autophagy as a cell death and tumor suppressor mechanism. Oncogene 2004; 23: 2891–2906
26. Choi KS. Autophagy and cancer. Experımental And Molecular Medıcıne 2012; 29; 44(2): 109-20
27. Yapislar H, Taskin E, Ozdas S, Akin D, Sonmez E. Counteraction of Apoptotic and Inflammatory Effects of Adriamycin in the Liver Cell Culture by Clinopitolite. Biol Trace Elem Res. 2016; 170(2): 373-81
28. Haanen C, Vermes I. Apoptosis and inflammation. Mediators of Inflammation 1995; 4(1): 5-15
29. Oka T. Hikoso S, Yamaguchi O, Taneike M, Takeda T, et al. Mitochondrial DNA That Escapes from Autophagy Causes Inflammation and Heart Failure. Nature 2012; 485(7397), 251–255
30. Toledano-Magaña Y, Flores-Santos L, Montes de Oca G, González-Montiel A, Laclette JP, Carrero JC. Effect of Clinoptilolite and Sepiolite Nanoclays on Human and Parasitic Highly Phagocytic Cells. Biomed Res Int. 2015; 164980
31. Delou JMA, Bıasolı D, Borges HL. The Complex Link between Apoptosis and Autophagy: a Promising New Role for RB. Annals of the Brazilian Academy of Sciences 2016; 88(4): 2257-2275
32. Saitoh Y, Ouchida R, Kayasuga A, Miwa N. Anti-Apoptotic Defense of bcl-2 Gene Against Hydroperoxide-Induced Cytotoxicity Together With Suppressed Lipid Peroxidation, Enhanced Ascorbate Uptake, and Upregulated Bcl-2 Protein. Journal of Cellular Biochemistry 2003; 89: 321–334

Correspondence:
Ata Onur Kepenek
Bahcesehir University, Medical Faculty, Turkey
Phone: +905320576482
atakepenek@hotmail.com

Progress in Nutrition 2020; Vol. 22, N. 1: 265-274 DOI: 10.23751/pn.v22i1.9041

Original article

Effects of (+) - catechin + quercetin usage before exhaustion exercise on free radical and antioxidant enzyme levels

Erdil Durukan

School of Physical Education and Sport, Balıkesir University, Balıkesir, Turkey - E-mail: erdurukan@hotmail.com

Summary. *Study Objectives:* The aim of this study was to determine the effects of the use of (+) - Catechin + Quercetin for 10 days before an exhaustion exercise on free radical and antioxidant enzyme levels. *Methods:* The study was performed on 12 male Wistar rats (260-320 gr.) from the same family and animals divided into two groups as a control and experimental group. After the first exhaustion exercise, rats in the experimental group used (+) - Catechin + Quercetin in addition to the standard laboratory diet and they performed the second exhaustion exercise. In the experimental group, 20 mg/kg (+) - Catechin + Quercetin substances were dissolved in dimethyl sulfoxide and given 1 ml/kg while the control group received 1 ml/kg 0.05% dimethyl sulfoxide by gavage in addition to standard laboratory diet daily for 10 days. SOD, CAT, GPx, GST, and MDA levels were measured by spectrophotometer. The IBM SPSS Statistics 24.0 was preferred for the statistical analysis. The repeated measures two-factor variance analysis was used to determine the difference between control and experimental groups. *Results:* It was determined that antioxidant enzyme levels (SOD, CAT, GPx, and GST levels) in rats using (+) - Catechin + Quercetin for 10 days before an exhaustion exercise were higher than those of the control group. Despite that, it was determined that MDA levels in rats using (+) - Catechin + Quercetin for 10 days before an exhaustion exercise were lower than those of the control group. *Conclusion:* It can be said that the use of (+) - Catechin + Quercetin can reduce the amount of MDA which is the end product of lipid peroxidation in exercise and may create a protective effect against free radicals and increase the levels of antioxidant enzymes and strengthen the antioxidant defense systems of the cells and have a positive effect on exercise performance.

Key words: antioxidants, catechin, exhaustion exercise, free radicals, quercetin

Introduction

Physical activity and exercise have exhaustion impact mechanisms according to the type of physical activity and exercise besides protecting human health. In both human and animal experiments, their bodies have been found to have the ability to adapt to internal and external sources of stress. In addition, Yagmur et al. (2019) stated that exercise is a physical stressor that causes hormonal, metabolic, cardiovascular and immunological changes and that stress can affect the body during heavy exercise (1).

Although physical exercise has many beneficial effects on health, there are some findings showing that reactive oxygen species and free radical formation are increased particularly during heavy exercise, and that oxidative damage occurs in muscles, liver, blood and other tissues (2-6). The degree of the oxidative damage that can occur during physical exercise is determined not only by the production of free radicals, but also by the defense capacity of antioxidants. Superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) provide the first line of defense against reactive oxygen species produced during exercise. Therefore, exercise is thought to directly affect these enzymes (7).

It is known that exercise is a source of stress that causes the formation of oxidative stress by increasing the production of free oxygen radicals, whereas it in-

creases resistance against oxidative stress by affecting the activity of antioxidant enzymes. Cells have enzymatic and non-enzymatic defense mechanisms in order to minimize or eliminate the damage caused by free radicals particularly the ones produced through physical activity and exercise. Antioxidant enzymes such as SOD, CAT, selenium-dependent glutathione peroxidase (GPx) glutathione-S-transferase (GST) constitute the enzymatic defense mechanisms. Vitamin C, vitamin A, vitamin E, flavanoids, melatonin, uric acid, haptoglobin, albumin, cysteine, ceruloplasmin, transferrin, lactoferrin, ferritin, oxypurinol, ubiquinone (coenzyme Q10), bilirubin, mannitol, lipoic acid, and hemopexin are non-enzymatic defense mechanisms having antioxidant contents (8). Although Flavanoid (+) - Catechin and Quercetin, which are among the non-enzymatic defense systems, are present in different forms, they have a structure preferred both in experimental studies and as ergogenic support due to their antioxidant effects. In addition to superoxide, lipid alkoxyl, peroxyl and nitric oxide radical scavenging, iron and copper chelation, α-tocopherol regeneration functions of flavonoids and other plant phenolics, they have also a vasodilator, immunostimulant, antiallergic, estrogenic, antiviral (against HSV, HIV, Influenza, and Rhinoviruses) effects (9, 10). In a study by Hayek et al. (1997) on (+) - Catechin and Quercetin, which are known to have different mechanisms of action in the literature, it was reported that (+) - Catechin and Quercetins bind to LDL particles by ether bonding, reducing susceptibility to oxidation and aggregation (11).

Phenolic antioxidants interact with lipid oxidation in such a way as to rapidly give H + to lipid radicals. Its function is to break down lipid peroxyl and alkoxyl radicals and thus terminate the lipid peroxidation chain reaction (12). As is known, MDA occurs as the final product in the peroxidation of fatty acids containing three or more double bonds. The effect of Catechin on lowering lipid peroxidation level is associated with the fact that Catechin has direct free radical scavenging activity due to the excess of hydroxyl groups (13, 14), its function of breaking down free radical chain reaction by working synergistically with alpha-tocopherol to give hydrogen molecule for the regeneration of alpha-tocopherol (15), its preventing oxidation of low-density lipoproteins (16) and/or it's preventing free radical formation by binding iron and copper by acting as a chelator (17).

Yu et al. (2010) stated that flavonoid supplementation not only reduces free radical formation but also removes free radicals and improves endurance exercise performance by reducing muscle fatigue (18). Quercetin is an important flavonoid that prevents the formation of free oxygen radicals in cells and provides protection against lipid peroxidation. Kocabaş et al., (2008) found that plasma MDA levels of rats given Quercetin were lower than those of the control group ($p<0.05$) (19). Duarte et al. (2001) observed a decrease in MDA levels in rats after 5 weeks of use of Quercetin (20).

There are various studies in the literature on the effects of (+) - Catechin and Quercetin given through diet on antioxidant defense systems (SOD, CAT, GPx, and GST) to prevent damage caused by exercise-induced free radicals (21-28). Göktepe and Günay (2014) examined the effects of Quercetin on MDA, SOD, GPx and GST levels in rats. They found that Quercetin, which was administered through diet for 10 days, had a protective effect against free radicals by reducing the amount of MDA under exhaustion exercise conditions and strengthened the antioxidant defense systems of the cells by increasing the antioxidant enzyme levels (SOD, CAT, GPx, GST) (29). In another study, it was found that (+) - Catechin, which was applied as a diet for 10 days, decreased MDA level under exhaustion swimming exercise conditions and increased antioxidant enzyme levels (SOD, CAT, GPx, GST) (30).

In the literature, there is no study in which (+) - Catechin and Quercetin flavonoids that have different effect mechanisms are used together with diet and which investigates their effects on exhaustion exercise-induced lipid peroxidation and antioxidant enzyme levels (SOD, CAT, GPx, GST). Although the bioavailability of (+) - Catechin and Quercetin is relatively well documented, data on the effect of a relationship of these two flavonoids on their absorption and metabolism in dietary intake is still lacking. In addition, previous studies have reported that flavonoids may interact with each other, and these interactions may demonstrate effective competition against drugs for syner-

gistic antioxidant properties or metabolic enzymes (31-35). Therefore, the current study is important in terms of determining the effects of dietary intake of (+) - Catechin + Quercetin together on free radical and antioxidant enzyme levels during exhaustion exercise. In this connection, the purpose of the current study is to reveal the protective effects of (+) - Catechin + Quercetin by comparing MDA values and antioxidant levels as the final product of lipid peroxidation, which is the indicator of the effects of free radicals on membrane lipids after exhaustion swimming exercise, between the control and experimental groups. The hypotheses of the study are given below;

a) The use of (+) -Catechin + Quercetin for 10 days before an exhaustion exercise increases SOD level.
b) The use of (+) -Catechin + Quercetin for 10 days before an exhaustion exercise increases CAT level.
c) The use of (+) -Catechin + Quercetin for 10 days before an exhaustion exercise increases GPx level.
d) The use of (+) -Catechin + Quercetin for 10 days before an exhaustion exercise increases GST level.
e) The use of (+) -Catechin + Quercetin for 10 days before an exhaustion exercise decreases MDA level.

Materials and Methods

Characteristics and Diet of the Rats

Twelve male Wistar Albino rats weighing 260-320 g from the same family were used in the current study. The rats were randomly divided into two groups as the control group (n = 6) and the experimental group (n = 6). The rats were quarantined for 10 days, six rates in one cage before the experiment. All the rats were fed in special cages with standard laboratory diet and water. The rats were subjected to 12 hours of light and 12 hours of dark photoperiod at room temperature of 18-22 ° C. In addition, the temperature in the laboratory was 18-22 ° C and the relative humidity was 50 ± 10%.

Application and Experimental Design

After the 10 day-quarantine, all the rats performed exhaustion swimming exercises twice at different times (at an interval of 10 days) in a water tank of 80 x 60 x 60 cm^3. The exhaustion criterion was determined as the initiation of uncoordinated movements of the rats and/or their are remaining motionless for 10 seconds underwater (29). The water temperature in the water tank was 28°C. The rats performed all the exercises between 09:00 and 10:00 in the morning when they were full. In both exercises, rats were dried by a towel and then blood was taken from them.

First exercise: After the 10-day quarantine, all the rats performed the exhaustion exercise without using any substance other than the standard laboratory diet. After the exhaustion exercise, blood was taken from their hearts.

Second exercise: After the first exhaustion exercise, the rats in the experimental group used (+) - Catechin + Quercetin in addition to the standard laboratory diet and they performed the second exhaustion exercise. In the experimental group, 20 mg/kg (+) - Catechin + Quercetin substances were dissolved in dimethyl sulfoxide and given 1 ml/kg while the control group received 1 ml/kg 0.05% dimethyl sulfoxide by gavage in addition to standard laboratory diet daily for 10 days. Sigma-Aldrich brand (+) - Catechin, Quercetin, and Dimethyl sulfoxide were used throughout the experiments.

Biochemical Analyses

Preparation of Erythrocytes and Taking Blood Samples from Rats

In the experiment, blood was taken from the hearts of the animals after both exhaustion exercises into heparinized tubes with the help of a vacutainer. In the blood samples, erythrocytes were separated from the plasma through centrifugation at 1600 rpm +4 ° C for 5 min. They were then washed in cold 0.9% NaCl solution. The supernatant was carefully separated after each wash. Erythrocytes were suspended in pH 7.4 phosphate buffer. Hemoglobin concentration was determined according to the Drabkin (1946) method (36). Cell mixtures were stored at -20 ° C for 24 hours. Cells were detonated by forming an osmotic pressure difference with water and centrifuged at 2500 rpm. For 10 minutes. MDA level, SOD, CAT, GPx and GST activities of the obtained supernatants were measured by using a spectrophotometer (Shimadzu UV-1700, Japan).

SOD enzyme: In the determination of the total SOD levels, increasing absorbance was measured by

autoxidation of pyrogallol at 440 nm in alkaline medium using the Marklund and Marklund (1974) method (37). One unit total SOD activity was calculated as the amount of protein that caused 50% inhibition of autoxidation of pyrogallol. The SOD activity was determined as U / mg hemoglobin.

CAT enzyme: The activity of the CAT enzyme was determined with the method specified by Aebi 1984 (38). Decreasing absorbance indicating the breakdown of H_2O_2 was measured at 240 nm. Changes in absorbance per unit time were taken as a measure of CAT activity. Enzyme activity was given in U / mg hemoglobin unit.

GPx enzyme: The determination of the GPx level was made according to the method specified by Paglia and Valentine 1967 (39). Oxidation of NADPH to Nicotinamide-adenine-dinucleotide phosphate causes a decrease in absorbance at 340 nm, thus indirectly used in the determination of the activity of GPx. Hydrogen peroxide was added to this mixture to initiate the enzymatic reaction and absorbances were read at 340 nm for 3 minutes. GPx activity was calculated as the amount of NADPH spent per unit time and the specific activity of the enzyme was determined as U / mg hemoglobin.

GST enzyme: GST determination was performed according to the method developed by Habig et al. and 1-chloro-2.4-dinitrobenzene (CDNB) was used as the substrate for all isozymes of GST (40). The absorbance at 340 nm was read for the determination of the enzyme activity. GST specific activity was given as U / mg hemoglobin.

Determination of the Amount of MDA: In order to determine the amount of MDA; based on the method used by Ohkawa et al. (1979), the amount of MDA, the end product of lipid peroxidation reacting with thiobarbituric acid (TBA) at 532 nm, was measured (41). The absorbance of the mixture added with TBA was read at 532 nm on the spectrophotometer. The amount of MDA was determined as nmol/mg hemoglobin.

Ethical Approval

The current study was conducted in the Biology Laboratory, Faculty of Science and Letters, Gazi University and the ethical approval for the study was given by Gazi University, Animal Experiments Local Ethics Committee under the number G.Ü.ET-10.091.

Statistical Analysis

Statistical analysis of the data was conducted with the IBM SPSS Statistics 24.0 program package. The repeated measures two-factor variance analysis (2 groups X 2 times) was used for the analysis of the obtained data. In addition, the percent difference of the variables between two exhaustion exercises was calculated using the formula "Δ% = ((Post-test – Pre-test) / Pre-test) × 100" (42). The confidence interval was 95% and the level of significance was set at $p< .05$.

Results

In this section, the effects of 10-day use of (+) - Catechin + Quercetin on rats' free radical markers and antioxidant enzyme levels are presented.

It was found that there was a difference in SOD levels according to the measurement times (F= 49.339; p= .001). However, there was also a difference between the SOD levels of the groups. (F= 8.810; p= .014). In addition, the interaction between the groups and the measurement times was statistically significant (F= 34.111; p= .001). Accordingly, it was determined that an increase of 20.21% was observed in the SOD levels of the experimental group (Table 1).

It was found that there was a difference in CAT levels according to the measurement times (F = 71.361; p = .001). However, there was also a difference between the CAT levels of the groups (F = 15.398; p= .003). In addition, the interaction between the groups and the measurement times was statistically significant (F= 52.758; p= .001). Accordingly, it was determined that an increase of 41.16% was observed in the CAT levels of the experimental group (Table 2).

It was found that there was a difference in GPx levels according to the measurement times (F= 16.633; p= .002). However, there was also a difference between the GPx levels of the groups (F= 25.875; p= .001). In addition, the interaction between the groups and the measurement times was statistically significant (F= 18.091; p= .002). Accordingly, it was determined that

an increase of 49.95% was observed in the GPx levels of the experimental group (Table 3).

It was found that there was a difference in GST levels according to the measurement times (F= 27.455; p= .001). However, there was also a difference between the GST levels of the groups (F= 15.357; p= .003). In addition, the interaction between the groups and the measurement times was statistically significant (F= 15.357; p= .003). Accordingly, it was determined that an increase of 33.80% was observed in the GST levels of the experimental group (Table 4).

It was found that there was a difference in MDA levels according to the measurement times (F= 105.311; p= .001). However, there was also a difference between the MDA levels of the groups (F= 117.788; p= .001). In addition, the interaction between the groups and

Table 1. Effect of using 10 daily (+) - Catechin + Quercetin on SOD (U/mg)

Groups / Times	N	First Exercise	Second Exercise	Total	Δ%	F	p
		$\bar{X}$±SD	$\bar{X}$±SD	$\bar{X}$±SE			
Control	6	593.40±23.73	605.23±24.74	599.31±8.74	1.99	8.810	.014*
Experimental	6	571.68±37.79	700.30±20.70	635.99±8.74	20.21		
Total	12	582.54±32.15	652.77±54.20		Interaction F= 34.111; p= .001**		
F= 49.339; p= .001**							

**p<0.05; **p<0.01; $\bar{X}$: Mean; S.D.: Standard Deviation; S.E.: Standard Error; Δ%: Percentage difference of the time points*

Table 2. Effect of using 10 daily (+) - Catechin + Quercetin on CAT (U/mg)

Groups / Times	First Exercise	Second Exercise	Total	Δ%	F	p
	$\bar{X}$±SD	$\bar{X}$±SD	$\bar{X}$±SE			
Control	249.47±27.36	257.37±24.66	258.16±8.71	3.17	15.398	.003**
Experimental	254.76±27.63	359.61±25.83	352.15±8.71	41.16		
Total	252.12±26.36	308.49±58.57		Interaction F= 52.758; p= .001**		
F= .71.361; p= .001**						

***p<0.01; $\bar{X}$: Mean; S.D.: Standard Deviation; S.E.: Standard Error; Δ%: Percentage difference of the time points*

Table 3. Effect of using 10 daily (+) - Catechin + Quercetin on GPx (U/mg)

Groups / Times	N	First Exercise	Second Exercise	Total	Δ%	F	p
		$\bar{X}$±SD	$\bar{X}$±SD	$\bar{X}$±SE			
Control	6	26.83±2.76	26.54±3.66	27.24± .98	-1.08	25.875	.001**
Experimental	6	27.65±3.91	41.46±5.04	34.00±1.27	49.95		
Total	12	27.24±3.25	34.00±8.82		Interaction F= 18.091; p= .002**		
F= 16.633; p= .002**							

***p<0.01; $\bar{X}$: Mean; S.D.: Standard Deviation; S.E.: Standard Error; Δ%: Percentage difference of the time points*

Table 4. Effect of using 10 daily (+) - Catechin + Quercetin on GST (U/mg)

Groups / Times	N	First Exercise	Second Exercise	Total	Δ%	F	p
		$\bar{X}$±SD	$\bar{X}$±SD	$\bar{X}$±SE			
Control	6	35.33±3.47	37.82±1.96	35.39± .96	7.05	15.357	.003**
Experimental	6	35.44±3.14	47.34±3.88	42.58± .89	33.80		
Total	12	35.38±3.15	42.58±5.78		Interaction F= 11.760; p= .006**		
F= 27.455; p= .001**							

***p<0.01; $\bar{X}$: Mean; S.D.: Standard Deviation; S.E.: Standard Error; Δ%: Percentage difference of the time points*

the measurement times was statistically significant (F= 68.723; p= .001). Accordingly, it was determined that a decrease of 53.89% was observed in the MDA levels of the experimental group (Table 5).

Discussion

In addition to superoxide, lipid alkoxyl, peroxyl and nitric oxide radical scavenging, iron and copper chelation, α-tocopherol regeneration functions of flavonoids and other plant phenolics, they have also a vasodilator, immune-stimulant, antiallergic, estrogenic, antiviral (against HSV, HIV, influenza, and rhinoviruses) effects (12). A member of the flavonoid family, catechin is a flavonoid that is abundant in beverages such as fruit juices, red wine, and green tea and chocolate. In addition, recent studies have shown that catechin has anticarcinogenic, antimutagenic and hypodermic effects (43). Another member of the flavonoid family, quercetin is a bioflavonoid that is widely available in fruits and vegetables. Quercetin directly removes free radicals, inhibits lipid peroxidation, iron chelation, and strengthens antioxidant defense (44). Quercetin is known to reduce or prevent oxidative damage by improving antioxidant enzyme activity or by reducing lipid peroxidation (45-47). Changes in the levels of antioxidant enzymes in the blood as an indirect result of oxidative stress induced by exhaustion exercise are evaluated either alone or in combination with other oxidative stress indicators, MDA.

In the current study, the levels of free radical and antioxidant enzymes before giving (+) - Catechin + Quercetin (first exercise) and free radical and antioxidant enzyme levels after (+) – giving Catechin + Quercetin (second exercise) were measured in the experimental and control groups. In this context, it was found that there were significant decreases in MDA, which is the end product of lipid peroxidation, in the experimental group (rats consuming (+) - Catechin + Quercetin for 10 days) compared to the control group ($p<0.001$). It was also found that antioxidant enzyme levels (SOD, CAT, GPx, and GST) increased significantly after (+) - Catechin + Quercetin intake compared to the control group ($p<0.001$).

The effects of flavonoids on lipid peroxidation have been studied by many researchers and it has been shown that flavonoids significantly lower MDA levels (48-52). It was observed that cisplatin caused an increase in the amount of MDA in the rat kidney, whereas Quercetin decreased the increase in this lipid peroxidation (53). Çiftçi (2013) reported that Quercetin administered to rats has a reducing effect on MDA and may also prevent degenerative changes in the heart vessels (54). Hollman et al. (1995) found that antioxidant capacity was significantly higher and lipid peroxidation was inhibited in rats fed with a diet containing 0.2% Quercetin compared to the control group (55). In another study, which tried to determine the antioxidative efficacy of Quercetin, they found that 2 weeks of Quercetin administration caused a decrease in MDA level in rats (56). Göktepe and Günay (2014) also concluded that Quercetin application had a protective effect against free radicals in rats by reducing the amount of MDA, the end product of lipid peroxidation (29).

Catechin, another flavonoid, is known to exhibit protective behaviour against pathologies such as cell toxicity, cancer development, and free radical oxidation. In a study, it was reported that Catechin administration in rats significantly reduced the consumption of α-tocopherol and the accumulation of lipid perox-

Table 5. Effect of using 10 daily (+) - Catechin + Quercetin on MDA (nmol/mg)

Groups / Times	N	First Exercise	Second Exercise	Total	Δ%	F	p
		$\bar{X}$±SD	$\bar{X}$±SD	$\bar{X}$±SE			
Control	6	18.94±1.00	17.87±1.36	18.89± .35	-5.81	117.788	.001**
Experimental	6	18.84±1.37	8.71±1.01	13.29± .35	-53.89		
Total	12	18.89±1.15	13.29±4.92		Interaction F= 68.723; p= .001**		
F= 105.311; p= .001**							

***p<0.01; $\bar{X}$: Mean; S.D.: Standard Deviation; S.E.: Standard Error; Δ%: Percentage difference of the time points*

ides in plasma (57). According to the result of the current study, the use of (+) - Catechin and Quercetin is similar to that of flavonoids in the literature on MDA.

Antioxidants remove free radicals in the environment. For this purpose, SOD, CAT, and GST are in antioxidative defense (58). Quercetin has a wide range of therapeutic properties such as antioxidant, antitoxic, anti-cancer, anti-variant, anti-diabetic, anti-inflammation, cardiovascular effects, which are particularly beneficial to health (59-65). It is also known that catechins act as antioxidants by removing free radicals from the environment (66). Indeed, Phachonpai et al. (2010) reported that quercetin administered to rats increased SOD, CAT and GPx enzyme levels (27). In another study, it was found that Quercetin, which was administered for 2 weeks, increased SOD, CAT, and GPx enzyme levels in rats (56). Gargouri et al. (2011) reported that quercetin administration increased SOD and CAT enzyme levels in human lymphocytes (67). Bu et al. (2011); in their study investigating the protective effect of Quercetin against cadmium-induced oxidative toxicity in testicular germinative cells of mice, reported that test animals were administered cadmium (4mg/kg/day) and quercetin (75mg/kg/day) for two weeks and at the end of the study antioxidant enzyme activities (SOD and GSH-Px) were significantly improved and lipid peroxidation and hydrogen peroxide production were controlled significantly (68).

In relation to the effects of Catechin on antioxidant enzyme levels, Chan et al. (2002) reported that Catechin is an effective antioxidant that increases SOD activity in rat astrocytes (69). In another study, it was concluded that catechin increased the levels of SOD, GPx, and CAT enzymes (70). Sadowska-Krępa et al. (2008) stated that exercise type and intensity may affect the response of antioxidant defense systems and as a result, they found that red grape extract with Catechin content slightly increased antioxidant enzyme levels (SOD, CAT, and GPx) in the experimental group after interval swimming test compared to the control group (71). Yu et al. (2010) examined CuZn-SOD and GPx activities in order to determine the effects of Cynomorium Songaricum as a flavonoid extract with Catechin content on swimming resistance and free radicals of rats and reported that the group's given flavonoid extract have higher antioxidant enzyme levels than the group not given (18). The results of the studies in the literature showed that Catechin and Quercetin increased antioxidant enzyme activities. The results of the current study are similar to the ones reported in the literature in terms of the effects of Catechin and Quercetin on antioxidant defense mechanisms.

Conclusion

It was found that at the increasing free radical level as a result of exhaustion exercise, the use of (+) - Catechin + Quercetin together resulted in a decrease of 36.79% in the level of MDA, the end product of lipid peroxidation. In addition, it resulted in an increase of 18.48%, 36.41%, 56.52%, and 26.50% in the levels of antioxidant enzymes SOD, CAT, GPx, and GST, respectively. This result shows that (+) - Catechin + Quercetin administered during exhaustion exercise will decrease free radical enzyme levels and increase antioxidant defense enzyme levels. Considering the results of the study, it is recommended that (+) - Catechin and Quercetin can be taken together through diet by individuals engaged in exhaustion exercise. In this way, the athletic performance of the individuals doing a exhaustion exercise can be enhanced.

References

1. Yagmur R, Isik O, Kilic Y, Akyuz, M. The hydration status and thyroid hormones levels among elite wrestlers. Prog Nutr 2019; 21(4): 952-7
2. Ji LL. Antioxidants and oxidative stress in exercise. Proc Soc Exp Biol Med 1999; 222(3): 283-92.
3. Ji LL, Leeuwenburgh C, Leichtweis S, et al. Oxidative stress and aging: role of exercise and its influences on antioxidant systems. Ann N Y Acad Sci 1998; 854(1): 102-17.
4. König D, Wagner KH, Elmadfa I, Berg A. Exercise and oxidative stress: significance of antioxidants with reference to inflammatory, muscular, and systemic stress. Exerc Immunol Rev 2001; 7: 108-33.
5. Packer L. Oxidants, antioxidant nutrients and the athlete. J Sports Sci 1997; 15(3): 353-63.
6. Urso ML, Clarkson PM. Oxidative stress, exercise, and antioxidant supplementation. Toxicology 2003; 189(1-2): 41-54.
7. Selçuk M. Sedanterler ile kuzey disiplini yapan antrene bireylerde programlı aerobik ve anaerobik egzersizlerin bazı

antioksidan profiller üzerine etkilerinin araştırılması. Sağlık Bilimleri Enstitüsü, Fizyoloji Anabilim Dalı, Doktora Tezi, Yüzüncü Yıl Üniversitesi, Van, Türkiye 2003.
8. Halliwell B, Aruoma OI. DNA damage by oxygen-derived species: Its mechanism and measurement in mammalian systems. FEBS Lett 1991; 281(1-2): 9-19.
9. Formica JV, Regelson W. Review of the biology of quercetin and related bioflavonoids. Food Chem Toxicol 1995; 33(12): 1061-80.
10. Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med 1996; 20(7): 933-56.
11. Hayek T, Fuhrman B, Vaya J, et al. Reduced progression of atherosclerosis in apolipoprotein E–deficient mice following consumption of red wine, or its polyphenols quercetin or catechin, is associated with reduced susceptibility of LDL to oxidation and aggregation. Arterioscler Thromb Vasc Biol 1997; 17(11): 2744-52.
12. Çimen MBY. Flavonoidler ve antioksidan özellikleri. Turkiye Klinikleri J Med Sci 1999; 19(5): 296-304.
13. Husain SR, Cillard J, Cillard P. Hydroxyl radical scavenging activity of flavonoids. Phytochemistry 1987; 26(9): 2489-91.
14. Kumari MR, Yoneda T, Hiramatsu M. Effect of "βCATECHIN" on the life span of senescence accelerated mice (SAM-P8 strain). IUBMB Life 1997; 41(5): 1005-11.
15. Rice-Evans C. Plant polyphenols: free radical scavengers or chain-breaking antioxidants?. In Biochemical Society Symposia 1995; 61: 103-16.
16. De Whalley CV, Rankin SM, Hoult JRS, Jessup W, Leake DS. Flavonoids inhibit the oxidative modification of low density lipoproteins by macrophages. Biochem Pharmacol 1990; 39(11): 1743-50.
17. Morel I, Lescoat G, Cillard P, Cillard J. Role of flavonoids and iron chelation in antioxidant action. In Methods in enzymology 1994; 234: 437-43. Academic Press.
18. Yu FR., Liu Y, Cui YZ, et al. Effects of a flavonoid extract from Cynomorium songaricum on the swimming endurance of rats. Am J Chin Med 2010; 38(01): 65-73.
19. Kocabaş, N. Homosisteinin indüklediği oksidatif stres üzerinde quercetinin koruyucu etkisi. Master›s thesis, Afyon Kocatepe Üniversitesi, Sağlık Bilimleri Enstitüsü, Afyon, Türkiye 2008.
20. Duarte J, Pérez-Palencia R, Vargas F, et al. Antihypertensive effects of the flavonoid quercetin in spontaneously hypertensive rats. Br J Pharmacol 2001; 133(1): 117-24.
21. Adewole SO, Caxton-Martins EA, Ojewole JA. Protective effect of quercetin on the morphology of pancreatic β-cells of streptozotocin-treated diabetic rats. Afr J Tradit Complement Altern Med 2007; 4(1): 64-74.
22. Agarwal R, Katiyar SK, Khan SG, Mukhtar H. Protection against ultraviolet B radiation-induced effects in the skin of SKH-1 hairless mice by a polyphenolic fraction isolated from green tea. Photochem Photobiol 1993; 58(5): 695-700.
23. Akyüz Ö. Effects of Different-Term Acute Swimming Exercises on Oxidative Stress Parameters in Rat Cerebellum Tissue. US-China Education Review B 2016; 6(10): 600-8.
24. Demirci N. Protective Effect of Prolonged Quercetin Supplement on Oxidative Stress and Antioxidant Enzyme Activities in Boxers. Studies on Ethno-Medicine 2017; 11(2): 168-73.
25. Sadowska-Krępa E, Domaszewski P, Pokora I, Żebrowska A, Gdańska A, Podgórski T. Effects of medium-term green tea extract supplementation combined with CrossFit workout on blood antioxidant status and serum brain-derived neurotrophic factor in young men: a pilot study. J Int Soc Sports Nutr 2019; 16(1): 1-9.
26. Park HJ, DiNatale DA, Chung MY, et al. Green tea extract attenuates hepatic steatosis by decreasing adipose lipogenesis and enhancing hepatic antioxidant defenses in ob/ob mice. J Nutr Biochem 2011; 22(4): 393-400.
27. Phachonpai W, Wattanathorn J, Muchimapura S, Tong-Un T, Preechagoon D. Neuroprotective effect of quercetin encapsulated liposomes: a novel therapeutic strategy against Alzheimer's disease. Am J Appl Sci 2010; 7(4): 480-5.
28. Vassalle C, Pingitore A, De RG, Vigna L, Bamonti F. Antioxidants in Sport Nutrition. In Biomarkers Part II: Biomarkers to estimate bioefficacy of dietary/supplemental antioxidants in sport. 2015; CRC Press/Taylor & Francis.
29. Göktepe M, Günay M. Quercetin uygulamasının egzersiz, serbest radikal ve antioksidan enzim düzeyleri üzerine etkisi. IntJSCS 2014; 2(Special Issue 1): 775-88.
30. Durukan E. Egzersizdeki Serbest Radikal ve Antioksidan Enzim Düzeylerine Catechin Uygulamasının Etkisi, Gazi Kitabevi, Ankara 2019.
31. Dresser GK, Bailey DG, Leake BF, et al. Fruit juices inhibit organic anion transporting polypeptide–mediated drug uptake to decrease the oral availability of fexofenadine. Clin Pharmacol Ther 2002; 71(1): 11-20.
32. Malien-Aubert C, Dangles O, Amiot MJ. Color stability of commercial anthocyanin-based extracts in relation to the phenolic composition. Protective effects by intra-and intermolecular copigmentation. J Agr Food Chem 2001; 49(1), 170-6.
33. Ohtani M, Kawabata S, Kariya S, et al. Effect of grapefruit pulp on the pharmacokinetics of the dihydropyridine calcium antagonists nifedipine and nisoldipine. YAKUGAKU ZASSHI 2002; 122(5): 323-9.
34. Silberberg M, Morand C, Manach C, Scalbert A, Remesy C. Co-administration of quercetin and catechin in rats alters their absorption but not their metabolism. Life Sci 2005; 77(25): 3156-67.
35. Violi F, Pignatelli P, Pulcinelli FM. Synergism among Flavonoids in Inhibiting Platelet Aggregation and H. Circulation 2002; 105: e53.
36. Drabkin DL. Spectrophotometric studies XIV. The crystallographic and optical properties of the hemoglobin of man in comparison with those of other species. J Biol Chem 1946; 164(2): 703-23.
37. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 1974; 47(3): 469-74.

38. Aebi H. Catalase in vitro. In Methods in enzymology 1984; 105: 121-6.
39. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967; 70(1): 158-69.
40. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases the first enzymatic step in mercapturic acid formation. J Biol Chem 1974; 249(22): 7130-9.
41. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95(2): 351-8.
42. Isik O, Dogan I. Effects of bilateral or unilateral lower body resistance exercises on markers of skeletal muscle damage. Biomed J 2018; 41(6): 364-368.
43. Koçyiğit A, Arslan SO, Erel Ö, Aktepe N, Avcı Ş, Gür S. Effects of dietary supplementation of catechin on antioxidant enzyme activities and lipid peroxidation levels in rats. Turkiye Klinikleri J Med Sci 2000; 20(1): 1-4.
44. Anjaneyulu M, Chopra K. Quercetin, a bioflavonoid, attenuates thermal hyperalgesia in a mouse model of diabetic neuropathic pain. Prog Neuropsychopharmacol Biol Psychiatry 2003; 27(6): 1001-5.
45. Abdallah FB, Fetoui H, Fakhfakh F, Keskes L. Caffeic acid and quercetin protect erythrocytes against the oxidative stress and the genotoxic effects of lambda-cyhalothrin in vitro. Hum Exp Toxicol 2012; 31(1): 92-100.
46. Molina MF, Sanchez-Reus I, Iglesias I, Benedi J. Quercetin, a flavonoid antioxidant, prevents and protects against ethanol-induced oxidative stress in mouse liver. Biol Pharm Bull 2003; 26(10): 1398-402.
47. Nabavi SF, Eslami S, Moghaddam AH, Jafari N, Nabavi SM, Ebrahimzadeh MA. Protective effects of quercetin against sodium fluoride-induced oxidative stress in rat erythrocytes. Toxicol Environ Chem 2011; 93(8): 1666-75.
48. Devi PS, Shyamala DC. Protective effect of quercetin in cisplatin-induced cell injury in the rat kidney. Indian J Pharmacol 1999; 31(6): 422-6.
49. Kahraman A, Erkasap N, Köken T, Serteser M, Aktepe F, Erkasap S. The antioxidative and antihistaminic properties of quercetin in ethanol-induced gastric lesions. Toxicology 2003; 183(1-3): 133-42.
50. Middleton E, Kandaswami C, Theoharides TC. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol Rev 2000; 52(4): 673-751.
51. Nijveldt RJ, Van Nood ELS, Van Hoorn DE, Boelens PG, Van Norren K, Van Leeuwen PA. Flavonoids: a review of probable mechanisms of action and potential applications. Am J Clin Nutr 2001; 74(4): 418-25.
52. Uygur R, Yagmurca M, Alkoc OA, et al. Effects of quercetin and fish n-3 fatty acids on testicular injury induced by ethanol in rats. Andrologia 2014; 46(4): 356-69.
53. Behling EB, Sendão MC, Francescato HD, Antunes LM, Costa RS, Bianchi MDLP. Comparative study of multiple dosage of quercetin against cisplatin-induced nephrotoxicity and oxidative stress in rat kidneys. Pharmacol Rep 2006); 58: 526-32.
54. Çiftçi R. Karaciğer fibrozisli ratlarda kuersetinin homosistein düzeyi ve koroner damar hasarı üzerine etkisi, Fırat Üniversitesi Sağlık Bilimleri Enstitüsü Fizyoloji Anabilim Dalı, Yükseklisans Tezi, Elazığ, Türkiye 2013.
55. Hollman PC, de Vries JH, van Leeuwen SD, Mengelers MJ, Katan MB. Absorption of dietary quercetin glycosides and quercetin in healthy ileostomy volunteers. Am J Clin Nutr 1995; 62(6): 1276-82.
56. Sriraksa N, Wattanathorn J, Muchimapura S, Tiamkao S, Brown K, Chaisiwamongkol K. Cognitive-enhancing effect of quercetin in a rat model of Parkinson's disease induced by 6-hydroxydopamine. Evid Based Complement Alternat Med 2012; 1-9.
57. Chander V, Singh D, Chopra K. Catechin, a natural antioxidant protects against rhabdomyolysis-induced myoglobinuric acute renal failure. Pharmacol Res 2003; 48(5): 503-9.
58. Gul M, Kutay FZ, Temocin S, Hanninen O. Cellular and clinical implication of glutathione. Indian J Exp Biol 2000; 38(7): 625-34.
59. Abarikwu SO, Pant AB, Farombi EO. Dietary antioxidant, quercetin, protects sertoli-germ cell coculture from atrazine-induced oxidative damage. J Biochem Mol Toxicol 2012; 26(11): 477-85.
60. Aguirre L, Arias N, Teresa Macarulla M, Gracia A, Portillo M. Beneficial effects of quercetin on obesity and diabetes. Open Nutraceuticals J 2011; 4(1): 189-98.
61. Nathiya S, Durga M, Devasena T. Quercetin, encapsulated quercetin and its application- a review. Int J Pharm Pharm Sci 2014; 6(10): 20-6.
62. Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. Int J Biomed Sci: IJBS 2008; 4(2): 89-96.
63. Porcu EP, Cossu M, Rassu G, et al. Aqueous injection of quercetin: An approach for confirmation of its direct in vivo cardiovascular effects. Int J Pharm 2018; 541(1-2): 224-33.
64. Ramadan MF, Selim Asker MM. Antimicrobical and antivirial impact of novel quercetin-enriched lecithin. J Food Biochem 2009; 33(4): 557-71.
65. Rauf A, Imran M, Khan IA, et al. Anticancer potential of quercetin: A comprehensive review. Phytother Res 2018; 32(11): 2109-30.
66. Veluri R, Weir TL, Bais HP, Stermitz FR, Vivanco JM. Phytotoxic and antimicrobial activities of catechin derivatives. J Agr Food Chem 2004; 52(5): 1077-82.
67. Gargouri B, Mansour RB, Abdallah FB, Elfekih A, Lassoued S, Khaled H. Protective effect of quercetin against oxidative stress caused by dimethoate in human peripheral blood lymphocytes. Lipids Health Dis 2011; 10(1): 1-4.
68. Bu T, Mi Y, Zeng W, Zhang C. Protective effect of quercetin on cadmium-induced oxidative toxicity on germ cells in male mice. Anat Rec 2011; 294(3): 520-6.
69. Chan P, Cheng JT, Tsai JC, et al. Effect of catechin on the activity and gene expression of superoxide dismutase in cultured rat brain astrocytes. Neurosci Lett 2002; 328(3): 281-4.

70. Kalender Y, Yel M, Kalender S. Doxorubicin hepatotoxicity and hepatic free radical metabolism in rats: the effects of vitamin E and catechin. Toxicology 2005; 209(1): 39-45.
71. Sadowska-Krępa E, Kłapcińska B, Kimsa E, Karpińsk R. Effects of supplementation with red grape skin polyphenolic extract and interval swimming test on the blood antioxidant status in healthy men. Med Sport 2008; 12(1): 1-7.

Correspondence:
Erdil Durukan
School of Physical Education and Sports, Balıkesir University, Balıkesir, Turkey
E-mail: erdurukan@hotmail.com

Progress in Nutrition 2020; Vol. 22: N. 1: 275-280 DOI: 10.23751/pn.v22i1.8746

Original article

The effect of short-term royal jelly supplement on testosterone levels in sedentary and healthy individuals

Ali Muhittin Taşdoğan[1], Zarife Pancar[2], Mustafa Özdal[2], Mehmet Vural[2], Serkan Pancar[3], Yakup Zühtü Birinci[3]

[1]Hasan Kalyoncu University, Health Science Faculty, Gaziantep, Turkey; [2]Gaziantep University, Sport Science Faculty, Gaziantep, Turkey - E-mail: ozdalm@hotmail.com; [3]Bursa Uludağ University, Physical Education and Sport Dept., Bursa, Turkey

Summary. This study with a placebo-controlled experimental design intends to investigate the effect of short-term Royal Jelly (RJ) on the testosterone levels in sedentary men at a dose of 1000 mg/day. For this purpose, a total of 20 adult sedentary men aged 21 to 23 were included in this study. The subjects visited the laboratory every day for 15 days between 08:00 and 10:00 to get their portion of royal jelly. The subjects were randomly divided into two groups, namely the experimental group (n = 10 individuals, 1000 mg/day of Royal Jelly) and the placebo group (n = 10 individuals, corn starch mixed with 1000 mg/day of water) and they took royal jelly in glass vials at the same time. Blood samples were taken from both groups of subjects one day before and one day after the study and analyzed to determine their testosterone levels. 2x2 mixed factor ANOVA and LSD tests were used to analyze data obtained from the experimental and the placebo group. A sharp increase in the testosterone levels of the experimental group that took RJ for a short time was found to be statistically significant ($p<0.05$). The pre- and post-test values of the placebo group were not found to be statistically significant ($p>0.05$). The study shows that a short-term 1000 mg/day dose of RJ supplements was effective in increasing testosterone levels in sedentary, healthy men.

Key words: testosterone, royal jelly, supplement

Introduction

Royal jelly is a special nutrient secreted by the hypopharyngeal and mandibular glands of young worker honey bees. The queen bee and the worker bee have the same genetic structure during hatching. However, dietary differences during the larval period leads to the distinction between the queen and the worker bee. All fertilized bee eggs are fed with royal jelly during the first three days of maturation (1-3). From the third day onwards, larvae fed with a mixture of royal jelly, honey and pollen produce worker bees while larvae fed with pure royal jelly that is a homogenous substance rich in water, and include rich water, proteins, carbohydrates, lipids, vitamins and minerals (4-9), produce queen bees. While the queen bee can produce eggs almost twice its own weight per day with exceptional productivity, worker bees, though female, cannot lay eggs (3). In the literature, the biological and pharmacological effects of royal jelly on humans, animals and culture cells have been studied. Studies that used rats and mice as test subjects to examine the antibacterial, fungal, antiviral and antiparasitic effects of royal jelly have demonstrated estrogenic and gonadotropic effects (10-12), effect on growth and development (13-15), impact on increased life expectancy (16), role in preventing hypoxia and increasing oxygen carrying capacity (17-19), role in increasing fertility in male rabbits, rats and mice (20-23), role in increasing reproductive capacity in sheep and rats (24-27), association with high sperm

quality, increased sperm concentration and motility (28), testicular protective effect (29), role in protecting the autoimmune system (30), preventing inflammation (31, 32), protecting against cancer (33), protecting the cardiovascular system (34, 35), minimizing neural damage, supporting memory (36), as well as antioxidative properties and role in mitigating osteoporosis, protecting the liver and preventing liver damage (18, 37-39). In a similar vein, studies that examined the effects of royal jelly on humans have demonstrated that it reduces fatigue, improves performance (10, 40), has a positive impact on blood parameters with regards to cancer, allergies and wound healing (41-46), causes a decrease in lipid metabolism, prevents cardiovascular occlusion, dilates the veins, regulates the blood pressure (47-50), has an antioxidant effect and protects against radiation (51-54), has positive effects on fertility in both men and women (55). However, there is limited information regarding the effect of short-term using the royal jelly, as 15-day intake, on serum testosterone levels in humans. At this scope, it is considered that the present study has an importance.

It is said that royal jelly has an estrogenic effect and a positive impact on reproduction in humans and animals. We hypothesized that short-term royal jelly intake increases testosterone levels in sedentary and healthy individuals. The purpose of this study was to investigate the effects of royal jelly supplements on the testosterone hormone.

Materials and methods

Participants

The study protocol was approved by the Ethics Committee of Gaziantep University (2017-311) and a voluntary consent form was obtained from all participants before the study. The power analysis was performed for sample size with GPower 3.1. with a priori test protocol.

A total of 20 healthy, sedentary men (Table 1) aged 21 to 23 participated voluntarily in the study. Healthy individuals without a chronic disease who do not smoke and who do not train regularly were included in the study. As per the exclusion criteria, individuals with a chronic disease, who smoke and train regularly were not included in the study. Besides, the reason for our age limitation was for reach to individuals who were in their first adulthood after puberty.

Study Design

This is a study with a placebo-controlled experimental design. A total of 20 male subjects were randomized with the stratified randomization method and divided into two equal groups. The subjects visited the laboratory every day for 15 days between 08:00 and 10:00 to get their portion of royal jelly. The placebo group (n = 10) took corn starch mixed with 1000 mg/day of water in glass vials for 15 days between 08:00 and 10:00, while the experimental group (n = 10) took a 1000 mg/day of pure royal jelly supplement during the same hours. Royal Jelly (Civan, Bee Farm, Bursa) was prepared in 1000 mg glass vials according to cold chain criteria and stored in a refrigerator. The subjects were instructed not to engage in any physical exercise or strenuous physical activity for 15 days.

All types of nutrients taken by the subjects along with their names and the quantities consumed were recorded during one-on-one interviews for seven days. The results of these records were calculated as daily average nutritional values. Energy levels, macronutrients (carbohydrates, fat, protein), micronutrients (vitamins, minerals) and fluid intake of the subjects who were asked to maintain their dietary habits throughout the experiment were analyzed with certain nutrients ruining the dietary balance of the group removed from the program to better monitor their diets. Testosterone hormone levels were measured in 5 ml of blood taken

Table 1. Descriptive parameters of the study subjects

		Mean	SD
Experimental Group (n=10)	**Age**	21.70	1.16
	Height	177.60	6.13
	Weight	71.53	6.42
	BMI	22.69	1.81
Placebo Group (n=10)	**Age**	23.00	1.16
	Height	174.30	6.53
	Weight	70.06	8.88
	BMI	23.08	2.58

SD. standard deviation; BMI. body mass index. There is no significant difference between groups in descriptive parameters ($p>0.05$)

Table 2. Statistical analysis of testosterone levels in the experimental and the placebo group

		Experimental Group (n=10)		Placebo Group (n=10)	
		Mean	**SD**	**Mean**	**SD**
Testosterone (ng/dL)	Pre-test	453.74	81.21	459.93	107.45
	Post-test	510.25[a]	70.50	466.03	108.09
	Difference	56.51[b]	34.59	6.10	45.53
	p (between pre-post tests)	0.003		0.546	
	p (between groups)	0.001			

a. significant difference between the pre-post tests (p<0.05); b. significant difference between the groups (p<0.05); SD. standard deviation

before and after the study.

Measurement of serum testosterone level

One day before and after taking royal jelly supplements, venous blood samples of 5 ml were taken from the right arms of fasting participants between 09:00 and 10:30 in the central laboratory of the Medical Faculty Hospital of Gaziantep University. The samples were placed in tubes with yellow covers. The blood samples collected were centrifuged for seven minutes at 4000 rpm in a Nüve-NF800 device to separate the serum. Serum testosterone levels were measured by electrochemiluminescence immunoassay (Hitachi Cobas 6000) (56).

Anthropometric Measurements

Weight has been measured to the nearest 0.1 kilogram using a digital weight scale. Height has been measured with a wall-mounted digital stadiometer. BMI was calculated as weight in kilograms divided by height in meters squared (57).

Statistical Analysis

SPSS 22.0 was used for statistical analysis. Data were provided in mean and standard deviation. Significance was defined as $p \leq 0.05$. 2x2 mixed factor ANOVA and LSD tests were used to analyze data obtained from the experimental and the placebo group.

Results

The effects of taking royal jelly at a dose of 1000 mg/day for a period of 15 days on testosterone levels are illustrated below in a table and diagram, for both the experimental and the placebo group. The groups consisted 20 subjects and there was no significant difference between groups in terms of descriptive parameters.

The pre-test value of the experimental group was 453.74 ng/dL and the post-test value was 510.25 ng/dL. According to the statistical analysis, the difference between the pre-and post-test values was 56.51 ng/dL. The pre-test value of the placebo group was 459.93 ng/dL and the post-test value was 466.03 ng/dL (Figure 1). According to the statistical analysis, the difference between the pre-and post-test values for the placebo group was 6.10 ng/dL (Table 2). Statistically, the increase between the pre- and post-test for the experimental group was found to be significant at p<0.05 level, whereas the values of the placebo group were not found to be statistically significant (p>0.05). Also a statistical significance was found between the groups in favor of the experimental group (p<0.05).

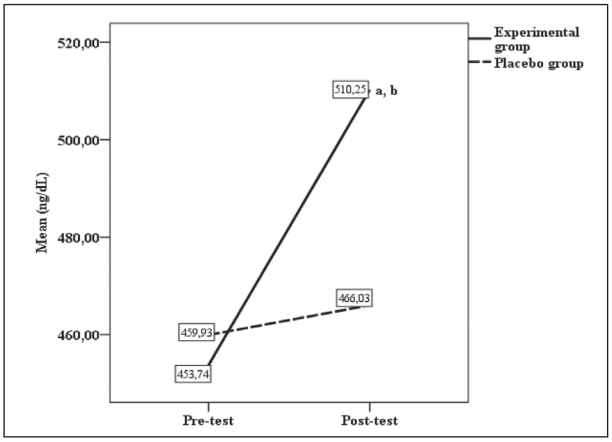


Figure 1. Variation in testosterone levels in the experimental and the placebo group. a, significant difference between the pre-post tests (p<0.05); b, significant difference between the groups (p<0.05).

Discussion

The purpose of this study was to determine the effects of short-term royal jelly supplements on testosterone levels in sedentary and healthy men. The strength of the study has come up with two major findings: [1] The royal jelly supplement taken by the experimental group increased testosterone levels in favor of the post-test ($p<0.05$), and [2] the difference between the pre-post tests was higher in the experimental group compared to the placebo group ($p<0.05$). The results of this study show that royal jelly supplement increases testosterone levels in sedentary and healthy men.

Our study showed an increase in testosterone levels of sedentary men in the experimental group taking royal jelly supplements for 15 days. The literature shows that royal jelly plays a role in increasing sperm count in infertility due to asthenozoospermia (58). Furthermore, royal jelly has been shown to have a positive effect on testosterone levels, live sperm count, ejaculation volume, sperm motility and fructose rate in seminal plasma in male rats (22).

Elnagar used royal jelly to prevent infertility in male rabbits due to temperature related stress. The study concluded that sperm motility, ejaculation volume, sperm concentration, seminal plasma's fructose rate and testosterone levels increased in groups of rabbits given royal jelly at different doses (200, 400, 800 mg/kg) compared to the control group (21). Another study found similarly that giving 100 mg/kg of royal jelly to diabetic rats over a period of six weeks increased testicular weight, sperm count and motility, sperm viability and testosterone levels (59). For women, taking royal jelly regularly has been shown to increase fertility. It was pointed out that this was due to the fact that royal jelly has an estrogenic effect. This estrogenic effect is mainly due to fatty acids in royal jelly. Royal jelly interacts with estrogen receptors via these fatty acids, leading to altered gene expression and cell proliferation (11). It is also argued that royal jelly accelerates oocyte maturation, and increases the fertilization, cleavage and blastocyst rate (60). All studies have confirmed the positive effects of royal jelly on the male reproductive system through experimental studies in animals and humans and through biochemical and histological findings.

In conclusion, intake of short-term royal jelly, which is used in many areas, is effective in increasing testosterone levels in sedentary healthy men. Royal jelly supports the development of bee larval cells and maintains the ovulation capacity, and this increases its possibility of having an important role in fertility. It is assumed that the possible physiological mechanism responsible for this effect works through polyunsaturated fatty acids and phospholipids contained in royal jelly protecting the sperm cell membrane from oxidative damage, interacting with testosterone receptors, causing cell proliferation and increasing testosterone levels.

Acknowledgments

There is no conflict of interest between the authors.

Limitations

The present study constructed on short-term intake of royal jelly. In order to obtain detailed data on this short-term intake, the limitation of our study is that three or more repeated measurements of the testosterone level were not taken. It could be suggested that daily or every two days measurements of serum testosterone level in order to sensitive determination for further research.

References

1. Stocker A, Schramel P, Kettrup A, and Bengsch E. Trace and mineral elements in royal jelly and homeostatic effects. Journal of Trace Elements in Medicine and Biology 2005; 19(2-3): 183-189.
2. Viuda-Martos M, Ruiz-Navajas Y, Fernández López J, and Pérez Álvarez JA. Functional properties of honey, propolis, and royal jelly. Journal of Food Science 2008; 73(9).
3. Kubo T, Sasaki M, Nakamura J, Sasagawa H, Ohashi K, Takeuchi H, and Natori S. Change in the expression of hypopharyngeal-gland proteins of the worker honeybees (Apis mellifera L.) with age and/or role. The Journal of Biochemistry 1996; 119(2):291-295.
4. Takenaka T, Yatsunami K, Echigo T. Changes in quality of royal jelly during storage. Nippon Shokuhin Kogyo Gakkaishi, 1986; 33(1): 1-7.
5. Howe SR, Dimick PS, and Benton AW. Composition of freshly harvested and commercial royal jelly. Journal of Apicultural Research 1985; 24(1): 52-61.
6. Lercker G, Capella P, Conte LS, Ruini F. Components

of royal jelly: I. Identification of the organic acids. Lipids 1981;16(12): 912-9.
7. Herbert EW, Graham JM. The hive and the honey bee. 1992; 197-233.
8. Boselli E, Caboni MF, Sabatini AG, Marcazzan GL, and Lercker G. Determination and changes of free amino acids in royal jelly during storage. Apidologie 2003; 34(2): 129-137.
9. Guo H, Kouzuma Y, and Yonekura M. Structures and properties of antioxidative peptides derived from royal jelly protein. Food Chemistry 2009; 113(1), 238-245.
10. Hinglais H, Hinglais M, Gautherıe J. Hormonal study of royal jelly; research on the gonadotrophic principles and substances of estrogenic action. Comptes rendus des Séances del' Academiedes Sciences 1956; 242 (20): 2482-2483.
11. Mıshıma S, Suzukı K M, Isohama Y, Kuratsu N, Arakı Y, Inoue M, Mıyata T. Royal jelly has estrogenic effects in vitro and in vivo. Journal of Ethnopharmacology 2005; 101 (1-3): 215-220.
12. Bonomi A. La gelatina reale nell'alimentazione dei suini in fase di svezzamento. Rivista di Suinicoltura 2001; 42 (4): 183-188.
12. Suzuki KM, Isohama Y, Maruyama H, Yamada Y, Narıta Y, Ohta S, Arakı Y, Mıyata T, Mıshıma S. Estrogenic activities of fatty acids and a sterol isolated from royal jelly. Evidence-Based Complementary and Alternative Medicine 2008; 5 (3): 295-302.
13. Bonomi A, Bonomı BM, Quarantellı A. Royal jelly in turkey feeding; Royal jelly in guinea-fowl feding. Rivista di Scienza dell'Alimentazione 2001; 30 (1): 49-60.
15. Dawson H, Rubottom R, Harrıs, L. The effect of administration of royal jelly on the differentiation and growth of newborn rats. The Anatomical record 1962; 142: 123-129.
16. Honda Y, Fujita Y, Maruyama H, Arakı Y, Ichıhara K, Sato A, Kojıma T, Tanaka M, Nozawa Y, Ito M, Honda S. Lifespan-Extending Effects of Royal Jelly and Its Related Substances on the Nematode Caenorhabditis elegans. Plos One 2011; 6 (8).
17. Gonnard P, N'guyen CC. Action of royal jelly on oxygen consumption in tissues in vitro. Annales pharmaceutiques francaises 1957; 15(6): 383-393.
18. Krylov V, Sokolskıı C. Royal jelly (in Russian). Agroprompoligrafist Krasnodar; 2000; pp.214.
19. Pejtscheff P, Belewa-Stajkowa P, Atanassoff N. Einfluss des Weiselfuttersaftes auf den Sauerstoffverbrauch und die Aktivität der Adenosintriphosphatase in den Geweben weisser Mäuse Der XXV. Internationale Bienenzüchterkongress Grenoble - Frankreich 1975, Apimondia-Verlag; Bukarest, Rumänien; pp 248-251.
20. El-Hanoun AM, Elkomy AE, Fares WA, Shahıen E. Impact of royal jelly to improve reproductive performance of male rabbits under hot summer conditions. World Rabbit Science 2014; 22: 241-248.
21. Elnagar SA. Royal jelly counteracts bucks'"summer infertility". Animal reproduction science 2010; 121(1-2), 174-180.
22. Hassan A. Effect of royal jelly on sexual efficiency in adult male rats. Iraq J Vet Sci 2009; (23)155-160.
23. Kohguchı M, Inoue S, Ushıo S, Iwakı K, Ikeda M, Kurımoto M. Effect of royal jelly diet on the testicular function of hamsters. Food Science and Technology Research 2004; 10 (4): 420-423.
24. Hüsein MQ, Haddad SG. A new approach to enhance reproductive performance in sheep using royal jelly in comparison with equine chorionic gonadotropin. Animal Reproduction Science 2006; 93 (1-2): 24-33.
25. Hüsein MQ, Krıdlı RT. Reproductive responses following royal jelly treatment administered orally or intramuscularly into progesterone-treated Awassi ewes. Animal Reproduction Science 2002; 74 (1-2): 45-53.
26. Kridli RT, Al Khetib SS. Reproductive responses in ewes treated with eCG or increasing doses of royal jelly. Animal Reproduction Science 2006; 92 (1-2): 75-85.
27. Kridli RT, Husein MQ, Humphrey WD. Effect of royal jelly and GnRH on the estrus synchronization and pregnancy rate in ewes using intravaginal sponges. Small Ruminant Research 2003; 49 (1):25-30.
28. Shahzada Q, Mehmoodb M, Khanc H, Husnad A, Qadeerd S, Azamd A, Naseere Z, Ahmadf E, Safdarg M, Ahmadb M. Royal jelly supplementation in semen extender enhances post-thaw quality and fertility of Nili-Ravi buffalo bull sperm. Animal Reproduction Science 2016; 167: 83-88.
29. Delkhoshe-Kasmaıe F, Malekınejad H, Khoramjouy M, Rezaeı-Golmısheh A, Janbazeacyabar H. Royal jelly protects from taxol-induced testicular damages via improvement ofantioxidant status and up-regulation of E2f1. Systems Biology in Reproductive Medicine 2014; 60 (2): 80-88.
30. Mannoor MK, Tsukamoto M, Watanabe H, Yamaguchı K, Sato Y. The Efficacy Of Royal jelly in the restoration of stress-induced disturbance of lymphocytes and granulocytes. Biomedical Research-India 2008; 19 (2): 69-77.
31. Arzı A, Olapour S, Yaghootı H, Karampour N. Effect of Royal Jelly on Formalin Induced-Inflammation in Rat Hind Paw. Jundishapur J Nat Pharm Prod 2015; 10(1): e22466.
32. Kohno K, Okamoto I, Sano O, Arai N, Iwakı K, Ikeda M, Kurımoto M. Royal Jelly inhibits the production of pro inflammatory cytokines by activated macrophages. Bioscience Biotechnology and Biochemistry 2004; 68 (1): 138-145.
33. Mannoor MK, Shımabukuro I, Tsukamotoa M, Watanabe H, Yamaguchı K. Honeybee royal jelly inhibits autoimmunity in SLE-prone NZB x NZW F1 mice. Lupus 2009; (1): 44-52.
34. Chauvın R. Action physiologique et therapeutique des produits de la ruche Traite de biologie de l'abeille 1968; Masson Paris; pp 116-154.
35. Chauvın R. La ruche et l'homme. Calmann-Lévy 1987, France.
36. Pyrzanowska J, Pıechal A, Blecharz-Klın K, Graıkou K, Wıdy-Tyszkıewıcz E, Chınou I. Chemical analysis of Greek royal jelly - Its influence of the long-term administration on spatial memory in aged rats. Planta medica 2012; 78 (11): 1248.

37. Kamakura M. Signal transduction mechanism leading to enhanced proliferation of primary cultured adult rat hepatocytes treated with royal jelly 57kDa protein. Journal of Biochemistry 2002; 132 (6): 911-919.
38. Kamakura M, Mıtanı N, Fukuda T, Fukushıma M. Antifatigue effect of fresh royal jelly in mice. Journal Of Nutrıtıonal Scıence And Vıtamınology 2001; 47 (6): 394-401.
39. Oka H, Emorı Y, Kobayashı N, Hayashı Y, Nomoto K. Suppression of allergic reactions by royal jelly in association with the restoration of macrophage function and the improvement of Th1/Th2 cell responses. International immunopharmacology 2001; 1(3): 521-532.
40. Kamakura M, Suenobu N, Fukushıma M. Fifty-seven-kDa protein in royal jelly enhances proliferation of primary cultured rat hepatocytes and increases albumin production in the absence of serum. Biochemical and Biophysical Research Communications 2001; 282 (4): 865-874.
41. Al-Mufarrej SI, El-Sarag MSA. Effects of royal jelly on the humoral antibody response and bloodchemistry of chickens. Journal of Applied Animal Research 1997; 12 (1): 41-47.
42. Gasic S, Vucevic D, Vasilijic S, Antunovic M, Chinou I, and Colic M. Evaluation of the immunomodulatory activities of royal jelly components in vitro. Immunopharmacology and Immunotoxicology 2007; 29(3-4), 521-536.
43. Kurkure NV, Kognole SM, Pawar SP, Ganorkar AG, Bhandarkar AG, Ingle VC, Kalorey DR. Effect of royal jelly as immunonomodulator in chicks. Journal of Immunology & Immunopathology 2000; 2(1/2): 84-87.
44. Vucevic D, Melliou E, Vasilijic S, Gasic S, Ivanovskı P, Chinou I, Colic M. Fatty acids isolated from royal jelly modulate dendritic cell-mediated immune response in vitro. International immunopharmacology 2007; 7 (9): 1211-1220.
45. Yamada K, Ikeda I, Sugahara T, Shırahata S, Murakamı H. Screening of immunoglobulin production stimulating factor (IPSF) in foodstuffs using human-human hybridoma HB4C5 cells 784. Agricultural and Biological Chemistry 1989; 53(11): 2987-2991.
46. Watanabe K, Shınmoto H, Koborı M, Tsushıda T, Shınohara K, Kanaeda J, Yonekura M Growth stimulation with honey royal jelly DIII protein of human lymphocytic cell lines in a serum-free medium. Biotechnology Techniques 1996 ; 10(12): 959-962.
47. Cho YT. Studies on royal jelly and abnormal cholesterol and triglycerides. American Bee Journal 1977; 117: 36-38.
48. Kuzina NG. Action of apilak on the electrolyte and catecholamine content of the wall of arterial vessels at different levels of systemic arterial pressure. Farmakologiia i Toksikologiia 1987; 50 (3): 46-49.
49. Ragab SS, Ibrahim MK. Evaluation of some chemical, antibacterial and biological properties of fresh and refrigerated royal jelly. Egyptian Journal of Microbiology 1999; 34 (1): 115-128.
51. Giordano A, Trenta A, Mazza L. Research on the eventual radioprotective action of royal jelly on the mouse. Experimental contribution. Radiobiologia, Radioterapia e Fisica Medica 1959;14: 423-439.
50. Shen X, Lu R, He G. Effects of lyophilized royal jelly on experimental hyperlipaemia and thrombosis. Zhonghua Yufang Yixue Zazhi 1995; 29 (1): 27-29.
52. Park HM, Cho MH, Cho Y, Kım SY. Royal Jelly Increases Collagen Production in Rat Skin After Ovariectomy. Journal of Medicinal Food 2012; 15 (6): 568-575.
53. Pejcev P, Bojadzıev S, Marovskı T. The Influence Of Royal Jelly On The Course Of Radıatıon Sıckness In White Rats. Folia Med 1965; Plovdiv 48: 69-73.
54. Wagner H, Dobler I, Thıem I. Effect of food-juice of the queen bee (royal jelly) on the peripheral blood and the survival rate of mice after whole body x-irradiation. Radiobiol Radiother 1970; (Berl) 11 (3): 323-328.
55. Al-Masrı A. The royal jelly. Honeybee kingdom and its derivaton, In Bartolome JAALFMP (ed.) Arabic Book House Publishers 2011; pp.291-300.
56. Shiota M, Fujimoto N, Takeuchi A, Kashiwagi E, Dejima T, Inokuchi J, Tatsugami K, Yokomizo A, Kajioka S, Uchiumi T, Eto M. The association of polymorphisms in the gene encoding gonadotropin-releasing hormone with serum testosterone level during androgen deprivation therapy and prognosis of metastatic prostate cancer. The Journal of urology. 2018; 199(3): 734-40.
57. Dobranowski K, Lloyd M, Côté P, Balogh R. Validity of proxy-reported height and weight to derive body mass index in adults participating in Special Olympics. Journal of Applied Research in Intellectual Disabilities. 2018; 31: 136-43.
58. Abdelhafiz AT, Muhamad JA. Midcycle pericoital intravaginal bee honey and royal jelly for male factor infertility. International Journal of Gynecology & Obstetrics 2008; 101(2), 146-149.
(59. Ghanbari E, Nejati V, Najafi G, Khazaei M, Babaei M. Study on the effect of royal jelly on reproductive parameters in streptozotocin-induced diabetic rats. International journal of fertility & sterility. 2015; 9(1): 113-120.
60. Eshtiyaghi M, Deldar H, Pirsaraei ZA and Shohreh B. Royal jelly may improve the metabolism of glucose and redox state of ovine oocytes matured in vitro and embryonic development following in vitro fertilization. Theriogenology 2016; 86(9). 2210-2221.

Correspondence:
Mustafa Özdal
Gaziantep University, Sport Science Faculty,
Gaziantep, Turkey
E-mail: ozdalm@hotmail.com

Progress in Nutrition 2020; Vol. 22, N. 1: 281-287 DOI: 10.23751/pn.v22i1.8747

Original article

Effect of royal jelly supplementation on aerobic power output and anaerobic power output

Ali Muhittin Taşdoğan[1], Mehmet Vural[2], Mustafa Özdal[2], Zarife Pancar[2]

[1]Hasan Kalyoncu University, Health Science Faculty, Gaziantep, Turkey; [2]Gaziantep University, Sport Science Faculty, Gaziantep, Turkey - E-mail: mozdalm@hotmail.com

Summary. This study with a placebo-controlled experimental design intends to investigate the effect of Royal Jelly (RJ) on the aerobic and anaerobic power output of sedentary men at a dose of 1000 mg/day. For this purpose, a total of 20 adult sedentary men aged 21 to 23 years were included in this study. The subjects visited the laboratory every day for 15 days between 08:00 and 10:00 to get their portion of royal jelly. The subjects were randomly divided into two groups, namely the experimental group (n = 10 individuals, 1000 mg/day Royal Jelly) and the placebo (n = 10 individuals, corn starch mixed with 1000 mg/day water) group and they took royal jelly in glass bottles at the same hour during these visits. In both groups, aerobic and anaerobic power measurements were performed in the laboratory one day before and after the 15-day period. 2×2 mixed factor ANOVA and LSD tests were used to analyze data obtained from the experimental and the placebo group. No significant difference was found in the analysis performed between the pre- and post tests for anaerobic power outputs of the placebo group ($p > 0.05$). Concerning the anaerobic power output of the experimental group, there was a significant difference in the fatigue index value in favor of the post-test ($p < 0.05$). The intergroup analysis of the difference between pre- and post-test in the same parameter showed a significant difference in favor of the experimental group ($p < 0.05$). In the pre-test and post-test analysis of the aerobic power outputs of the placebo group, no significant difference was found ($p > 0.05$). A significant difference was found in the pre- and post-test analysis of the experimental group in favor of the post-test in terms of aerobic power output ($p < 0.05$). The intergroup analysis of the difference between pre- and post-test showed a significant difference in favor of the experimental group in terms of aerobic power outputs ($p< 0.05$). Consequently, it can be argued that royal jelly supplementation taken daily for 15 days at 1000 mg has a positive effect on the aerobic capacity of sedentary men.

Key words: Supplement, performance, power

Introduction

Royal jelly, a sticky, jelly-like matter, is excreted from the glands (hypopharyngeal and mandibular) of bees. This secreted substance is used to feed queen bees and larvae. The color of this nutrient ranges from light cream to dark yellow. Royal jelly is acidic and has a pungent taste (1). Royal jelly impacts the organism in many ways. Studies have shown positive effects on blood parameters (2).

Studies on the physiological effects of royal jelly in rats and mice have shown positive results. Many studies show it helps with suppressing humoral immunity in rats, stimulating proliferation of immune component cells and antibody production in mice (3), raising hematopoietic stem cell production (4) and lowering cholesterol levels (5). It has also been reported that it has a positive effect on skeletal muscle weight, muscle strength and recovery of injured muscle groups (6).

Studies in humans have revealed the positive effects of royal jelly. One study reported that taking 6 g of royal jelly per day for a period of 4 weeks lowered LDL cholesterol levels (7*)*. In addition, royal jelly has been acknowledged for its anti-aging properties (8, 9). It has been shown that it has a positive influence on the endurance of athletes (10), chronic fatigue and vagosympathetic balance of swimmers, regulation of heart rhythm and energy deficiency (11), regulation of the fat-muscle ratio and the body mass index in footballers (12). Another study has shown that royal jelly increases physical performance and dilates blood vessels (13). In a study on elderly people, royal jelly has been shown to have a positive effect on muscle strength and physical performance (6-minute walking test) (14).

Taking into account the contribution of royal jelly to animals and humans, the hypothesis is posited that controlled use of royal jelly supplements can have positive effects on aerobic and anaerobic power. The present study is important since the studies on the effect of royal jelly on athletic performance in general, including aerobic and anaerobic power, are not comprehensive enough in scope. The aim of this study is to investigate the effects of royal jelly supplements on aerobic and anaerobic power outputs in sedentary male subjects, taking into account the positive effects of royal jelly on human health in line with the abovementioned information and studies.

Materials and Methods

Experimental Design

This is a study with a placebo-controlled experimental design. A total of 20 male subjects were randomized with the stratified randomization method and divided into two equal groups. The subjects visited the laboratory every day for fifteen days between 08:00 and 10:00 to get their portion of royal jelly. The placebo group (n = 10) took corn starch mixed with 1000 mg/day water in glass vials for 15 days between 08:00 and 10:00 on an empty stomach, while the experimental group (n = 10) took a 1000 mg/day pure Royal Jelly supplement during the same hours. They did not drink anything before royal jelly intake. Royal Jelly (Civan, Bee Farm, Bursa) was prepared in 1000 mg glass vials according to cold chain criteria and stored in a refrigerator. The subjects were instructed not to engage in any physical exercise or strenuous physical activity for 15 days. The groups carried out royal jelly intake only in the morning. At the other time of the day, they carried on a normal diet. Subjects did not receive any energy drink or coffe before measurement. Royal jelly was kept in the freezer and given to the subjects.

All types of nutrients taken by the subjects along with their names and the quantities consumed were recorded during one-on-one interview for 7 days. The results of these records were calculated as daily average nutritional values. Energy levels, macronutrients (carbohydrates, fat, protein), micronutrients (vitamins, minerals) and fluid intake of the subjects who were asked to maintain their dietary habits throughout the experiment were analyzed with certain nutrients ruining the dietary balance of the group removed from the program to better monitor their diets. One day before and one day after the study, the subjects underwent measurements in a lab environment to determine the aerobic and anaerobic powers of the experimental group and the placebo group with everyone participation in the groups.

Subjects

A total of 20 healthy sedentary men (Table 1) aged 21 to 23 participated voluntarily in the study. Healthy individuals without a chronic disease who do not smoke and who do not train regularly were included in the study. As per the exclusion criteria, patients with chronic disease, who smoke and train regularly were not included in the study. The study protocol was approved by the Ethics Committee of Gaziantep University (2017-311) and a voluntary consent form was obtained from all participants before the study.

Determination of aerobic power outputs in an exercise with increased load

The aerobic power outputs of the subjects were measured directly with an ergoline bicycle (Sana Bike 450F, Ergosana GMBH, Bitz, Germany) and an ergospirometer (Figure 14, Ergo100 PFT Systems, Medical Electronic Construction R&D, Brussel, Belgium).

The measurement was carried out by using the "breath by breath" method on ergospirometer during

Table 1. Descriptive parameters of the study subjects

Variables	Experimental Group (n=10)		Placebo Group (n=10)	
	M	SD	M	SD
Age	21.70	1.16	23.00	1.16
Height	177.60	6.13	174.30	6.53
Weight	71.53	6.42	70.06	8,88
BMI	22.69	1.81	23.08	2.58

exercise with increased load to determine the amount of O_2-CO_2 in the expiratory air. Heart rate, O_2 saturation and blood pressure data were recorded during exercise by making subjects wear an O_2 saturation probe and an arm manometer before the test. At the beginning of the test, the bicycle pedal load was set to 50 watts and the test was continued with an increase of 25 watts per minute. During the test, the subject had a bike screen as reference and tried to pedal at 60 rpm. When the subject realized that he could no longer continue, the exercise was stopped after checking the Respiratory Exchange Rate (RER) value (15). Peak oxygen consumption (VO_{2PEAK}), relative peak oxygen consumption (rVO_{2PEAK}), workload VO_2 ratio ($\Delta VO2/\Delta WR$) minute ventilation (VE_{PEAK}), respiratory rate (RR_{PEAK}), peak carbon dioxide release (VCO_{2PEAK}) and the respiratory exchange rate (RER_{PEAK}) was measured as aerobic power output parameters.

Determination of the anaerobic power outputs with the Wingate anaerobic power test

The Wingate test protocol with a bicycle ergometer with scale (894E Peak Bike, Monark Exercise AB, Vansbro, Sweden) was used to determine the anaerobic power outputs. Subjects did warm-up exercises for ten minutes before starting the test. Weights corresponding to 7.5% of the subject's body weight were put on the scales of the bicycle. The subject was told he could start the test at any time by pressing the button controlling the scale. When the subject felt ready, he pressed the button that controlled the scales to lower the weight on the scales to put more weight on the pedals. From that moment on, he cycled with maximum effort for 30 seconds. The test was ended when the time was out (16). Anaerobic power output parameters were measured as in peak power (PP), relative peak power (rPP), average power (AP), relative average power (rAP), minimum power (MP), relative minimum power (rMP), fatigue index (FI) and time to peak (TTP).

Royal Jelly

Freshness has been attributed a great importance for RJ quality. Royal jelly can be spoiled easily if not properly stored. Immediately after harvest it should be placed in dark vessel and stored 0 - 5°C. Stored under these conditions its quality remains OK for half an year. Deterioration of royal jelly can be prevented by storing RJ in Argon after harvesting (19). After longer storage it will turn rancid. Frozen royal jelly can be lyophilised as it can be transported more easily in the dry state. If frozen, it can be stored for 2-3 years without loosing of its quality. Chauvin states that the physical properties of RJ change after 20 hours after harvest, if left at ambient temperature (29).

Statistical Analysis

SPSS 20 package program was used to analyze the data obtained from the study (SPSS Inc., Chicago, IL, USA). Data were presented as mean, standard deviation values. The Shapiro-Wilk test was used for the normality test. 2×2 mixed factor ANOVA and LSD tests were used to analyze data obtained from the groups. The level of significance was accepted as $p < 0.05$.

Results

Table 2 illustrates the analysis of participants' aerobic measurements between pre-test and post-test. As a result of the analysis, no significant difference was found between the pre- and post-test values of the placebo group ($p > 0.05$). In the analysis for the

Table 2. Analysis of participants' aerobic measurements between pre-test and post-test

M		Experimental Group (n = 10)		Placebo Group (n = 10)	
		SD	M	SD	
VO_{2PEAK} (L/min)	Pre-test	2.46	1.35	1.74	0.64
	Post-test	2.96[a]	0.64	1.61	0.51
	Difference	0.49[b]	1.13	-0.13	0.49
rVO_{2PEAK} (ml/kg/min)	Pre-test	34.85	19.77	25.17	10.77
	Post-test	41.40[a]	7.52	22.69	6.16
	Difference	6.55[b]	15.80	-2.48	7.93
VO_2/ WR ($mlO_2.min^{-1}.W^{-1}$)	Pre-test	11.50	6.08	10.62	4.17
	Post-test	11.30	2.88	9.78	3.49
	Difference	-0.20	5.60	-0.84	3.16
VE_{PEAK} (L/min)	Pre-test	47.19	28.30	35.29	10.71
	Post-test	81.71[a]	27.04	32.97	7.86
	Difference	34.52[b]	21.55	-2.32	8.73
RR_{PEAK} (breath/min)	Pre-test	36.40	8.98	38.30	12.55
	Post-test	42.30[a]	6.09	34.00	10.84
	Difference	5.90[b]	8.44	-4.30	10.81
VCO_{2PEAK} (L/min)	Pre-test	2.39	1.35	1.70	0.57
	Post-test	3.31[a]	0.87	1.55	0.44
	Difference	0.91[b]	1.05	-0.15	0.54
RER_{PEAK} (VCO_2/VO_2)	Pre-test	1.05	0.12	1.09	0.09
	Post-test	1.16[a]	0.09	1.04	0.07
	Difference	0.113[b]	0.14	-0.05	0.11

SD-standard deviation, VO_{2PEAK}-peak oxygen uptake, rVO_{2PEAK}-relative peak oxygen uptake, ΔVO2/ΔWR-oxygen uptake to work rate slope, VE_{PEAK}-peak minute ventilation, RR_{PEAK}-peak respiratory rate, VCO_{2PEAK}-peak carbon dioxide output, RER_{PEAK}-peak respiratory exchange ratio. a-significant difference between pre- and post-tests of group, b-significant difference between the experimental and placebo groups.

experimental group between pre-test and post-test values in aerobic measurements, there was a meaningful difference in favor of the post-test concerning the VO_{2PEAK}, rVO_{2PEAK}, VE_{PEAK}, RR_{PEAK}, VCO_{2PEAK} and RER_{PEAK} values ($p < 0.05$). Concerning the VO_{2PEAK}, rVO_{2PEAK}, VE_{PEAK}, RR_{PEAK}, VCO_{2PEAK} and RER_{PEAK} values, a significant difference was found in favor of the experimental group in the intergroup comparison of the differences between the pre-test and post-test ($p < 0.05$).

Table **3** illustrates the analysis of participants' anaerobic measurements between pre-test and post-test. As a result of the analysis, no significant difference was found between the pre- and post-test values of the placebo group concerning anaerobic measurement results ($p > 0.05$). In the analysis of the anaerobic measurements of the experimental group, a significant difference was found concerning the FI value in favor of the final test ($p < 0.05$). In the intergroup analysis of the difference between pre-test and post-test of the measurements, a significant difference was found in favor of the experimental group concerning the FI value ($p < 0.05$).

Discussion and Conclusion

This study investigated the effect of a daily dose of 1000 mg royal jelly on the aerobic and anaerobic power of sedentary men. Immediately after the preliminary measurements before starting the royal jelly supplements, the 15-day long process for royal jelly supplements was initiated. At the end of the process, the final measurements were taken to evaluate the aerobic and anaerobic performance of the individuals. Our study

Table 3. Analysis of participants' anaerobic measurements between pre-test and post-test

M		Experimental Group (n = 10)		Placebo Group (n = 10)	
		SD	M	SD	
PP (W)	Pre-test	727.97	139.67	695.99	123.56
	Post-test	730.05	132.77	728.52	125.70
	Difference	2.078	52.54	32.53	79.44
rPP (W/kg)	Pre-test	10.14	1.65	9.84	1.65
	Post-test	10.25	1.52	10.30	1.64
	Difference	0.12	0.78	0.46	1.11
AP (W)	Pre-test	507.58	77.48	471.50	69.95
	Post-test	509.29	71.56	488.96	78.39
	Difference	1.71	31.67	17.46	22.31
rAP (W/kg)	Pre-test	7.07	0.80	6.65	0.86
	Post-test	7.16	0.71	6.89	0.80
	Difference	0.09	0.40	.239	0.28
MP (W)	Pre-test	269.75	33.61	247.04	43.07
	Post-test	293.81	46.46	240.97	94.82
	Difference	24.05	50.60	-6.07	83.31
rMP (W/kg)	Pre-test	3.78	0.52	3.48	0.55
	Post-test	4.15	0.68	3.39	1.27
	Difference	0.37	0.74	-0.09	1.18
FI (%)	Pre-test	62.24	5.43	64.04	5.73
	Post-test	59.23[a]	6.39	66.85	11.63
	Difference	-3.01[b]	4.94	2.816	8.84
TTP (msec)	Pre-test	3.01	1.39	2.90	1.56
	Post-test	2.66	1.59	3.07	1.29
	Difference	-0.353	1.45	0.17	1.67

SD-standard deviation, PP-peak power, rPP-relative peak power, AP-average power, rAP-relative average power, MP-minimum power, rMP-relative minimum power, FI-fatigue index, TTP-time to peak. a-significant difference between pre- and post-tests of group, b-significant difference between the experimental and placebo groups.

came up with two major findings. Firstly, Royal Jelly was positively affect the aerobic power], and secondly anaerobic power values. In the analysis of the data of the experimental group, a statistically significant difference was found in the parameters VO_2, relative VO_2, VE, RR, VCO_2 and RER in favor of the final test in the analysis of the difference between the pre- and post-test in aerobic measurements. In the anaerobic measurements of the experimental group, a significant difference was found concerning the FI value in favor of the final test. According to the results obtained by this study, using royal jelly supplements for 15 days can have a positive effect on aerobic and anaerobic power in sedentary men.

Previous studies have shown that royal jelly has beneficial effects on animals and humans. Due to its easy applicability and the convenience it affords with regards to monitoring of the results, the positive effect of royal jelly on animals in a clinical setting has been demonstrated in almost all studies. It is assumed that the positive results from animal experiments may also apply to the human organism. Research on humans has shown that royal jelly intake has contributed positively to physical performance **(in elders),** a strong memory (13,17), overall health, insomnia, vigor, heart health and vascular ailments (18), renewal of energy spent, faster recovery from fatigue (19), physical endurance and energy efficiency **(in athletes)** (11), reducing high choles-

terol levels (7), and posture and body mass index (12). Researchers also found that royal jelly has a positive effect on the body of **footballers**; and contributes to the growth and development of the body (20, 21).

Studies have shown that royal jelly has positive effects on physical performance (14), muscle strength and the fat-muscle ratio (12). Aerobic power is adversely affected by the increase in lipid hydroperoxides in the blood (22). As an antioxidant, royal jelly can inhibit lipid peroxidation (23). A previously conducted study revealed that royal jelly supplementation stimulates lipid peroxidation and inhibits its increase. After the supplementation, a significant difference was obtained by ensuring a decrease in the amount of lipid peroxidation (10). Based on the results of this study, it can be assumed that in our study, a certain part of the increase in aerobic power may be due to this factor.

Anaerobic power is affected by a number of hormonal changes in the organism. It has been shown that individuals with high values of total testosterone and androstenedione have low fat in their bodies and a high bone density and that such individuals have high performance values and a maximum oxygen consumption capacity (24). Testosterone can directly stimulate glycogen synthesis, and a previous study has shown that circulating testosterone levels increase during short-term intensive exercise (25). Since royal jelly increases testosterone production (26, 27, 28), in our study, the effect of royal jelly on testosterone can be put forward as the physiological basis of the increase in the fatigue index value that occurs in anaerobic power.

It can thus be argued that royal jelly taken at a dose of 1000 mg for 15 days has positive effects on aerobic and anaerobic capacity in sedentary healthy male subjects. On the basis of the research results, it can be argued that the increase in the aerobic and anaerobic values of the subjects is due to the positive effects of royal jelly on physical performance, overall health and fatigue, and to the presence of highly nutritious components in royal jelly.

References

1. Takenaka T, Takashi E. General chemical composition of the royal jelly. Bull Fac Agr Tamagawa Univ 1980; 20: 71-9.
2. Krylov V, Sokolskıı C. Royal jelly. Agroprompoligrafist, Krasnodar, Russia 2000; 214.3. Sver L, Orsolic N, Tadic Z, Njari B, Valpotic I, Basic I. A royal jelly as a new potential immunomodulator in rats and mice. Comp Immunol Microbiol Infect Dis 1996; 19: 31- 38.
4. Okamoto I, Taniguchi Y, Kunikata T, Kohno K, Iwaki K, Ikeda M, Kurimoto M. Major royal jelly protein 3 modulates immune responses in vitro and in vivo. Life Sci 2003; 5: 2029-2045.
5. Taniguchi Y, Kohno K, Inoue S, Koya-Miyata S, Okamoto I, Arai N, Iwaki K, Ikeda M, Kurimoto M. Oral administration of royal jelly inhibits the development of atopic dermatitis-like skin lesions in NC/Nga mice. International immune pharmacology 2003; 3 (9): 1313-24.
6. Niu KJ, Guo H, Guo YT, Ebihara S, Asada M, Ohrui T, Furukawa K, Ichinose M, Yanai K, Kudo Y, Arai H, Okazaki T, Nagatomi R. Royal jelly prevents the progression of sarcopenia in aged mice in vivo and in vitro. Journals of Gerontology Series A-Biological Sciences and Medical Sciences 2013; 68 (12): 1482-92.
7. Guo H, Saiga A, Sato M, Miyazawa I, Shibata M, Takahata Y, Morimatsu F. Royal jelly supplementation improves lipoprotein metabolism in humans. Journal of Nutritional Science and Vitaminology 2007; 53 (4): 345-348.
8. Ramadan MF, Al-Ghamdi A. Bioactive compounds and health-promoting properties of royal jelly: A review. Journal of Functional Foods 2012; 4:39-52.
9. Fratini F, Cilia G, Mancini S, Felicioli A. Royal Jelly: An ancient remedy with remarkable antibacterial properties. Microbiological Research 2016; 192: 130-141.
10. Krylov V, Agafonov A, Krıvtsov N, Lebedev V, Burımıstrova L, Oshevenskı L, Sokolskı S. Theory and agents of apitherapy (in Russian. 2007, Moscow.
11. Krylov V, Kopylova S, Kuznetsova C, Ovchınnıkov N. The Effects of royal jelly and ubıquınone-10 on heart rate varıabılıty of qualıfied swımmers durıng physıcal exertıon. Theory and Practice of Sport 2015; 1: 23-26.
12. Joksimovic A, Stankovic D, Joskimovic I, Molnar S, Joksimovic S. Royal jelly as supplement for young football players. Sport Science 2011; 1: 62-67.
13. Haddadin MS, Haddadin J, Benguiar R. The effect of royal jelly on growth and short-chain fatty acid production of probiotic bacteria and activity of bacterial procarcinogenic enzymes in rat faeces. Polish Journal of Food and Nutrition Sciences 2012; 62: 251-258.
14. Meng G, Wang H, Pei Y, Li Y, Wu H, Song Y, Guo Q, Guo H, Fukushima S, Tatefuji T, Wang J, Du H, Su Q, Zhang W, Shen S, Wang X, Dong R, Han P, Okazaki T, Nagatomi R, Wang J, Huang G, Sun Z, Song K, Niu K. Effects of protease-treated royal jelly on muscle strength in elderly nursing home residents: A randomized, double-blind, placebo controlled, dose-response study. Scientific Report 2017; 7: 11416: 1-9.
15. Buchfuhrer MJ, Hansen JE, Robinson TE, Sue DY, Wasser KA, Whipp BJ. Optimizing the exercise protocol for car- 342 diopulmonary assessment. J Appl Physiol 1983; 55: 1558-64.
16. Oded Bar-Or. The wingate anaerobic test an update on

methodology, reliability and validity. Sport Medicine 1987; 4(6): 381-394.
17. Shkenderov S, Ivanov T. Pcelni Produkti, The Bee Products (in Bulgarian). Zemizdat (Abstract in Honey bibliography) 1983; 1-238.
18. Pavero A, Cavıglıa E. Royal jelly and its applications in therapy. Archivio E.Maragliano di patologia e clinica 1957; 13(4): 1023-1033.
19. European Parliament and Council Regulation (EC) No 1924/2006 Of The European Parliament and of The Council of 20 December 2006 on nutrition and health claims made on foods. Official Journal of the European Union L 2007; 404: L 12-3-L 12/17. (20). Bonomi, A. (1983) Acquisizioni in tema di composizione chimica e di attivita' biologica della pappa reale. Apitalia 1983; 10(15): 7-13.
21. Takahashi M, Matsuo I, Ohkido M. Contact dermatitis due to honeybee royal jelly. Contact dermatitis 1983; 9(6): 452.
22. Devasagayam TPA, Tilak JC, Boloor KK, Ketaki SS, Saroj SG, Lele RD. Free radicals and antioxidants in human health: current status and future prospects. J Assoc Physicians India 2004; 52: 794-804.
23. Stefan B. Royal jelly, bee brood: composition, health, medicine: A Review. Bee Product Science 2017.
24. Rickenlund A, Carlstrom K, Ekblom B, Brismar TB, Schoultz B, Hirschberg AL. Hyperandrogenicity is an alternative mechanism underlying oligomenorrhea or amenorrhea in female athletes and may improve physical performance. Fertil Steril 2003; 79(4): 947-55.
25. Cumming DC, Brunsting LA, Strich G, Ries AL, Rebar RW. Reproductive hormone increases ın response to acute exercise in man, med. Sci. Sports Exerc 1986; l8: 369-73.
26. Vittek J, Slomiany B. Testosterone in royal jelly. Cellular and Molecular Life Sciences 1987; 40: 104-106.
27. Gawish AM, ElFiky S, Therase M, AbdElraaof A, Khalil W, Mohamed KA. Sperm abnormality toxicity due to cyclosporine A and the ameliorative effect of royal jelly in male rats. The Journal of Basic & Applied Zoology 2016; 76: 60-73.
28. Shahzad Q, Mehmood MU, Khan H, ul Husna A, Qadeer S, Azam A, Naseer Z, Ahmad E, Safdar M, Ahmad M. Royal jelly supplementation in semen extender enhances post-thaw quality and fertilty of Nili-Ravi buffalo bull sperm. Animal Reproduction Science 2016; 167: 83-88.
29. Chauvin, R (1987) La ruche et l'homme. Calmann-Lévy, France

Correspondence:
Mustafa Özdal
Gaziantep University, Sport Science Faculty,
Gaziantep, Turkey
E-mail: ozdalm@hotmail.com

Progress in Nutrition 2020; Vol. 22, N. 1: 288-296 DOI: 10.23751/pn.v22i1.7220

Original article

Effect of soy protein on hypercholesterolemia and hypertension to reduce the risk of cardiovascular diseases

Saman Zahid[1], Tahir Zahoor[1], Muhammad Nadeem[2], Muhammad Farhan Jahangir Chughtai[3], Adnan Khaliq[3], Samreen Ahsan[3], Ambreen Sagheer[4]

[1]National Institute of Food Science and Technology, University of Agriculture, Faisalabad-Pakistan; [2]Department of Environmental Sciences, COMSATS Institute of Information Technology, Vehari-Pakistan - E-mail: mnadeem11@gmail.com; [3]Department of Food Science and Technology, Khawaja Fareed University of Engineering and Information Technology, Rahim Yar Khan-Pakistan; [4]Government College of Home Economics, Gulberg, Lahore- Pakistan

Summary. High blood cholesterol and hypertension are the leading causes of cardiovascular diseases which are one of the leading causes of death worldwide. The current research study was designed to isolate soy protein to be used as nutraceutical agents against hypercholesterolemia and hypertension. Soy protein was extracted and isolate was given to rats for 4 weeks after inducing hypercholesterolemic and hypertensive conditions. Blood samples were collected and analyzed for lipid profile (cholesterol, triglycerides, low density lipoprotein and high density lipoprotein) and serum nitric oxide on 14th and 42nd days of study. The results re vealed significant decrease in total cholesterol (120.00 to 110.00 ml/dL), triglycerides (87.66 to 74.00 mg/dL), and low density lipoprotein (67.00 to 47.66 mg/dL) while significant increase in high density lipoprotein (29.33 to 50.00 mg/dL) and nitric oxide (24.00 to 50.33 mg/dL) due to the uptake of soy protein isolate for a period of 28 days was observed.

Key words: soy protein, hypercholesterolemia, hypertension, cardiovascular diseases

Introduction

Globally most of the deaths are caused by heart diseases, liver diseases and cancers. Among these, cardiac ailments are major contributors (1). Main CVD risk factors are high blood pressure, diabetes, hyperlipidemia, obesity and inflammation. Dietary modifications are advised for cardio-protective effects, for that plant foods are recommended because they can regulate blood plasma concentrations (2). Healthy changes in diet can lower the incidence of cardiovascular disease by lowering risk factors and in specific, legumes are highlighted as part of heart friendly diet, so augmented ingestion can lower weight and blood glucose, high blood pressure, and can manage dyslipidemia (3).

Soybeans are certainly the topmost oilseed produced globally as it is extensively cultured for their lipid content. Moreover, soybeans are familiar as a valued source of nutrients as they comprise high-quality protein (~40%); carbohydrates, poly unsaturated fatty acids (PUSFA, 18%) and dietary fibers. Soybean is a cherished legume because it contains all essential amino acids which human body can't produce; but it is comparatively deficient in sulfur containing amino acids, cysteine and methionine. It can be ingested as a complete protein. Soybean contains about 37 to 42% of protein. The two chief proteins of soybean are 11S glycinin, and 7S β-conglycinin, both have globular structure (4).

Due to having all essential nutrients, soybeans are known to be health friendly. These health benefits are attributed to essential amino acids, bioactive peptides, unsaturated fatty acids, secondary metabolites such as isoflavones, anthocyanins etc. The protein part of soybeans is comprised of 37-45%, of which 70 to 83 are glycinin and beta-conglycinin that is storage proteins. Besides providing basic functions such as maintenance, healing, soy derived proteins also prevent from developing dangerous diseases. Some proteolytic enzyme inhibitors prevent and treat the cancers of colon and rectum without developing toxicity in normal body cells (5).

Soybeans can reduce bad cholesterol and this effect is proven by several research studies. It can lower 12.9% of this bad cholesterol. Food and drug administration has approved a health claim regarding soybean consumption which states that daily consumption of 25g of soy prevent can lower the incidence of heart diseases (2). Soy protein is usually used to substitute animal proteins in diet. The ingestion of soy protein is considered to lower down high blood cholesterol and LDL-cholesterol levels (6). Soy protein hence prevents from cardiovascular diseases as it reduces intestinal cholesterol absorption and increases fecal cholesterol excretion (7).

The proposed mechanisms for decreasing the incidence of cardiovascular diseases are dilation of blood vessels, inhibition of platelets accumulation with vessels walls, check the relocation and propagation of smooth muscle cells, and lessening the sticking of some compounds to the vascular endothelium such as cholesterol by enhancing the excretion of bile through feces or reducing the absorption of cholesterol in small intestine. Undigested soy peptides carry with them the bile acids out of the human body and prevent cholesterol absorption. Beta- conglycinin, one of the major proteins of soy, lowers blood triglyceride level and keep body fat mass lowers. These biologically active peptides also possess effects against high blood pressure, oxidative stress, obesity, immune system diseases, diabetes, high blood cholesterol and cancer (8-10)

Keeping in view the above described themes, this research study was preliminary designed to isolate soy protein to be used as nutraceutical agents and to conduct in vivo studies for effectiveness of soy protein against hypercholesterolemia and hypertension

Materials and Methods

The study was conducted in Food Microbiology and Biotechnology Laboratory, National Institute of Food Science and Technology (NIFSAT), University of Agriculture, Faisalabad (UAF). Soy beans were obtained from local market. NaOH, HCl, H_2SO_4, digestion tablets, Hexane and Cholesterol were obtained from Food Microbiology Lab, NIFSAT, UAF.

Preparation of soy protein isolates

Soy protein was isolated from soy beans following the protocol given by L'hocine (11). Intact soybeans were first broken down into three or four pieces to separate the husk and then ground into fine pieces. Then ground soy meal 250g were mixed with hexane in the ratio of 1:2. The meal and solvent mixture was stirred for half an hour and then allowed to get settled after which the solvent layer was drained off. The hexane was used to de fat the soy meal. After removal of hexane, the mixture was washed with fresh water and then left overnight to dry at room temperature.

Water was added in defatted meal in the ratio of 1:15, heated up at 55°C, and the pH 9.0 was adjusted by adding 2N NaOH and stirred continuously for 40 minutes. The mixture was then allowed to cool down at room temperature. The slurry was then centrifuged at 14300g for half an hour and the temperature of centrifuge was maintained at 4°C. The supernatant was collected in a separate flask and the pH was set at 4.5 by using 2N HCl and was stirred for 45 minutes at 25°C. It was again centrifuged at 2830g for 15 minutes at 4°C. The precipitate thus obtained was washed with water and again centrifuged at 2830g for 10 minutes twice. The washed precipitate was suspended in water and pH was adjusted at 7.0 by using 2N NaOH. The precipitates were then freeze dried and stored at refrigeration temperature.

Crude protein of soy powder and soy protein isolate

Protein content of soy powder was determined by Kjeldhal method by using Kjeltech Apparatus (Model: D-40599, Behr Labor Technik, Gmbh-Germany, Method No: 984-13) as per procedure described in AOAC (12).

Efficacy Study/Experimental protocol

To evaluate the role of soy protein on hypercholesterolemia and hypertension, rats as an experimental model were used. Purposely, fifteen rats were acquired and housed in animal room of National Institute of Food Science and Technology, University of Agriculture, Faisalabad. Rats were acclimatized for a period of 1 week by the provision of regular diet and water ad libitum. Afterwards rats were divided into three groups; each comprising of five rats. The group G_1 was given normal diet, G_2

and G_3 were given high cholesterol diet for specific period to induce hypercholesterolemia and hypertension. After induction of hypercholesterolemia and hypertension, the baseline values for different biomarkers (total cholesterol, triglycerides, low density lipoprotein, high density lipoprotein and nitric oxide) were recorded. Then, the G_2 was started to feed normal diet whereas G_3 was given soy protein isolate diet for a period of one month. After one month, the above mentioned parameters were again recorded. During entire experimental period, animal room was maintained at a temperature and relative humidity of 23±2°C and 55±5% respectively, with 12:12 hours light: dark cycle (13).

Study parameters

Body weight and feed intake were measured on weekly basis. Lipid profile and nitric oxide levels were observed twice during whole study *i.e.* at 14th and 42nd days. For this purpose, rats were fasted overnight. Blood samples of rats were collected through cardiac puncture in EDTA coated tubes for study and non-coated tubes to measure serum lipid profile and nitric oxide level through Microlab-300, Merck, Germany.

Body weight gain

Increase in body weight of rats from all experimental groups was measured weekly throughout the study period to analyze the effect of soy diet on body weight.

Feed intake

Feed intake of rats of all groups was measured weekly throughout the study period to analyze any effect of high cholesterol diet or soy protein diet.

Determination of serum lipid profile

Serum lipid profile of rats including cholesterol, high density lipoproteins (HDL), low density lipoproteins (LDL), and triglycerides were measured by following protocols.

Estimation of triglycerides

Triglycerides in serum sample were estimated by liquid triglycerides (GPO-PAP) method as illustrated by (14). Three test tubes were taken and labeled as blank, standard and sample. Triglyceride reagent (1mL) was added in these tubes and heated at 37°C for 4 minutes. Then 0.01 mL of blood sample was added. The mixture of reagent and blood was mixed properly and then placed in incubator at 37°C for 5 minutes. Then absorbance of all solutions was observed. For this, spectrophotometer was used and its wavelength was adjusted at 520 nm.

Estimation of total cholesterol

Serum cholesterol level of rats was measured using CHOD-PAP method following the protocol of Kim (15). Three test tubes were taken and labeled as blank, standard and sample. One mL of reagent was added in these tubes and heated up to 37°C for 2 min. Then blood sample (0.01ml) was transferred in these tubes, shaken and placed in incubator at 37°C for 10 min. The absorbance by contents of all tubes was then observed by using spectrophotometer whose wavelength was adjusted at 250nm.

HDL

High density lipoproteins (HDL) in serum samples were calculated by method as mentioned by Alshatwi (16). Two tubes were taken and labeled control and sample. Equal amounts of serum and HDL cholesterol reagent was added in these tubes and mixed them thoroughly. Then the mixture was centrifuged at 1500-2000 rpm for 10 min. and separated the supernatant. The supernatant was considered as sample and processed further to calculate high density lipoprotein cholesterol. The absorbance by supernatant was measured by placing in spectrophotometer whose wavelength was adjusted at 520nm.

LDL

Low density lipoproteins (LDL) in serum samples were calculated by method as mentioned by Alshatwi

Table 1. Composition of diet g/100g

Ingredient	Normal diet	High fat diet
Wheat flour	65 g	45.5 g
Chickpea flour	20 g	14 g
Milk powder	15 g	10.5 g
Fat	----	30 g *
Energy	360.5 kcal	522.4 kcal

** Animal fat 20g, vegetable fat 10g*

(16). Blank, Standard, and Sample tubes were labeled appropriately. Reagent (1000µL) was transferred to all tubes and pre-warmed at 37°C for 2 min. Then sample (100 µL) was added to all the tubes, mixed and returned to 37°C. All the tubes were incubated at 37°C for 5 min. After that, absorbance of all tubes was measured by using spectrophotometer whose wavelength was set at 546 nm.

Nitric oxide (NO)

NO determination was carried out by Griess assay presented by (17). Firstly, serum proteins were removed by ultrafiltration with 10 kd rnicron at 4°C, 130,000 rpm. Then, nitrate in the serum was reduced with nitrate reductase and β-NADPH for 3 h. After that, β-NADPH was removed by 10 min incubation with 80 mM α-ketoglutaric acid and 1 M NH_4Cl. The amount of nitrite was measured by adding 150 µL of Griess reagent (Promega, USA). At last, the absorbance of the chromophores formed was read by using spectrophotometer at wavelength 540 nm.

Statistical analysis

All the data attained during the study was analyzed using a software Statistic 8.1 as described by Montgomery (18).

Results and Discussion

Crude protein of soy meal and soy protein isolate

The concentration of protein was 34.32% in soy powder as it contained all other nutrients such as carbohydrates, fats, minerals and vitamins. When soy protein was isolated from soy meal, the percentage of protein got enhanced as it was only protein. The concentration of protein in soy protein isolate was 81.2% as shown in Figure 3. It indicates that percentage of protein got enhanced by two folds in soy protein isolate as compared to that of soy meal.

Weight of rats

The weight of rats was observed on weekly basis. Rats who were fed normal diet gained more weight from 131.00g to 196.20g which was 65.2g during six weeks of study, then comes the group of rats who fed high cho-

Figure 1. Preparation of soy protein isolates

Figure 2. Efficacy study to determine the effect of protein isolates on health.

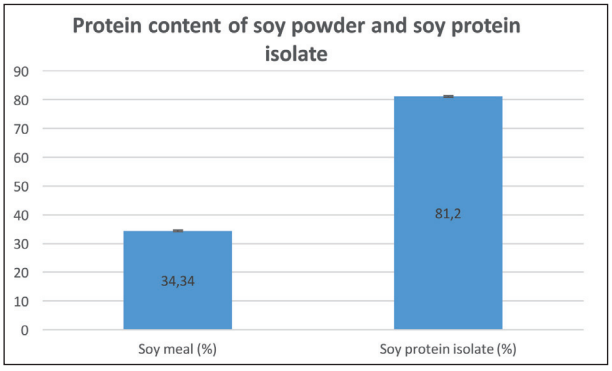


Figure 3. Protein content of soy powder and soy protein isolate

lesterol diet whose weight increased from 121.00g (on 1st week) to 201.00g (on 2nd week) which was 80g. The lowest body weight was observed in the group who was treated with soy protein isolate (Table 2). These readings have shown that soy protein isolate had significant effect in reducing weight and high cholesterol diet had remarkable effect in gaining weight.

The rats given normal diet, high cholesterol diet and high cholesterol plus soy protein isolate shown highly significant increase in weight. But the trend of increase was same with the passage of time that may be due to the increase in body size. The change in weight was consistent. This study has shown that as rats get older with days their weight got increased. But the effect of treatment on weight of rats shows that rats who fed normal diet gain more weight, then comes the group of rats who fed high cholesterol diet and the least weight gain was recorded in group which was fed soy protein isolate diet. It indicates that G_2 who took soy protein isolate diet gained less weight as compared to those who fed normal and high cholesterol diet.

Result of this study can't be co-related with previous research work mentioned by Kobayashi (19). Work by this scientist showed no significant change in body weight of rats either by consuming high cholesterol diet or soy protein isolate diet.

Feed intake

The purpose of this observation was to find out the effect of cholesterol and soy protein isolate supplementation in normal diet on diet consumption pattern. The values of feed intake of rats are given in Table 3. Effects of days and combined effect of days and treatment are highly significant as they increased the uptake of feed but effect of treatment on feed intake of rats is non-significant as it remained almost constant in all groups.

First group who received normal diet had minimum consumption of 19.800g at day 1 and maximum consumption of 23.400g at day 42 which showed increment of 3.6g during six weeks of study. Second group who received high cholesterol diet had minimum consumption of 21.20g recorded at day 1 and maximum of 22.80g on day 42 which showed increase of 1.6g during entire study. Third group who received soy protein isolate diet had minimum consumption of 22.80g on day 21 and maximum of 23.00g on day 42 which showed increase of 0.2 g during 4 weeks. The effect of treatment on diet consumption pattern was non-significant which indicates that high cholesterol, soy protein isolate diet has no effect on consumption pattern.

A slight increase in feed intake of rats was observed as they get older day by day. The results regarding effect of treatments on rats feed intake shows that there was no change in feed intake of rats either the diet was sup-

Table 2. Effect of treatments and days on weight of rats

Treatment	Days							Mean
	0	7	14	21	28	35	42	
G_0	131.00±1.00^{o}	146.00±1.58^{m}	167.00±1.58^{i}	182.80±1.30^{f}	188.80±1.30^{d}	196.20±0.83^{c}	211.20±0.83^{a}	174.71^{a}
G_1	121.00±1.58^{p}	136.00±1.58^{n}	154.00±1.58^{l}	160.00±1.58^{k}	173.00±1.58^{h}	186.00±1.58^{e}	201.00±1.58^{b}	161.57^{b}
G_2	118.80±5.01^{p}	131.80±2.58^{o}	144.00±1.58^{m}	162.40±2.70^{j}	165.40±1.51^{i}	175.80±1.09^{g}	191.00±0.70^{d}	155.60^{c}
Mean	123.60^{g}	137.93^{f}	155.00^{e}	168.40^{d}	175.73^{c}	186.00^{b}	201.07^{a}	

Values are given as Mean ± Standard deviation, Different small letters in rows and columns show significance

Table 3. Effect of treatments and days on feed intake of rats

Treatments	Days							Mean
	0	7	14	21	28	35	42	
G_0	19.80±0.83^{hi}	20.60±0.54^{gh}	20.20±0.83^{g-i}	3.40±0.54^{ab}	23.400±0.54^{ab}	23.800±0.83^{a}	23.400±1.14^{ab}	22.200^{a}
G_1	21.20±0.83^{e-g}	21.00±0.70^{fg}	21.80±0.83^{d-f}	23.200±0.83^{a-c}	22.400±1.14^{b-d}	23.000±0.70^{abc}	22.800±0.83^{abcd}	22.086^{a}
G_2	19.20±0.83^{i}	22.20±0.83^{c-e}	21.80±0.83^{d-f}	22.800±0.83^{a-d}	22.800±0.83^{a-d}	22.800±0.83^{abcd}	23.000±0.70^{abc}	22.086^{a}
Mean	20.06^{c}	21.26^{b}	21.26^{b}	23.133^{a}	22.867a	23.200^{a}	23.067^{a}	

Values are given as Mean ± Standard deviation, Different small letters in rows and columns show significance

plemented with cholesterol or soy protein isolate. Current results highly correlate with earlier research findings of Kobayashi who reported non-significant effect on food intake, food efficiency and total energy intake after supplementing the diet with either cholesterol or soy protein isolate (9).

Total cholesterol

Soy protein possesses hypocholesterolemic properties in both normal and hypertensive subjects. Serum levels of total cholesterol are presented in Table 4. The highest value after 14 days for cholesterol 123.33ml/dl and 120.00ml/dl was measured in groups provided with high cholesterol diet trailed along by normal cholesterol level 90.00ml/dl in rats given normal diet. A decrease from 123.33±3.055 to 121.33±3.055 (2ml/dl) was observed in second group and decrease from 120.00±5.00 to 110.00±3.00 (ml/dl) was observed in third group which was fed soy protein isolate. Results revealed 8.51% decrease in total cholesterol of rats of third group due to intake of soy protein isolate.

After inducing hypercholesterolemia, when rats were given soy protein diet there was reduction in their serum cholesterol levels. By the 2nd week of study, group 2nd and 3rd had highest blood cholesterol levels but by the 6th week the serum cholesterol of third group i.e. the group given soy protein diet reduced noticeably while that of members of second group remained same.

Cholesterol level must be below 200mg/dl as this concentration is considered as ideal. People having cholesterol 240mg/dl or more are at high risk of developing heart diseases. Current results are highly correlate with earlier research findings presented by kawakami (19) which showed a decrease in plasma cholesterol level by 10% in hypercholesterolemic subjects after ingesting soy protein isolate. Another finding by Kawakami (19) showed decrease in serum cholesterol which was attributed to soy protein isolate intake. Kobayashi (9) supported the current investigation of total cholesterol depended on the amount of isoflavone aglycones ingested along with soy protein. Wang (20) found significant reductions in plasma total cholesterol (8.4%, $P<0.001$) when subjects having high cholesterol levels ingested the diets having soy protein compared with the animal protein diets.

Triglycerides

The highest value of triglycerides was 87.66 mg/dl in group third after that second highest value was 85.00 mg/dl in group second as both these groups were fed high cholesterol diet. The lowest value of triglyceride was 72.66 mg/dl in group first because it was fed with normal diet. After 28 days, the lowest value for TG was 74.00 and 74.66 mg/dl in first and third groups respectively. This decrease from 87.66 mg/dl to 74.00 mg/dl in third group was due to treatment with soy protein isolate. Triglyceride was also decrease in second group from 85.00 mg/dl to 84.00 mg/dl. Results revealed that triglycerides level was decreased by 9.60% in third group due to consumption of soy protein isolate (Table 5).

Rats given high cholesterol diet had more triglycerides while the rats given treatment after inducing hypercholesterolemia showed decrease in TG levels almost near to group consumed normal diet. Trend of increase or decrease in serum TG due to effect of days is shown in Graph 4.8. The TGs of 2nd group members remained highest at the 2nd and 6th week but that of the animals of 3rd group got decreased at week 6 after getting treatment.

Current data is in collaboration with previous research work illustrated by Yoon (21) which showed

Table 4. Effect of treatments and days on total cholesterol (TC) of rats

Treatments	Days		Means
	14	42	
G_0	90.00±5.00^{d}	99.67±4.50^{c}	94.83^{c}
G_1	123.33±3.05^{a}	121.33±3.05^{a}	122.33a
G_2	120.00±5.00^{a}	110.00±3.00^{b}	115.00^{b}
Means	111.11^{a}	110.33^{a}	

Values are given as Mean ± Standard deviation, Different small letters in rows and columns show significance

Table 5. Effect of treatments and days on triglycerides (TG) contents

Treatments	Days		Means
	14	42	
G_0	72.667±2.51^{b}	74.000±1.00^{b}	73.333^{b}
G_1	85.000±5.00^{a}	84.000±5.00^{a}	84.500^{a}
G_2	87.667±5.03^{a}	74.667±1.52^{b}	81.167^{a}
Means	81.778^{a}	77.556^{b}	

Values are given as Mean ± Standard deviation, Different small letters in rows and columns show significance

that plasma TG levels were significantly decrease in the group given soy protein isolate. When subjects were provided soy protein isolate for consumption by quitting high cholesterol diet the serum triglycerides level decreased significantly (11.9 %, P<0.0001). Another study presented by Wang (20) showed decrease in serum triacylglycerol by 12% when subjects were given soy diet. The reduction in plasma TG levels was consistent, decreased by 12.3% (P = 0.018) after subjects consumed the soy protein diet relative to animal protein containing diets.

High density lipoprotein (HDL)

There is an inverse relationship between high density lipoprotein cholesterol (HDL) concentration and development of coronary artery disease. More the amount of HDL in plasma, lesser will be the chances of getting cardiac ailments. In the current study, at 14th day, the highest HDL was 52.00 mg/dl in 1st group. The second highest was 29.33 mg/dl in 3rd group while lowest was 27.66 mg/dl in 2nd group. Third group showed 22.66% decline in HDL while second group showed 24.33% decline in HDL after consuming high cholesterol diet. As HDL is termed as good cholesterol, so consumption of high cholesterol diet declined its level in Group 2 and Group 3 as members of both groups ingested high cholesterol diet. But HDL remained normal in Group 1 because they were on normal diet. At 42nd day, the group 3 showed remarkable increase in HDL from 29.33 mg/dl to 50.00 mg/dl which was 20.67%. The Group 2 also showed increase in HDL from 27.66 mg/dl to 30.00 mg/dl which was 2.33% and may be due to cessation of high cholesterol diet and shifting to normal diet (Table 6).

In the current study, the group three after getting treatment for hypercholesterolemia shown increase in serum HDL while that of group two remained same because this group didn't get any treatment. Group one also showed normal range as it was on normal diet balance in all nutrients. After giving high cholesterol diet, the HDL levels of both group second and group third got decreased as compared to that of group first. But by the 6th week, group 3 shown remarkable increase in HDL comparable with that of normal group.

Kobayashi found that daily soy beans consumption alleviates bad cholesterol due to the formation of equol from soy isoflavone daidzein (9). The ingestion of high soy protein diet for a period of one month resulted in 17% increase in HDL level in hypercholesterolemic rats.

Table 6. Effect of treatments and days on high density lipoprotein (HDL) contents

Treatments	Days		Means
	14	42	
G_0	52.00±3.00[a]	52.00±2.64[a]	52.00[a]
G_1	27.66±2.51[b]	30.00±2.00[b]	28.83[c]
G_2	29.333\±2.51[b]	50.00±2.00[a]	39.66[b]
Means	36.33[b]	44.00[a]	

Values are given as Mean ± Standard deviation, Different small letters in rows and columns show significance

Low density lipoprotein (LDL)

Soy protein decreases low density lipoprotein in hypercholesterolemic subjects. The results showed a highly significant decrease in low density lipoprotein. The effect of days, treatment and combined effect of days and treatment was highly significant in reducing low density lipoprotein in hypercholesterolemic subjects in which soy protein isolate was ingested (Table 7). At 14th day, the highest LDL level 67.00 mg/dl was observed in third group, the second highest 64.66 mg/dl was observed in second group and lowest 48.00 mg/dl was observed in first group. The first two highest values were due to consumption of high cholesterol diet. Then at 42nd day, the highest mean 64.33 mg/dl was observed in 2nd group while lowest means 47.66 mg/dl and 47.66 mg/dl were observed in 1st and 3rd group respectively. Third group showed a remarkable decrease from 67.00 mg/dl to 47.66 mg/dl and this was due to shift from high cholesterol diet to diet containing soy protein iso-

Table 7. Effect of treatments and days on low density lipoprotein (LDL) content

Treatments	Days		Means
	14	42	
G_0	48.00±1.00[b]	47.66±2.08[b]	47.83[c]
G_1	64.66±5.50[a]	64.33±4.50[a]	64.50[a]
G_2	67.00±6.24[a]	47.66±1.52[b]	57.33[a]
Means	59.88[a]	53.22[b]	

Values are given as Mean ± Standard deviation, Different small letters in rows and columns show significance

late. Means of Group 2 at 42nd day were remained almost same 64.66 mg/dl and 64.33 mg/dl because it was not given soy protein isolate diet. 14.42% decrease was observed after treatment with soy protein isolate diet in group 3.

The ingestion of high cholesterol diet increased the level of LDL in both Group 2 and Group 3. The level dropped down in Group 3 when given soy protein while remained high in Group 2. The results further showed that at 2nd week, LDL was high in Group 2 and group 3. At 6th week, the level got decreased in Group 3 after getting treatment for 4 weeks.

Results of present project are comparable to earlier studies of Wang (20) who reported that when subjects having high cholesterol were fed with soy diet the serum low density lipoprotein showed a significant decrease (17%, P = 0.003). Kobayashi found that equol, a metabolite of daidzein decreased plasma low density lipoprotein cholesterol (9). But this reduction was depended upon the amount of soy foods consumed daily. In his study, serum LDL cholesterol got elevated after provision of high cholesterol diet. But when treated with diet containing 10% and 15% soy protein its level got decreased by 16% and 18% respectively.

Serum nitric oxide (NO)

Nitric oxide is an inter and intra-cellular signaling molecule that plays important roles in many physiological and pathological processes, including vasodilation, transmission of signals along neurons, modulation of immune system, cardiac contraction, inhibition of platelet accumulation, stem cell differentiation into macrophages and their proliferation (22).

Serum nitric oxide levels are given in Table 8. At day 14th, the lowest mean 24.00 mg/dl was observed in Group 3, the second lowest 27.66 mg/dl was observed in Group 2 while highest 49.33 mg/dl was observed in Group 1. The lowest means were due to high cholesterol diet which decreased serum NO level and highest was due to normal diet as it didn't contain high cholesterol. At day 42nd, the highest mean 50.66 mg/dl was observed in first group which was on normal diet throughout the project. The 2nd highest 50.33 mg/dl was observed in third group. The significant increase from 24.00 mg/dl to 50.33 mg/dl was due to provision of diet containing soy protein isolate and decline in level of high cholesterol in diet. The means of 2nd group were changed from 27.66 mg/dl to 29.00 mg/dl which was due to shifting on normal diet from high cholesterol diet. This indicates that normal cholesterol in diet can also improve serum NO but significant increase happened when diet was high in soy protein. Almost 23.9% increase in serum NO was observed as compared to that of control group after feeding soy protein isolate. Till 2nd week, serum NO levels fell down in Group 2 and group 3 but at 6th week the level increased in Group 2. When Group 2 and Group 3 were given high cholesterol diet serum NO levels got decreased, but when Group 3 was given soy protein diet its level increased comparable to that of normal group.

Hypertension is caused when systolic blood pressure (SBP) get increased to 140 mm·Hg and/or diastolic blood pressure (DBP) to 90 mm·Hg. Primary hypertension accounts for 90% of all cases and although the root cause is unclear, the vital issues are unhealthy diet, smoking, stress, obesity, and possibly genetics. Hypertension increases the risk of blood vessels injury through Inflammation, hence aggregate the risk for cardiovascular diseases. Angiotensin II causes constriction of blood vessels; its level gets increased due to high blood pressure and elevates the peroxidation of lipids, which in turn generates low density lipoprotein cholesterol and free radicals. Soy beans have some constituents who can alleviate the chances of developing high blood pressure by producing vasodilator. Soy isoflavone is considered to form nitric oxide which is a key vasodilator (3).

Results of present project are comparable to earlier studies. Park (17) found that NO concentration was significantly elevated in the soy protein isolate group, compared to the control group, a difference of 40% (48.4±8.9 v 29.8±2.0, $p<0.05$).

Table 8. Effect of treatments and days on Nitric Oxide (NO) contents

Treatments	Days		Means
	14	42	
G_0	49.333±1.52[a]	50.667±1.52[a]	50.000[a]
G_1	27.667±2.51[b]	29.000±2.00[b]	28.333[c]
G_2	24.000±2.00[c]	50.333±2.51[a]	37.167[b]
Means	33.667[b]	43.333[a]	

Values are given as Mean ± Standard deviation, Different small letters in rows and columns show significance

Conclusion

Serum cholesterol, triglycerides and low density lipoprotein levels were significantly reduced while serum high density lipoprotein and nitric oxide levels were significantly increased after getting treatment. From present research work it is concluded that high cholesterol diet raises blood cholesterol and blood pressure but when consumption of cholesterol is decreased and diet is supplemented with soy protein isolate it causes decrease in blood cholesterol and blood pressure near normal levels.

References

1. Irazola VE, Gutierrez L, Bloomfield G, Carrillo-Larco RM, Prabhakaran D, Gaziano T, et al. Hypertension prevalence, awareness, treatment, and control in selected LMIC communities: results from the NHLBI/UHG Network of centers of excellence for chronic diseases. Glob Heart 2016;11(1):47-59.
2. Padhi EM, Blewett HJ, Duncan AM, Guzman RP, Hawke A, Seetharaman K, et al. Whole soy flour incorporated into a muffin and consumed at 2 doses of soy protein does not lower LDL cholesterol in a randomized, double-blind controlled trial of hypercholesterolemic adults, 2. J Nutr 2015;145(12):2665-74.
3. Ramdath DD, Padhi EM, Sarfaraz S, Renwick S, Duncan AM. Beyond the cholesterol-lowering effect of soy protein: a review of the effects of dietary soy and its constituents on risk factors for cardiovascular disease. Nutrients 2017;9(4):324.
4. O'Keefe S, Bianchi L, Sharman J. Soybean nutrition. SM J Nutr Metab 2015;1(1):1006.
5. Gomes LS, Senna R, Sandim V, Silva-Neto MrA, Perales JE, Zingali RB, et al. Four conventional soybean *[Glycine max (L.) Merrill]* seeds exhibit different protein profiles as revealed by proteomic analysis. J Agric Food Chem 2014;62(6):1283-93.
6. Malla S, Hobbs JE, Sogah EK. Estimating the Potential Benefits of New Health Claims in Canada: The Case of Soluble Fiber and Soy Protein. Can J Agr Econ 2016;64(2):173-97.
7. Sacks FM, Lichtenstein A, Van Horn L, Harris W, Kris-Etherton P, Winston M. Soy protein, isoflavones, and cardiovascular health: an American Heart Association Science Advisory for professionals from the Nutrition Committee. Circulation 2006;113(7):1034-44.
8. Talaei M, Koh W-P, van Dam RM, Yuan J-M, Pan A. Dietary soy intake is not associated with risk of cardiovascular disease mortality in Singapore Chinese Adults[-3]. J Nutr 2014;144(6):921-8.
9. Kobayashi M, Egusa S, Fukuda M. Isoflavone and protein constituents of lactic acid-fermented soy milk combine to prevent dyslipidemia in rats fed a high cholesterol diet. Nutrients 2014;6(12):5704-23.
10. Singh BP, Vij S, Hati S. Functional significance of bioactive peptides derived from soybean. Pep 2014;54:171-9.
11. L'hocine L, Boye JI, Arcand Y. Composition and functional properties of soy protein isolates prepared using alternative defatting and extraction procedures. J Food Sci 2006;71(3):137-45.
12. AOAC. Official Methods of Analysis of Association of Official Analytical Chemists International. In: Horwitz, W (Ed), 20th Ed AOAC Press, Arlington, VA, USA 2016.
13. Yang J-H, Mau J-L, Ko P-T, Huang L-C. Antioxidant properties of fermented soybean broth. Food Chem 2000;71(2):249-54.
14. Kuo K-L, Weng M-S, Chiang C-T, Tsai Y-J, Lin-Shiau S-Y, Lin J-K. Comparative studies on the hypolipidemic and growth suppressive effects of oolong, black, pu-erh, and green tea leaves in rats. J Agric Food Chem 2005;53(2):480-9.
15. Kim A, Chiu A, Barone MK, Avino D, Wang F, Coleman CI, et al. Green tea catechins decrease total and low-density lipoprotein cholesterol: a systematic review and meta-analysis. J Am Diet Assoc 2011;111(11):1720-9.
16. Alshatwi AA, Al Obaaid MA, Al Sedairy SA, Al-Assaf AH, Zhang JJ, Lei KY. Tomato powder is more protective than lycopene supplement against lipid peroxidation in rats. J Nutr Res 2010;30(1):66-73.
17. Park E, Shin J-I, Park O-J, KANG M-H. Soy isoflavone supplementation alleviates oxidative stress and improves systolic blood pressure in male spontaneously hypertensive rats. J Nutr Sci Vitaminol 2005;51(4):254-9.
18. Montgomery DC. Design and analysis of experiments: John wiley & sons; 2008.
19. Kawakami Y, Kiyosawa T, Nakamura S, Osada K. Effects of isoflavone supplementation on disturbances in lipid metabolism and antioxidant system due to exogenous cholesterol oxidation products in rats. J Funct Foods 2014;7:212-8.
20. Wang Y, Jones PJ, Ausman LM, Lichtenstein AH. Soy protein reduces triglyceride levels and triglyceride fatty acid fractional synthesis rate in hypercholesterolemic subjects. Atherosclerosis 2004;173(2):269-75.
21. Yoon M, Won SB, Kwon YH. Altered lipid metabolism in rat offspring of dams fed a low-protein diet containing soy protein isolate. Life Sci 2017;174:1-7.
22. Lei J, Vodovotz Y, Tzeng E, Billiar TR. Nitric oxide, a protective molecule in the cardiovascular system. Nitric Oxide 2013;35:175-85.

Correspondence:
Muhammad Nadeem
Department of Environmental Sciences, COMSATS Institute of Information Technology, Vehari-Pakistan
Email: mnadeem11@gmail.com

Progress in Nutrition 2020; Vol. 22, N. 1: 297-303 DOI: 10.23751/pn.v22i1.9189

Original article

A folk remedy: royal jelly improves lung capacity in smokers

Ali Muhittin Taşdoğan[1], Ebru Tarıkçı Kılıç[2], Zarife Pancar[2], Mustafa Özdal[3]

[1]Hasan Kalyoncu University, Medical Faculty Anesthesiology and reanimation, Gaziantep, Turkey; [2]Health Sciences University Ümraniye Training and Research Hospital Anesthesiology and Reanimation, Ümraniye İstanbul - E-mail: ebru.tarkc@yahoo.com; [3]Gaziantep University, Sport Science Faculty, Gaziantep, Turkey

Summary. *Background and Aim:* Royal jelly (RJ) is one of the natural, valuable curative bee productdue to its promising health-beneficial and nutritional properties. This healthy diet possesses anti-inflammatory, antimicrobial, antioxidants, antitumor, and immunomodulatory functions which benefit in humans health and welfare,resulting in its widespread medical use. The aim of this randomized case controlled study was to determine the effect of royal jelly on the lung capacity of sedentary male smokers. *Materials and Methods:* The study was approved by the ethics committee of the university and consisted of 83 sedentary male and single participants aged 25-30 years without any health problems. Participation was voluntary. This case controlled design study was conducted in accordance with the ethical principles outlined by the World Medical Association's Declaration of Helsinki. Written informed consent was obtained from participants. The sample was divided into four groups: smoker experimental (Group I: 22), non-smoker experimental (Group II: 21), smoker control (Group III: 20), and non-smoker control (Group IV: 20). The experimental groups consumed 1000 mg/day pure royal jelly between 08.00 and 10.00 am for 21 days. The control groups consumed a placebo liquid between 08.00 and 10.00 am for 21 days. Pre- and post-pulmonary function tests (PFTs) were performed. *Results:* Group I had significantly higher mean posttestforced expiratory volume in one second(FEV1) (1.86±0.19 L) than mean pretest FEV1 (1.76±0.05 L) while Group II had significantly higher mean posttest FEV1 (2.25±0.27 L) than mean pretest FEV1 (2.18±0.17 L) ($p < 0.000$). No statistically significant difference was observed in the control groups. *Conclusion*: PFT results before and after 1000mg/day royal jelly supplement revealed positive and significant differences.

Key words: royal jelly, sedentary, smoking, pulmonary function tests, FEV1

Introduction

Royal jelly is a substance secreted by honey bees to feed especially the queen larvae, hence the name royal jelly. Its health benefits were discovered in the 1600s. Royal jelly is a secretion of the hypopharyngeal glands of 5-10-day-old honeybee workers. It is pellet-like and has a distinct smell and bitter taste. Worker bees begin to synthesize protein in the hypopharyngeal glands about four days after they are hatched. Protein synthesis continues to increase for eight days, reaches its maximum level on the fourteenth day and starts to decrease from the seventeenth day on (1, 2). Royal jelly is milky when secreted and delivered to the oral cavity but it turns dark and creamy after being placed in honeycomb cells. It is a yogurt-like homogeneous substance with the consistency of a fluid paste which, however, becomes more viscous when stored at room temperature or in a refrigerator at 5°C. It contains proteins, lipids, carbohydrates, ash, P, Na, K, Ca, Mg, pollen, and C, D and E and B, and many other vitamins (3, 4).

It also contains 10-hydroxydecanoic acid (10-HDAA), 10-hydroxydecanoic acid (10-HDAA) and sebacic acid (SEA) which exhibit broad-spectrum activity against numerous bacteria and fungi (5). The proteins in royal jelly are antioxidants used in the treatment of such diseases as cancer, atherosclerosis, hypertension, infertility, asthma, depression, and diabetes mellitus resulting

from oxidative stress caused by the imbalance between reactive oxygen species (6). It also plays a key role in cell renewal, regeneration, and organization, and antiaging, and lowers blood cholesterol, total lipid, phospholipid, triglyceride, beta lipoprotein levels, and blood pressure, and dilates vessels. It has also been reported for many years that royal jelly exhibits antimicrobial and insulin-like hypoglycemic and immunological activities, has therapeutic properties for skin and hair, and reproductive diseases, regulates sexual functions, and repairs and rejuvenates cells (7-9).

Royal jelly is used more and more in daily diet and in the treatment of many diseases. With these superior properties, royal jelly is becoming increasingly important for health and is studied more extensively.

Smoking increases airway resistance during respiration. Thenicotine in cigarettes causes bronchioles to contract, and carbon monoxide in the smoke is bound by hemoglobin and hence reduces blood oxygen carrying capacity. Research shows that smoking is a dominant predictor of airway obstruction. Pulmonary function test (PFT) values should be measured to determine airway obstruction. PFTsare widely used to understand and rate the abnormalities in respiratory system functions. The air entering and leaving the lungs should be able to move fast enough and play a decisive role in physical capacity.Air velocity depends on theairway resistanceof chest and lung tissues, affecting dynamic measurements (10, 11).

1) FVC (forced vital capacity) is the maximum amount of air exhaledafter a maximum inhalation.There is little or no difference between VC and FVC in normal subjects.
2) FEV1 (forced expiratory volume in one second) is the maximum amount of air expelled during the first-second after a maximum inhalation.This is the most commonly used value to measure lung function. Exercise-induced bronchoconstriction (EIB) diagnosis requires a ≥ 10% fall in FEV1 after exercise.
3) FEV1/FVC ratio is the ratio of the forced expiratory volume in the first second to the forced vital capacity of the lungs. It is normally 80–90% and goes below 70% in the case of obstructive pulmonary diseases.

The aim of this study was to determine the effect of royal jelly on the pulmonary functions of sedentary male smokers aged 25-30 years.

Material and Method

The study was approved by the ethics committee of the university. The study sample consisted of 83 sedentary single male participants aged 25-30 years without any health problems between January and February 2019. Participants were asked whetherthey had any lung disease in the past or now, whether they smoked and how many cigarettes they smoked per day, how long they had been smoking, and whether they were performing physical activity or not. The exclusion criteria were (1) being diagnosed with a disease affecting respiratory functions, (2) receiving bronchodilator therapy, (3) having a neuromuscular and/or cardiopulmonary disease, (4) having undergone abdominal and thoracic surgery, (5) using alcohol and drugs, and (6) refusing to participate in the study. The smoker groups consisted of those who had been smoking for at least 5 years and 10 to 20 cigarettes per day. The non-smoker groups consisted of those who had never smoked before. Participants were randomly assigned to four groups: smoker experimental (Group I: 22), non-smoker experimental (Group II: 21), smoker control (Group III: 20), and non-smoker control (Group IV: 20). Anthropometric measurements were performed before intervention. Body weight was recorded in light clothing and height without shoes. Body mass index (BMI) was calculated by dividing weight (Kg) by the square of height (m). The experimental groups consumed 1000 mg/day pure royal jelly in glass vials between 08.00 and 10.00 am for 21 days. The control groups consumed a placebo liquid in glass vials between 08.00 and 10.00 am for 21 days. The medications were stored in boxes labeled as A and B. The participants were blinded to the allocation throughout the study period and were educated to follow a healthy lifestyle. Participants were instructed not to engage in any exercise or activities requiring physical strength for 21 days. Daily dietary intake data were evaluated at baseline and at the end of the study by 2 days; 1 in the week day 1 in the weekend days by a dietician blinded to the study. They were followed up every week to check the side effects if any and for the compliance. Pre- and post- PFTs were performed using spirometry. VC (L), FVC (L), FEV1 (1), and FEV1/FVC (%) were calculated.

During the measurements, participants were seated in an upright position on a fixed chair, and their nostrils were occluded with a nose clip to prevent them from breathing through their nose. They were asked to inhale and exhale through their mouth three times followed by a maximum inspiration and then maximum expiration. The best value was recorded after three consecutive repetitions. The PFTs were performed by another researcher blinded to the study protocol.

Statistical Analysis

Scale parameters were described by means and standard deviations. Normality of parameters were tested with Kolmogorov Smirnov Test. One Way ANOVA test was used for normally distributed parameters, and Kruskal Wallis Test was used for non-normal distributed parameters. Mann Whitney U test was used for post hoc test of nonparametric differences. All analysis were performed at SPSS 17.0 for windows at 95% confidence interval.

Results

Of participants, 42 were smokers (at least five years and 10-20 cigarettes per day) while the remaining 41 were non-smokers. All the participants were male, single and all of them have academic educational status. In the study flow diagram; the randomization and the loss to follow up are presented (Fig1).

In the study period no participants reported any side effects.

Table 1 shows the groups' demographic data, indicating no statistically significant difference in age, BMI, or in daily dietary intake between the groups ($p > 0.05$).When the pretest and post test results (PFTs) were examined in terms of lung function parameters such as VC, FVC, FEV_1, it was found that the measured values improved significantly in Group I and Group II (p: 0.000) Table 2

Pretest results of Group I were; 2.31 ± 0.08 L for VC, 2.34 ± 0.08L for FVC, 1.76 ± 0.05 for FEV_1 whereas the improved results were found as 2.41 ± 0.19 L, 2.43 ± 0.21L, and 1.76 ± 0.05 as shown in Table 3.

The pretest results were 2.83 ± 0.07 L for VC, 2.71 ± 0.04L for FVC, 2.18 ± 0.17 for FEV_1 for Group II whereas improved results after royal jelly were found

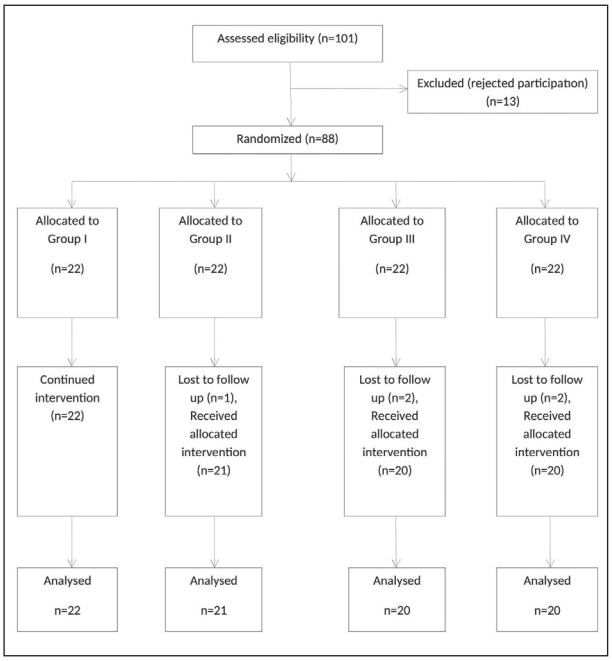


Figure 1: The flow Diagram

Table 1. Demographic parameters of the groups

Pretest, Mean±SD	Group I (n=22)	Group II (n=21)	Group III (n=20)	Group IV (n=20)	P value
Age	27,27±1,64	26,62±1,28	27,20±1,67	26,60±1,35	0.369[b]
BMI	23,73±1,49	23,71±1,71	23,55±1,70	23,20±1,58	0.702[b]
Energy	2328,77±22,25	2324,10±22,23	2326,50±24,38	2325,60±25,42	0.872[b]
Carbonhydrate	243,23±8,28	242,81±6,07	242,05±7,78	243,25±8,36	0.955[a]
Protein	66,82±1,65	67,19±1,78	66,95±1,73	67,15±1,98	0.894[b]
Total fat	115,73±1,86	115,71±1,93	115,45±2,06	115,90±1,71	0.934[b]

a. One Way ANOVA Test, b. Kruskal Wallis H Test, SD: Standard Deviation. BMI: Body Mass Index. P value < 0.05 is considered statistically significant.

as 2.86 ± 0.05L for VC, 2.78 ± 0.09 L for FVC, and 2.25 ± 0.27 for $FEV_{1.}$ respectively.

In other words, Royal Jelly reduced the difference between the initial pulmonary function capacities of smokers and nonsmokers.

Table 4 presents the pretest results of PFT.The main comparison was made between the smokers and nonsmokers. There was no statistically significant difference in pretest measured VC, FVC, and FEV1 values between Group II and Group IV and Group I and Group III (p >0.05).

There was no statistically significant difference in posttest measured VC, FVC, and FEV_1 values between Group I and Group III (p > 0.05). However, Group II had significantly higher FVC value than Group IV (p < 0.05) Table 5.

Figure II shows the pretest FEV1/FVC ratios of the groups. Non-smokers (Groups II and IV) had higher pretest FEV1/FVC ratio than smokers (Groups I and III). Group II had higher pretestFEV1/FVC ratio than Group IV.

Figure III shows the pretest FEV1/FVC ratios of the groupsNon-smokers (Groups II and IV) had higher posttest FEV1/FVC ratio than smokers (Groups I and III). Among non - smokers Group II had higher posttestFEV1/FVC ratio than Group IV whereas Groups I and III had high range.

Discussion

Research shows that cigarette smoking causes respiratory impairment not only at older ages but also at younger ages. Smoking leads to two physiopathological changes in the lungs; (1) the proteolytic destruction of lung parenchyma and emphysema, which is abnormal

Table 2. Pre-test PFT results of the groups

Pretest, Mean±SD	Group I (n=22)	Group II (n=21)	Group III (n=20)	Group IV (n=20)	P value
VC	2.31±0.08	2.83±0.07	2.29±0.07	2.84±0.07	0.000[b]
FVC	2.34±0.08	2.71±0.04	2.32±0.08	2.71±0.03	0.000[b]
FEV_1	1.76±0.05	2.18±0.17	1.74±0.05	2.14±0.05	0.000[b]

a. One Way ANOVA Test, b. Kruskal Wallis H Test, SD: Standard Deviation.

Table -3: Post-test results of the groups

Final, Mean±SD	Group I (n=22)	Group II (n=21)	Group III (n=20)	Group IV (n=20)	p
VC	2.41±0.19	2.86±0.05	2.31±0.07	2.83±0.07	0.000[b]
FVC	2.43±0.21	2.78±0.09	2.34±0.10	2.72±0.04	0.000[b]
FEV_1	1.86±0.19	2.25±0.27	1.77±0.06	2.13±0.08	0.000[b]

a. One Way ANOVA Test, b. Kruskal Wallis H Test, SD: Standard Deviation. VC: Vital capacity, FVC: Forced vital capacity, FEV_1: Forced expiratory volume in one second. P value < 0.05 is considered statistically significant

Table 4. Pretest Comparison of the groups

Pre-test , p values	VC	FVC	FEV1	FEV1/FVC
Group I- Group II	0.000	0.000	0.000	0.000
Group I- Group III	0.399	0.472	0.367	0.658
Group I- Group IV	0.000	0.000	0.000	0.000
Group II- Group III	0.000	0.000	0.000	0.000
Group II-Group IV	0.606	0.679	0.548	0.712
Group III- Group IV	0.000	0.000	0.000	0.000

VC: Vital capacity, FVC: Forced vital capacity, FEV_1: Forced expiratory volume in one second.

Table 5. Posttest Comparison of the groups

Post-test, p values	VC	FVC	FEV1	FEV1/FVC
Group I- Group II	0.000	0.000	0.000	0.171
Group I - Group III	0.180	0.323	0.253	0.867
Group I - Group IV	0.000	0.000	0.000	0.068
Group II - Group III	0.000	0.000	0.000	0.084
Group II - Group IV	0.197	0.048	0.260	0.894
Group III- Group IV	0.000	0.000	0.000	0.005

VC: Vital capacity, FVC: Forced vital capacity, FEV_1: Forced expiratory volume in one second.

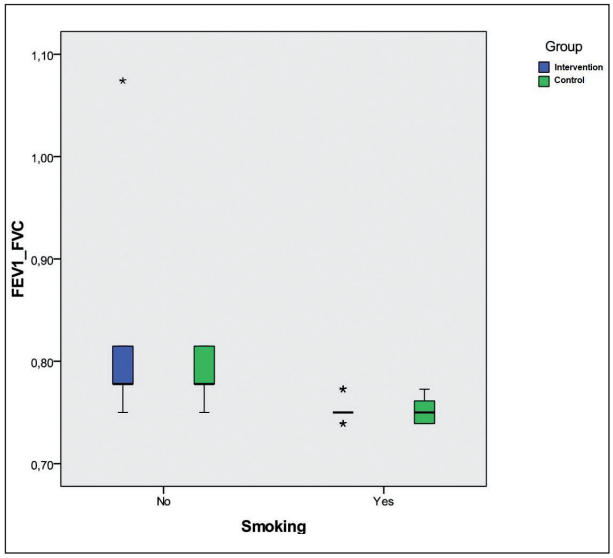


Figure 2: Pretest values of FEV_1/FVC

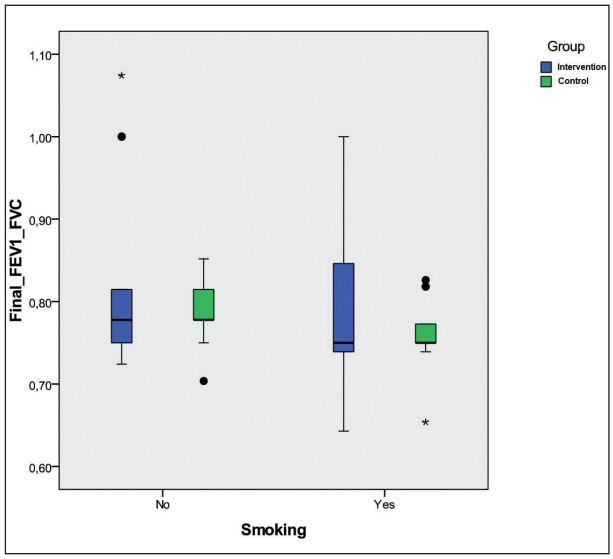


Figure 3: Posttest values of FEV_1/FVC

permanent enlargement of air spaces, and chronic obstructive lung damage and (2) inflammatory constriction of peripheral airways characterized by excessive edema, mucus release and peripheral airway fibrosis. Smoking increases airway resistance and causes prolonged expiration (12, 13). Free radicals increase during chronic inflammation, and the balance between oxidant and antioxidant is adversely affected, resulting in oxidative stress. Smokers have low blood antioxidant capacity. This may not only be due to oxidative stress, but also because it circulates, and therefore, has a systemic effect. Smoking is associated with a reduction in erythrocyte glutathione peroxidase activity, serum antioxidant activity, and plasma ascorbic acid, vitamin E, B carotene, uric acid and selenium levels. There is an increase in the amount of superoxide produced by neutrophils in peripheral blood and a decrease in total antioxidant capacity, especially during the attack and exacerbation episodes of COPD and asthma (14-16).

The harmful effects of smoking on the respiratory system emerge at later ages and is directly proportional to pack-year. Our participants were young and had low pack-year and therefore had normal PFT values. It is known that PFT cannot be used to detect early changes in the small airways, which is supported by some studies. Karimi et al. (17) reported that the prevalence of asymptomatic people with normal PFT values was high among smokers and that early changes in the small airways could be detected by assessing the levels of air trapping in the lungs using computed tomography (CT).

Present analysis demonstrates that there is a positive correlation between royal jelly consumption and lung functions. In vitro studies show that 10H2DA,10-HDAA, and SEA fatty acids in royal jelly reduce the release of nitric oxide, IL 10 and TNF alpha (dose-dependent major inflammatory mediators) and that C, D, A, and E vitamins in it protect lung tissue from harmful oxidative damage. AEOL150 was intratracheally administered 6 hours a day, 3 days a week to rats exposed to filtered air (control group) or cigarette smoke (experimental group) to test whether cigarette-induced inflammation would be reduced by a catalytic antioxidant. The number of cells in the bronchoalveolar lavage of the experimental group rats was significantly reduced. A significant reduction was observed in necrophils and lymphocytes in two days and in macrophages and lymphocytes in eight weeks. At 8 weeks, squamous cell metaplasia was 12% and 2% of the total airway epithelial area in the control and experimental rats, respectively (18).

Antioxidants protect tissues from harmful oxidative damage. Diet is the most important source of antioxidants. The combined activity of dietary antioxidants is probably superior to the individual effect of each antioxidant drug, supporting the hypothesis that royal jelly shows strong antioxidant properties due to the additive effect of its antioxidants (19, 8).

Vitamin C, which is found in tissues and liquids with high potential for free radical production, contributes to antioxidant defense by primarily removing peroxyl and oxygen radicals. One of the mechanisms explaining the protective effects of vitamin C on lung function is as follows: Vitamin C is an important antioxidant in the liquid that covers the surface of the airways. Proteases and antiproteases in that fluid protect the epithelial and immune cells from oxidant attack. Low vitamin C content affects the activity of pulmonary antioxidant defense systems negatively. Royal jelly is a rich source of vitamin C and therefore an important bioactive compound. Vitamin C protects the body from smoke-induced airway inflammation and lung damage and from oxidant air pollutants such as ozone and nitrogen dioxide and also slows down the rate of lung function (FEV1) decline in adults. It has recently been reported that vitamins A and E also slow down the rate of decline in FEV1 and protect respiratory functions in smokers. Royal jelly is rich in vitamins A and E (20-22).

Our literature review showed that there are many studies reporting positive effects of royal jelly on the lungs.El Aidy et al. (23) found that royal jelly and propolis had a positive anti-inflammatory effect on allergic asthma and pulmonary fibrosis in albino rats.Arajua et al. (24) also showed that 1 gram/kg/day royal jellyled to a decrease in the number of Th1-mediated cells and an increase in the number of Th2-mediated cells in the peripheral blood and lungs in people with asthma. Studies argue that royal jelly does that by scavenging free radicals.

Zargar et al. (25) investigated the effect of royal jelly on pulmonary fibrosis induced by bleomycin. They reported that 50-100 mg/kg royal jelly acted as a protective mechanism in bronchoalveolar lavage samples by reducing TGF-β, TNF-α cytokines, and chemotaxis of inflammatory cells and by increasing INF-γ, an antifibrotic cytokine. They also showed histopathologically that royal jelly provided macroscopic improvement.

TNF-α has both inflammatory and fibrogenic properties and is responsible for the development of airway obstruction, inflammation and pulmonary fibrosis. Intracellular studies have shown that MRJP3, a major protein in royal jelly, reduces TNF-α production. IFN-γ prevents fibroblast activation. In vitro studies have shown that IFN-γ has an inhibitory effect on TGF-β signaling pathways. Zargar (25) showed that TGF-β level decreased in royal jelly consumers.

Kamiya et al. (26) conducted an intracellular study of the proapoptoticactivities of royal jelly and reported that HPO-DAEE, a fatty acid of royal jelly, induced apoptosis of A549 cells.They argued that intracellular ROS activated by HPO-DAEE played a role in the destruction of cancer cells. Further research is warranted detailing the chemical structure and clinical application of HPO-DAEE in royal jelly.

In conclusion, proteins, proapoptoticfatty acids, vitamins, and numerous antioxidants in royal jelly can prevent smoking-induced airway obstruction and fibrosis in the early period, and PFTs can yield positive results before there arises a shift in the balance between oxidants and antioxidants in favor of oxidants.

The strength of our clinical trial is randomized and controlled design without inter individual differences however has two main limitations; (1) the sample consisted only of young male participants and (2) smoking participants had a low pack-year history in a short intervention time. Further studies with larger sample sizes with different dosages and durations are needed to provide more evidence regarding the positive effects of royal jelly on pulmonary function.

Conclusion

Royal Jelly is associated with the improvement in lung function even in young smokers with intact oxidant/antioxidant balance and a low pack-year smoking history, however, it is misleading.

Finally, Royal jelly at moderate concentrations has come into promising interest with many health benefits in medicine. Future in vitro trials on respiratory physiology are needed.

Author Contribution

Concept – ETK; MÖ Design – ETK; Supervision – ZP; Materials – AMT; Data collection and/or processing – AMT; ZP Analysis and/or interpretation – MÖ; LitErature review – ETK; ZP Writing – ETK; Critical review – ETK.

Acknowledgments

We all thank Kadir Yılmaz for his valuable statistical contribution.

References

1. Dobritzsch D, Aumer D, Fuszard M, Erler S. The rise and fall of major royal jelly proteins during a honeybee (Apismellifera) workers' life.Ecology and Evolution.2019;9:8771–8782.
2. Abdelnour SA, Hack ME,Alagawany AET, Elnesr SS, ElmonemAbd MO, Swelum AA. Useful impacts of royal jelly on reproductive sides, fertility rate and sperm traits on animalsJ AnimPhysiolAnimNutr (Berl). 2020 Jan 9.doi: 10.1111/jpn.13303. [Epub ahead of print]
3. Petelin A, Kenig S, Kopinč R, Deželak M, ČerneličBizjak M, JenkoPražnikar Z.Effects of Royal Jelly Administration on Lipid Profile, Safety, Inflammation, and Antioxidant Capacity in Asymptomatic Overweight Adults.Evid Based Complement Alternat Med. 2019 Jun 13:4969720.
4. Mureşan CI, Buttstedt A.pH-dependent stability of honey bee (Apismellifera) major royal jelly proteins.Sci Rep. 2019 Jun 21;9(1):9014. doi: 10.1038/s41598-019-45460-0.
5. Šedivá M, Laho M, Kohútová L, Mojžišová A, Majtán J, Klaudiny J.10-HDA, A Major Fatty Acid of Royal Jelly, Exhibits pH Dependent Growth-Inhibitory Activity Against Different Strains of Paenibacillus larvae.Molecules.2018 Dec 7;23(12).pii: E3236. doi: 10.3390/molecules23123236.
6. Miyata Y, Sakai H.Anti-Cancer and Protective Effects of Royal Jelly for Therapy-Induced Toxicities in Malignancies. Int J Mol Sci. 2018 Oct 21;19(10). pii: E3270. doi: 10.3390/ijms19103270. Review.
7. Yang YC, Chou WM, Widowati DA, Lin IP, Peng CC. 10-hydroxy-2-decenoic acid of royal jelly exhibits bactericide and anti-inflammatory activity in human colon cancer cells.BMC Complement Altern Med. 2018 Jul 3;18(1):202.
8. Kocot J, Kiełczykowska M, Luchowska-Kocot D, Kurzepa J, Musik I.Antioxidant Potential of Propolis, Bee Pollen, and Royal Jelly: Possible Medical Application.Oxid Med Cell Longev. 2018 May 2;2018:7074209.
9. P. M. Fratellone, F. Tsimis, and G. Fratellone, "Apitherapyproductsformedicinaluse," Journal of AlternativeandComplementaryMedicine, 2016; 22 (2):1020–1022
10. Chen X, Li Y, Hua C, et al. Establishment of rapid risk assessment model for cigarette smoke extract exposure in chronic obstructive pulmonary disease.Toxicol Lett. 2019 Nov;316:10-19.
11. EnrightPaul L, LebowitzMichael D.,W.Donald Cockroft. Physiologic Measures: Pulmonary Function Tests. Am J RespirCrit Care Med 1994(149): 59-518.
12. Ilumets H, Mazur W, Toljamo T, et al. Ageing and smoking contribute to plasma surfactant proteins and protease imbalance with correlations to airway obstruction. BMC Pulmonary Medicine 2011, 11:19
13. Jawed S, Ejaz S, Rehman R. Influence of smoking on lung functions in young adults. J Pak Med Asoc. 2012;62:772–775.
14. Hogg JC. Pathophysiology of airflow limitation in chronic obstructive pulmonary disease. Lancet. 2004;364(9435):709–721.
15. Jin H, Webb-Robertson BJ, Peterson ES, et al. Smoking, COPD, and 3-Nitrotyrosine levels of plasma proteins. Environ Health Perspect. 2011;119(9):1314–1320.
16. Erdogan A, Ucar EY, Araz O, Saglam L, Mirici NA. Contribution of spirometry to early diagnosis of chronic obstructive pulmonary disease in primary health care centers. Turk J Med Sci. 2013;43:690–694.
17. Karimi R, Tornling G, Forslund H, et al. Differences in regionalairtrapping in currentsmokerswith normal spirometry. European Respiratory Journal 2017 49: 1600345
18. Smith KR, Uyeminami DL, Kodavanti UP, Crapo JD, Chang LY, Pinkerton KE Inhibition of tobacco smoke-induced lung inflammation by a catalytic antioxidant. Free RadicBiol Med 2002; 33: 1106-1114.
19. McKeever TM, Scrivener S, Broadfield E, Jones Z, Britton J, Lewis SA. Prospective study of diet and decline in lung function in a general population. Am J RespirCrit Care Med 2002; 165: 1299-1303.
20. Nieman DC, Henson DA, McAnulty SR, et al. Influence of vitamin C supplementation on oxidative and immune changes after an ultramarathon. J ApplPhysiol 2002; 92: 1970-1977.
21. Kelly Y, Sacker A, Marmot M. Nutrition and respiratory health in adults: findings from the health survey for Scotland. EurRespir J 2003;21: 664-671.
22. Calikoglu M, Unlu A, Tamer L, Ercan B, Bugdayci R, Atik U. The levels of serum vitamin C, malonyldialdehyde and erythrocyte reduced glutathione in chronic obstructive pulmonary disease and in healthy smokers. ClinChem Lab Med. 2002; 40: 1028-1031.
23. Waleed K. El-Aidy, Ahmad A. Ebeid, Sayed SartajSohrab. Evaluation of propolis, honey, androyaljelly in amelioration of peripheralbloodleukocytesandlunginflammation in mouseconalbumin-inducedasthma model. SaudiJournalBio.Sci.2015 Nov22(6): 780-8
24. Araujo M.A.R.,Libério S.A., Guerra R.N.M., Ribeiro M.N.S., Nascimento F.R.F. Mechanisms of action underlying the anti-inflammatory and immunomodulatory effects of propolis: a brief review. Rev. Bras. Farm. 2012;22:208–219.
25. Zargar HR, Hemmati AA, Ghafourian M, Arzi A, Rezaie A, Javad-Moosavi SA. Long-termtreatmentwithroyaljelly-improvesbleomycin-inducedpulmonaryfibrosis in rats.Can J PhysiolPharmacol. 2017 Jan;95(1):23-31.
26. Kamiya T, Watanabe M, Hara H, Mitsugi Y, Yamaguchi E, Itoh A, Adachi T. Induction of Human-Lung-Cancer-A549-Cell Apoptosis by 4-Hydroperoxy-2-decenoic Acid Ethyl Ester through Intracellular ROS Accumulation and the Induction of Proapoptotic CHOP Expression.J Agric Food Chem. 2018 Oct 17;66(41):10741-10747.

Correspondence:
Ebru Tarıkçı Kılıç
Health Sciences University Ümraniye Training and Research Hospital Anesthesiology and Reanimation, Ümraniye stanbul E-mail: ebru.tarkc@yahoo.com;

Progress in Nutrition 2020; Vol. 22, N. 1: 304-315 DOI: 10.23751/pn.v22i1.9148

Original article

Sleep quality and its relationship with night eating syndrome, the risk of diabetes, and nutritional status among university students

Yasemin Akdevelioglu, Teslime Ozge Sahin, Ozge Yesildemir

Department of Nutrition and Dietetics, Faculty of Health Sciences, Gazi University, 06560, Ankara/Turkey - E-mail: dytozgeozay@gmail.com

Summary. Changes in sleep quality increase the risk of diabetes and obesity by affecting nutrition. This study was conducted to find the correlation between sleep quality and night eating syndrome in addition to obesity predisposition and the risk of diabetes. 550 university students including 330 women and 220 men between the ages of 17-42 years participated in the study. A face to face questionnaire was conducted in order to collect information about their personal characteristics, nutritional habits, and physical activities. Their anthropometric measurements were taken and food consumption in the last 24 hours were recorded. The Night Eating Syndrome Questionnaire, the Pittsburg Sleep Quality Index, and the Finnish Diabetes Risk Score were conducted on the participants. 40% of the students were found to have good sleep quality, while 60% were found to have poor sleep quality. The median values of night eating syndrome, the risk of diabetes, and the sleep quality scores of participants with good sleep quality were significantly lower than that of the participants with poor sleep quality. Also, a positive correlation was found between the sleep quality score along with the night eating and diabetes risk scores. Moreover, night eating syndrome and sleep duration were positively correlated with waist circumference and waist/height ratio which are indicators of obesity. As a result, the study found that poor sleep quality increased night eating syndrome, obesity predisposition, and the risk of diabetes, a metabolic disease. University students may be recommended to improve their sleep quality in order to prevent the above-mentioned metabolic diseases.

Key words: sleep quality, night eating syndrome, diabetes, obesity, nutrition

Introduction

Humans have biological, psychological, social, and cultural needs. One of our basic needs, sleep is a state of physical and mental rest in which people are inactive and unaware of their surroundings (1). Sleep is a natural process which maintains energy homeostasis. It also improves and restores the nervous system. It is correlated with many components of the biological structure, mainly the nervous system which controls stimulation, motor-skills, behaviors and cognitive functions (2). Generally, healthy sleep consists of two dimensions including sleep duration and sleep quality (3). Sleep quality is about feeling energetic, fit, and ready for a new day after waking up. It is also related to quantitative aspects of sleep such as sleep latency, sleep duration, and the number of times one wakes up at night in addition to subjective aspects such as sleep depth and restfulness (4). Although sleep is one of the basic physiological needs to maintain health, sleep disorders have currently become an important issue due to living conditions or physiological and psychological problems. According to the Epidemiology of Sleep Disorders in the Adult Population Survey which drew

up the sleep map of Turkey, 13% of the population has difficulty falling asleep, 30% sleep less than eight hours, and 11% sleep less than six hours (5).

Hunger is correlated with wakefulness. Distortions in sleep duration and quality may cause physiological and behavioral changes resulting in eating disorders (6). Night eating syndrome has been among the most prominent disorders recently (7). It is defined as consuming more than 25% of the daily food intake after dinner, waking up to eat at least twice a week, appetite suppression during morning hours, and appetite increase during evening hours in addition to having difficulty in falling asleep or maintaining the state of sleep at least four times a week (8). It was suggested that sleep disorders take an active role in the occurrence of night eating syndrome, the sleep quality of individuals with the syndrome is significantly low, they often wake up to eat, and have difficulty falling asleep (9).

Distortions in sleep duration and quality may significantly affect appetite, nutrition, in addition to energy balance and thus may trigger obesity, insulin resistance, and diabetes (10-12). Several prospective studies showed that poor sleep quality patterns such as excessive sleep duration (13), difficulty falling asleep (14), and sleep management (14, 15) increase the risk of impaired glucose tolerance or type 2 diabetes. Pyykkönen et al. (16) indicated that sleeping less than 6 hours or more than 9 hours increases the risk of type 2 diabetes. Even though the mechanisms which are correlated with sleep duration, quality, and the risk of diabetes are not completely clarified, it was suggested that short sleep duration may lead to insulin resistance by increasing sympathetic nervous system activity (17, 18) along with evening cortisol levels (19), and reducing cerebral glucose use (20). Increasing load on the pancreas due to insulin resistance may surpass β-cell function and cause type 2 diabetes in time. In addition, along with sleep disorder, increase in systemic inflammation indicators such as C-reactive protein and interleukin-6 play an important role in the formation of diabetes (21, 22).

Night eating syndrome is regarded as a key mechanism caused by the correlation between poor sleep quality and metabolic diseases such as diabetes. However, any study which examines the relationship between night eating syndrome, sleep quality, and diabetes risk together is not available in literature. This study was planned and carried out to find the correlation between sleep quality and night eating syndrome in addition to diabetes risk and nutritional status.

Method

The study was carried out with 550 volunteers including 330 women and 220 men between the ages of 18-42 years who studied at universities in Ankara, Turkey between May-November 2019. Sample size calculated according to a previous research examining night eating syndrome (23), nutritional habits and sleep quality among university students and considering 95% power and 5% α-error, 392 adolescents had been determined. This study, coded 2019-081, was approved by the Ethics Committee of Gazi University on 30.04.2019 under document no E.55336.

Participants

Individuals who did not meet exclusion criteria were not included in the study. Exclusion Criteria included being under 18, having physical or mental incapacity, undergoing psychiatric drug use in the last 6 months, and type 2 diabetes mellitus diagnosis.

The face to face interview method was applied to collect data from the participants with a questionnaire form consisting of three sections. The first section included questions regarding personal characteristics of the participants. The second was about nutritional habits and physical activities. The last part included anthropometric measurements.

Data Collection

Food Consumption Record

The 24-Hour Dietary Recall Form was recorded to determine their energy and food intake. The average energy and nutrient intake were analyzed by means of the " Nutrition Information System 7.2" (Turkish Version), a computer-aided nutrition program (24).

The Night Eating Syndrome Questionnaire

The Night Eating Questionnaire is used to assess the risk of night eating syndrome. Consisting of 16

questions, the Night Eating Syndrome Questionnaire was developed by Allison et al. (25) and adapted into Turkish by Atasoy et al. (26). The questionnaire consists of questions about morning appetite and the first meal of the day, evening and nocturnal ingestion, ratio of food intake after dinner, food cravings, control over night eating behavior, difficulty in falling asleep, frequency of waking up to eat, awareness and mood during nocturnal ingestions. Items except the 7th are rated between 0-4 with 5 point likert scale and total score ranges between 0-52. Scores not less than 25 are evaluated as "with night eating syndrome", while the scores under 25 are "no night eating syndrome" (26).

The Pittsburg Sleep Quality Index (PSQI)

The Pittsburgh Sleep Quality Index (PSQI) is an effective instrument used to measure the quality and patterns of sleep in the adults. The index was developed by Buysse et al. in 1989 (26) and Ağargün et al. carried out validity and reliability study of the index (27) in Turkey in 1999. It consists of 19 questions each of which has 7 items rated between 0-3. These items are subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disorder, sleeping pill use and daytime dysfunction. PSQI score is obtained by the total score of seven items ranging between 0-21. High scores are associated with poor sleep quality and high level of sleep disorder. More specifically, the scores above 5 mean clinically poor sleep quality (28).

The Finnish Diabetes Risk Score (FINDRISC)

FINDRISC was developed by the Finnish Diabetes Association in order to scan the risk of diabetes within the scope of the Program for the Prevention of Type 2 Diabetes in Finland (29). It is widely used in Turkey and highly recommended by the Turkish Journal of Endocrinology and Metabolism (30). Providing information about the risk of diabetes within 10 years, the FINDRISC consists of 8 questions including (age, BMI, waist circumference, exercise, vegetable and fruit consumption, hypertension, blood sugar level, diabetes history in family). Total FINDRISC score ranges between 0-26 and the individuals whose risk score is above 20 are at diabetes risk and required to be taken in a protection program (30, 31).

Body Composition and Anthropometric Measurements

The anthropometric (height, waist circumference) and body composition measurements of participants were carried out in compliance with applied techniques (32). The "Tanita BC 532" body analysis device was used to measure the body composition (body weight) of participants. In addition, their BMI (kg/m^2) values and waist/height ratios were calculated (33).

Statistical Analyses

The normality of distribution of continuous variables was tested by one-sample Kolmogorov-Smirnov test. Continuous variables with normal distribution were presented as mean (standard deviation [SD]); non-normal variables were reported as median (interquartile range [IQR]). Qualitative variables were expressed with frequency (f) and percentages (%) and Chi-square test was used to compare this data. For the comparison of quantitative variables and the Mann Whitney U test was used in other cases. Pearson correlation coefficient and Spearman correlation coefficient were used in the examination of the correlation among variables (34). All statistical calculations were evaluated within the 95% reliability range and at the $p<0.05$ significance level.

Results

Sleep Quality

The age average of participants was 21.6±2.43 year and the median and interquartile range of their sleep quality scores was 6.61(3.0).The distribution of some characteristics of students by their sleep quality is given in Table 1. 40% of the students were found to have good sleep quality, while 60% were found to have poor sleep quality. The mean age of poor sleeper students was found 21.6±2.39 year and mean age of good sleepers was found 21.7±2.49 year. Gender distribution of students with good sleep quality was 55% women and 45% men. 28.2% of the students with good sleep quality lived with their parents, while the ratio was 18.5% in students with poor sleep quality. 54.1% of the students with good sleep quality stayed in a dormitory, while the ratio was 64.8% in students with poor sleep quality ($p<0.05$). There were significant dif-

Table 1. Distribution of some characteristics of students by their sleep quality

	Sleep Quality				Total (n:550)		χ^2	p
	Good (n:220)		Poor (n:330)					
	f	%	f	%	f	%		
Gender								
Female	121	55.0	201	60.9	322	58.5	1.899	.168
Male	99	45.0	129	39.1	228	41.5		
Residence								
With family	62	28.2[a]	61	18.5[b]	123	22.4	8.67	**.034**
Alone at home	11	5.0	12	3.6	23	4.2		
With flatmates	28	12.7	43	13.1	71	12.9		
Dormitory	119	54.1[a]	214	64.8[b]	333	60.5		
Main meals								
1-2	104	47.3	195	59.1	299	54.4	7.431	**.004**
3	116	52.7	135	40.9	251	45.6		
Snack Meals								
1	103	46.8	118	35.8	221	40.2	6.759	.060
2	96	43.6	172	52.1	268	48.7		
≥3	21	9.5	40	12.1	61	11.1		
Meal skipping								
Yes	179	81.3	287	87.0	466	84.7	12.470	**.002**
No	41	18.7	43	13.0	84	15.3		
Skipped Meals								
Breakfast	76	40.6	130	44.4	206	42.9	0.669	.716
Lunch	103	55.1	152	51.9	255	53.1		
Dinner	8	4.3	11	3.8	19	4.0		
Sleep duration in weekdays (hour/day)								
≤5	3	1.4[a]	56	17.0[b]	59	10.7	35.476	**.000**
6-8	201	91.4[a]	259	78.5[b]	461	83.8		
≥9	16	7.3	15	4.5	30	5.5		
Sleep duration in weekends (hour/day)								
≤5	-	-[a]	11	3.3[b]	11	2.0	11.266	**.010**
6-8	114	51.8	189	57.3	303	55.1		
≥9	106	48.2[a]	130	39.4[b]	236	42.9		
Night eating syndrome								
Available	1	0.5	25	7.6	26	4.7	14.863	**.000**
N/A	219	99.5	305	92.4	524	95.3		
Diabetes risk level								
Low	213	96.8	313	94.8	528	95.6	1.227	.268
Medium/high	7	3.2	17	5.2	24	4.4		
BMI classification								
Underweight	12	5.5	27	8.2	39	7.1	1.526	.466
Normal	156	70.9	225	68.2	381	69.3		
Slightly overweight/overweight	52	23.6	78	23.6	130	23.6		

a-b: These letters denotes a subset of sleep quality categories whose column proportions differ significantly from each other at the .05 level.

ferences in terms of the sleep duration of students with good and poor sleep quality on weekdays and weekends. 1.4% of the students with good sleep quality and 17% of the students with poor sleep quality slept less than 5 hours on weekdays (p<0.001). Additionally, the sleep duration of participants with good sleep quality was a minimum of 6 hours, while 3.3% of the students with poor sleep quality slept a maximum of 6 hours.

There were also significant differences between the number of their main and snack meals. 40.9% of the students with poor sleep quality and 52.7% of the students with good sleep quality had three main meals a day (p<0.05).

The percentage of protein intake (15.5%) of the participants with good sleep quality was higher than in those with poor sleep quality (14.0%). On the contrary, the percentage of carbohydrate intake of participants with good sleep quality was lower (44.0% and 45.0%) (p<0.05) (Table 2).

Correlation between sleep quality score, night eating syndrome and diabetes risk scores and dietary energy, macronutrient intake and some anthropometric measurements was shown Table 3. The sleep quality score had positive correlation with the carbohydrate-based energy intake rate. However, there was a negative correlation with the protein-based energy intake rate and sleep quality score ($r_{CHO(\%)}$:.100, $r_{protein(\%)}$:-.151; p<0.01). The examination of the correlation with subcomponents of sleep quality and nutrition showed that there was a positive correlation between dietary CHO intake and sleep latency, a negative correlation between habitual sleep efficiency and dietary protein intake amount, contribution of protein-based energy to daily energy intake, and protein intake amount per body weight. In addition, even though sleep disorders had negative correlation with protein-based energy intake rates, there was a positive correlation with the CHO rate. There was a negative correlation between daytime dysfunction and the protein-based energy intake rate. There was no correlation between the sleep quality

Table 2. Median and interquartile range values of several anthropometric measurements, daily energy and nutrient intake in addition to scale scores according to sleep quality

	Sleep Quality		
	Good (n:220) Median (IQR)	**Poor (n:330) Median (IQR)**	**p**
Anthropometric measurements			
Body weight (kg)	61.5 (15.0)	60.0 (17.0)	.376
Waist Circumference (cm)	74.5 (12.0)	75.0 (14.0)	.975
Waist/height	0.4 (0.0)	0.4 (0.0)	.573
BMI ($kg/m^{2)}$	22.1 (4.6)	22.1 (4.4)	.756
Energy and nutrients			
Energy (kcal)	1655.2 (721.7)	1645.2 (760.4)	.965
Carbohydrate (g)	170.7 (98.1)	182.0 (92.4)	.177
Carbohydrate (%)	44.0 (11.0)	45.0 (11.0)	**.018***
Protein (g)	60.9 (31.2)	58.4 (31.8)	.148
Protein (%)	15.5 (5.0)	14.0 (5.0)	**.002***
Protein (g/kg)	1.0 (5.0)	0.97 (0.5)	.287
Fat (g)	76.6 (38.8)	74.0 (37.0)	.275
Fat (%)	41.0 (11.0)	40.0 (11.0)	.302
Scale scores			
Sleep Quality	4.0 (2.0)	8.0 (3.0)	**.000****
Night Eating Syndrome	12.0 (5.0)	15.0 (5.0)	**.000****
Diabetes risk	4.0 (4.0)	5.0 (4.0)	**.020****

** Mann-Whitney U is significant at the 0.05 level.** Mann-Whitney U is significant at the 0.001 level.*

score along with the waist circumference, waist/height ratio, or BMI of participants. However, sleep duration, one of the sub-components of sleep quality, was positively correlated with waist circumference and waist/height ratio.

Median values of night eating ($p<0.001$), risk of diabetes ($p<0.05$), and sleep quality scores ($p<0.001$) of participants with good sleep quality were significantly lower than in those with poor sleep quality (Table 2). In addition, a positive correlation was found between the sleep quality score and night eating in addition to diabetes risk scores ($r_{findrisk}$: .111, r_{GYS}:.428). There was also a positive correlation between the risk of diabetes as well as subjective sleep quality, sleep latency, sleep duration, and night eating syndrome ($p<0.05$) (Table 3).

Night Eating Syndrome

The night eating syndrome rates of participants with poor sleep quality (7.6%) were significantly higher than in those with good sleep quality (0.5%) ($p<0.001$) (Table 1). There was a positive correlation between night eating syndrome and all sub-components of sleep quality ($p<0.05$) (Table 3). A positive correlation was observed between night eating syndrome and dietary energy intake, carbohydrates, fat, and dietary fiber amount, while it was negatively correlated with the protein-based energy intake rate ($p<0.05$) (Table 3). Meal skipping habits of participants showed that 61.1% of participants with night eating syndrome skip breakfast, 34.6% lunch, and 3.8% dinner. However, meal skipping rates of individuals not having night eating syndrome were 41.9%, 54.2%, and 4.0% respectively ($\chi 2$: 3.995, $p=.136$) (Table 1). Night eating syndrome was positively correlated with diabetes risk, as well ($r=.225$, $p<0.001$).

In addition, there was another positive correlation between night eating syndrome scores and waist/height, BMI, waist circumference measurements ($r_{w/h}$:131, r_{BMI}: .170, r_w:.125) ($p<0.05$).

Diabetes Risk

3.2% of the participants with good sleep quality and 5.2% of the participants with poor sleep quality had a high risk of diabetes ($p>0.05$) (Table 1). The median value of diabetes risk was significantly high in participants with poor sleep quality (Table-2). There was a significantly positive correlation between diabetes risk and waist/height along with BMI and waist circumference measurements ($p<0.05$; .460; .490; .509 respectively) (Table 3).

Discussion

There is a general opinion that university students have poor sleep quality and inadequate sleep duration (35). In our study, supporting the above-stated opinion, the median value of the sleep quality scores of students was 6.61, 60% of the students (n: 330) had poor sleep quality, and 40% had good sleep quality (n: 220). In another study, similarly, the median value of the sleep quality scores of university students was 6.9±2.4 (36). This study showed that the residence and flatmates of students affected their sleep quality. It was found that the rate of students living with their families was lower and rate of staying dormitory was higher among the students with poor sleep quality than in students with good sleep quality ($p<0.05$). In another study, 75% of the students staying in the dormitory were found to have poor sleep quality (37). These results can be related to the impact of the sleep environment on sleep quality. Additionally, there was a significant difference between weekday and weekend sleep durations of students in terms of their sleep quality ($p<0.05$) (Table 1). 91.4% of students with good sleep quality and 78.5% of students with poor sleep quality sleep for 6-8 hours on weekdays ($p<0.001$) (Table 1). Considering that the ideal sleep duration is a minimum of 7 hours for this age group, (38)nearly all participants with good sleep quality got adequate sleep (38). In this study, the poor sleep quality of students (PSQI>5) can be attributed to various factors such as living away from their families, living alone or with friends, having the freedom to go to bed at any time, increasing anxiety of academic success, difficulty in adapting to the dormitory environment, and increasing time spent on out-of-school social activities.

In this study, a significant positive correlation was found between night eating syndrome and sleep quality scores ($p<0.05$). Also, the rate of night eating syndrome (7.6%) was found to be significantly higher in patients with poor sleep quality than in those with good sleep quality (0.5%) ($p<0.001$) (Table 1). Simi-

Table 3. Correlation between sleep quality score, night eating syndrome and diabetes risk scores and dietary energy, macronutrient intake and some anthropometric measurements

	NES	FINDRISC	BMİ (kg/m²)	Waist (cm)	Waist/ height	Energy (kcal)	Protein (g)	Protein (%)	Protein (g/kg)	Fat (g)	Fat (%)	n-6/n-3	CHO (g)	CHO (%)	Fiber (g)
Sleep Quality (PSQI)	**.428****	**.111****	.004	.012	.042	.051	-.031	**-.151****	-.015	.015	-.022	.009	**.102***	**.100***	.064
Subjective sleep quality	**.112****	**.096***	.041	.043	.058	-.030	.016	.047	.016	-.047	-.024	-.014	-.026	-.015	-.040
Sleep latency	**.367****	**.108***	.042	.055	.070	.065	.035	-.037	.027	.042	.007	.064	**.086***	.032	.066
Sleep duration	**.180****	.073	.063	**.126****	**.129****	.069	.029	-.062	.002	.048	-.035	-.056	.081	.066	.078
Sleep efficiency	**.126****	.028	.059	.070	.077	-.050	**-.102***	**-.114****	**-.104***	-.070	-.033	.035	.000	**.090***	.064
Sleep disturbance	**.291****	.012	-.044	**-.086***	-.050	-.012	-.049	**-.085***	-.018	-.029	-.040	.016	.041	**.092***	.004
Use of sleep medication	**.202****	.039	-.071	-.083	-.055	.057	-.004	-.080	.031	.031	-.015	-.062	.075	.050	.005
Daytime dysfunction	**.347****	.076	-.038	-.064	-.046	.025	-.027	**-.100***	-.001	-.002	-.027	.029	.066	.076	.048
NES	1.000	**.225****	**.170****	**.125****	**.131****	**.170****	.078	**-.104***	.003	**.120****	-.016	.063	**.190****	.079	**.092***
FINDRISC		1.000	**.460****	**.490****	**.509****	.050	.015	-.030	**-.150****	.078	.062	.070	.029	-.052	-.026
BMI (kg/m²)			1.000	**.753****	**.745****	.073	.070	.069	**-.283****	.057	.000	-.044	.055	-.036	.002
Waist circumference (cm)				1.000	**.909****	**.115****	**.130****	.075	**-.215****	**.104***	.001	-.024	.072	-.053	.000
Waist/height					1.000	.054	.073	.059	**-.185****	.061	.010	.014	.027	-.052	-.032
Energy (kcal)						1.000	**.744****	**-.154****	**.650****	**.856****	**.047***	.077	**.873****	.023	**.551****
Protein (g)							1.000	**.487****	**.892****	**.619****	-.016	-.060	**.538****	**-.222****	**.344****
Protein (%)								1.000	**.449****	**-.162****	-.082	**-.214****	**-.299****	**-.367****	**-.212****
Protein (g/kg)									1.000	**.538****	-.022	-.024	**.475****	**-.196****	**.317****
Fat (g)										1.000	**.504****	**.087***	**.545****	**-.395****	**.364****
Fat (%)											1.000	.058	**-.339****	**-.867****	**-.204****
n-6/n-3 fatty acids												1.000	.099	.040	.076
CHO (g)													1.000	**.459****	**.618****
CHO (%)														1.000	**.295***
Dietary fiber (g)															1.000

larly, another study conducted with university students found that those with a higher rate of night eating syndrome symptoms had worse sleep quality (39). In addition, there was a significant positive correlation between night eating syndrome and sub-components of sleep quality ($p<0.05$) (Table 1). Similar to our study, sleep disorders, sleep medication use, and daytime dysfunction were significantly higher in individuals with night eating disorder, according to the Pittsburgh Sleep Quality Index (40). Also, in a study conducted with 144 morbid obese individuals in England, it was found that the total night eating score is weakly correlated with sleep duration and strongly correlated with sleep disorders (41). The correlation between poor sleep quality and night eating syndrome is explained by the decrease in sleep duration due to frequent nocturnal ingestion in individuals with night eating syndrome (9, 42, 43).

On the contrary, night eating syndrome may also occur due to the deterioration of sleep quality. Since the triggering factor is unknown, there is a vicious cycle between poor sleep quality and night eating syndrome (44).

In our study that the number of main meals decreased significantly with the deterioration of sleep quality, whereas the number of intermediate meals increased ($p<0.05$) (Table 1). As sleep duration in people with poor sleep quality is shorter, they constantly need snacks. This may increase the number of intermediate meals and deteriorate their appetite. For this reason, they may skip breakfast. In furtherance, the rate of skipping breakfast was higher in the participants whose sleep durations were shorter (45). Sleep quality is also associated with dietary patterns as well as meal order. It was found that individuals with short sleep durations tend towards high-calorie and high-carbohydrate foods (46). Our study found that the ratio of dietary energy from carbohydrate was higher and had a positive correlation with sleep quality scores in participants with poor sleep quality ($p<0.05$) (Table 3). Another study showed that sleep quality deteriorated with the increase in dietary carbohydrate intake, and those with a higher consumption of confectionery in addition to noodles had worse sleep quality and those with a higher consumption amount of fish along with vegetables had better sleep quality (47). On the contrary, in a study conducted with Chinese adults, the carbohydrate rate in diet was significantly lower in adults with shorter sleep durations than in normal adults (48). Dietary carbohydrates contain a large variety of sugar chains with different metabolisms. Thus, it is not surprising that the amount of any individual carbohydrate has no consistent influence on sleep parameters. In addition to carbohydrates, there is a correlation between the dietary protein ratio and sleep quality. Our study found that the ratio of dietary energy from protein was lower and had a negative correlation with sleep quality scores in participants with poor sleep quality ($p<0.05$) (Table 3). Another study demonstrated that short or long sleep duration in adults decreased the protein-based dietary energy rate compared to normal sleep durations (49). This is explained as tryptophan, a precursor to the neurotransmitter serotonin and the neuro-secretory hormone melatonin, both of which are linked to sleep and alertness.

Dietary patterns were closely correlated with night eating syndrome as well as sleep quality. Our study results showed that night eating syndrome had a positive correlation with the amount of dietary energy, carbohydrates, fat, and dietary fiber intake in addition to a negative correlation with protein-based energy intake ($p<0.05$) (Table 3). However, in a study, adolescents with night eating syndrome were found to have high dietary fat and low carbohydrate intake (50). In addition, there are several studies in literature which find no difference among night eating syndrome, sleep quality, and nutrient intake (51, 52). This may be caused by the difference in dietary carbohydrate content (mono-polysaccharide, low-high glycemic index etc.) and tryptophan amounts, timing of carbohydrate ingestion which impacts serotonin synthesis, and circadian disruption (53). It was considered that the effect of night eating syndrome on increasing energy intake may be correlated with the hormones regulating food intake. In a study examining the effect of sleep duration on hormones regulating food intake, it was found that a 4-hour sleep duration decreased leptin levels by 18% on average and increased ghrelin levels by 28%. Accordingly, the hunger of individuals with inadequate sleep durations increased by 23% and their craving for high-carbohydrate food increased by 30% (54). In addition, orexin A and orexin B peptides released from

the lateral region of the hypothalamus play a role in the neuroendocrine control of appetite in the state of sleep-wake. Orexin-containing neurons is active during wakefulness and quiescent during sleep (55) The short sleep duration in humans has been shown to decrease GLP-1 levels. Sleep restriction can also trigger reward-driven eating behavior, which can lead to excessive eating and cause emotional stress in addition to impulsive behavior. These changes may contribute to the increase in food intake, as well (56).Anthropometric measurements which are indicators of obesity may change with the increase of energy intake as a result of the effects of these mechanisms.

This study found that night eating syndrome and sleep duration were positively correlated with waist circumference and waist/height ratio which are indicators of obesity ($p<0.05$) (Table 3). Additionally, in parallel with the literature, there was a significantly positive correlation between night eating and BMI (57, 58). Another study reported that individuals were at normal weight before having night eating syndrome and they put on weight after getting the syndrome (59). Similarly, another study found that short sleep duration increased obesity in adults by 1.55 times (60). In a study conducted with university students, a positive correlation was found between poor sleep quality and short sleep duration in addition to obesity (23). Systematic review similarly concluded that short sleep duration seems to be independently associated with weight gain, particularly in young age groups (61).

Distortions in sleep duration and quality may significantly affect appetite, nutrition in addition to energy balance, and thus may trigger obesity, insulin resistance, and diabetes (10-12). Poor sleep quality as well as its complaints are associated with alterations in diurnal cortisol levels consistent with alteration of neuroendocrine functioning, in particular the hypothalamic-pituitary-adrenal (HPA). Plasma glucose levels of nocturnal individuals were consistently at a high level between midnight and early morning, whereas insulin secretion was markedly decreased during this time period (i.e., a pronounced mismatch of glucose and insulin levels). In contrast, there was a strong positive correlation between the plasma levels of glucose along with insulin during the daytime and evening in the diurnal individuals. For this reason, night meals cause disruption of insulin response against glucose. Accordingly, obesity and diabetes are higher in patients with night eating syndrome (62, 63). Also systematic review by Irwin confirmed the presence of the association between sleep disturbance and markers of systemic inflammation such as C-reactive protein and interleukin-6 which are also related to obesity. It is suggested that increased inflammation markers play an important role in the development of chronic diseases such as diabetes, and dyslipidemia (3). Night eating syndrome delays meal times along with glucose, and insulin levels. Ghrelin levels change similarly to the deterioration in sleep quality as a result of delayed energy intake. In parallel with this information, our study showed that the risk of diabetes increases, as night eating syndrome increases and sleep quality deteriorates. The correlation between sleep duration and diabetes risk is supported by a number of epidemiological and clinical studies. Epidemiological studies in the United States and around the world have shown that the risk of diabetes and the outcome of diabetes are positively correlated with short (≤6h/24h) and long sleep (≥9h/24h) durations (64).In a meta-analysis of prospective cohort studies, short sleep duration was associated with high risk of diabetes (relative risk=1.37; 95%CI, 1.22-1.53) (65). Gangwisch et al. (61)Q indicated in a meta-analysis of seven studies that diabetes type 2 risk in individuals with inadequate/short sleep duration was 28% higher. Engeda et al. (66) found that individuals who sleep ≤5 hours/day have 2 times the rate of pre-diabetes than those who sleep 9 hours. In summary, repeated bouts of restricted sleep may induce chronic hyperinsulinemia, stimulating downstream pathways like pancreatic beta cell failure and lipogenesis, leading to development of diabetes and obesity (64).

Results and recommendations

In this study conducted on 550 university students with a mean age of 21.6, more than half of the participants were found to have poor sleep quality. It was found that deterioration in sleep quality increased obesity predisposition and night eating syndrome which play a role in the etiology of metabolic diseases

and even increase the risk of diabetes which is also a metabolic disease. University students should improve their sleep quality in order to prevent themselves from getting such diseases. It is recommended to ensure a suitable sleep environment and adequate sleep duration which is one of the most important factors which improve sleep quality.

References

1. Peuhkuri K, Sihvola N, Korpela R. Diet promotes sleep duration and quality. Nutr Res 2012;32(5):309-19.
2. Sahin L, Ascioglu M, Taskin E. Sleep and regulation of sleep. Journal of Health Sciences 2013;22(1):93-8.
3. Lian Y, Yuan Q, Wang G, Tang F. Association between sleep quality and metabolic syndrome: A systematic review and meta-analysis. Psychiatry Res 2019; 274:66-74.
4. Ustün Y, Yücel SC. Hemşirelerin uyku kalitesinin incelenmesi. Maltepe Üniversitesi Hemşirelik Bilim ve Sanatı Dergisi 2011;4(1):29-38.
5. Ardic S, Demir AU, Ucar ZZ, Firat H, Itil O, Karadeniz D, et al. Prevalence and associated factors of sleep-disordered breathing in the Turkish adult population. Sleep Biol Rhythms 2013;11(1):29-39.
6. Miller AL, Miller SE, LeBourgeois MK, Sturza J, Rosenblum KL, Lumeng JC. Sleep duration and quality are associated with eating behavior in low-income toddlers. Appetite 2019;135:100-7.
7. Orhan FO, Tuncel D. Night eating disorders. Current Approaches in Psychiatry 2009;1(2):132-54.
8. Allison KC, Lundgren JD, O'Reardon JP, Geliebter A, Gluck ME, Vinai P, et al. Proposed diagnostic criteria for night eating syndrome. Int J Eat Disord 2010;43(3):241-7.
9. Rogers NL, Dinges DF, Allison KC, Maislin G, Martino N, O'Reardon JP, et al. Assessment of sleep in women with night eating syndrome. Sleep 2006;29(6):814-9.
10. Zimberg IZ, Dâmaso A, Del Re M, Carneiro AM, de Sá Souza H, de Lira FS, et al. Short sleep duration and obesity: mechanisms and future perspectives. Cell Biochem Funct 2012;30(6):524-9.
11. Hasler G, Buysse DJ, Klaghofer R, Gamma A, Ajdacic V, Eich D, et al. The association between short sleep duration and obesity in young adults: a 13-year prospective study. Sleep 2004;27(4):661-6.
12. Gottlieb DJ, Punjabi NM, Newman AB, Resnick HE, Redline S, Baldwin CM, et al. Association of sleep time with diabetes mellitus and impaired glucose tolerance. Arch Intern Med 2005;165(8):863-7.
13. Ayas NT, White DP, Al-Delaimy WK, Manson JE, Stampfer MJ, Speizer FE, et al. A prospective study of self-reported sleep duration and incident diabetes in women. Diabetes Care 2003;26(2):380-4.
14. Kawakami N, Takatsuka N, Shimizu H. Sleep disturbance and onset of type 2 diabetes. Diabetes Care 2004;27(1):282-3.
15. Mallon L, Broman JE, Hetta J. High incidence of diabetes in men with sleep complaints or short sleep duration: a 12-year follow-up study of a middle-aged population. Diabetes Care 2005;28(11):2762-7.
16. Pyykkönen A-J, Isomaa B, Pesonen A-K, Eriksson JG, Groop L, Tuomi T, et al. Sleep duration and insulin resistance in individuals without type 2 diabetes: the PPP-Botnia study. Ann Med 2014;46(5):324-9.
17. Tochikubo O, Ikeda A, Miyajima E, Ishii M. Effects of insufficient sleep on blood pressure monitored by a new multibiomedical recorder. Hypertension 1996;27(6):1318-24.
18. Irwin M, Thompson J, Miller C, Gillin JC, Ziegler M. Effects of sleep and sleep deprivation on catecholamine and interleukin-2 levels in humans: clinical implications. J Clin Endocrinol Metab 1999;84(6):1979-85.
19. Leproult R, Copinschi G, Buxton O, Van Cauter E. Sleep loss results in an elevation of cortisol levels the next evening. Sleep 1997;20(10):865-70.
20. Spiegel K, Knutson K, Leproult R, Tasali E, Van Cauter E. Sleep loss: a novel risk factor for insulin resistance and Type 2 diabetes. J Appl Physiol (1985) 2005;99(5):2008-19.
21. Irwin MR, Olmstead R, Carroll JE. Sleep disturbance, sleep duration, and inflammation: A systematic review and meta-analysis of cohort studies and experimental sleep deprivation. Biol Psychiatry 2016;80(1):40-52.
22. Kanagasabai T, Ardern CI. Contribution of inflammation, oxidative stress, and antioxidants to the relationship between sleep duration and cardiometabolic health. Sleep 2015;38(12):1905-12.
23. Yahia N, Brown C, Potter S, Szymanski H, Smith K, Pringle L, et al. Night eating syndrome and its association with weight status, physical activity, eating habits, smoking status, and sleep patterns among college students. Eat Weight Disord 2017;22(3):421-33.
24. BeBis (Beslenme Bilgi Sistemi) bilgisayar yazılım programı versiyon 7.2. 2010.
25. Allison KC, Lundgren JD, O'Reardon JP, Martino NS, Sarwer DB, Wadden TA, et al. The Night Eating Questionnaire (NEQ): Properties of a measure of severity of the night eating syndrome. Eat Behav 2008;9(1):62-72.
26. Buysse DJ, Reynolds CF, 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res 1989;28(2):193-213.
27. Agargun M. Pittsburgh uyku kalitesi indeksinin gecerligi ve guvenirligi. Turk Psikiyatri Derg 1996;7:107-15.
28. Fiorentini A, Valente R, Perciaccante A, Tubani L. Sleep's quality disorders in patients with hypertension and type 2 diabetes mellitus. Int J Cardiol 2007;114(2):50-2.
29. Finnish Diabetes Association. Programme for the Prevention of Type 2 Diabetes in Finland (2003-2010). Finland, 2003. [cited 2020 March 5]. Available from: http://www.diabetes.fi/files/1108/Programme_for_the_Prevention_of_Type_2_Diabetes_in_Finland_2003-2010.pdf

30. Turkiye Endokrinoloji ve Metabolizma Dernegi. TEMD Diabetes Mellitus ve Komplikasyonlarının Tanı, Tedavi ve İzlem Kılavuzu (2018). Ankara, 2018. [cited 2020 March 5]. Available from: http://temd.org.tr/admin/uploads/tbl_kilavuz/20180814161019-2018tbl_kilavuz6c373c6010.pdf
31. Lindstrom J, Eriksson JG, Valle TT, Aunola S, Cepaitis Z, Hakumaki M, et al. Prevention of diabetes mellitus in subjects with impaired glucose tolerance in the Finnish Diabetes Prevention Study: results from a randomized clinical trial. J Am Soc Nephrol 2003;14(7):108-13.
32. Lohman TG, Roche AF, Martorell R. Anthropometric standardization reference manual. Human kinetics books. Champaign, IL:1988.
33. Word Health Organization. Diet, nutrition and the prevention of chronic diseases. WHO Technical Report Series. Geneva, 916, 2003. [cited 2020 March 5]. Available from: https://apps.who.int/iris/bitstream/handle/10665/42665/WHO_TRS_916.pdf;jsessionid=FCA97C4681041AE5D3BF3AE0B8DFE8FB?sequence=1
34. Statistical Package for the Social Sciences (SPSS Inc.). 2001.
35. Orzech KM, Salafsky DB, Hamilton LA. The state of sleep among college students at a large public university. J Am Coll Health 2011;59(7):612-9.
36. Saygılı S, Akıncı AÇ, Arıkan H, Dereli E. Üniversite öğrencilerinde uyku kalitesi ve yorgunluk. Ejovoc 2011;1(1):88-94.
37. Orsal O, Orsal O, Alparslan GB, Unsal A. Evaluation of the relation between quality of sleep and anxiety among university students. HealthMED 2012;6(7):2244-55.
38. Hirshkowitz M, Whiton K, Albert SM, Alessi C, Bruni O, DonCarlos L, et al. National Sleep Foundation's sleep time duration recommendations: methodology and results summary. Sleep health 2015;1(1):40-3.
39. Nolan LJ, Geliebter A. Night eating is associated with emotional and external eating in college students. Eat Behav 2012;13(3):202-6.
40. Lundgren JD, Allison KC, O'Reardon JP, Stunkard AJ. A descriptive study of non-obese persons with night eating syndrome and a weight-matched comparison group. Eat Behav 2008;9(3):343-51.
41. Cleator J, Abbott J, Judd P, Wilding JP, Sutton CJ. Correlations between night eating, sleep quality, and excessive daytime sleepiness in a severely obese UK population. Sleep Med 2013;14(11):1151-6.
42. Spaggiari MC, Granella F, Parrino L, Marchesi C, Melli I, Terzano MG. Nocturnal eating syndrome in adults. Sleep 1994;17(4):339-44.
43. Manni R, Ratti MT, Tartara A. Nocturnal eating: prevalence and features in 120 insomniac referrals. Sleep 1997;20(9):734-8.
44. Rogers NL, Dinges DF, Allison KC, Maislin G, Martino N, O'Reardon JP, et al. Assessment of sleep in women with night eating syndrome. Sleep 2006;29(6):814-9.
45. Kim S, DeRoo LA, Sandler DP. Eating patterns and nutritional characteristics associated with sleep duration. Public Health Nutr 2011;14(5):889-95.
46. McNeil J, Doucet E, Chaput J-P. Inadequate sleep as a contributor to obesity and type 2 diabetes. Can J Diabetes 2013;37(2):103-8.
47. Katagiri R, Asakura K, Kobayashi S, Suga H, Sasaki S. Low intake of vegetables, high intake of confectionary, and unhealthy eating habits are associated with poor sleep quality among middle-aged female Japanese workers. J Occup Health 2014;56(5):359-68.
48. Shi Z, McEvoy M, Luu J, Attia J. Dietary fat and sleep duration in Chinese men and women. Int J Obes (Lond) 2008;32(12):1835.
49. Kant AK, Graubard BI. Association of self-reported sleep duration with eating behaviors of American adults: NHANES 2005–2010. Am J Clin Nutr 2014;100(3):938-47.
50. Farhangi MA. Night Eating Syndrome and Its Relationship with Emotional Eating, Sleep Quality and Nutritional Status Among Adolescents' Boys. Community Ment Health J 2019:1-8.
51. Allison KC, Ahima RS, O'Reardon JP, Dinges DF, Sharma V, Cummings DE, et al. Neuroendocrine profiles associated with energy intake, sleep, and stress in the night eating syndrome. J Clin Endocrinol Metab 2005;90(11):6214-7.
52. Türközü D, Aksoydan E. Uyku süresi ve kalitesinin beslenme ve vücut bileşimine etkisi. Sted 2015; 24(1):10.
53. Smith I, Saed K, St-Onge M-P. Sleep and food intake. Sleep and Health: Elsevier. 2019; 243-55.
54. Spiegel K, Tasali E, Penev P, Van Cauter E. Brief communication: sleep curtailment in healthy young men is associated with decreased leptin levels, elevated ghrelin levels, and increased hunger and appetite. Ann Intern Med 2004;141(11):846-50.
55. Spiegel K, Tasali E, Leproult R, Van Cauter E. Effects of poor and short sleep on glucose metabolism and obesity risk. Nat Rev Endocrinol 2009;5(5):253.
56. Zhu B, Shi C, Park CG, Zhao X, Reutrakul S. Effects of sleep restriction on metabolism-related parameters in healthy adults: A comprehensive review and meta-analysis of randomized controlled trials. Sleep Med Rev 2019;45:18-30.
57. Poggiogalle E, Lubrano C, Gnessi L, Marocco C, Di Lazzaro L, Polidoro G, et al. Reduced sleep duration affects body composition, dietary intake and quality of life in obese subjects. Eat Weight Disord 2016;21(3):501-5.
58. Nolan LJ, Geliebter A. "Food addiction" is associated with night eating severity. Appetite 2016;98:89-94.
59. Marshall HM, Allison KC, O'Reardon JP, Birketvedt G, Stunkard AJ. Night eating syndrome among nonobese persons. Int J Eat Disord 2004;35(2):217-22.
60. Cappuccio FP, Taggart FM, Kandala N-B, Currie A, Peile E, Stranges S, et al. Meta-analysis of short sleep duration and obesity in children and adults. Sleep 2008;31(5):619-26.
61. Gangwisch JE, Malaspina D, Boden-Albala B, Heymsfield SB. Inadequate sleep as a risk factor for obesity: analyses of the NHANES I. Sleep 2005;28(10):1289-96.
62. Howell MJ, Schenck CH, Crow SJ. A review of nighttime

eating disorders. Sleep Med Rev 2009;13(1):23-34.
63. Schenck CH. A study of circadian eating and sleeping patterns in night eating syndrome (NES) points the way to future studies on NES and sleep-related eating disorder. Sleep Med 2006;8(7):653-6.
64. Seixas AA, Robbins R, Chung A, Popp C, Donley T, McFarlane SI, et al. Sleep health and diabetes: The role of sleep duration, subjective sleep, sleep disorders, and circadian rhythms on diabetes. Sleep and Health: Elsevier 2019; 213-25.
65. Itani O, Jike M, Watanabe N, Kaneita Y. Short sleep duration and health outcomes: a systematic review, meta-analysis, and meta-regression. Sleep Med 2017;32:246-56.
66. Engeda J, Mezuk B, Ratliff S, Ning Y. Association between duration and quality of sleep and the risk of pre-diabetes: evidence from NHANES. Diabetic Med 2013;30(6):676-80.

Correspondence:
Ozge Yeşildemir, Research Asisstant
Gazi University, Faculty of Health Sciences
Department of Nutrition and Dietetics
Postcode: 06560
Besevler/Ankara/TURKEY
Tel: +90 312 2162976
E-mail: dytozgeozay@gmail.com

Progress in Nutrition 2020; Vol. 22, N. 1: 316-322 DOI: 10.23751/pn.v22i1.9311

Original article

The effect of 8-week Zumba® fitness on body composition of turkish womens

Aziz Güçlüöver

Kırıkkale University, Faculty of Sport Sciences, Physical Education and Sports Teaching Department, Kırıkkale/TURKEY - E-mail: azizgucluover@hotmail.com

Summary. The aim of this study is to evaluate the effect of 8-week zumba fitness on body composition of Turkish women between the ages of 18-35. The sample of the study consists of 28 (n=14 experimental group and n=14 control group) women who are between the ages of 18-35 and living in the city centre of Kırıkkale. The women in the experimental group participated in the zumba fitness which lasted about 60 minutes 3 days a week for 8 weeks. The women in the control group continued their daily life. Body composition of the participants in both experimental and control groups was determined by Bioelectric Impedance Analysis Method before and after 8 week zumba fitness. The data obtained from applications to determine body composition were evaluated in SPSS 25.0 package program with 95% confidence interval and 0.05 significance level. In statistical analyses, statistically significant difference has been observed in the mean values obtained from the applications of pre-test and post-test regarding variables of BMI (kg/cm^2), body fat percentage (% fat), body fat mass (kg), lean body mass (kg), right leg fat percentage (% fat), right leg fat mass (kg) of the women in the experimental group ($p<0.05$). Within the lights of the findings obtained in the study, it has been concluded that the 8-week zumba fitness caused a decrease in body weight, body fat percentage and BMI values of Turkish women between the ages of 18-35. In the regional body analysis done in the study, it has been understood that the effect of the zumba fitness program was on the lower extremity.

Key words: turkish women, zumba fitness program, body composition

Introduction

In the international studies on health improvement, regular physical activity in women was reported to reduce and prevent problems related to the musculoskeletal system. Participating in regular exercise and avoiding sedentary activity have been highly recommended (1, 2). Recently, the significance of preventive rehabilitation has gradually increased. Health professionals have been focused on participation in regular and sufficient physical activities and prevention of injuries (3). It has been known that adult women participate in different types of exercise, especially the demand of women for spine stabilization exercises which are known as pilates or core trainings are high (4). It has been reported in the literature that these relevant exercises have many positive effects from individuals' flexibility to functional capacities (5, 6).

Due to the fact that women have different physiological structures compared to men and their roles in society change according to cultures, their responsibilities and daily activities (housework, child care, etc.) increase. They prefer to rest rather than regular physical activity or exercise. Therefore, the rate of sedentary life tendency is high. For this reason, regular exercise is significant for women (7, 8).

It was reported in the literature that women were affected more by the negativities caused by sedentary life (7, 9). Thus it is significant to encourage women to exercise and to ensure them to benefit from the ad-

vantages of exercise (7, 8). Exercise in women was reported to be effective on musculoskeletal pain (10), depression, life quality, posture, body composition (such as body mass index and waist-to-hip ratio) (11, 12). Many women who wish to do exercise today prefer zumba fitness.

Zumba fitness which combines exercise with the most active and entertaining figures of Latin dance is one of the most preferred fitness programs by women. Zumba fitness is based on different Latin American rhythms such as, bachata, reggaetón, salsa, merengue, cumbia and samba. Hip hop, belly dancing, Indian and African figures also enrich the content of Zumba fitness (13). Zumba fitness which is one of the most popular fitness programs today was developed in the mid 90's by the famous fitness trainer Alberto "Beto" Perez. One day, Beto Perez notices that he has forgotten the CDs that he always brings to his lessons. At that time, he takes the CD containing salsa and merengue music that he has prepared for himself in his bag and uses that CD in his class. Beto Perez completes the class with his energy and his own choreographies. Everybody who attends the course is amazed. Thus, Zumba fitness is born (14). Zumba fitness session takes an average of 1 hour. The session starts with warm-up music. Then, songs are lined up to be an intermittent exercise. Zumba fitness session ends with the cooling music (15).

Zumba fitness which not only entertains while exercising, but also gives flexibility to body activates effectively all body muscles. While it increases calorie expenditure with aerobic interval loading and strength exercises, it also enables the strength of whole body and cardiovascular system to increase (13). While all the muscles of the body are functioning, calorie burning is between 600 and 1000 in 45 and 60 minute courses and it plays an active role in the weight loss process (15). This modern fitness program provides contributions such as prevention of posture disorders, development of coordination and strengthening of bone joint segments (16). It speeds up weight loss with a balanced diet and helps shape all parts of the body (15). The researches confirm that the implementation of zumba fitness contributed to statistically significant effects in improving functional and motoric abilities of a woman (17, 18, 19, 20) and changes in women body composition. Referring to the results obtained from similar studies, the hypothesis of the study was determined that 8 weeks of zumba fitness had an effect on body composition in Turkish women between the ages of 18-35. Therefore the aim of the study is to evaluate the effect of Zumba fitness on the body composition of Turkish women between the ages of 18-35, who regularly participated in the Zumba fitness program for 8 weeks.

Method

Participants

The sample of the study consists of 28 women who live in the city center of Kırıkkale and between the ages of 18-35 (n = 14 experimental groups and n = 14 control groups).

Zumba Fitness Program

Zumba fitness was practiced 3 times a week for 8 weeks. Each session, which lasted approximately 60 minutes, consisted of warming, cooling with a main section and stretching sections. The intensity of the exercise was determined by the tempo of the music. The warm-up section continued for 8-10 minutes (tempo 125-140 bpm). In the main part of the session, the participants aimed to dance and exercise with their favourite music. The main part of the session was exercised accompanied by 8-10 original fitness Zumba songs. The dance choreographies and intensity of the movements were adapted to the tempo of the music ranging from 140-160 bpm. All Latin American dance choreographies (such as merengue, salsa, samba, belly dance, cha cha cha, tango) were utilized. Each dance song continued for 3-5 minutes with 15-20 seconds rest between the songs. During the cooling phase, which is the last part of the session, the participants were aimed to relax physically and mentally. Stretching exercises were performed to relax muscles, increase muscle flexibility and avoid muscle pain. No jumping and squat movement were allowed during the cooling phase. All of the movements were performed in a standing, sitting and stretching position (tempo of music is 100 Beats Per Minute (BPM)) (13).

Height and Body Weight Measurements

The tape measure with a 0.01 cm degree of accuracy was used in the height measurements of the participants. The measurements were performed when the participants' feet were bare. While height measurements were performed when the participants' heads were upright, the soles of feet stood on the ground flatly, the knees were stretched, the heels were adjacent and body was upright; body weights were measured with a device of Bioelectrical Impedance Analysis (BIA) with a sensitivity of 0.1 and with barefoot and minimal clothing (21).

Bioelectrical Impedance Analysis

Bioelectrical impedance analysis to determine the body composition of the participants was performed with the device of "Tanita-BC 418 MA". The device of Tanita has 8 electrodes and uses high frequency constant current source (50 kHz, 500A). The individuals who participated in the measurement were asked not to eat anything until at least 4 hours before the measurement, not to drink anything including caffeine-containing drinks, not to take a bath or use sauna, not to drink alcohol until 24 hours before the measurement and not to do sports on the day of the measurement. Individuals were asked to stand on bare feet on the metal surface of the device, to hold the parts of the device that should be handled with both hands, and to release their arms free in a position parallel to the body. The measurements lasted approximately 1-2 minutes for each subject, and the percentage of body fat detected by the device of bioelectrical impedance analysis was printed out from the device (21).

Table 1. Comparison of the pretest and posttest averages of the body composition of women in the experimental group

Variables	Pre-test			Post-test			t	p
	N	$\bar{X}$	Sd±	N	$\bar{X}$	Sd±		
Body Weight (kg)	14	60.64	10.08	14	58.43	11.47	-2.232	0.003*
Body Mass Index (kg/cm^2)	14	23.96	3.36	14	22.58	3.56	-1.476	0.005*
Basal Metabolic Rate	14	1346.6	115.4	14	1352.7	121.2	-1.186	0.238
Total Body Water (kg)	14	31.12	2.56	14	31.20	2.52	-0.994	0.342
Fat Percentage (% fat)	14	31.66	7.34	14	30.22	7.16	-2.458	0.002*
Fat Mass (kg)	14	20.82	7.29	14	19.56	8.15	-2.776	0.016*
Free Fat Mass (kg)	14	41.42	4.71	14	42.52	5.17	-1.145	0.028*
Right Arm Fat Percentage (% fat)	14	33.92	8.26	14	33.96	9.26	0.614	0.596
Right Arm Fat Mass (kg)	14	1.12	0.62	14	1.09	0.58	-0.489	0.512
Right Arm Free Fat Mass (kg)	14	1.10	0.24	14	1.24	0.28	-1.108	0.256
Left Arm Fat Percentage (% fat)	14	34.58	8.10	14	33.46	8.22	1.424	0.424
Left Arm Fat Mass (kg)	14	1.07	0.48	14	1.11	0.54	1.378	0.486
Right Arm Free Fat Mass (kg)	14	1.90	0.22	14	1.92	0.26	-3.402	0.286
Fat Percentage (% fat)	14	43.86	6.79	14	43.42	7.12	0.916	0.268
Fat Mass (kg)	14	9.92	5.74	14	9.78	5.68	-1.405	0.214
Free fat Mass (kg)	14	22.52	1.65	14	23.04	1.61	-1.504	0.166
Right Leg Fat Percentage (% fat)	14	34.66	5.32	14	34.49	5.41	-1.124	0.268
Right Leg Fat Mass (kg)	14	3.95	1.29	14	4.14	1.30	-4.067	0.001*
Right Leg Free Fat Mass (kg)	14	4.28	071	14	4.18	0.72	2.385	0.033*
Left Leg Fat Percentage (% fat)	14	34.56	5.16	14	35.81	5.10	-4.564	0.001*
Left Leg Fat Mass (kg)	14	3.90	1.26	14	4.05	1.28	-4.048	0.001*
Left Leg Free Fat Mass (kg)	14	4.13	0.74	14	4.12	0.70	1.992	0.005*

**0.05 significance level*

Data Analysis

The statistical analysis of the data obtained from the measurements carried out to determine the body composition of women in the experimental and control groups participating in the study were performed in the SPSS 25.0 package program with 95% confidence interval and 0.05 error level. Paired Sample T Test was utilized to compare the averages of the values observed in two different cases of a variable.

Results

When Table was examined it was observed that there was a statistically significant difference between the average values obtained from the pre-test and post-test applications regarding body mass index (kg/cm^2), body fat percentage (% fat), body fat mass (kg), body free fat mass (kg), right leg fat percentage (% fat), right leg fat mass (kg), right leg free fat mass (kg), left leg fat percentage (% fat), left leg fat mass (kg) and left leg free fat mass (kg) variables of women in the experimental group ($p < 0.05$). In Table 2, it was observed that there was no statistically significant difference between the average values obtained from the pre-test and post-test applications to determine the body composition of women in the control group ($p > 0.05$).

Discussion and Conclusion

In this study, the zumba fitness applied to women in the experimental group caused changes in the body composition of women. Variables in which these changes have been observed are body weight (kg), BMI (kg/cm^2), body fat percentage (% fat), body fat

Table 2. Comparison of the pretest and posttest averages of the body composition of women in the control group

Variables	Pre-test			Post-test			t	p
	N	$\bar{X}$	Sd±	N	$\bar{X}$	Sd±		
Body Weight (kg)	14	60.64	12.08	14	61.43	12.47	-2.113	0.055
Body Mass Index (kg/cm^2)	14	23.89	4.40	14	24.12	4.46	-1.870	0.084
Basal Metabolic Rate	14	1312.9	117.9	14	1319.9	120.5	-1.192	0.255
Total Body Water (kg)	14	30.02	2.45	14	30.20	2.48	-0.998	0.336
Fat Percentage (% fat)	14	30.78	8.38	14	31.40	8.41	-1.753	0.103
Fat Mass (kg)	14	19.77	9.22	14	20.23	9.37	-1.882	0.082
Free Fat Mass (kg)	14	40.40	4.83	14	69.30	8.26	-1.033	0.320
Right Arm Fat Percentage (% fat)	14	33.84	9.17	14	33.60	9.14	0.602	0.557
Right Arm Fat Mass (kg)	14	1.04	0.51	14	1.05	0.53	-0.694	0.500
Right Arm Free Fat Mass (kg)	14	1.87	0.19	14	1.89	0.20	-1.482	0.128
Left Arm Fat Percentage (% fat)	14	34.27	9.06	14	33.96	9.22	0.673	0.513
Left Arm Fat Mass (kg)	14	1.10	0.56	14	1.09	0.59	0.618	0.547
Right Arm Free Fat Mass (kg)	14	1.90	0.24	14	1.92	0.26	-1.309	0.126
Fat Percentage (% fat)	14	44.94	6.96	14	27.87	9.80	0.963	0.353
Fat Mass (kg)	14	9.60	5.61	14	9.89	5.71	-1.358	0.198
Free Fat Mass (kg)	14	22.95	1.57	14	23.18	1.59	-1.510	0.155
Right Leg Fat Percentage (% fat)	14	34.48	5.21	14	34.52	5.19	-1.728	0.167
Right Leg Fat Mass (kg)	14	3.95	1.29	14	4.14	1.30	-1.162	0.214
Right Leg Free Fat Mass (kg)	14	4.28	0.71	14	4.18	0.72	-2.385	0.367
Left Leg Fat Percentage (% fat)	14	34.56	5.16	14	35.81	5.10	-2.564	0.412
Left Leg Fat Mass (kg)	14	3.90	1.26	14	4.05	1.28	-3.314	0.324
Left Leg Free Fat Mass (kg)	14	4.13	0.74	14	4.12	0.70	1.992	0.068

mass (kg), body free fat mass (kg) and right leg fat percentage (% fat), right leg fat mass (kg), right leg free fat mass (kg), left leg fat percentage (% fat), left leg fat mass (kg), left leg free fat mass (kg). Similar findings can be found when the literature is examined.

Barene et al. (22) have stated that the 12-week zumba fitness reduced the fat percentage and fat mass values of women working in the health sector. Ljubojevi et al. (23) have suggested that zumba fitness program caused a decrease in overall body weight, fat percentage and fat mass values of women who are between the ages of 25-35. Micallef (24) has confirmed the effect of Zumba fitness on body composition stating that it decreases fat mass, BMI and fat mass values. Cugusi et al. (25) have stated that the 12-week zumba fitness caused a significant change in body weight and BMI values and decreased the number of heart beats during resting. In their studies, Jain and Nigudkar (26) divided 60 women who are between the ages of 20-50 into two groups, who participated in the 12-week zumba fitness and who both participated in the zumba fitness and also went on a diet during this period. After the 12-week program, differences have been observed in both groups in terms of anthropometric characteristics, body composition and components of physical fitness. No significant difference has been observed in fat percentage and waist-to-hip ratio values in the group participating only in the zumba fitness after the application. Baştu et al. (27) have concluded that there was a significant decrease in BMI and body weight values of women who participated in the study after 12 weeks of pilates, crossfit, zumba fitness. In the study done in 2009, Biçer et al. (28) investigated the effects of 8-week (3 days a week, 60 minutes a day) aerobic dance exercises on cardiovascular fitness, recovery pulse rate, blood pressure, flexibility and body weight. At the end of the research, the finding that the difference between cardiovascular fitness, recovery pulse rate, systolic blood pressure, flexibility and body weight was statistically significant has been reached. Krishan et al. (29) have stated that the 12-week Zumba fitness ensured the development of aerobic fitness of women who were overweight, obese and have type 2 diabetes, and decreased their body weight and body fat percentage.

60 healthy sedentary mid-fat and young women participated in the study conducted by Akdur et al. (30). The groups were asked to do exercise for an hour, 3 days a week, for 10 weeks. Following the research, a positive significant difference in body fat ratio values was observed. They attempted to determine the contribution of dance and walking activities to performance in 60 men and women between the ages of 24-48. Dividing the experimental group into two, they practiced dance for the first group and walking exercises for the second group for 8 weeks. At the end of the research, no significant difference was found between the groups. Nindl et al. (31) practised a physical activity program consisting of resistance and aerobic activities for 31 healthy women 5 days a week for 6 months. It was found that their body weights averages before exercise were 66.5 kg and 64.8 kg after exercise, their body fat weight averages were 24.7-22.1 kg, and free fat body weight averages were 41.8-42.7 kg. At the end of the exercise program, they observed 2.2% decrease in body weight, 10% decrease in body fat and also 2.2% improvement in free fat body weight. In the study Çolako lu and Karacan (32) applied aerobic exercise with 50-75% intensity for middle aged and young women for 12 weeks, 3 days a week, 45-60 minutes, they found a decrease in body weight in both groups. Sucu (33) determined that at the end of 10 weeks BMI values of those who exercised were lower than those who did not perform physical activity. Moreover, it was determined that participants' body weight, chest, waist, hip, arm and BMI values before the exercise program decreased positively at the end of the exercise program. In the study by Güneş (34), when the anthropometric measurements of women who were doing and not doing sports were compared, a difference was observed in favour of women who were doing sports in terms of measurement of chest and shoulder. Additionally, it was concluded that waist-hip ratio and hip circumference measurement scores were higher in the groups doing sports. Özeno lu et al. (35) observed that for women who have been doing aerobic exercise alone for 3 months and 3 hours a week for 3 months, the average weight before exercise decreased from 70.33±11.53 kg to 69.06±10.94 kg, BMI values average from 27.14±4.27 kg/cm^2 to 26.58±4.20 kg/. In the study, it was observed that exercise caused a significant decrease in the weight, BMI, waist circumference, waist-height ratio, body fat percentage, hip circumference measurements of adult women ($p<0.05$). In the

study Amano et al. (36) practised aerobic exercise to 18 obese individuals during 30 minutes 3 times a week for 12 weeks, the mean values of body weight, BMI, body fat percentage before and after training was observed respectively as 74.1±2.6 kg, 70.3±2.9 kg; 27.3±0.4 kg/cm^2, 25.9±0.5 kg/cm^2; %29.6±1.3, %26.6±1.3. It was reported that the reductions observed in anthropometric measurements were significant. In the study by Dalleck et al. (37), it was observed that exercise for 5 days / week, 30 minutes and 45 minutes for 12 weeks ensured a decrease in BMI, body composition and waist circumference of postmenopausal women compared to the control group who do not exercise.

Within the findings obtained in the study, it has been concluded that the 8-week zumba fitness caused a decrease in body weight, body fat percentage and BMI values of Turkish women between the ages of 18-35. In the regional body analysis done in the study, it has been understood that the effect of the zumba fitness program was on the lower extremity. It has been foreseen that if Zumba fitness is performed for a longer period and is supported by diet, it can contribute more to the body composition to reach the desired level.

References

1. Warburton DE, Nicol CW, Bredin SS. Health benefits of physical activity: The evidence. CMAJ, 2006; 174: 801-809.
2. Brown WJ, Burton NW, Rowan PJ. Updating the evidence on physical activity and health in women. Am J Prev Med, 2007; 33: 404-411.
3. Cruz-Ferreira A, Fernandes J, Laranjo L, Bernardo LM, Silva A. A systematic review of the effects of Pilates method of exercise in healthy people. Archives of Physical Medicine and Rehabilitation, 2011; 92: 2071-2081.
4. Düzgün , Kaya DÖ, Baltacı G, Karacan S, Çolako lu F. Sedanter kadınlarda hamstring-quadriseps geli imi: 6 ay takipli stabilizasyon ve aerobik e itimin kar ıla tırılması. Clin Exp Health Sci, 2017; 7: 45-51.
5. Wells C, Kolt GS, Bialocerkowski A. Defining Pilates exercise: A systematic review. Complement Ther Med, 2012; 20(4): 253-262.
6. Papavasiliou KA. Egzersiz ve ya am kalitesi. Trakya Univ Tip Fak Derg, 2010; 27:54-56.
7. Kaya DÖ, Celenay T . Fluctations of state anxiety, spinal structure and postural stability across the menstural cycle in active women. Turk J Med Sci, 2016; 46: 977-984.
8. Cımbız A, Uzgören N, Aras Ö. Kas iskelet sisteminde a rıya ait risk faktörlerinin lojistik regresyon analizi ile belirlenmesi: Pilot çalı ma. Fizyoterapi Rehabilitasyon, 2007; 18:20-27.
9. Kaya DÖ, Celenay T . Health-related physical fitness profiles of women who attend regular activity classes. Orthop J Sports Med, 2014; 2:232596711 4S00285.
10. Lim EC, Poh RL, Low AY, Wong WP. Effects of Pilates based exercises on pain and disability in individuals with persistent nonspecific low back pain: A systematic review with meta-analysis. J Orthop Sports Phys Ther, 2011; 41:70-80.
11. Eyigor S, Karapolat H, Ye il H, Uslu R, Durmaz B. Effects of Pilates exercises on functional capacity, flexibility, fatigue, depression and quality of life in female breast cancer patients: A randomized controlled study. Eur J Phys Rehabil Med, 2010; 46:481-487.
12. Yılmaz Yelvar GD, Baltacı G, Bayrakcı Tunay V, Atay AO. The effect of postural stabilization exercises on pain and function in females with patello femoral pain syndrome. Acta Orthop Traumatol Turc, 2015;49:166-174.
13. Perez B, Greenwood-Robinson M. Zumba: Ditch the workout, join the party! The zumba weight loss program. Grand Central Life & Style, 2014.
14. Thompson WR. Worldwide survey of fitness trends for 2012. ACSM's Health & Fitness Journal, 2011; 15(6): 9-18.
15. Gök D, Özbal AF, Ak D. Kastamonu üniversitesi ö rencilerinin zumba katılımları ile ilgili görü leri. II. International Physical Education, Sport, Recreation and Dance Congress, April 20-21, 2019; Alanya, Turkey.
16. Furjan-Mandi G, Kosalec V, Vlaši J. The effects of aerobic exercise on the increase of repetitive strength in women. 3th International Aspects of Sports, Physical Education and Recreation, 2011; 75-83.
17. Mandari S, Sibinovi A, Mikala ki M, Stojiljkovi S. The effects of the program HI-Low aerobics on morphological characteristics and functional ability students in the eight grade. Journal of Sports Science and Health, 2011; 1(1): 18-23.
18. Oreb G, Matkovi B, Vlaši J, Kosti R. The structure of the functional abilities of the dancers. Croatian Sports Herald, 2007; 9(1): 16-23
19. Park SK, Park JH, Kwon YC, Yoon MS, Kim CS. The effects of long-term aerobic exercise on maximal oxygen consumption, left ventricular function and serum lipids in elderly women. Journal of Physiological Anthropology and Applied Human Science, 2003; 22(1): 11-17. doi: 10.2114/jpa.22.11; PMid: 12672978
20. Šebi L, Šahat S, Zukovi A, Luki A. Coordination tests predictive value on success during the performance of dance and aerobics motion structures. Homosporticus, 2012; 14(1): 22-26.
21. Sarıta N, Özkarafakı , Pepe O, Büyükipekci S. Üniversiteli erkek ö rencilerin vücut fat yüzdelerinin üç farklı yöntemle de erlendirilmesi. Sa lık Bilimleri Dergisi, 2011; 20(2): 107-115.
22. Barene S, Krustrup P, Brekke OL, Holtermann A. Soccer and Zumba as health-promoting activities among female

hospital employees: A 40-weeks cluster randomised intervention study. Journal of Sports Sciences, 2014; 32(16): 1539-1549.
23. Ljubojevi A, Jovanovi S, Zrni R, Šebi L. Zumba fitness cardio exercise: the effects on body fat mass reduction of woman. Homo Sporticus, 2016; 18(1).
24. Micallef C. The effectiveness of an 8-week Zumba programme for weight reduction in a group of Maltese overweight and obese women. Sport Sciences for Health, 2014; 10(3): 211-217.
25. Cugusi L, Wilson B, Serpe R, Medda A, Deidda M, Gabba S, ... Mercuro G. Cardiovascular effects, body composition, quality of life and pain after a Zumba fitness program in Italian overweight women. The Journal of Sports Medicine and Physical Fitness, 2016; 56(3): 328-335.
26. Jain PK, Nigudkar MR. Effect of 12week zumba program and healthy diet on anthropometry, body composition and fitness parameters in working women. J Nutr Heal Food Eng, 2016; 5(4): 672-7.
27. Ba tu G, Özcan R, Gültekin D, Günay Ö. The effects of cross-fit, pilates and zumba exercise on body composition and body image of women. Uluslararası Spor Egzersiz ve Antrenman Bilimi Dergisi, 2016; 2(1): 22-29.
28. Biçer B, Yükta ır B, Yalçın HB, Kaya F. Yeti kin bayanlarda 8 haftalık aerobik dans egzersizlerinin bazı fizyolojik parametreler üzerine etkisi. Beden E itimi ve Spor Bilimleri Dergisi, 2009; 11(3).
29. Krishnan S, Tokar TN, Boylan MM, Griffin K, Feng D, Mcmurry L, ... Cooper JA. Zumba® dance improves health in overweight/obese or type 2 diabetic women. American Journal of Health Behavior, 2015; 39(1), 109-120.
30. Akdur H, Sözen AB, Yi it Z, Balota N, Güven O. The effect of walking and step aerobic exercise on physical fitness parameters in obese women. J Ist Faculty Med, 2007; 70(3): 6467.
31. Nindl BC, Harman EA, Marx JO, Gotshalk LA, Frykman PN, Lammi E, Kraemer WJ. Regional body composition changes in women after 6 months of periodized physical training. Journal of Applied Physiology, 2000; 88(6): 2251-2259.
32. Çolako lu FF, Karacan S. Genç bayanlar ile orta ya bayanlarda aerobik egzersizin bazı fizyolojik parametrelere etkisi. Kastamonu E itim Dergisi, 2006; 14(1): 277-284.
33. Sucu N. Düzenli egzersiz yapan ve yapmayan kadınların bazı antropometrik özellikler ile beden imgesi ve sosyal fizik kaygı düzeyleri arasındaki ili kinin incelenmesi. Yüksek Lisans Tezi, Aksaray Üniversitesi, Sosyal Bilimleri Enstitüsü, Aksaray, 2018; 56-57.
34. Güne . Medyada yer alan kadın bedeni imgeleri ve kadınlarda beden imgesi ho nutsuzlu u. Yüksek Lisans Tezi, Ege Üniversitesi Sa lık Bilimleri Enstitüsü, zmir, 2009; 42-44.
35. Özeno lu A, Uzdil Z, Sevde Y. Kadınlarda tek ba ına planlı egzersizin antropometrik ölçümler ve vücut kompozisyonu üzerine etkisi. Samsun Sa lık Bilimleri Dergisi, 2016; 1(1): 1-10.
36. Amano M, Kanda T, Maritani T. Exercise training and autonomic nervous system activity in obese individuals. Medicine Science in Sports Exercise, 2001; 33(8): 1287-1291.
37. Dalleck LC, Allen BA, Hanson BA, Borresen EC, Erickson ME, Lap SL. Dose response relationship between moderate-intensity exercise duration and coronary heart disease risk factors in postmenopausal women. Journal of Wome's Health, 2009; 18(1): 105-113.

Correspondence:
Prof., Aziz Güçlüöver
Kırıkkale University, Faculty of Sport Sciences,
71450, Kırıkkale / TURKEY
Phone: + 90 318 357 42 42 ext.2507,
Fax: + 90 318 567 42 20
E-mail: azizgucluover@hotmail.com

Progress in Nutrition 2020; Vol. 22, N. 1: 323-329 DOI: 10.23751/pn.v22i1.9023

Original article

Compositional and nutritional inventory of naturally mutant strain *Auricularia cornea* var. Li. edible mushroom from China

Rebecca Roy

Institute of Plant and Environment Protection, Beijing Academy of Agriculture and Forestry Sciences, Beijing, 100097, China
E-mail: rebecca@ipepbaafs.cn

Summary. Auricularia cornea var. Li. is a natural mutation strain of A. cornea which has been preferred by consumers for its white colour, good taste with their pharmacological properties. Even though there are many reports about the cultivation technologies, to the best of our knowledge, very few studies have been performed on nutritional compositions of white species of Auricularia. Therefore, this study aimed at determining the nutritional compositions of A. cornea var. Li..Eighty percent of sawdust, 18% of wheat bran and 2% of lime were used as cultivation substrate. Results were obtained by the mean ± S.E of three independent determinations based on the dry weight. Total dietary fiber was the dominant compound (78.94 g/100g), followed by protein (8.68 g/100g), carbohydrates (6.31 g/100g), ash (2.43 g/100g) and fat (0.91 g/100g). Potassium (1121.66 mg/100g) was the most abundant mineral, followed by magnesium (143.23 mg/100g), calcium (108.97 mg/100g) and sodium (29.22 mg/100g) in tested samples. A. cornea var. Li. has been found to contain all the essential amino acids. Glutamic acid was recorded more than 13% of the total detected amino acid content and linoleic acid was recorded more than 43% of total detected fatty acid content in this study. Although this mushroom desired for its color and flavor, it has found to be a good source of total dietary fibers, proteins, trace functional minerals, and low fat content, making it an ideal component in healthy diets.

Key words: amino acid profile, *Auricularia fuscosuccinea*, *Auricularia polyticha*, fatty acid profile, macro nutrient, nutritional composition

Introduction

Auricularia is a widely-reaching species of edible fungus. This species is recognized by its earlike shape. *Auricularia* belongs to family Auriculariaceae. It can be observed mostly on dead woods and decaying logs (1). *Auricularia* is the fourth largest cultivated mushroom species in the world as well as routinely used ingredient in Chinese dishes and also in Chinese medicine (2). China is one of the largest producers of *Auricularia* (3). China Edible Fungi Association has been reported the annual production in 2017 reached nearly 75.2 and 16.9 million tons for *A. auricula* and *A. cornea*, respectively. *Auricularia* produce many types of polysaccharides (4) and these kinds of polysaccharides have the ability to stimulate the inner systems in human body that can stop proliferation of cancer cells (5). It has also been reported to have antitumor (6), antioxidant (7), anticoagulant (8),antibacterial, anti-parasitic (9,10),anti-inflammatory(11), immunomodulatory (12), prevention of alcohol-related liver diseases (13) and hypo glycemic (14) properties. Additionally, *Auricularia* has been reported to contain high level of crude protein, low level of fat and 60% of fatty acids are unsaturated (15) which contributes to treat cardiovascular, hypo cholesterol (16) and obesity (17) *Auricularia* species contain more fiber (18). Hence, they have the ability to medicate constipation (19).

Auricularia cornea var. *Li.* is a natural mutation strain of *A. cornea* and it has been favoured by customers for its white colour, flavour, and medicinal properties.

However, there are insufficient studies were carried out about nutritional composition of *A. cornea* var. *Li.*. Therefore, this study was conducted to determine the nutrition compositions and the mineral elements of *A. cornea* var. *Li.* expecting that this information could be used by academics, medicines, and consumption market.

A. cornea, *A. cornea* var. *Li.* on a decaying trunk in nature shown in Figure 1 and Figure 2 respectively. Artificially cultivated *A. cornea* var. *Li***.** shown in Figure 3.

Figure 1. *Auricularia cornea* in nature

Figure 2. Naturally mutation white strain *Auricularia cornea var. Li.* in nature.

Figure 3. Naturally mutation white strain *Auricularia cornea* var. *Li.* in nature.

Materials and methods

Spawn Preparation and Fruiting Body Production

Auricularia cornea var. *Li.* used in this study was provided by Beijing Engineering Research Center for Edible Mushrooms. The strain was cultured and maintained on potato dextrose broth at 25 °C. When required, 1.5% (w/v) agar was added to the appropriate medium. Eighty percentage of oak (*Quercus* spp) sawdust, 18% of wheat bran, and 2% of lime were used to prepare substrate and the water content of the substrate was adjusted to 62% (W/W). One kilogram of substrate was filled in polyethylene bags (16 cm × 32 cm × 0.04 cm) which were then, autoclaved at 121 °C for 120 min. Autoclaved substrate was inoculated with *A. cornea* var. *Li.* spawn by 2% (w/w) of substrate fresh weight. Inoculated polyethylene bags were kept in the spawn running room at 25 °C and 70% RH under dark condition. After the mycelium completely colonized, bags were unfolded in order to facilitate the fruiting body development and maintained at 25 °C and 85-90% RH. Fruiting bodies were harvested at mature stage.

General Chemical Analysis

Fresh mushrooms were collected randomly after the first flush and dried at 60 °C to a constant weight. Mushroom nutrition compositional analyze were carried out according to the AOAC (20) standard procedures for moisture, ash, crude protein, fat and fiber. Moisture content (%) was determined by drying 10.0

g of dried mushroom in oven. Crude protein (%) was analyzed using dried, ground mushrooms (1.0 g) by micro-Kjeldahl method; the nitrogen factor used for crude protein calculation was 6.25. The level of crude fat (%) was determined by Soxhlet extraction of dried mushrooms (3.0 g). Total dietary fiber (%) was determined by taking approx. 3.0 g dried mushroom with H_2SO_4 (1.25%) and NaOH (1.25%) followed by heating at 105 ± 5 °C in hot air oven up to the constant weight. Total ash (%) content was determined by burning dried mushrooms (3.0 g) in a muffle furnace at 55 °C for 8 hrs until ashing was completed. Total carbohydrates (%) were estimated by determining the difference as follow:

Total carbohydrates (%) = 100 – (% Moisture + % Crudeprotein+%Crudefat+%Totaldietaryfiber+%Ash) (Eq.1)

Total energy was calculated as in (21) formula:

Energy (kcal) = 4× (g Protein) + 9× (g Fat) +4× (g Carbohydrates) + 2 × (g Total dietary fiber) (Eq.2)

Mineral Elements Analyses

Five hundred milligrams of mushroom samples were burned to ash in a muffle furnace at 450 °C. Then residue was dissolved with 0.5 mL/mL of HNO_3, 0.5 mL/mL of HCl (20) and added proper amount of distilled water to which were directly weighed iron (Fe), copper (Cu), zinc (Zn), manganese (Mn). It was diluted by 25 ml of distilled water to measure the other elements (22). The concentrations of iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), potassium (K), sodium (Na), calcium (Ca), and magnesium (Mg) were determined in an flame atomic absorption spectrometry in Analyst 200 Perkin Elmer equipment.(Perkin Elmer, Waltham, MA, USA).

After the crude fat extraction procedure, fatty acids were determined following the methods described in (23), by gas chromatography (DANI 1000) with split/splitless injector with flame ionization detection (GC-FID) (DANI Instrument SpA., Cologno Manzese, Italy). A high polar chromatography column HP-88 was used (100 m × 0.25 mm × 0.2 μm) (Agilent Technologies, CO, Santa Clara, USA). Hydrogen flow rate was 4.mL/min. Split injection was carried out at 250 °C (1:40). Relative percentage of each fatty acid was expressed by FAME mixture with standard. The amino acid composition was determined by a high performance liquid chromatograph (HPLC)-based amino acid analyzer (Agilent 1120 Compact LC) as detailed in (24), Vitamin-E was determined via spectrophotometry detailed in (25).

Statistical Analysis

Three replicates of *A. cornea* var. *Li.* samples were used for all the analyses. Nutrition compositional values were calculated as the mean ± S.E of three independent determinants on dry weight basis.

Results

Chemical compositions of analyzed *A. cornea* var. *Li.* are shown in Table 1. All the values were calculated based on their composition in 100 g of dry matter. The *A. cornea* var. *Li*. has been recorded to contain 219.89 Kcal of energetic value, 2.74 g of moisture, and 2.43 g of ash. Total dietary fiber was the dominant compound and it showed 78.94 g, followed by protein (8.68 g), carbohydrates (6.31 g), and ash (2.43 g). Fat showed the lowest value and recorded 0.91 g.

The mineral contents of the *A. cornea* var. *Li* obtained from the experiment are given in the Table 2. The macro element potassium was the most abundant among all and recorded 1121.66 mg, followed by magnesium (143.23 mg), calcium (108.97 mg) and finally Sodium (29.22 mg) in sample. Among microelements, zinc was the most abundant and it recorded as 10.13 mg, followed by iron (3.82 mg), copper (1.41 mg) and manganese (0.50mg) in *A.cornea* var. *Li.*. The vitamin E was also recorded as 0.59 mg.

Table 1. Chemical compositions of *A.cornea* var. *Li.*

Chemical component (per 100 g)	Value
Energetic value (kcal)	219.89±1.19
Total dietary fiber (g)	78.94±0.64
Protein (g)	8.68 ±0.03
Carbohydrates (g)	6.31±0.54
Ash (g)	2.43±0.04
Moisture (g)	2.74± 0.05
Fat (g)	0.91±0.02

Values are expressed as the mean ± S.E of three independent determinations on dry weight basis.

Table 2. Mineral content of *A.cornea var. Li.*

Mineral content (mg/100g)	Value
Sodium	29.22± 0.77
Vitamin E	0.59±0.00
Potassium	1121.66± 30.53
Magnesium	143.23±2.16
Calcium	108.97±0.94
Iron	3.82±0.13
Zinc	10.13±0.31
Copper	1.41±0.03
Manganese	0.50±0.00

Values are expressed as the mean ± S.E of three independent determinations on dry weight basis.

Free amino acid of the mushroom studied is shown in Table 3. Eighteen amino acids were identified in *A. cornea* var. *Li*. Glutamic acid showed more than 13% of the total detected amino acid content and it was recorded 0.87 g. Aspartic acid recorded as 0.76 g. The lowest value was noted in methionine and it was 0.03 g.

Table 3. Amino acid profile of *A. cornea var. Li.*

Detected free Amino acids content (g/100g)	*A .cornea* var. *Li.*	Percentage of Amino acids content
Aspartic acid (ASP)	0.76±0.007	11.74
Threonine (THR)	0.41±0.009	6.33
Serine (SER)	0.41±0.006	6.34
Glutamic acid (GLU)	0.87±0.006	13.48
Glycine (GLY)	0.34±0.006	5.19
Alanine (ALA)	0.52±0.003	8.09
Valine(VAL)	0.37±0.004	5.70
Methionine (MET)	0.03±0.002	0.40
Isoleucine (ILE)	0.20±0.007	3.09
Leucine (LEU)	0.53±0.008	8.15
Tyrosine (TYR)	0.20±0.009	3.03
Phenylalanine (PHE)	0.33±0.013	5.03
Lysine (LYS)	0.41±0.007	6.32
Histidine (HIS)	0.16±0.003	2.43
Arginine (ARG)	0.39 ±0.004	5.96
Proline (PRO)	0.36±0.007	5.48
Tryptophan(TRP)	0.12±0.002	1.82
Cysteine (CYS)	0.09±0.002	1.43

Values are expressed as the mean ± S.E of three independent determinations on dry weight basis

Fatty acid and their percentage of detection in *A. cornea* var. *Li* presented in Table 4. Linoleic was predominating over the other fatty acids constituents and recorded as 0.36 g. Linoleic acid showed more than 43% of total fatty acid content in *A. cornea* var. *Li*. Considerable amount of *cis*-9-octadecenoic acid and palmitic acid were observed and values recorded as 0.25 g and 0.11 g, respectively. Other fatty acids, such as myristic acid, pentadecanoic acid, hexadecanoic acid, octadecanoic acid and behenic acid were found in minor amounts.

Table 5 shows the previous studies records of white strains in *Auricularia* spp.

Free amino acid of the *A. cornea var. Li*, *A. polyticha* and *A. fuscosuccinea* were compared with the current study and previous records and presented in the Table 6.

Table 4. Fatty acid profile of *A. cornea var. Li.*

Detected fatty acids (g/100g)	Number of carbon	*A. cornea* var. *Li.*	Percentage of Fatty acid content
Myristic acid	C14:0	0.01±0.002	0.94
pentadecanoic acid	C15:0	0.02 ±0.000	2.42
palmitic acid	C16:0	0.11±0.000	13.25
stearic acid	C18:0	0.06±0.005	7.83
cis-9-octadecenoic acid	C18:1n9c	0.25 ±0.012	29.83
linoleic acid	C18:2n6c	0.36±0.001	43.54
-linolenic acid	C18:3n3	0.01±0.003	1.52
behenic acid	C22:0	0.01±0.001	1.14

Values are expressed as the mean ± S.E of three independent determinations on dry weight basis.

Table 5. Previous studies of white strains recorded in *Auricularia* spp.

White strains of *Auricularia* spp.	Ash (g/100g)	Protein (g/100g)	Total dietary Fiber (g/100g)	Fat (g/100g)
A. polyticha (26)	2.49	12.33	24.82	-
A. polyticha (27)	2.1	7.7	-	0.45
A. fuscosuccinea (28)	5.5	17.83*	-	4.5
A. cornea var. *Li.* (our study)	2.43	8.68	78.94	0.91

** means the nitrogen factor used for crude protein calculation was 6.25, replaced the original factor 4.38.*

Table 6. Free amino acid contents of *A. cornea var. Li.* , *A. polyticha* and *A. fuscosuccinea*.

Detected free Amino acids (g/100g)	*A .cornea* var. *Li.* (our study)	*A. polyticha* (26)	*A. fuscosuccinea* (28)
Aspartic acid (ASP)	0.76±0.007	1.12	0.06
Threonine (THR)	0.41±0.009	0.63	0.16
Serine (SER)	0.41±0.006	0.57	0.16
Glutamic acid (GLU)	0.87±0.006	1.28	0.16
Glycine (GLY)	0.34±0.006	0.52	0.02
Alanine (ALA)	0.52±0.003	0.80	0.04
Valine(VAL)	0.37±0.004	0.62	0.03
Methionine (MET)	0.03±0.002	0.64	-
Isoleucine (ILE)	0.20±0.007	0.43	0.03
Leucine (LEU)	0.53±0.008	0.84	-
Tyrosine (TYR)	0.20±0.009	0.33	0.06
Phenylalanine (PHE)	0.33±0.013	0.57	0.02
Lysine (LYS)	0.41±0.007	0.61	-
Histidine (HIS)	0.16±0.003	0.26	-
Arginine (ARG)	0.39 ±0.004	0.67	0.08
Proline (PRO)	0.36±0.007	0.55	-
Tryptophan(TRP)	0.12±0.002	0.13	0.01
Cysteine (CYS)	0.09±0.002	0.07	-

Discussion

The *A. cornea* var. *Li*. white strain is enormously preferred by consumers in China for their white colour and flavour with their nutritional and pharmaceutical characters. As in (29), the chemical composition of edible mushrooms defines their nutritional value and sensory properties. Therefore, its timely needed to analyze the nutritional compositions of *A. cornea* var. *Li.* which have the benefits of making healthy food decisions and maintaining healthy weights for consumers, food production industries, medicines, and research based fields.

The nutrition compositions of mushroom are easily influenced by the growing substrate and environment (30,31,32,33). In our study, Oak sawdust was used as the main substrate. The nutrient values of mushroom can be different regarding to their substrate.

The *A. polyticha* (26, 27) (Table 5) shown more similar amounts of ash content with *A. cornea* var. *Li.*, although *A. fuscosuccinea* (28) contain it fairly high. *Auricularia* species generally rich in fiber than other cultivated mushroom species such as *Agaricus bisporus, Tremella fuciformis* (19). According to (2), more than 50% of fiber content has been reported in *A. auricula-judae*. Total dietary fiber of this study showed (78.94 ± 0.64 g) higher value than *A. polyticha* (26) (24.82 g) (Table 5). *Auricularia* species produce two kinds of -D-glucans, acidic hetero types polysaccharides (4), *A. cornea* var. *Li.* also has been reported to contain several types of polysaccharides (13) and these non-starchy polysaccharides could be a higher source of dietary fiber which was observed in *A. cornea* var. *Li.*.

Fiber is often mentioning to as the seventh nutrient and mainly consists of cellulose, hemicellulose, and lignin (34). They have the ability to promote intestinal absorption and digestion, medicate constipation (19), lowering blood sugar, prevents circulatory and intestinal cancer (35). Therefore, intake of *A. cornea* var. *Li.* with high level of fiber has great impacts on healthy diets.

Our study indicates that, protein value of *A. cornea* var. *Li*. was slightly higher than *A. polyticha* (27), while it's slightly lower than *A. polyticha* (26) and deviated from *A. fuscosuccinea* (28) (Table 5). *A. cornea* var. *Li.* showed the values in between values among the white strains. Eighteen amino acids were identified in this study. All the detected amino acids of *A. cornea* var. *Li.* values were higher than *A. fuscosuccinea* (28). Compositions of amino acid was consistent with the previous study of *A. polyticha* (26) with some quantity differences. As reported in (36,37) about edible mushrooms, *A. cornea* var. *Li.* is also especially rich in glutamic acid (0.87 g) and considerable amount of aspartic acid (0.76 g). Glutamic acid recorded more than 13% of the total detected amino acid contents. It is well known the glutamic acid contribute to the flavour properties of mushroom (38) could be associated with desires flavour of *A. cornea* var. *Li.*. Sulphur-containing amino acids are typically lacking in mushrooms (39,40). The *A. cornea* var. *Li*. found to be a good source of protein with includes all of the nine essential amino acids among the other white strains of *Auricularia.*

In our study, *A. cornea* var. *Li.* contains lower fat value (0.91 g) than *A. fuscosuccinea* (4.5 g) (28) and higher value than *A. polyticha* (27) (0.45 g) (Table 3). Mushrooms are low in fat which have been recom-

mended as ideal vegetable for the fatness (30). Hence, *A. cornea* var. *Li* has low fat value, is advisable for treating cardiovascular, hypo cholesterol and obesity related diseases. Linoleic acid recorded more than 43% of total detected fatty acids. Linoleic acid is an essential fatty acid to mammals; therefore, could be supplied by *A. cornea var. Li.*. Unsaturated fatty acids are probable forerunner flavour compound source in fungi, these fatty acids are the precursor of 1-*octen*-3-*ol,* recognized as the alcohol of fungi, which is the primary aromatic compound in most fungi (38). Hence, the linoleic acid in *A. cornea* var. *Li.* could be narrowly related to their preferred flavour.

A. cornea var. Li. analyzed in the present study appeared to be rich in minerals; especially, potassium, magnesium, calcium, sodium, zinc and iron (Table 2). Minerals are necessary for metabolic reactions, ruling of water and ions balance, strong bone formation which relates with calcium, coordination of nerve impulses particularly sodium, helping to control blood pressure and maintain muscle and nerve functions specially potassium (41) and supports curing iron-deficiency anemia conditions by iron (42, 43).

Conclusion

A. cornea var. *Li* was found to be a rich sources of dietary fiber, proteins, minerals with having low fat value. Even though consumers prefer the flavour, taste and medicinal properties, they have enriched with nutritional contents which making them in well-balanced diets.

Acknowledgement

This work was supported by Beijing Agriculture Innovation Consortium (BAIC05-2019), the Open Research Fund Program of Beijing Key Lab of Plant Resource Research and Development, Beijing Technology and Business University (PRRD-2019-YB3).

Conflict of interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

1. Harding & Patric. Mushroom Miscellany 2008; 1st edn: London, Harper Collins Publisher. ISBN: 100007284640.
2. Sekara A, Kalisz A, Grabowska A, Siwulski M. *Auricularia* spp.—mushrooms as novel food and therapeutic agents—a review. Sydowia 2015; 67: 1–10.
3. Zhang Y, Geng W, Shen Y, Wang Y, Dai YC. Edible mushroom cultivation for food security and rural development in China: bio-innovation, technological dissemination and marketing. Sustainability 2014; 6(5): 2961–2973.
4. Sone Y, Kakuta M, Misaki A. Isolation and characterisation of polysaccharides of "Kikurage," fruit body of *Auricularia auricula-judae. Agricultural and Biological Chemistry* 1978; 42**:** 417-425.
5. Wasser SP, Weis AL. Therapeutic effects of substances occurring in higher basidiomycetes mushrooms: A modern perspective. Critical Reviews in Immunology 1999; 19: 65-96.
6. Wang J, Li X, Song G. An efficient separation method of polysaccharides: preparation of an antitumor polysaccharide APS-2 from *Auricularia polytricha* by radial flow chromatography. Chromatographia 2013;19:629-33.
7. Elisashvili V. Submerged cultivation of medicinal mushrooms: bioprocesses and products (review). International Journal of Medecinal Mushrooms 2012; 14(3): 211–239.
8. Chen G, Luo YC, Ji BP, Li B, Su W, Xiao ZL, Zhang GZ. Hypo cholesterolemic effects of *Auricularia auricula* ethanol extract in ICR mice fed a cholesterol-enriched diet. Food Science and Technology 2011; 48(6): 692–698.
9. Yu M, Xu X, Qing Y, Luo X, Yang Z, Zheng L. Isolation of an anti-tomor polysaccharide from *Auricularia polytricha* (jew's ear) and its effects on macrophage activation. European Food Research and Technology 2009; 228: 447-485.
10. Song G, Du Q. Structure characterization and antitumor activity of an -glucan polysaccharide from *Auricularia polytricha*. Food Research International 2012; 45:381-387.
11. Damte D, Reza MA, Lee SJ, Jo WS, Park SC. Anti-inflammatory activity of dichloromethane extract of *Auricularia auricula-judaein* RAW264.7cells. Toxicol Research 2011; 27(1): 11–14.
12. Sheu F, Chien PJ, Chien AL, Chen YF, Chin KL. Isolation and characterization of an immunomodulatory protein (APP) from the Jew's Ear mushroom *Auricularia polytricha*. Food Chemistry 2004; 87(4): 593–600.
13. Wang X, Lan Y, Zhu Y, et al. Hepatoprotective effects of *Auricularia cornea* var. *Li.* polysaccharides against the alcoholic liver diseases through different metabolic pathways. Scientific Reports 2018; *8*:7574.
14. Wu NJ, Chiou FJ, Weng YM, Yu ZR. In vitro hypoglycemic effects of hot water extract from (wood ear mushroom). International Journal of Food Science and Nutrition 2014; 65: 502-6.
15. Kavishree S, Hemavathy J, Lokesh BR, Shashirekha MN, Rajarathnam S. Fat and fatty acids of Indian edible mushrooms. Food Chemistry 2008;106(2):597–602.
16. Zhao S, Rong C, Liu Y, Xu F. Extraction of a soluble poly-

saccharide from *Auricularia polytricha* and evaluation of its anti-hyper cholesterolemic effect in rats. Carbohydrate Polymers 2015; 122: 39-45.
17. Reza MA, Hossain MA, Damte D, Jo WS, Hsu WH, Park SC. Hypolipidemic and hepatic steatosis preventing activities of the wood ear medicinal mushroom *Auricularia auricula-judae* (higher Basidiomycetes) ethanol extract in vivo and in vitro. International Journal of Medecinal Mushrooms, 2015; 17(8): 723–734.
18. Cheung PCK. Dietary fiber content and composition of some edible fungi determined by two methods of analysis. Science of Food and Agriculture 1997; 73(2): 255–260.
19. Kim TI, Park SJ, Choi CH, Lee SK, Kim WH. Effect of ear mushroom (*Auricularia*) on functional constipation. Korean Journal of Gastroenterol 2004*;* 44(1):34–41.
20. Association of Official Analytical Chemists (AOAC). Official methods of analysis of the Association of Official Analytical Chemists AOAC, 1995; 16th edn: Arlington,USA. ISBN: 0935584544.
21. Manzi P, Marconi S, Aguzzi A, Pizzoferrato L. Commercial mushrooms: nutritional quality and effects of cooking. Food chemistry 2004;84:201-206.
22. Ruiz-Rodriguez B, Morales P, Fernandez-Ruzi V, et al. Valorization of wild strawberry tree fruits (*Arbutus unedo* L.) through nutritional assessment and natural production data. Food Research International 2011; 44: 1244-1253.
23. Barreira JCM, Antonio AL, Gunaydi T, et al. Chemometric characterization of gamma irradiated chestnuts from Turkey. Radiation Physics and Chemistry 2012; 81: 1520-1524.
24. Partil SS, Ahmed SA, Telang SM, Baig MMV. The nutritional value of *pleurotus ostreatus* (JACQ.:FR.) Kumm cultivated on different lignocellulosic agro wastes. Innovative Romanian Food Biotechnology 2010;7:66-76.
25. Rishi L, Jadoon S, Waseem A, Yaqoob M, Nabi A. Flow injection methods for the determination of -tocopherol with spectrophotometric detection. Chemical Society of Pakistan, 2011; 33(4): 508–514.
26. Yao Q, Chen G, Yan S, Lin X, Lin Q. (Nutritional analysis and evaluation of two varieties of *Auricularia polytricha*). Acta Nutrimenta Sinica 2018; 40(2): 197-199. DOI: 10.13325/j.cnki.acta.nutr.sin.2018.02.023.
27. Zhang X, Jia J, Zhang AG, Li C, Zhou L. (Nutritional detection and analysis of white *Auricularia polytricha* and *A. auricular)*. China Food Safety Magazine 2017; 10: 97-97. DOI:10.16043/j.cnki.cfs.2017.30.073.
28. Mau JL, Wu KT, Wu YH, Lin YP. Nonvolatile taste components of ear mushrooms. Agricultural Food Chemistry 1998; 46(11): 4583–4586.
29. Manzi P, Aguzzi A, Pizzoferrato L. Nutritional value of mushrooms widely consumed in Italy. Food Chemistry 2001; 73: 321-325.
30. Kurtzman RH. A review mushrooms: sources for modern western medicine. Micologia Aplicada International 2005; 17: 21–33.
31. Gregori A, vagelj M, Pohleven J. Cultivation techniques and medicinal properties of Pleurotus spp. Food Technology and Biotechnology 2007; 45: 238–249.
32. Wang D, Sakoda A, Suzuki M. Biological efficiency and nutritional value of *Pleurotus ostreatus* cultivated on spent beet grain. Bioresources technology 2001; 78: 293-300.
33. Ahmed SA, Kadam JA, Mane VP, Patil SS, Baig MMV. Biological efficiency and nutritional contents of *Pleurotus* florida (Mont.) singer cultivated on different agro-wastes. Nature and Science 2009; 7: 44–48.
34. Anita FP, Abraham P. Clinical dietetics and nutrition 1997; 4th edn: Calcutta, Delhi Oxford University Press. IBSN: 0192641565.
35. Cheung PCK. Mini-review on edible mushrooms as source of dietary fiber: preparation and health benefits. Food Science and Human Wellness 2013;2(3–4):162–166.
36. Kim MY, Chung MI, Lee SJ, et al. Comparison of free amino acid, carbohydrate concentrations in Korean edible and medicinal mushrooms. Food Chemistry 2009; 113: 386 – 393.
37. Madachi SJM, Nkunya MHH, Nyigo VA, Urasa IT. Amino acid composition of some Tanzanian wild mushrooms. Food Chemistry 2004;86:179-182.
38. Maga JA. Mushroom flavour. Agricultural Food Chemistry 1981; 29(1): 1–4.
39. Senatore F, Dini A, Marino A. Chemical constituents of some basidiomycetes. Science of Food and Agriculture 1988; 45: 337–345.
40. Senatore F. Fatty acid and free amino acid content of some mushrooms. Science of Food and Agriculture 1990; 51: 91–96.
41. Kalac P, Svoboda L. A review of trace element concentrations in edible mushrooms. Food Chemistry 2000; 69: 273- 281.
42. Anderson GJ, Frazer DM, McLaren GD. Iron absorption and metabolism. Curr Opin Gastroenterol 2009; 25: 129–135.
43. Byrnes V, Barrett S, Ryan E, et al. Increased duodenal DMT-1 expression and unchanged HFE mRNA levels in HFE-associated hereditary hemochromatosis and iron deficiency. Blood Cells, Molecules & Diseases 2002; 29: 251–260.

Correspondence:
Reading PhD student,
Institute of Plant and Environment Protection,
Beijing Academy of Agriculture and Forestry Sciences,
Beijing, 100097, China.
E-mail: rebecca@ipepbaafs.cn

Progress in Nutrition 2020; Vol. 22, N. 1: 330-342 DOI: 10.23751/pn.v22i1.9177

Original article

Comparing video and poster based education for improving 6-17 months children feeding practices: a cluster randomized trial in rural Benin

Fifali Sam Ulrich Bodjrenou[1,2], Waliou Amoussa Hounkpatin[1], Irène Mitchodigni[1,2], Valère Salako[1], Romain Glele Kakai[1], Ysé Dadele[1], Gervais Ntandou Bouzitou[2], Lauriina Schneider[3], Marja Mutanen[3], Mathilde Savy[4], Gina Kennedy[5], Joseph D. Hounhouigan[1], Céline Termote[6]

[1]University of Abomey-Calavi, Faculty of Agronomic Sciences, Cotonou Benin - bodjrenousam@gmail.com; [2]Bioversity International West and Central Africa. Benin, Cotonou Benin, [3]University of Helsinki, Department of Food and Environmental Sciences, Finland, University of Helsinki, Finland; [4]Nutripass Research Unit, Institut de Recherche pour le Développement (IRD), University of Montpellier, SupAgro, Montpellier, France; [5]Bioversity International Headquarters. Maccarese (Fiumicino), Rome, Italy; [6]Bioversity International Kenya, Nairobi, Kenya

Summary. *Objective:* This study aimed to assess whether short nutrition educational videos were more effective to improve child feeding practices compared to posters in a highly food insecure rural area in Southern Benin. *Materials and Methods:* A two-arm cluster-randomized trial was implemented in two districts of the Mono region, Benin. Over a 6 month period, eight villages received nutrition education sessions using either short videos (n=4 villages) or posters and flyers (n=4 villages). Dietary practices were collected among 6-17 months children (n=155) before and at the end of the nutrition education program using a qualitative 24 hours recall. UNICEF/WHO indicators for dietary diversification and meal frequency assessing were derived for each child. The videos versus poster effect was assessed by the difference-in-differences (DID) estimator using generalized estimated equations (GEE). *Results:* Overall, respectively 49% and 72% of children had achieved Minimum Dietary Diversity (MDD) and Minimum Meal Frequency (MMF) at baseline. Results from DID analysis showed that videos did not have significant advantage in terms of improving children compared to posters neither for dietary diversity (DID = -0,036; p-value=0,651) nor for meal frequency (DID = -0,048; p-value=0,574). However, others factors namely children age, mother age and districts, had significant influence on these feeding practices. *Conclusions:* The nutrition education program using posters and videos performed the same in improving complementary feeding practices. The conditions of the utilisation of videos might be improved and other factors determining children feeding practices taken into account to allow mothers and other participants to benefit from nutrition sessions.

Key words: nutrition education, communication, complementary feeding practices, dietary diversity, meal frequency, 6-23 months children.

Abbreviations

DDS: Dietary Diversity Score
DHS: Demographic Health Survey
DID: Difference-in-Differences
GEE: Generalized Estimating Equations
MDD: Minimum Dietary Diversity
MMF: Minimum Meal Frequency
NGO: Non-Governmental Organization
UNICEF: United Nations International Children's Emergency Fund
WHO: World Health Organization

Introduction

Poor child feeding practices are an issue globally. According to a review of Demographic Health Surveys (DHS) from 60 countries, only 36% of children aged 6 to 23 months achieved the minimum dietary diversity (MDD) for children. Only 22% of children achieved MDD and minimum meal frequency (MMF) (1). In Benin, complementary feeding practices are sub-optimal. In 2014, children who reached MDD, MMF and Minimum Acceptable Diet were respectively 26%, 45% and 16% (2). There is a large burden of malnutrition, 32% of children under five are stunted, with 12% severely stunted (2). Stunting prevalence increases significantly during the period of 6-23 months indicating that poor complementary feeding practices contribute to the high burden of malnutrition among children in Benin (2,3,4). At the same time, a large diversity of cultivated and wild foods are locally available and accessible (5,6).

To better understand this contradictory situation, Bioversity International investigated the current and potential role of local foods in meeting nutritional requirements from complementary foods in a highly food insecure area, the department of Mono, Benin. The research was implemented in the two districts with highest percentage of households affected by food insecurity, namely *Bopa* (40%) and *Houéyogbé* (34%). Cross-sectional dietary intake studies showed that complementary feeding practices were not optimal. Only 49% and 39% of children between 6 and 11 months in *Bopa* and *Houéyogbé*, respectively, achieved MDD. Consumption of nutritious foods such as eggs (2.1%) and milk (2.7%) was very low (7,8).

According to the national DHS, the proportion of stunted children decreases significantly from illiterate mothers to instructed ones (2,3,4). This positive relationship between mother instruction and child nutritional status was confirmed by several studies in low and middle income countries in Africa (9-12). Furthermore, even if formal instruction is important, mother's knowledge on child feeding practices and nutrition has been shown to be more crucial in certain situations (13). Several studies in different countries found that nutrition education used alone or combined with others strategies could contribute to better feeding practices and/or improved nutritional status (14-23).

Furthermore, in spite of large diversity of cultivated and non-cultivated foods being locally available and accessible in the study areas, as stated previously, complementary feeding practices and children nutritional status were still inadequate. This highlights the importance of educational pursuits in the areas.

An intervention study providing nutrition education was carried out, whereby we capitalized on the WHO and UNICEF guidelines (24,25) as well as knowledge on locally available agrobiodiversity, to develop the educational materials. During a diagnostic project phase, the current and potential role of local foods in meeting nutritional requirements from complementary foods in the districts of *Bopa* and *Houéyogbé* in Mono department, a highly food insecure area, was investigated. Subsequently, key nutrition education messages were developed in accordance with WHO and UNICEF guidelines (24,25). Recommendations were adapted to the local context, for example, foods from local biodiversity were suggested to improve complementary feeding practices.

The education program was organized as a combination of principles from two theories used to promote health behavioural changes: the Health Belief Model and the Social Ecological Theory. It aimed to capitalize on the advantages of videos especially in rural and low literacy context (26-28). Communication tools were designed to influence mothers' perceptions and practices regarding complementary feeding. Allowing every household or community member, furthermore, to participate in the education sessions and encouraging all to share their experiences and worries makes it potentially easier for mothers to adopt healthy behaviours through the creation of an environment conducive to change (29).

In Benin, similarly to many low income countries, visual aids such as posters, flyers or image boxes have been traditionally and largely used, while the use of videos are relatively new in education program (28,30). However, videos present many advantages in terms of standardization of the message, facilitation of understanding and remembrance etc (26-28,31). Results from a study in Benin showed that videos were more attractive and facilitated memory compared to other communications tools (28). According to the Benin National Council of Nutrition (30), videos represent the best education tools for nutri-

tion program at communities, households and schools levels followed by others audio and visual supports. The objective of the present study was to assess whether short educational videos used in nutrition education programs were more effective to improve child feeding practices and care compared to traditional ways of nutrition education using posters and flyers with same messages in rural villages in Southern-Benin.

Materials and Methods

Settings

The study was part of a research project implemented in the districts of *Bopa* and *Houéyogbé* in Southern-Benin. The area is predominantly rural, food insecure, with low literacy levels, particularly for women. The research project included food consumption, household food security, ethnobiology and market surveys, as well as nutritional status assessments in a first phase. This paper presents the results of the subsequent nutrition education intervention conducted in the same area.

Design and sampling

Seventeen villages participated in the nutritional diagnostic survey (7,8) and then received the nutrition education program. In each district, villages were paired considering their socioeconomic and demographic characteristics; child feeding practices and nutritional status. For each pair (two villages with similar characteristics), videos were attributed to one village and posters and flyers to the other. Distance between villages was also considered. Neighbouring villages received the same intervention (poster-flyer or video) in order to avoid contamination. Distance between villages with different education tools was about 8km in *Bopa* and 5km in *Houéyogbé*.

The evaluation of the program was done with a sample of height villages randomly selected among the seventeen villages using casting lot technique: four in *Bopa* (*Hounviatouin*, *Tèkozouin*, *Djidjozoun* and *Kpavé*) and four in *Houéyogbé* (*Gbadagli*, *Dahè-Kpodji*, *Zoungbonou* and *Aguêhon*) districts. The design was a cluster-randomized trial with two intervention arms (video messages and poster-flyer messages). In each District, two villages receiving video messages and two receiving poster-flyer messages were selected.

Eventual contamination was assessed through asking the participants whether they heard about or participated in the education program in another village; no positive case was reported.

This study is a part of a nutrition education program aiming to improve not only complementary feeding but also breastfeeding practices and children health care. Therefore the overall target group is children aged 0-23 months.

At baseline (prior to the nutrition programme), we considered children aged 0-17 months aiming to have the same children aged 6-23 months at endline (at the end of the nutrition programme). Within each village, all mothers with a child aged 0-17 months were listed. From this list, 45 mother-child pairs were randomly selected using casting lots technique and surveyed. In total (for the eight villages), 360 pairs were interviewed at baseline. Due to some technical constraints, the nutrition programme started not just after the baseline but two months later. Therefore, from the baseline sample, 257 mother-child pairs (136 in poster-flyer group and 121 in video group) were interviewed at endline, 103 mother-child pairs (29%) had to be replaced because of non-availability of informants or because of children having outgrown the target age bracket (>23 months). Since education sessions were organised for the whole cluster/village, i.e. all community members were invited no matter whether they participated in the baseline survey or not, the number of replacement mother-child pairs had been sampled randomly from the updated lists of mothers with child between 6 and 23 months for each village. However, only mothers with children aged 6-17months and who participated both baseline and endline surveys (n=155) have been considered for analysis in the present paper. We focused on this age group since, majority of children continued to benefit from breastfeeding and receive also complementary foods during this period according to national DHS (2). Analysis among the whole sample (including replacement cases) had also been performed but showed similar trend.

Ethical considerations

The research protocol covering the whole study was approved by the Benin National Ethics Commit-

tee for Health Research (N°45/MS/DC/SGM/DFR/CNERS/SA). Written informed consent was also obtained from all participants before collecting data.

Production of educational tools

Results from the diagnostic surveys described previously (7,8) revealed gaps in nutrition knowledge and practices from which we derived the main education topics: 1) Mothers' diet during pregnancy and breastfeeding practices, 2) Complementary feeding practices, 3) Strategies to improve the nutritional value of complementary foods of children, 4) Hygiene and Diarrhoea, Prevention of micronutrient deficiencies and supplementation, 5) Stunting - Support and prevention of malnutrition. For each topic, key messages were derived in accordance with the WHO and UNICEF nutritional guidelines (24,25).

GloCal Videos from an existing nutrition education program, implemented in Eastern Africa (www.glocalnutrition.com), which correspond to our key messages for Benin, were selected. Transcripts and pictures from the GloCal videos were adapted to the local context (e.g. foods not available in our study area were replaced). However, there was no Glocal video for key message relative to stunting. More, key messages relative to complementary feeding practices and the need of good protein sources for infants and children were not adequately covered by Glocal videos. For these three key messages, three new videos were produced by the research team and used during education sessions. For the new video among the need of proteins, the scenario was written and a sketch was realized in a local school using teacher and pupils. The video relative to Stunting was an animated cartoon for which we wrote the text and a professional designer drew pictures. The video relative to complementary feeding practices was a real life situation filmed in one of the study villages.

All videos used during education sessions, basically in English or French, have been translated in the three most spoken local languages of the study area, namely: *Fon*, *Saɦouè* and *Kotafon*. The research team made sure that messages in the videos were preserved when translating. Table 1 presents the list of videos.

Posters, the comparative method to the videos, were produced in line with the WHO and UNICEF

Table 1. List of videos used during nutrition education sessions

Topics	Videos topics	Sources
Topic 1: Mothers' diet during pregnancy and breastfeeding practices, Part 1	Additional needs during pregnancy and breastfeeding	Adapted from Glocal
	Colostrum	Glocal
	Exclusive breastfeeding	Glocal
Topic 1: Mothers' diet during pregnancy and breastfeeding practices, Part 2	Advantages of breastfeeding	Glocal
	Sufficiency of breastfeeding	Glocal
	Breastfeeding problems	Glocal
	Mothers breast milk versus cow milk	Glocal
Topic 2: Complementary feeding practices	Why starting complementary feeding at 6 months?	Adapted from Glocal
	Dietary diversity	Adapted from Glocal
	Complementary feeding practices	New video
Topic 3: Strategies to improve the nutritional value of complementary foods of children	Recipes to improve complementary foods nutritional value	Adapted from Glocal
	Infants and children need good protein sources	New video
Topic 4: Hygiene and Diarrhoea, Prevention of micronutrient deficiencies and supplementation	Hygiene	Glocal
	Worms	Glocal
	Anaemia	Adapted from Glocal
	Sources of iron	Adapted from Glocal
	Folate	Adapted from Glocal
	Vitamin A	Adapted from Glocal
	Malaria	Glocal
Topic 5: Stunting and Support and prevention of malnutrition	Stunting	New video

nutrition guidelines using pictures (24,25,32). First, pictures used in nutritional education programs in Benin and other countries were reviewed and used when matching with the key messages. Where necessary, new pictures were designed; the key message was presented to a professional designer and discussed. The newly produced picture drafts were corrected and validated by nutrition experts before finalisation.

Posters were designed around the same key messages as the videos. Based on the posters, we made small flyers summarizing the information to distribute to the mothers after the poster sessions only. Posters were mainly based on pictures and small messages in French. However, discussions were made in local languages.

Organization of nutritional education meetings

Sessions were organized in a public area using local language twice a month per village. All community members (not only mothers) were invited to attend. A nutritionist/moderator presented the poster or video and subsequently invited the participants to ask questions and share their experiences and worries regarding the topic.

Poster sessions were organized during the day in a hall or under a tree or in a hut. The different pictures on the posters were discussed. Video sessions were organized outdoors by night (starting between 7.30 and 8 pm). Videos were projected on a white screen and a loud speaker was used. During each session, each video was presented at least twice. Subsequently, the video content was discussed part by part. Then, we moved for the next video. No flyer was given.

Each session lasted between one to two hours depending on the duration of the discussion.

Nutrition programme lasted six months. We grouped posters and videos around the five main topics described above (Table 1). Topic 1 was developed during two sessions since it encompassed many videos. Each of other topics (2, 3, 4 and 5) were developed during one education session. In each village, two education sessions were organized per month. Therefore, the first round of the six sessions (Topic 1 part 1, Topic 1 part 2, Topic 2, Topic 3, Topic 4 and Topic 5) lasted three months. Then, we organised a second round (revision round) of all sessions. This means that each education topic had been developed twice in a village over the period of intervention, once during each round of sessions.

Sessions were organized at community level meaning that all community members (not only mothers) were invited to attend.

Data collection

Mothers or primary caregivers were interviewed. Data collected were: (1) mothers' socio-economic characteristics (activities, matrimonial status, instruction level etc.) and (2) child aged 6-17 months feeding practices and food consumption data using a 24 hour recall. For each child, all foods/recipes (including drinks) consumed the previous day were listed. Then, for each recipe, all the ingredients were recorded.

Data analysis and statistics

From the 24 hour recall data, foods consumed by the children were split into ingredients and categorized into seven food groups. Meal Frequency (MF), Dietary Diversity Score (DDS), Minimum Dietary Diversity (MDD) and Minimum Meal Frequency (MMF) indicators were estimated following WHO and UNICEF guidelines (24,25,33). Feeding practices and socio-economic characteristics were compared (poster-flyer vs video villages) using univariate statistical tests, Pearson's chi square (34). Student t-tests or Mann Whitney test were used for continuous variables.

Univariate chi-square tests were used to examine whether the proportion of children that achieved each of the indicators differed between the poster (considered as reference group, coded 0) and video group (coded 1 and considered as intervention group) at baseline.

Results from the above univariate chi-square tests may be biased due to confounding effects related to care-givers' characteristics (age, ethnicity, instruction level, matrimonial status, number of nutrition training sessions attended, number of income generating activities), having participated in nutrition education in the past, child age and sex, as well as District (*Houéyogbé/Bopa*). To account from this, the intervention effect (video compared to poster-flyer) from the baseline to endline surveys was assessed by the difference-in-differences (DID) estimator using DDS and MF rather

than MDD and MMF since continuous variables usually allow strongest analysis than derived categorised variables. However analysis were also performed for MDD and MMF. These results, not presented in this paper, showed similar trend.

The DID is an impact evaluation approach applicable whenever one has access to panel data and/or repeated cross-sectional data. DID estimator removes unobserved fixed effects via within-person comparisons over time as well as common period and ageing effects by comparing the trends of an intervention and reference group. The between-comparison with the trend of a reference group additionally removes any common period effects that affect the intervention and reference group in identical ways as well as any ageing effects (35).

To account also from data structure (panel data), the DID analysis was run under a generalized estimating equations (GEE) framework (36,37,38) similarly as in Waswa et al (23). We used Poisson loglinear distribution considering the type of dependent variables. Data were analysis following Intention To Treat approach.

To allow comparison between baseline and endline, data used in this paper were related to complementary feeding among children aged 6 to 17 months, which is the age group common to the two surveys. Further analysis had been performed among all children aged 6-23 months and results (not presented) showed similar trend.

All statistical analyses were implemented in SPSS version 23.

Results

Socio-economic characteristics of informants

Most of socioeconomic characteristics relative to mothers and children were similar in the two groups (Table 2). Even if majority of mothers (84,5%) were from *Sahouè* ethnic group, significant differences were observed between groups, the percentage of *Sahouè* in Poster group is higher than video group. Mothers in video group were involving in more income generating activities than poster-flyer group.

Globally, about half of mothers participated the nutrition sessions organised. Participation to nutrition

Table 2. Socioeconomic characteristics of children and mothers and participation to nutrition education programme.

Characteristics	Total (n=155)	Poster (n=83)	Video (n=72)	p-value
Children				
Age of children	10.0 ± 2.6	9.9 ± 2.7	10.1 ± 2.4	0.573[s]
Children sex				
Boys	53.5	55.4	51.4	0.616[c]
Girls	46.5	44.6	48.6	
Mothers				
Age of mothers	28.2 ± 6.1	27.6 ± 6.1	28.9 ± 5.9	0.161[s]
Matrimonial status of primary caregiver				
Living alone	18.1	18.1	18.1	0.998[c]
Living with husband	81.9	81.9	81.9	
Instruction recoded				
Illiterate	69.0	69.9	68.1	0.892[c]
Literate or Primary school	20.6	19.3	22.2	
Secondary school and more	10.3	10.8	9.7	
Ethnic group				
Sahoué	84.5	95.2	72.2	<0.001[c]
Autre	15.5	4.8	27.8	
Income Generating Activities	91.0	84.3	98.6	0.002[c]
Number of Income Generating Activities	1.5 ± 0.9	1.4 ± 1.0	1.7 ± 0.9	0.040[M]
Nutrition education				
Participation to a nutrition education programme in the past	3.9	2.4	5.6	0.311[c]
Participation to at least one nutrition session	53.5	54.2	52.8	0.858[c]
Participation to all nutrition sessions	13.5	12.0	15.3	0.558[c]
Number of nutrition sessions attended	1.5 ± 1.8	1.5 ± 1.8	1.7 ± 1.9	0.735[M]

Statistical tests: C, Chi square; S, Student t test; M, Mann Whitney.

**Values presented are percentages or Mean ± Standard deviation. Explanations among Matrimonial status of care givers: 'Living with husband' corresponds to woman married who lives with her husband and 'Living alone' to woman who is married but doesn't live with her husband, divorced or widow.*

education programme even if in the past did not differ from one group to another.

Description of feeding practices at baseline

Cereals, roots and tubers were the most important food group and were consumed by almost all children (Table 3). Food groups consumed by more than 50% of children were: flesh foods, vitamin A products (including leafy vegetables, red palm oil etc.) and others fruits and vegetables. Eggs and milk products were less consumed (< 5% of children).

Percentages of children who consumed legumes and nuts in one hand and fruits and vegetables different from those rich in vitamin A differed from poster-flyer to video group (p=0.005 and p=0.044 respectively).

About half of children achieved the MDD in the two groups. However, DDS showed slightly significant advantage for video group. MMF percentages were about 72% with no significant difference between the two groups (Table 3).

Table 3. Complementary feeding practices among 6-17 months children at baseline

Parameters	Groups			p-value
	All (n=149)	Poster (n=77)	Video (n=72)	
Food groups				
Cereals, roots and tubers	99.3	100.0	98.6	0.299[C]
Legumes and nuts	24.2	33.8	13.9	0.005[C]
Vitamin A rich products	61.1	62.3	59.7	0.744[C]
Other fruits and vegetables	62.4	70.1	54.2	0.044[C]
Milk and milk products	0.7	1.3	0.0	0.332[C]
Eggs	4.0	2.6	5.6	0.359[C]
Fishes and meat	67.8	71.4	63.9	0.325[C]
Feeding practices indicators				
MF	2,7±1,0	2,7±1,0	2,7±1,0	0.542[M]
DDS	3.2 ± 1.4	3.4 ± 1.3	2.9 ± 1.4	0.034[M]
MDD	49.3	56.6	41.7	0.070[C]
MMF	71.6	73.7	69.4	0.567[C]

Statistical tests: C, Chi square; M, Mann Whitney.
**Values presented are percentages or Mean ± Standard deviation*

Effect of the intervention on achieving recommendations

Video was associated to a better likelihood to increase DDS. However, the DID estimates from the GEE models assessing the effect of using video compared to poster-flyer on the likelihood to increase DDS and MF (Table 4) showed no statistically significant difference. This indicates that video did not significantly decrease or increase the likelihood to have a greater DDS or MF compared to poster-flyer.

Regarding the other explanatory variables included in the DID models, none of the following variables related to care-givers (education level, number of training session attended, having or not an income generating activity and having participated in nutrition education in the past) significantly influenced the feeding practices. However, child age was significantly and positively associated to both DDS and MF indicating that the likelihood to reach a better dietary diversity and meal frequency increased with children age. Younger mothers were also less likely to increase the dietary diversity of their children than older ones whereas mothers living in *Bopa* were more likely to increase the meal frequency than those living in the district of *Houéyogbé*.

Globally, the intercepts in the two models were significant indicating that there were others variables explaining feeding practices that were not taken into account.

Discussion

Foods given to children in this study were mainly cereals, roots and tubers based corroborating with the literature on households' diets in the country (3,39-43).

Results showed that, overall, about half of children had achieved MDD at baseline and more than 60% reached MMF. Very similar values were presented by Mitchodigni et al (7,8) for the same districts. However, these trends were very different and high compared to results from DHS. Indeed, our survey was conducted during harvesting period. In general, seasons affect the availability of different foods and therefore, could influence dietary diversity (41,44). In a rural poor household context, where financial power to purchase food is low, results from two African coun-

Table 4. Results from GEE analysis among 6-17 months children dietary diversity score and meal frequency

Parameters	B	Std. Error	Hypothesis Test		
			Wald Chi-2	df	p-value
Dietary Diversity Score					
Intercept	0,595	0,1724	11,904	1	0,001
Survey: Baseline vs Endline (R)	0,051	0,0676	0,566	1	0,452
Districts: *Bopa* vs *Houéyoghé* (R)	-0,004	0,0470	0,007	1	0,934
Sex of children: Boys vs Girls (R)	-0,082	0,0421	3,785	1	0,052
Group: Poster vs Video (R)	0,167	0,0685	5,957	1	0,015
DID	**-0,036**	**0,0798**	**0,204**	**1**	**0,651**
Nutrition education past: No vs Yes (R)	0,004	0,0559	0,006	1	0,936
Matrimonial status: LA vs LWH (R)	0,080	0,0507	2,507	1	0,113
Ethnic group: *Sahouè* vs Others (R)	-0,042	0,0621	0,450	1	0,502
Age of children	0,024	0,0084	8,356	1	0,004
Number of training sessions attended	0,019	0,0139	1,967	1	0,161
Mothers instruction level	0,047	0,0343	1,889	1	0,169
Age of mothers	0,009	0,0039	5,059	1	0,024
Number of activities	-0,019	0,0237	0,667	1	0,414
Meal Frequency					
Intercept	0,938	0,1763	28,272	1	<0,001
Survey: Baseline vs Endline (R)	-0,114	0,0652	3,045	1	0,081
Districts: *Bopa* vs *Houéyoghé* (R)	-0,109	0,0475	5,222	1	0,022
Sex of children: Boys vs Girls (R)	-0,022	0,0441	0,253	1	0,615
Group: Poster vs Video (R)	0,025	0,0604	0,169	1	0,681
DID	**-0,048**	**0,0861**	**0,316**	**1**	**0,574**
Nutrition education past: No vs Yes (R)	0,021	0,0650	0,100	1	0,752
Matrimonial status: LA vs LWH (R)	-0,003	0,0601	0,002	1	0,963
Ethnic group: *Sahouè* vs Others (R)	-0,072	0,0629	1,313	1	0,252
Age of children	0,023	0,0084	7,218	1	0,007
Number of training sessions attended	0,008	0,0129	0,351	1	0,553
Mothers instruction level	0,061	0,0335	3,310	1	0,069
Age of mothers	0,002	0,0040	0,275	1	0,600
Number of activities	-0,010	0,0213	0,233	1	0,630

LA=Living Alone. Woman who is married but doesn't live with her husband, or divorced or widow. LWH=Living with husband. Woman married who lives with her husband; R=Reference category

tries, with one having similar agro ecological characteristics with Benin, showed that the harvesting period corresponds to good food availability (45,46).

Results from DID analysis showed that children in video arm and those in poster-flyer arm performed the same with dietary diversity and meal frequency. So, video did not have a significant better likelihood of increasing DDS and MF compared to poster-flyer arm.

Some studies have compared the effect of videos used in nutrition education to other communication tools. A systematic review on the effectiveness of video-based education (47) showed that interventions using video were variably effective for modifying health behaviour. Significant improvements in behavioural outcomes were not reported uniformly across all studies. The effectivity depends on the target behaviours to be influenced; video-based education is effective in influencing certain types of health behaviours and non-effective for others. Authors (47) underlined the fact that videos shall be tailored, meaning adapted to the message, the target population and the context of diffusion. Nevertheless, the majority of the studies included in this review were from developed or high income countries; results may thus not be systematically applicable to low income countries like Benin. Unfortunately, literature from developing

or African countries on this specific domain is scarce. Videos have, however, some advantages compared to other methods. They are more attractive and facilitate mobilisation, e.g. in the present study, the number of people attending the video sessions was twice as much than for the poster sessions. In 2008, an NGO showed videos to farmers in 19 villages in Benin. These videos had attracted large audiences of community members, including youth and women (28). In our study, videos featuring fellow village inhabitants as actors received more appreciation.

Videos do not necessarily need to be facilitated by an expert who knows the subject; sometimes the video can speak for itself (28). It removes inconsistencies across educators and balances the presentation of information to provide more standardized education (31). Videos are also suitable for individuals or populations with low health literacy or illiterate audiences (26,27) and they facilitate message recall. Bentley et al. (28) showed that farmers were able to remember education videos they had seen five years later. This paper, however, only analysed behaviours and did not look into changes in knowledge and attitudes.

The fact that participants in this study received individual flyers at the end of the poster sessions and not after the video sessions might have mitigated the advantage of videos. According to Glanz and Rimer (48), using multiple communications channels (here poster + flyer vs video) increases the efficacy. Flyers allowed people to set their own pace in reviewing information when back home (47). Combining videos with flyers could increase their efficacy. Another possibility would be to share videos with participants by copying them on their smartphones after the sessions. However, most rural dwellers in Benin do not own smartphones.

Another issue is that posters were presented during the day, but videos had to be presented at night to improve quality of projection. During the night, after work, people could be more available and more focus on to watch video with no stress among their activities (to go market or to farm for example). However, after a whole day of activities, mothers could have been tired and less concentrated on the content presented and discussed. People in remote villages with lack of basic services such as power or running water, could have been more attracted to the technology (electricity, big screens, projectors, etc.) than the content of the videos.

Results showed that dietary diversity and meal frequency are influenced by some other socioeconomic factors. DDS and MF increased with children age. Results from national DHS (2,3,4) and literature for other African countries (49,50,51) confirmed these trend. Indeed, when children are growing, they move from eating cereal (especially maize) porridge to extracts from family dishes which are more diversified. Mother's age was positively associated with dietary diversity. Similar results were found in Ethiopia for child dietary diversity (52). As the age increases, mothers gain experience in child feeding.

We observed also that mothers in the district of *Bopa* were more likely to increase the meal frequency than those living in *Houéyogbé*. This difference was also observed by Mithodigni et al (7) even if not significant. Mother occupation could be pointed out here. The occupation could provide or not an opportunity for mothers to be with their children while working and nurse them properly (53,54). According to our field observations, mothers in *Houéyogbé* were mostly involving in out-of-home activities. However, most of mothers in *Bopa* were involving in agriculture or home based small activities. Even if they had to go to farm, they used to move with their children and this allowed them to continue to feed them. In the same direction, Mitchodogni et al (8), found when mothers were involving in income-generating activities, their children were less likely to meet MMF.

Limitations

Feeding practices could not be changed rapidly; therefore, there are two challenges: using a sample too small to detect the effects produced or intervention duration being too short to measure an impact (55).

The period of 6 months is usually considered and recommended as the minimum to observe outcomes from interventions targeting complementary feeding even if on growth performance (56,57). However, some complementary feeding practices, such as dietary diversity could change rapidly if interventions reach the specific constraints of the target group (58). Inter-

ventions implemented during 3 to 6 months and based on behavior change approaches could lead to improved complementary feeding practices (58).

Sample size is actually a major issue in assessing effectiveness of nutrition education intervention. However, sample size varies greatly across studies, for example from only n=8 to about n=1424 mainly depending on the objectives of the study and the level of difficulty to collect the outcome data. We have: n=8 for Bauer & Capra (59); n=60 for Isenring et al. (60); n=80 for Ha & Caine-Bish (61); n=99 for McAleese & Rankin (62); n=198-207 for Waswa et al. (23) and n=1424 for Hoddinott et al. (63). While our sample size (n=360 for the whole sample, n=257 for both baseline and endline and n=155 considered in the present study) could be seen as low compared to other studies, it is higher than that of Waswa et al. (23) who did similar studies in Kenya and we believed it was sufficient to make accurate inference (15 villages and n=150). We could have used some formulas to calculate the sample size a priori but this would have implied speculations on parameters such as variance, effect size, and we deliberately chose to not do such speculations.

Conclusion

The present study compared videos and posters-flyers used as communication tools in a nutrition education program implemented in a rural area in Southern-Benin. Results didn't show a significant advantage of videos in terms of improving child dietary diversity nor meal frequency compared to posters and flyers. More investigations are needed in order to disentangle the effects of videos and posters on mothers' nutrition knowledge and attitudes as these factors could influence feeding practices. The contribution of each video, either Glocal videos or newly designed ones, could also be considered. Furthermore, we recommend the videos being tested for use in different settings, such as health centres and for different audience such as extension workers as the conditions in rural villages in Benin (no electricity, no smartphones, etc.) are logistically challenging.

References

1. International Food Policy Research Institute. Global Nutrition Report 2016: From Promise to Impact: Ending Malnutrition by 2030. 2016. Washington, DC, USA: IFPRI.
2. Institut National de la Statistique et de l'Analyse Économique (INSAE), ICF. Demographic Health Survey of Benin, 2017-2018. 2019. Cotonou, Benin and Rockville, Maryland, USA: INSAE and ICF. P675.
3. Institut National de la Statistique et de l'Analyse Économique (INSAE) and Macro International Inc. Demographic Health Survey (DHS-III) - Benin 2006. 2007. Calverton, Maryland, USA: INSAE Benin and Macro International Inc.
4. Institut National de la Statistique et de l'Analyse Economique (INSAE). Enquête par grappes à indicateurs multiples 2014, Rapport final [Multiple Indicator Cluster Survey 2014]. 2015. Cotonou, Bénin : INSAE. http://www.insae-bj.org/autres_enquetes.html
5. Achigan-Dako EG, Pasquini MW, Assogba Komlan F, N'danikou S, Yédomonhan H, Dansi A, Ambrose Oji B. Traditional vegetables in Benin: Diversity, distribution, ecology, agronomy, and utilisation. 2010. Cotonou, Benin: Imprimeries du CENAP/Institut National des Recherches Agricoles du Bénin (INRAB).
6. Achigan-Dako EG, N'danikou S, Assogba Komlan F, Ambrose-Oji B, Pasquini MW, Ahanchede A. Diversity, geographical and consumption patterns of traditional vegetables in sociolinguistic communities in Benin: implication for domestication utilization. Economic Botany 2011; 10 (20): 1-17. Doi: 10.1007/s12231-011-9153-4
7. Mitchodigni IM, Amoussa Hounkpatin W, Ntandou-Bouzitou G, Termote C, Kennedy G, Hounhouigan DJ (a). Complementary Feeding Practices of Children Aged 6-23 Months in Rural Area, Southern-Benin: Challenges and Opportunities. International Journal of TROPICAL DISEASE & Health 2017; 24(2): 1-12. Article no.IJTDH.33877. ISSN: 2278–1005, NLM ID: 101632866
8. Mitchodigni IM, Amoussa Hounkpatin W, Ntandou-Bouzitou G, Avohou H, Termote C, Kennedy G, Hounhouigan DJ (b). Complementary feeding practices: Determinants of dietary diversity and meal frequency among children aged 6-23 months in Southern-Benin. Food Security 2017 ; 9: 1117–1130. https://doi.org/10.1007/s12571-017-0722-y
9. El Ati J, Alouane L, Mokni R, Béji C, Hsaïri M, Oueslati A, Maire B. Le retard de croissance chez les enfants tunisiens d'âge préscolaire : Analyse des causes probables et interprétation de son évolution au cours des 25 dernières années [Stunting among Tunisian preschool children: Analysis of probable causes and interpretation of its evolution over the last 25 years]. Options Méditerranéennes : Série B. Etudes et Recherches 2002 ; 41:51-70.
10. Badji MS. Analyse de l'évolution des déterminants de la santé nutritionnelle des enfants âgés de moins de cinq ans au Sénégal [Analysis of the evolution of the determinant factors of the nutritional health of under five years children

in Senegal]. Perspective Afrique 2. 2006; No. 2-3, 2006-07: Article1. www.perspaf.org
11. Ajao KO, Ojofeitimi EO, Adebayo AA, Fatusi AO, Afolabi OT. Influence of Family Size, Household Food Security Status, and Child Care Practices on the Nutritional Status of Under-five Children in Ile-Ife, Nigeria. African Journal of Reproductive Health 2010; 14(4): 123-132
12. Senbanjo IO, Olayiwola IO, Afolabi WA, Senbanjo OC. 2013. Maternal and child under-nutrition in rural and urban communities of Lagos state, Nigeria: the relationship and risk factors. BMC Research Notes. Published online: 23 July 2013; 6, 286. doi: 10.1186/1756-0500-6-286
13. Jesmin A, Yamamoto SS, Malik AA, Haque A. Prevalence and Determinants of Chronic Malnutrition among Preschool Children: A Cross-sectional Study in Dhaka City, Bangladesh. Journal of Health, Population and Nutrition 2011; 29(5): 494-499.
14. Santos I, Victora CG, Martines J, Gonçalves H, Gigante DP, Valle NJ, Pelto G. Nutrition Counseling Increases Weight Gain among Brazilian Children. The Journal of Nutrition, November 2001; 131 (11): 2866–2873. https://doi.org/10.1093/jn/131.11.2866
15. Reyes H, Pérez-Cuevas R, Sandoval A, Castillo R, Santos JI, Doubova SV, Gutiérrez G. The family as a determinant of stunting in children living in conditions of extreme poverty: a case-control study. BMC Public Health. Published online: 30 November 2004; 4:57. Doi: 10.1186/1471-2458-4-57
16. Kilaru A, Griffiths PL, Ganapathy S, Ghosh S. Community-based nutrition education for improving infant growth in rural Karnataka. Indian pediatrics June 2005; 42(5):425-32
17. Roy SK1, Fuchs GJ, Mahmud Z, Ara G, Islam S, Shafique S, Akter SS, Chakraborty B. Intensive nutrition education with or without supplementary feeding improves the nutritional status of moderately-malnourished children in Bangladesh. J Health Popul Nutr; December 2005; 23(4):320-30.
18. Shi L, Zhang J. Recent Evidence of the Effectiveness of Educational Interventions for Improving Complementary Feeding Practices in Developing Countries. Journal of Tropical Pediatrics April 2011; 57(2):91-8. DOI: 10.1093/tropej/fmq053
19. Shahnaz V, Engle P, Balakrishna N, Griffiths PL, Johnson SL, Creed-Kanashiro H, Fernandez Rao S, Shroff MR, Bentley ME. Cluster-randomized trial on complementary and responsive feeding education to caregivers found improved dietary intake, growth and development among rural Indian toddlers. Maternal and Child Nutrition 2013; 9: 99–117. DOI: 10.1111/j.1740-8709.2012.00413.x
20. Khan AZ, Rafique G, Qureshi H, Badruddin SH. A Nutrition Education Intervention to Combat Under nutrition: Experience from a Developing Country. Hindawi Publishing Corporation ISRN Nutrition 2013; Article ID 210287. https://doi.org/10.5402/2013/210287.
21. Lassi ZS, Das JK, Zahid G, Imdad A, Bhutta ZA.. Impact of education and provision of complementary feeding on growth and morbidity in children less than 2 years of age in developing countries: a systematic review. BMC Public Health 2013; 13(Suppl3): S13. http://www.biomedcentral.com/1471-2458/13/S3/S13
22. 22 Sunguya BF, Poudel KC, Mlunde LB, Shakya P, Urassa DP, Jimba M, Yasuoka J. Effectiveness of nutrition training of health workers toward improving caregivers' feeding practices for children aged six months to two years: a systematic review. Nutrition Journal 2013; 12:66. http://www.nutritionj.com/content/12/1/66
23. Waswa LM, Jordan I, Herrmann J, Krawinkel MB, Keding GB. Community-based educational intervention improved the diversity of complementary diets in western Kenya: results from a randomized controlled trial. Public Health Nutrition 2015; 18(18): 3406-19. DOI: 10.1017/S1368980015000920
24. WHO, UNICEF. Indicators for assessing infant and young child feeding practices. Part 1: Definitions. Conclusions of a consensus meeting held 6–8 november 2007 in Washington, DC, USA. 2009. Washington, D.C., USA: WHO and UNICEF.
25. UNICEF. Programming Guide: Infant and Young Child Feeding. 2012. New York, USA: IYCF Unit, Nutrition Section, UNICEF. Available at: iycn@unicef.org.
26. Davis TC, Michielutte R, Askov EN, Williams MV, Weiss BD. Practical assessment of adult literacy in health care. Health Education & Behavior 1998; 25: 613–624.
27. Sobel RM, Paasche-Orlow MK, Waite KR, Rittner SS, Wilson EA, Wolf MS. Asthma 1-2-3: A low literacy multimedia tool to educate African American adults about asthma. Journal of Community Health 2009; 34: 321–327.
28. Bentley J, Van Mele P, Okry F, Zossou E. Videos that speak for themselves: when non-extensionists show agricultural videos to large audiences. Development in Practice 2014; 24(7): 921-929. DOI: 10.1080/09614524.2014.942216
29. Glanz K, Rimer BK, Viswanath K. Health behaviour and health education: Theory, Research, and Practice. 4th edition. 2008. San Francisco, USA: John Wiley & Sons, Inc.
30. CAN. Stratégie Nationale de Communication pour le Changement Social et Comportemental pour la promotion de la Nutrition au Bénin et son Plan Opérationnel 2017-2021. 2017. Cotonou, Bénin : CAN/Nouvelles Presses Industries Graphiques.
31. Gagliano ME. A literature review on the efficacy of video in patient education. Journal of Medical Education 1988; 63: 785-792.
32. UNICEF. Le Paquet de Counseling Communautaire pour l'Alimentation du Nourrisson et du Jeune Enfant (ANJE) : Livret de Messages Clés et Outils de formation [The Community Counseling Package for Infant and Young Child Feeding (IYCF): Key Messages Booklet and Training Tools]. 2010. New York, USA: UNICEF.
33. Kennedy G, Ballard T, Dop MC. Guidelines for Measuring Household and Individual Dietary Diversity. 2010. Roma, Italy: Nutrition and Consumer Protection Division, FAO.
34. Thomas R, Vaughan I, Lello J. Data analysis with R statistical software. A guidebook for scientists, 1st edition. 2013.

Eco-explore: Wales.
35. Gebel M, Voßemer J. The impact of employment transitions on health in Germany. A difference-in-differences propensity score matching approach. Social Science & Medicine 2014; 108:128-136.
36. Zuur A, Ieno e, Walker N, Saveliev A, Smith G. Mixed effects models and extensions in ecology. [Gail M, Krickeberg K, Samet JM, Tsiatis A, Wong W, editors]. 2009. New York, NY: Spring Science and Business Media.
37. Ballinger GA. Using generalized estimating equations for longitudinal data analysis. Organizational research methods 2004; 7 (2): 127-150.
38. Wooldridge JM. Econometric Analysis of Cross Section and Panel Data. 2nd ed. 2010. Cambridge, MA: MIT Press.
39. Atègbo EAD. Food and Nutrition insecurity in Nothern Benin: impact on growth performance of children and on year to year nutritional status of adults. PhD Thesis. 1993. Wageningen, The Netherlands: Wageningen University.
40. Kayodé APP. Diversity, users' perception and food processing of sorghum: Implications for dietary iron and zinc supply. PhD Thesis. 2006. Wageningen, The Netherlands: Wageningen University.
41. Mitchikpe CES, Dossa RAM, Ategbo EAD, Van Raaij JMA, Kok FJ. Seasonal variation in food pattern but not in energy and nutrient intakes of rural Beninese school-aged children. Public Health Nutrition 2008; 12(3): 414-422. DOI: 10.1017/S1368980008002929.
42. FAO. Profil nutritionnel de pays: République du Bénin. 2011. Roma, Italy: FAO.
43. Amoussa-Hounkpatin W, Blum L, Ledain AS, Badou A. Rapport final de l'enquête qualitative: Etude des déterminants des pratiques d'alimentation complémentaire des enfants de moins de 2 ans, Cas de 3 localités au Bénin: Boukoumbé, Djakotomey et Kétou [Final report of the qualitative survey: Study of the determinants of complementary feeding practices of under 2 years children; Case study of 3 localities in Benin: Boukoumbé, Djakotomey and Kétou]. 2014. Cotonou, Benin: Ministry of Health of Benin, Faculty of Agricultural Sciences of the University of Abomey-Calavi, UNICEF-Benin.
44. Alladayè F, Mitchikpè CES, Adégbola PY. Variation saisonnière des apports nutritionnels des enfants âgés de 24 à 59 mois dans les zones agroécologiques des départements du Borgou et de l'Alibori au nord-est du Bénin [Seasonal variation in nutritional intake of 24 to 59 months children in the agroecological zones of the departments of Borgou and Alibori in North Eastern Benin]. Bulletin de la Recherche Agronomique du Bénin (BRAB) December 2017: 46-56. Available at http://www.slire.net & http://www.inrab.org
45. Abizari AR, Azupogo F, Nagasu M, Creemers N, Brouwer ID. Seasonality affects dietary diversity of school-age children in northern Ghana. PLoS ONE 2017; 12(8): e0183206. https://doi.org/10.1371/journal.pone.0183206
46. Powell B, Kerr RB, Young SL, Johns T. The determinants of dietary diversity and nutrition: ethnonutrition knowledge of local people in the East Usambara Mountains, Tanzania. Journal of Ethnobiology and Ethnomedicine 2017; 13(23). DOI 10.1186/s13002-017-0150-2
47. Tuong W, Larsen ER, Armstrong AW. Videos to influence: a systematic review of effectiveness of videobased education in modifying health behaviors. J Behav Med 2014; 37: 218-233. DOI: 10.1007/s10865-012-9480-7
48. Glanz K, Rimer BK. Theory at a Glance: A Guide for Health Promotion Practice. 2nd edition. 2005. US: National Cancer Institute, National Institutes of Health, Department of Health and Human Services.
49. Beyene M, Worku AG, Wassie MM. Dietary diversity, meal frequency and associated factors among infant and young children in Northwest Ethiopia: a cross- sectional study. BMC Public Health Published online: 3 October 2015; 15, 1007. DOI:10.1186/s12889-015-2333-x
50. Belew AK, Ali BM, Abebe Z, Dachew BA. Dietary diversity and meal frequency among infant and young children: a community based study. Italian Journal of Pediatrics. Published online: 15 August 2017; 43(73). DOI:10.1186/s13052-017-0384-6
51. Mekonnen TC, Workie SB, Yimer TM, Mersha WF. Meal frequency and dietary diversity feeding practices among children 6–23 months of age in Wolaita Sodo town, Southern Ethiopia. Journal of Health, Population and Nutrition 2017; 36(18). DOI 10.1186/s41043-017-0097-x
52. Agize A, Jara D, Dejenu G. Level of Knowledge and Practice of Mothers on Minimum Dietary Diversity Practices and Associated Factors for 6–23-Month-Old Children in Adea Woreda, Oromia, Ethiopia. Hindawi BioMed Research International. Published online: 2 December 2017; ID 7204562. https://doi.org/10.1155/2017/7204562
53. Netshandama V. Breastfeeding practices of working women. Curationis February 2002; 25(1):21-7. DOI:10.4102/curationis.v25i1.698
54. Nkrumah J. Maternal work and exclusive breastfeeding practice: a community based crosssectional study in Efutu Municipal, Ghana. International Breastfeeding Journal 2017; 12(10). DOI 10.1186/s13006-017-0100-6
55. IYCN/USAID. 2011. Behaviour change interventions and child nutritional status: Evidence from the promotion of improved complementary feeding practices. Literature review. June 2011. Massachusetts Ave., NW Washington, DC 20001, USA. www.iycn.org
56. Dewey KG, Adu-Afarwuah S. Review Article Systematic review of the efficacy and effectiveness of complementary feeding interventions in developing countries. Maternal and Child Nutrition. 2008; 4:24–85.
57. Panjwani A, Heidkamp R. Complementary Feeding Interventions Have a Small but Significant Impact on Linear and Ponderal Growth of Children in Low- and Middle-Income Countries: A Systematic Review and Meta-Analysis. J Nutr 2017; 147(Suppl):2169S–78S. doi: https://doi.org/10.3945/jn.116.243857
58. Sanghvi T, Seidel R, Baker J, Jimerson A. Using behavior change approaches to improve complementary feeding practices. Matern Child Nutr. 2017; 13(S2):e12406. DOI:

10.1111/mcn.12406
59. Bauer JD, Capra S. Nutrition intervention improves outcomes in patients with cancer cachexia receiving chemotherapy—a pilot study. Supportive care in cancer 2005; 13(4): 270-274.
60. Isenring EA, Capra S, Bauer JD. Nutrition intervention is beneficial in oncology outpatients receiving radiotherapy to the gastrointestinal or head and neck area. British journal of cancer 2004; 91(3), 447.
61. Ha EJ, Caine-Bish N. Effect of nutrition intervention using a general nutrition course for promoting fruit and vegetable consumption among college students. Journal of nutrition education and behavior 2009; 41(2):103-109.
62. McAleese JD, Rankin LL. Garden-based nutrition education affects fruit and vegetable consumption in sixth-grade adolescents. Journal of the American Dietetic Association 2007; 107(4):662-665.
63. Hoddinott J, Maluccio JA, Behrman JR, Flores R, Martorell R. Effect of a nutrition intervention during early childhood on economic productivity in Guatemalan adults. The lancet 2008; 371(9610): 411-416.

Correspondence:
Fifali Sam Ulrich Bodjrenou
University of Abomey-Calavi, Faculty of Agronomic Sciences, 03 BP 2819 Jéricho Cotonou Benin.
Phone: +22961001440.
E-mail: bodjrenousam@gmail.com

Progress in Nutrition 2020; Vol. 22, N. 1: 343-350 DOI: 10.23751/pn.v22i1.7691

Case report

Nutrition planning and hydration control during a six-stage Pirineos FIT Endurance trail-running race. A case report

Eraci Drehmer[1], Mª Ángeles Navarro[1], Sandra Carrera[2], Roberto Herrera[1], Carlos Sanchis[1], Mari Luz Moreno[1]

[1]Universidad Católica de Valencia "San Vicente Mártir", Faculty of Physical Activity and Sport Sciences Email: eraci.drehmer@ucv.es;
[2]Universidad Católica de Valencia "San Vicente Mártir" - Faculty of Medicine & Odontology

Summary. Ultra-endurance competitions are highly demanding sport events for athletes and require a carefully controlled nutrition, hydration and supplementation before, during and after the physical effort. Scientific research has shown a positive relationship between dietetic (caloric and macronutrient ingestion) recommendations and sport performance. This study describes the nutritional and hydration planning applied to an athlete competing at the Pirineos FIT (a semi-self-sufficient trail-running multi-stage event). Diary caloric ingestion was around 4000 Kcal, 550 Kcal were consumed during the race. In general, the athlete maintained the minimal recommendable levels of hydration (2.5% Body Weight Loss) and Borg's Scale of Exertion (RPE) was used to report subjective perception of fatigue after each stage. Hematological and biochemical parameters showed a normal response to endurance physical exercise. Therefore, the nutrition and hydration planning were successfully applied.

Key words*:* trail-running, hydration, nutrition planning, Spanish Pyrenees

Introduction

"Pirineos Fit" is an international event in Jaca (Spain), a 234 km and 15.075 m elevation gain six-day stage trail-running race. In this competition, participants have to complete the stages (34-41 km) in semi-self-sufficient conditions (athletes carry food and equipment, only providing two points of liquid provisioning during each stage). Participation in long lasting single and multi-stage endurance events has been growing over the last decades (1-3) despite their high physiological (4), nutritional (5) and psychological (6) demands. Although athlete physiological and hydric response has been recently described in single-stage ultra-endurance trail running races (4,7), little is known about its biochemical response and nutrition control during trail-running multi-stage races, with only a few studies assessing hydration status and heart rate in short (three days) races (8,9).

Macronutrient and fluid intake during endurance events should be a major concern for athletes, coaches and nutritionists to ensure both performance and health during competition. Correct nutrition reduces energy depletion, physiological stress and gastrointestinal problems, increases performance and accelerates recovery (10,11); while adequate hydration avoids hyponatremia, hyperthermia and central nervous system dysfunction (12,13) that leads to low performance and health damage.

The aim of this study is to describe a successful case of nutritional and hydration planning of a six-stage trail-running race in the Spanish Pyrenees.

Interventions and methods

Athlete

Our participant was a highly trained (65.3 ml·kg·min VO_2max) and fit (Weight = 66.15 kg; BMI = 22.49 kg/m^2; Fat Mass = 11.05 %; Free Fat Mass = 42.28 %; ∑ 8 Skinfolds[1] = 55.4 mm; Basal metabolism[2] = 1664 Kcal) 29-years-old male with significant training and racing history in trail-running: 14 years specific training, more than 300 endurance flat running events, various podiums in short trail races (2nd place at "Liga Serranía" 2017, Spain), and participations in ultra-trail events of 200-330 km in semi-self-sufficient conditions. Informed consent approved by Universidad Católica de Valencia "San Vicente Mártir" was obtained.

Race

"Pirineos FIT" is a six-day stage race, from Panticosa to Jaca where the athletes (> 18 y/o) compete covering the distance of each stage. Officially[3], stages range from 34 to 41 km with 1460-3565 m of positive accumulated ascension (m+). Organization ensures correct marking of the stages but is the athlete the responsible for localization and orientation. Event requires athletes carry all food and equipment, only providing two points of liquid provisioning during each stage.

Training

Training preparation for "Pirineos Fit" started August'16 and implied 295 training sessions, 405 h of running, 105625 m+ and 3429.1 km. During this period, maximum training volume ranged between 15h 29' and 21h 10' (including a 110 km ultra-endurance event in September'16); while minimum training volume was established in April with 5h 47' of training. Last training was programmed for the last four months before the race, with two months of general preparation (March-April) and two months of specific ultra-endurance training (May-June). The best performance results accomplished by the athlete during the preparation process where at "Maratón de Alcublas" (21.9 km; 1h 48'; 705 m+; 2nd overall), "Tail Vielha-Molieres" (40 km; 4000 m+; 7h 17'; 14th overall) and "UTES" (106 km; 6200 m+; 14h 58'; 1st overall).

Using Skinner & McLellan's triphasic model (14), during preparation process, 27.9% of the time was spent in Phase I, 54.2% in Phase II and 18.4% in Phase III.

Nutrition planning and record

Key recommendations for the competition were given to the athlete, aiming to reduce dehydration, hyponatremia and to minimize body weight loss (BWL) to 1-3 %, considered a minimal level of dehydration (15). Main recommendations were to control hydric and electrolytic reposition ingesting 400-600 mL/h of fluid, 460-1150 mg/L of Na^+ and ensure periodic ingestion of carbohydrates (30-60 g/h) (15-17). During "Pirineos FIT", the method used for dietary assessment prior (breakfast), during (intra-competition) and after (post-race, lunch, afternoon, snacks and dinner) each stage was registered based on Food Record as described on Larson-Meyer et al. (18). This record includes food, supplement and fluid intake. Nutrients register was calibrated with the software "*Programa Alimentación y Salud*" v.2 (Granada University, Spain). Additionally, subjective effort perception and feeling, along with the heart rate (HR) were reported by the athlete.

24 hours before competition the athlete ingested 4032 kcal/16854 kJ, 598 g of carbohydrates (9 g/kg), 167 g of proteins (2.52 g/kg) and 124 g of lipids. BWL was registered by the athlete, measuring body weight immediately before and after each stage using a portable scale (*Model 876 Seca,* United Kingdom).

Blood samples were taken before and after the event. 28 biochemical markers were assessed by Megalab S.A. (*Madrid, Spain*): total, HDL and LDL cholesterol, triglycerides, transaminases (GOT-AST, GPT-ALT), iron, ferritin, urea, ureic nitrogen (BUN), creatin kinase, isoenzyme MB, Na^+, K^+, Cl^-, Ca^{++}, thyroxine, thyroid stimulating hormone, testosterone, cortisol, glucose, vitamin B12, reactive protein C, erythrocytes, hemoglobin, hematocrit, mean corpuscular hemoglobin and lymphocytes.

1 Skinfolds measured following International Society for the Advancement of Kinanthropometry (ISAK) methodology: tricipital, subscapular, chest, axillar, abdominal, thigh, suprailiac, rear thigh.
2 Basal metabolism was calculated based on Harris Benedict formula: 66.5 + (13.75 x weight (kg)) + (5 x height (cm)) – (6.78 x age (years old)).
3 Distance and positive accumulated ascension were modified day-to-day by the race organization due to necessary track modifications.

Statistical analysis

Measurement data are presented as mean ± SD. The software used to analyze the data was Microsoft Excel.

Observations and outcomes

Race-day intakes

Athlete's nutrients and supplements intake, together with its nutritional value, during the six stages are shown in Table 1. Breakfast (three hours before competition) was always the same (Table 2). Immediately before each stage (within the last 20-30 min) one antioxidant capsule and two mineral capsules were ingested, providing 50 mg of coenzyme Q, 40 mg of vitamin C, 20 mg of phosphatidylserine and 5 mg of NADH in order to improve physical performance and reduce oxidative stress associated to exercise (19, 20) and 128 mg of Na^+, 180 mg K^+ and 151 Mg^{++} in order to rebalance the acidified medium and keep cellular homeostasis respectively. During the first two stages, a 100 kcal reposition beverage (protein: 6 g; carbohydrates: 21 g; Na^+: 240 mg) diluted in 500 mL of water was added.

Table 2 shows nutrient and supplement intake during each day of competition (before, during and after each stage). Differences between stages in nutrient, supplements intake and fluid ingestion (water and reposition beverage) were due to specific characteristics of the stage (Table 3). Caffeine ingestion in the first stage was due to the ingestion of a sport gel containing 22.5 mg of anhydride caffeine and 31.5 g of carbohydrates. The reposition beverage provided a mix of different high glycemic index carbohydrates (glucose, maltodextrin, sucrose: 21 g/30 g), Na^+ (240 mg/30 g), Mg^{++} (151 mg/30 g) and branched chain amino acids (BCAAs) (6 g/30 g). Complementarily, aiming to optimize sodium intake, salt capsules were used which provided 430 mg of Na^+, 5.04 mg of Ca^{++} and 10.09 mg Mg^{++}.

During stage four, nutrient and supplement intake was markedly lower than in the others due to an orientation mistake (see table 2) that focused the athlete on finding the correct path to the finish line.

Table 1. Athlete nutritional intake during each stage

Stage	Intake	Energy (kcal/kJ)	CH (g)	P (g)	F (g)	Na^+ (mg)	Caf (mg)	Antioxidants (mg)
One	45 g reposition beverage in 700 ml of water, 1x sport bar, 150g banana, 5x salt capsule, 1x sport gel, 1.5 L water, 1x antioxidant capsule	717/2996	165.98	12.90	1.07	1442	22.5	50mg Coenzyme Q, 40mg vitamin C, 200mg phosphatidylserine, 5mg NADH
Two	30 g reposition beverage in 500 ml of water, 2x sport bar, 4x salt capsule, 1.5 L water, 1x antioxidant capsule	390/1630	87.83	7.15	0.27	1109	-	50mg Coenzyme Q, 40mg vitamin C, 200mg phosphatidylserine, 5mg NADH
Three	60g reposition beverage in 1000 ml of water, 2x sport bar, 150 g banana, 20 g raisins, 8x salt capsule, 4 L water, 1x antioxidant capsule	689/2082	154.03	15.50	0.90	2216	-	50mg Coenzyme Q, 40mg vitamin C, 200mg phosphatidylserine, 5mg NADH
Four	60g reposition beverage in 1000 ml of water, 75g banana, 8x salt capsule, 4 L water, 1x antioxidant capsule	297/1240	60.23	13.02	0.51	2201	-	50mg Coenzyme Q, 40mg vitamin C, 200mg phosphatidylserine, 5mg NADH
Five	60g reposition beverage in 1000 ml of water, 2x sport bar, 62.5 g sweet potato and honey, 8x salt capsule, 3 L water, 1x antioxidant capsule	587/2452	106.41	19.28	7.92	2228	-	50mg Coenzyme Q, 40mg vitamin C, 200mg phosphatidylserine, 5mg NADH
Six	30g reposition beverage in 500 ml of water, 2x sport bar, 62.5 g sweet potato and honey, 8x salt capsule, 2 L water, 1x antioxidant capsule	613/2562	118	13.82	7.95	1993.6	-	50mg Coenzyme Q, 40mg vitamin C, 200mg phosphatidylserine, 5mg NADH

Note. CH = Carbohydrates; F = Fat; P = Proteins; Caf = Caffeine.

Table 2. Athlete nutritional intake before, during and after competition

Timing	Energy & Macronutrient	Stage number						Mean ± SD
		One	Two	Three	Four	Five	Six	
Breakfast	E (kcal/kJ)	1078/4506	1078/4506	1078/4506	1078/4506	1078/4506	1078/4506	1078 ± 0/4506 ± 0
	CH (g)	164.5	164.5	164.5	164.5	164.5	164.5	164.5 ± 0.0
	P (g)	50.3	50.3	50.3	50.3	50.3	50.3	50.3 ± 0.0
	F (g)	25.2	25.2	25.2	25.2	25.2	25.2	25.2 ± 0.0
During	E (kcal/kJ)	717/2996	390/1630	690/2882	297/1241	587/2453	613/2562	549 ± 169/2294 ± 705
	CH (g)	166.0	87.8	154.0	60.2	106.4	118	115.4 ± 39.9
	P (g)	12.9	7.2	15.5	13.0	19.3	13.8	13.6 ± 3.9
	F (g)	1.1	0.3	0.9	0.5	7.9	8.0	3.1 ± 3.8
	Na^+ (mg)	1442	1109	2216	2201	2228	1994	1865 ± 460
	Caffeine (mg)	22.5	0.0	0.0	0.0	0.0	0.0	3.8 ± 9.2
	CoenzQ (mg)	50	50	50	50	50	50	50 ± 0
	Vitamine C (mg)	40	40	40	40	40	40	40 ± 0
	PS (mg)	200	200	200	200	200	200	200 ± 0
	NADH (mg)	5	5	5	5	5	5	5 ± 0
Post-Stage[a]	E (kcal/kJ)	213/966	671/2805	477/1994	213/890	282/1179	213/890	348 ± 187/1454 ± 783
	CH (g)	32.9	88.6	98.2	32.9	50.8	32.9	56.1 ± 29.9
	P (g)	20.4	39.9	21.7	20.4	22.4	20.4	24.2 ± 7.7
	F (g)	0.3	12.2	0.5	0.3	0.9	0.4	2.4 ± 4.8
Lunch	E (kcal/kJ)	1050/4389	760/3177	675/2822	1045/4368	1145/ 4786	908/3795	931 ± 183/3890 ± 767
	CH (g)	108.5	68.7	63.9	216.2	178.7	160.3	132.7 ± 62.1
	P (g)	56.9	52.3	37.2	32.3	32.0	55.7	44.4 ± 11.8
	F (g)	46.3	33.5	31.4	11.6	38.7	10.3	28.6 ± 14.6
Snacks	E (kcal/kJ)	720/3010	663/2771	623/2604	0	0	0	334 ± 367/1398 ± 1536
	CH (g)	77.7	99.4	68.1	0.0	0.0	0.0	40.9 ± 45.9
	P (g)	18.4	14.9	35.5	0.0	0.0	0.0	11.5 ± 14.4
	F (g)	36.3	23.6	25.1	0.0	0.0	0.0	14.2 ± 16.1
Dinner	E (kcal/kJ)	821/3432	578/2416	510/2132	822/3436	669/2796	1247/5212	775 ± 264/3237 ± 1102
	CH (g)	113.5	94.4	68.3	97.3	18.9	23.3	69.3 ± 40.0
	P (g)	68.7	9.5	22.4	43.4	45.7	128.3	53.0 ± 42.2
	F (g)	13.4	20.8	18.2	31.5	45.2	72.0	33.6 ± 22.0

Note. PS = phosphatidylserine; CH = carbohydrates; E = energy; F = fat; P = proteins; CoenzQ = coenzyme Q

[a] *Energy intake immediately after each stage.*

Immediately after crossing the finish line, the athlete ingested two salt capsules plus 15 g of glutamine and 10 g of BCAA's. This was completed with food and liquid provided by the race organization. Specific nutritional value of the post-race nutrient intake is described in Table 2 along with nutritional information of each day lunch (after finishing competition), afternoon snacks (only completed in the first three stages) and dinner. Total daily nutritional intake by the athlete is summarized in Table 3 for each day of competition (breakfast, before-race, during-race, post-race, afternoon snacks and dinner). The greatest ingestion of proteins was the last day of competition, explained by the final celebration dinner.

Table 3. Total nutritional energy and macronutrients intake for each competition day

Energy & Macronutrient	Competition day					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
E (kcal)	4617	4140	4053	3455	3761	4059
E (kJ)	19298	17305	16940	14441	15720	16966
CH (g)	663.1	603.4	617.0	571.0	519.3	499.0
CH (g/kg/day)	10.0	9.1	9.3	8.6	7.8	7.5
P (g)	227.6	147.1	182.6	159.4	169.7	268.5
P (g/kg/day)	3.4	2.6	2.8	2.4	2.6	4.1
F (g)	122.6	115.6	101.3	69.1	117.9	115.9

Note. CH = Carbohydrates; E = Energy; F = Fat; P = Proteins;

Hydration and weight loss

The athlete began the competition with a body weight of 66.2 kg and finished it with 64.9 kg. After the conclusion of each stage, a reduction in body weight was observed (Table 4). The BWL average during "Pirineos FIT" was 2.4%.

Total fluid ingestion during the stages was 2000-5000 mL provided by the reposition beverage and water and was accompanied by a Na^+ intake of 1109 – 2228 mg provided by the reposition beverage and the salt capsules (Table 4). Carbohydrate ingestion during the stages with food and supplements ranged between 1.20 and 7.74 g/100 mL and 9.27–40.65 g/h, with the minimum ingestion in the fourth stage. Details of meteorological conditions, weight loss and evolution, fluid ingestion, Na^+ and carbohydrates are provided in Table 4.

Effort perception and athlete's performance

Borg's Scale of Perceive Exertion (RPE) (21) was used to report the subjective effort perception of the athlete during each stage (Table 4). Mean heart rate

Table 4. Meteorological conditions, carbohydrate, fluid, sodium and weight control during the six competition stages

		Stage number					
		One	Two	Three	Four	Five	Six
Meteorology	T_{min} (ºC)	6.0	12.0	10.0	16.7	16.1	14.9
	T_{max} (ºC)	24	28	22	30.5	33.2	28.7
	Humidity (%)	98	94	71	77	89	98
	$Wind_{max}$ (km/h)	24	25	29	40	41	44
Weight	Weight $_{PRE}$ (kg) [a]	66.2	66.6	66.5	66.8	66.7	67.3
	Weight $_{POST}$ (kg) [b]	64.5	65.6	64.5	65.0	66.0	64.9
	BWL (kg)	1.7	1.0	2.0	1.8	0.7	2.4
	BWL (%)	2.6	1.5	3.0	2.7	1.0	3.6
Nutrients intake	Fluid intake (mL)	2200	2000	5000	5000	4000	2500
	Fluid/ hour (mL/h)	538.8	732.6	819.7	769.2	808.1	536.8
	CH (g/100 mL)	7.6	4.4	3.1	1.2	2.7	4.7
	CH intake (g/h)	40.7	32.1	25.3	9.3	21.5	25.3
	Na^+ (mg)	1442	1109	2216	2201	2228	1994
Performance	Stage result	6th	6th	7th	6th	4th	8th
	Race time (h:min)	4:05	2:44	6:06	6:30	4:57	4:40
	Distance (km) [c]	29.9	24.3	41	35	35	40
	m+	2000	1500	2600	2700	2874	1850
	RPE	13	11	15	11	17	20
	Mean HR (bpm)	150	148	141	131	142	138
	Max HR (bpm)	170	175	180	172	182	183
	Time Phase I (%)	3	23	34	74	44	68
	Time Phase II (%)	94	75	66	24	56	30
	Time Phase II (%)	3	0	0	1	0	2

Note. CH = Carbohydrates; T = temperature; $Wind_{max}$ = maximum registered wind; m+ = accumulated positive ascension; BWL = body weight loss; RPE = Borg's Scale of Perceived Exertion score. [a] Weight before each stage; [b] Weight after each stage; [c] Real distance completed by the athlete during the stages. Differences with the official distance is due to day-to-day track changes by the race organization.

(HR), maximum HR, RPE and final stage position are also described in Table 4. In the last km of stage one, the athlete suffered from light cramps and RPE was 13 ("somewhat hard"). For Stage two and four RPE score was 11 ("moderate"). Stage three was perceived with a RPE of 15 ("hard") but he had great feelings, being able to maintain pace during the stage and complete nutrient intake. Stage five was reported as the day with the best feelings, finishing in a 4th position despite a RPE of 17 ("very hard)". The athlete reported good feelings during the first km of the last stage, but the final RPE was 20 ("extenuating"), forcing him to slow down the pace.

Biochemical, hematological and hormonal parameters

Comparing blood analysis before and after the competition showed an increase in GOT-AST (71.86 %), GPT-ALT (117.86 %), iron (56.30 %), ferritin (55 %), creatine kinase (83.57 %), total testosterone (40.49 %) and reactive protein C (40 %). A reduction was observed in urea concentration (12.79 %) and BUN (12.39 %). No modifications were observed in the rest of the parameters (total cholesterol, HDL, LDL, triglycerides, thyroid hormones, cortisol, glucose, white blood cells and red blood cells).

Discussion

The aim of this study was to assess several nutrition and hydration strategies applied to an athlete that participated in an endurance trail-running race that finally led to a successfully implementation and a good competition performance.

Literature has shown a positive relationship between dietetic recommendations and sport performance (22). Adequate energetic intake improves endurance, strength and FFM (Fat-Free Mass); while uncontrolled caloric restriction depletes glycogen, critical for training and physical exercise (23). Many factors must be considered for an adequate nutritional and hydration plan: athlete's characteristics, equipment to be carried, race modality and details, environmental characteristics and the solid and liquid intake possibilities.

"Pirineos FIT" represents a nutritional and sport challenge. Therefore, it is crucial to minimize the total food carriage and provide the optimal energy and macronutrients intake in the competition. The athlete arrived to the competition well prepared, both from the nutritional and physical point of view, being completed an adequate diet and training program designed by professionals. The foods and supplements that were chosen to carry out the dietary-nutritional planning were those that the athlete usually takes and therefore is familiar to them, in order to avoid gastrointestinal problems. For the nutritional planning, energetic and nutritional recommendations were applied following specific evidence for endurance sports (24). It is worth mentioning that there were some differences between dietary carbohydrate prescription (30-60 g/h), based on literature recommendations (15-17), and the carbohydrate quantity ingested by the athlete during the race. Even the athlete accomplished the minimum carbohydrate recommended intake, he wasn't able to increase it due to the effort of self-management of food intake and race orienting. Despite that, the athlete's perceptions were always optimal and didn't prevent him of accomplish a good final position. Furthermore, no hunger or cramps were reported by the athlete, and the feelings were described as "better than in previous races".

Maybe, subjective perception of fatigue of the athlete was reduced by the ingestion of antioxidants due to the athlete's decision of avoiding caffeine intake after the first stage. As it's well documented, long duration and high intensity exercise increases the participation of oxidative metabolism, consequently increasing reactive oxidative species and oxidative stress (25). Regarding hydration, the athlete exceeded the recommendations (400-600 mL/h) in the race, avoiding dehydration with a minimal BWL except in the last stage (26,27).

Blood analysis assessment before and after competition showed habitual physiological responses to high demanding endurance efforts. Increased transaminases, testosterone, iron, ferritin and protein C are normal physiological responses after exercise (28). In fact, trained subjects tend to have significantly higher concentrations of transaminases than sedentary people (29, 30). Moreover, a high level of testosterone is a marker for an adequate physical load and long-term adaptation to exercise (28), while the increase in protein C reactive indicated an increase of physical stress

during the race (31). Additionally, several studies have shown that after a moderate or intense exercise, an increase in serum iron and ferritin is observed (32). However, reduction in urea and BUN levels demonstrated that there wasn't FFM loss (33).

The good response in biochemical parameters, without no changes after the competition, the good final position of the athlete in the race (always within the first ten) and the good feelings of the athlete could be attributed (at least in part) to the appropriate dietetic-nutritional planning carried out in this study, as well as to the good body composition of the athlete, an adequate training load and an optimal age. This study adds complementary information to the existing literature (34, 35) regarding the positive influence of adequate dietary, nutritional, supplementation and hydration planning in trail-running. In addition, the dietary-nutritional strategy applied in this study can serve as a guideline for other professionals preparing ultra-endurance athletes to achieve optimal performance.

Conclusions

The application of nutrition and hydration strategies are crucial for successful performance at "Pirineos FIT", where it is necessary to reach a balance between the amount of food (weight) that the athlete has to carry and optimizing the intake of nutrients and drink. This study demonstrates the importance of an adequate dietary-nutritional planning to successfully compete in a race like "Pirineos FIT". This case study can be a useful tool for other dietitians-nutritionists who advise athletes disputing ultra-endurance trail-running events.

Acknowledgments

We wish to thank the athlete described in this case study, who provided consent and all the data for this publication.

References

1. Hoffman MD, Ong JC, Wang G. Historical Analysis of Participation in 161 km Ultramarathons in North America. Eur Sport Hist Rev. 2010;27(11):1877-1891. doi:10.1080/09523367.2010.494385.
2. Knechtle B, Abou Shoak, Rüst, Lepers R, Rosemann T. European dominance in multistage ultramarathons: an analysis of finisher rate and performance trends from 1992 to 2010. Open Access J Sports Med. 2013;4:9-18. doi:10.2147/OAJSM.S39619.
3. Knoth C, Knechtle B, Rüst CA, Rosemann T, Lepers R. Participation and performance trends in multistage ultramarathons—the "Marathon des Sables" 2003–2012. extrem physiol med. 2012;1(1):13. doi:10.1186/2046-7648-1-13.
4. Ramos-Campo DJ, Ávila-Gandía V, Alacid F, et al. Muscle damage, physiological changes, and energy balance in ultra-endurance mountain-event athletes. Appl Physiol Nutr Metab. 2016;41(8):872-878.
5. Chlíbková D. Pre- and Post-Race Hydration Status in Hyponatremic and Non-Hyponatremic Ultra-Endurance Athletes. Chin J Physiol. 2016;59(3):173-183. doi:10.4077/CJP.2016.BAE391.
6. Lane AM, Wilson M. Emotions and trait emotional intelligence among ultra-endurance runners. J Sci Med Sport. 2011;14(4):358-362. doi:10.1016/j.jsams.2011.03.001.
7. López-Gómez JA, Martínez-Sanz JM, Martínez-Rodríguez A, Ortiz-Moncada R. Planificación dietético-nutricional para llevar a cabo una Ultramaratón, la Transvulcania: Informe de caso. Rev Esp Nutr Hum Diet. 2016;20(2):120-127. doi:10.14306/renhyd.20.2.205.
8. Singh NR, Peters EM. Markers of Hydration Status in a 3-Day Trail Running Event. Clin J Sport Med. 2013;23(5):354-364. doi:10.1097/jsm.0b013e318286c2c3.
9. Singh NR, Denissen EC, McKune AJ, Peters EM. Intestinal temperature, heart rate, and hydration status in multiday trail runners. Clin J Sport Med. 2012;22(4):311-318. doi:10.1097/JSM.0b013e318248e27f.
10. Pfeiffer B, Stellingwerff T, Hodgson AB, et al. Nutritional intake and gastrointestinal problems during competitive endurance events. Med Sci Sports Exerc. 2012;44(2):344-351. doi:10.1249/MSS.0b013e31822dc809.
11. Williamson E. Nutritional implications for ultra-endurance walking and running events. Extrem Physiol Med. 2016;5(1):200.
12. Jeukendrup AE. Nutrition for endurance sports: marathon, triathlon, and road cycling. J Sports Sci. 2011;29 Suppl 1(sup1):S91-S99. doi:10.1080/02640414.2011.610348.
13. Peters EM. Nutritional aspects in ultra-endurance exercise. Curr Opin Clin Nutr Metab Care. 2003;6(4):427-434. doi:10.1097/01.mco.0000078986.18774.90.
14. Skinner JS, Mclellan TH. The Transition from Aerobic to Anaerobic Metabolism. Res Q Exerc Sport. 1980;51(1):234-248. doi:10.1080/02701367.1980.10609285.
15. Blasco R. Hydroelectrolic replacement in extreme thermal situations. Archivos de medicina del deporte: revista de la Federación Española de Medicina del Deporte y de la Confederación Iberoamericana de Medicina del Deporte. 2012;29(152):991-1003.
16. Burke LM, Hawley JA, Wong SHS, Jeukendrup AE. Carbohydrates for training and competition. J Sports Sci.

2011;29(sup1):S17-S27. doi:10.1080/02640414.2011.585473.
17. Palacios N, Bonafonte L, Manonelles P, Manuz B, Villegas JA. Consensus on drinks for the sportsman. Composition and guidelines of replacement of liquids. Document of consensus of the Spanish Federation of …. 2008;25. doi:10.1093/jama/9780195176339.021.15.
18. Larson-Meyer DE, Woolf K, Burke L. Assessment of Nutrient Status in Athletes and the Need for Supplementation. Int J Sport Nutr Exerc Metab. 2018;28(2):139-158. doi:10.1123/ijsnem.2017-0338.
19. Pingitore A, Lima GPP, Mastorci F, Quinones A, Iervasi G, Vassalle C. Exercise and oxidative stress: potential effects of antioxidant dietary strategies in sports. Nutrition (Burbank, Los Angeles County, Calif). 2015;31(7-8):916-922. doi:10.1016/j.nut.2015.02.005.
20. Kawamura T, Muraoka I. Exercise-Induced Oxidative Stress and the Effects of Antioxidant Intake from a Physiological Viewpoint. Antioxidants. 2018;7(9):119. doi:10.3390/antiox7090119.
21. Borg G. Perceived exertion as an indicator of somatic stress. Scand J Rehabil Med. 1970;2(2):92-98.
22. Thomas DT, Erdman KA, Burke LM. Position of the Academy of Nutrition and Dietetics, Dietitians of Canada, and the American College of Sports Medicine: Nutrition and Athletic Performance. J Acad Nutr Diet. 2016;116(3):501-528. doi:10.1016/j.jand.2015.12.006.
23. Kerksick CM, Kulovitz M. Requirements of Energy, Carbohydrates, Proteins and Fats for Athletes. In: Nutrition and Enhanced Sports Performance. Elsevier; 2013:355-366. doi:10.1016/B978-0-12-396454-0.00036-9.
24. Jeukendrup A. A step towards personalized sports nutrition: carbohydrate intake during exercise. Sports Med. 2014;44 Suppl 1(S1):25-33. doi:10.1007/s40279-014-0148-z.
25. Morillas-Ruiz JM, Villegas García JA, López FJ, Vidal-Guevara ML, Zafrilla P. Effects of polyphenolic antioxidants on exercise-induced oxidative stress. Clin Nutr (Edinburgh, Scotland). 2006;25(3):444-453. doi:10.1016/j.clnu.2005.11.007.
26. Goulet ED. Dehydration and endurance performance in competitive athletes. Nutr Rev. 2012;70:S132-S136. doi:10.1111/j.1753-4887.2012.00530.x.
27. Goulet EDB. Effect of exercise-induced dehydration on endurance performance: evaluating the impact of exercise protocols on outcomes using a meta-analytic procedure. Br J Sports Med. 2013;47(11):679-686. doi:10.1136/bjsports-2012-090958.
28. Urdampilleta A, López R, Martínez JM, Mielgo J. Basic biochemical, hematological and hormonal parameters for monitoring the health and nutritional status in athletes. Rev Esp Nutr Hum Diet. 2014;18(3):155-171.
29. Barranco T, Tvarijonaviciute A, Tecles F, Carrillo JM, Sánchez-Resalt C, Jimenez-Reyes P, Rubio M, García-Balletbó M, Cerón JJ, Cugat R. Changes in CK, LDH and AST in saliva samples after an intense exercise: a pilot study. J Sports Med Phys Fitness. 2017
30. Romagnoli M, Alis R, Aloe R, Salvagno GL, Basterra J, Pareja-Galeano H, Sanchis-Gomar F, Lippi G. Influence of training and a maximal exercise test in analytical variability of muscular, hepatic, and cardiovascular biochemical variables. Scand J Clin Lab Invest. 2014 Apr;74(3):192-8.
31. Pournot H, Bieuzen F, Louis J, Mounier R, Fillard JR, Barbiche E, Hausswirth C. Time-course of changes in inflammatory response after whole-body cryotherapy multi exposures following severe exercise. PLoS One. 2011;6(7):e22748.
32. Sim M, Dawson B, Landers G, Swinkels DW, Tjalsma H, Trinder D, Peeling P. Iron regulation in athletes: exploring the menstrual cycle and effects of different exercise modalities on hepcidin production. Int J Sport Nutr Exerc Metab. 2014 Apr;24(2):177-87.
33. Kyung-A Shin, PhD, Ki Deok Park, MD, PhD, Jaeki Ahn, MD, PhD, Yongbum Park, MD, and Young-Joo Kim, PhD. Comparison of Changes in Biochemical Markers for Skeletal Muscles, Hepatic Metabolism, and Renal Function after Three Types of Long-distance Running. Observational Study. Medicine (Baltimore). 2016 May; 95(20): e3657.
34. Knechtle B. Relationship of anthropometric and training characteristics with race performance in endurance and ultra-endurance athletes. Asian journal of sports medicine. 2014;5(2):73-90.
35. Shirreffs SM, Sawka MN. Fluid and electrolyte needs for training, competition, and recovery. J Sports Sci. 2011;29(sup1):S39-S46. doi:10.1080/02640414.2011.614269.

Correspondence:
Eraci Drehmer
Universidad Católica de Valencia "San Vicente Mártir"
Ramiro de Maetzu, 14
46900, Torrent (Valencia)
+ 96 363 74 12 (Extension 4251)
Email: eraci.drehmer@ucv.es