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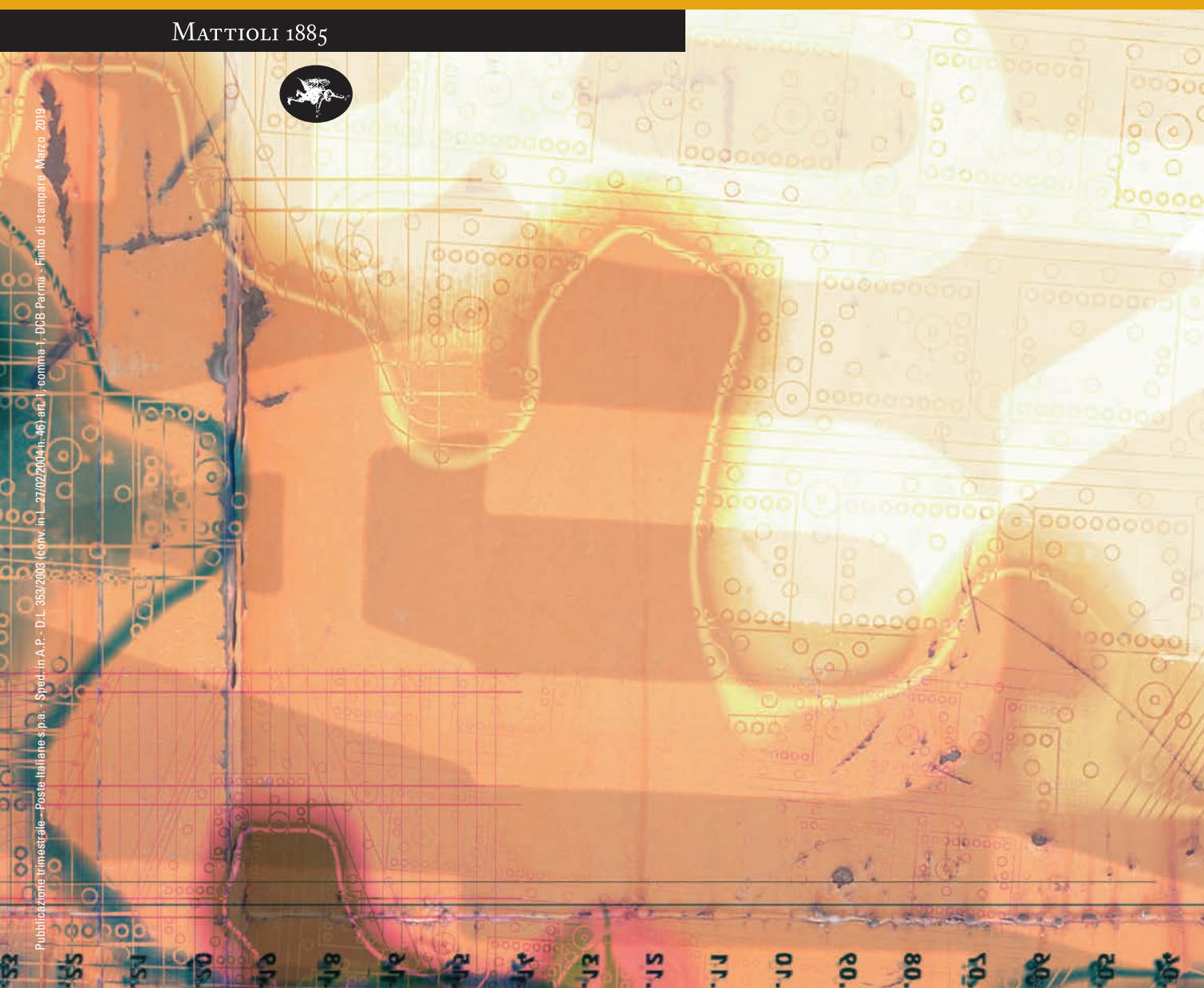

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Original article

The effect of acute L-arginine supplementation on repeated sprint ability performance

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Summary. *Aim*: The aim of this study is to determine the effect of acute L-arginine supplementation on repeated sprint ability performance in football players aged between 18-21 years. *Methods:* The study was conducted on 20 volunteer healthy male football players playing in the under-21 football team in the 1st league of Turkey. General characteristics of football players were questioned and their anthropometric measurements were taken. The study was performed as a double-blind placebo-controlled design. Players were randomly given 0.15 g/kg/day relative dosage L-arginine or placebo with 500 ml of water 1 hour before repeated sprint ability test (RSAT). The 12x20m RSAT protocol was applied in the synthetic turf football field with a recovery interval of 30 seconds between each sprint and the photocell system was used to determine running time. *Results:* The mean age of the arginine group is 18.30±0.48 years and the mean age of placebo group is 18.33±0.50 years. 85% of the players never used L-arginine, and any dietary supplements. Only the ninth sprint time of the 12 sprints performed after the supplementation was 5.24% faster than the placebo group in the arginine group ($p<0.05$). However, no difference was detected between the groups in terms of sprint decrement score, total sprint time, blood pressure and heart rate (HR) ($p>0.05$). *Conclusion:* In this study, the supplementation of acute L-arginine administered to players had no significant effect on HR, blood pressure and RSAT total sprint time and sprint decrement score.

Key words: Acute supplementation, football, L-arginine, repeated sprint ability.

Introduction

Athletes use a variety of ingredients or supplements to minimize the decrease or deterioration in performance due to the fatigue that occurs during exercise (1). L-arginine is an amino acid commonly used by athletes for the claim that it accelerates recovery in intensive exercise. L-arginine, one of the nutritional ergogenic supplements, is a precursor of nitric oxide (NO) and is used by athletes because of its vasodilator effect, accelerated recovery, increased blood flow to muscles, improved mitochondria biogenesis and increasing efficiency by effecting oxidative phosphorylation (2-4).

Although L-arginine, thought to have a positive effect on performance, is widely used by athletes, there are conflicting results showing that it has a sportive performance enhancing effect (5-7) or not (8-11). This study was conducted to examine the acute effect of relative dosage (0.15 g/kg/day) L-arginine supplementation on the performance of repeated sprint ability test (RSAT) of football players aged 18-21 years.

Material and Methods

This study was carried out with 20 healthy volunteer male players playing in U21 category (arginine group: n=10 and placebo group: n=9) of a football team in the 1st Professional Football League in Turkey in 2017-2018 season in Ankara, without any chronic disease, non-smokers, and have 4-6 trainings per week.

A player in the placebo group was left out of the study due to the injury he suffered during RSAT and data obtained from 19 players were assessed. Participants were asked to sign the informed consent form, and Helsinki Declaration was complied with during the study. Clinical Research Ethics Committee of Kirikkale University (No:12/16, 16 May 2017) was approved this study.

Procedure

During the execution phase of the study, football players were met two weeks before the test and all necessary information about the study was given. In this briefing, players were asked to continue their team nutrition and training programs until the morning of the test and not to use any supplements. In addition, they were asked not to consume caffeinated or alcoholic beverages during the last 24 hours before the test and not to perform exercise with high intensity during the last 48 hours. On the test morning, the players were met at the club facilities and their anthropometric measurements were taken before the supplementation. TANITA BC 418 (Japan) scale was used for body weight and TANITA portable stadiometer was used height measurements, and all measurements were made in accordance with the technique (12). Afterwards, heart rate measurements of players were taken with Polar brand short-range radio telemetry (Polar Team, Kempele, Finland). Blood pressure measurements were measured on the right arm twice after resting in sitting position after 10 minutes using Erka (Erka, Germany) brand manual blood pressure device by a nurse and the average of two measurements were taken.

Football players were given L-arginine or placebo orally at a dosage of 0.15 g/kg/day. They are randomly selected from the list numbered in the order of anthropometric measurement and divided into two groups as arginine and placebo groups. The study was conducted as a double-blind placebo-controlled design. L-arginine/placebo was prepared at a dosage of 0.15 g/kg/day by the dietitians to be given to the players and the participant number was labelled with supplementation code. Players were allowed to consume L-arginine/placebo supplementation with 500 ml of water one hour before RSAT. Dietitians who prepared L-arginine and placebo supplementation, did not share the list of supplementation with other researchers until all measurements were completed in order to ensure impartiality of measurements.

A standardized warm-up was made for the football player with oncoming test time, 45 minutes after the L-arginine/placebo supplementation. After self-paced low intensity running, the players were applied a warm-up that includes two sets of active dynamic stretching for the lower extremities (high knees, butt flicks, hamstring swings, carioca and sprint lunge) and three brief sprints. After warm-up, the football players performed a three minutes passive rest. Photocell system (Fusion Sport Smart Speed Photocell, Australia) was used for RSAT measurements. The starting and ending points of the 20 m distance determined for the test were measured and marked by the researcher using a tape measure. Photocells are positioned at both marked points at the waist level. RSAT was applied one hour after the supplementation in the surf synthetic turf football field with 30 seconds of recovery intervals between each sprint (jog run up to the last five seconds). Each sprint was started with a voice command, with a verbal stimulus of five seconds before the completion of the 30-second recovery interval after the sprint, ensuring that the football player is ready at the starting point. In RSAT, 30 second recovery interval between each sprint was followed by a digital stopwatch. After each sprint, the players' sprint times and heart rate (HR) values were recorded in the test data form. Football players did not consume food and beverages throughout RSAT. After finishing the test, the football player actively rested for five minutes and then rested in sitting position with soles of feet in contact with the floor for ten minutes. After fifteen minutes from RSAT, HR and blood pressure measurements were repeated. In order to determine the percentage of performance decrement (%) occurring in RSAT, the sprint decrement score formula was used. To assess the repeated sprint performance of the players in the study, 12x20m RSAT protocol, sprint decrement score and total sprint time comparisons were used (13,14).

Statistical Analysis of Data

Statistical analysis of the data was performed using the SPSS Windows 20 package program. Descriptive statistics are shown as mean± standard deviation

in normal distributed variables and the number (n) and percent (%) in nominal variables. The normal distribution of data was tested using Shapiro-Wilk test because the number of participants was below 50 in both groups. Data in text and figures are presented as mean and 95% confidence interval of the mean. In the data where the distribution in the statistics between the groups was normal, the mean differences were assessed by the independent samples t-test, which is a parametric test and where there is non-normal distribution in the data was assessed by non-parametric Mann-Whitney U-test. The results were considered statistically significant on the level of $p<0.05$.

Results

The players in the study (n=19) are all males and the mean age is 18.30±0.48 years in the arginine group (n=10) and 18.33±0.50 years in the placebo group (n=9). Of the football players; 85% have not used L-arginine before, 70% have no knowledge of L-arginine and have never used any nutritional supplements before. Arginine and placebo groups have similar characteristics in terms of general properties ($p>0.05$) (Table 1).

There was no significant change observed in systolic blood pressure (SBP) and diastolic blood pressure (DBP) values of football players in arginine and placebo groups before supplementation and 15 minutes after the test ($p>0.05$) (Table 2).

When the HR means of the football players arginine and placebo groups during resting, 12 sprint results and 15 minutes after the test were compared, no difference was found between the two groups ($p>0.05$) (Table 3, Figure 1).

When each sprint time for RSAT was compared between arginine and placebo group, there was no significant difference observed ($p>0.05$) between all sprint times (Figure 2), the fastest sprint time, the total sprint time (Figure 3), and the sprint decrement score (Figure 4), except for the 9th sprint.

Difference between RSAT 9th sprint time of arginine and placebo groups (Arginine: 2.941±0.092 sec vs. Placebo: 3.095±0.137 sec; difference: 0.154±0.045 sec) was significant ($p<0.05$). The 9th sprint time difference between the two groups was 5.24% less in favour of the arginine group. In addition, when the slowest sprint times (arginine: 3.015±0.067 sec vs. placebo: 3.172±0.170 sec) during 12 sprints in the arginine and placebo groups were compared, the difference between the two groups was significant ($p<0.05$).

Table 1. General characteristics of football players in Arginine and Placebo groups.

General Characteristics	Arginine Group (n=10)	Placebo Group (n=9)	p
Age (years)	18.30±0.48	18.33±0.50	0.879
Body Weight (kg)	72.04±6.82	75.06±9.42	0.441
Height (cm)	175.30±6.46	179.11±8.82	0.305
BMI (kg/m^2)	23.45±1.94	23.31±1.50	0.863
Body Surface Area (m^2)	1.87±0.11	1.94±0.17	0.343
Training Age (years)	9.40±2.07	10.44±2.35	0.321

Mean ± SD; BMI: Body Mass Index

Table 2. Blood pressure findings of football players in Arginine and Placebo groups before supplementation and 15 minutes after the test.

Blood Pressure	Arginine Group (n=10)	Placebo Group (n=9)	p
Before SBP	106.50±11.56	111.67±15.81	0.433
Before DBP	71.00±9.66	73.33±10.31	0.417
After SBP	105.00±5.27	105.00±7.07	0.820
After DBP	63.00±10.59	66.67±10.00	0.449

Mean ± SD; SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure

Table 3. Heart Rate findings of football players in Arginine and Placebo groups.

Heart Rate (HR)	Arginine Group (n=10)	Placebo Group (n=9)	p
Resting HR (beat/min)	66.10±2.73	65.56±3.88	0.731
12 Sprint Average HR (beat/min)	160.30±9.09	163.00±13.35	0.618
HR 15 minutes later (beat/min)	90.70±8.83	96.44±12.29	0.285

Discussion

This study is among the few studies (11) in the relevant literature evaluating acute effect of L-arginine

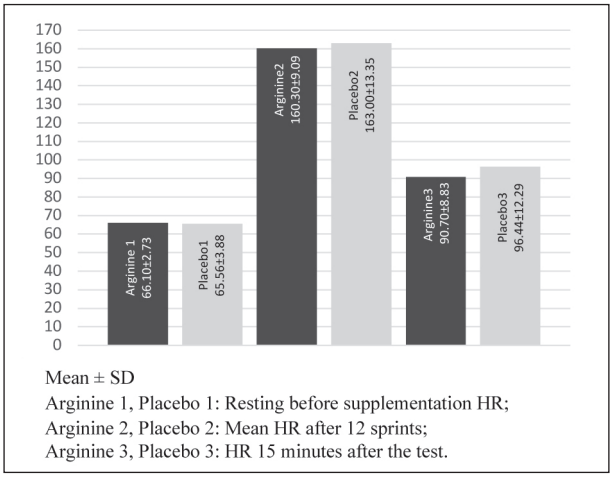

Figure 1. Heart Rate findings of football players in Arginine and Placebo groups.

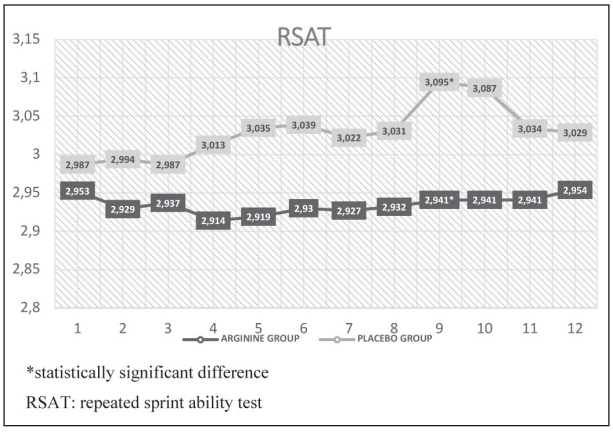

Figure 2. Each sprint times of football players in Arginine and Placebo groups.

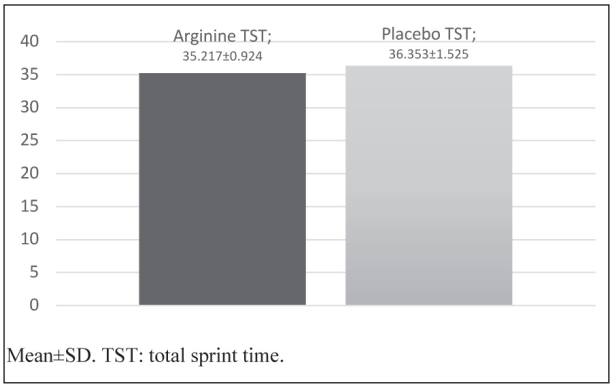

Figure 3. Total Sprint times of the players in Arginine and Placebo group.

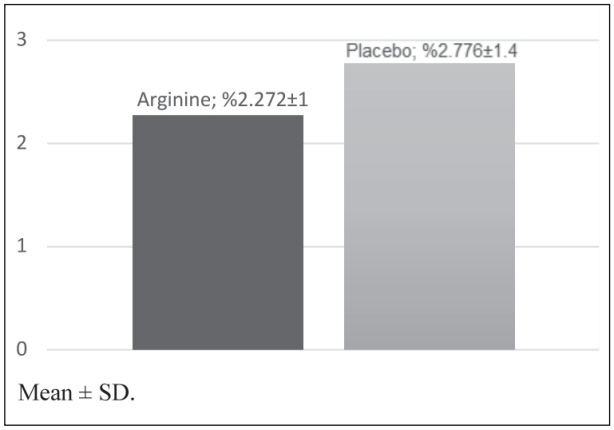

Figure 4. Sprint decrement score of football players in Arginine and Placebo groups.

supplementation on repeated sprint ability performance with a field based RSAT protocol.

When all HR measurements of the arginine and placebo groups were compared, there was no significant difference between the two groups. Similar to the results of this study, in the studies conducted by Yavuz et al.[7] on trained male wrestlers (0.15 g/kg/day L-arginine/ placebo); Sandbakk et al. 2015[10] on trained skiers (6 g L-arginine+614 mg nitrate, 614 mg nitrate or placebo); and Ermolao et al.[11] on trained football players (placebo=carbohydrate 26.7 g, placebo+caffeine 300 mg, placebo+arginine 3 g, placebo+BCCA 5 g and the mixture of all) reported no change in HR among the attempts. Although the dosages of arginine used in the aforementioned studies were different, the results showed that acute supplementation had no effect on HR in trained individuals.

In this study, there was no significant difference between arginine and placebo groups in blood pressure before supplementation and 15 minutes after RSAT, and L-arginine supplementation did not cause significant changes in blood pressure. Similarly, in a study on young elite male skiers, whom were given 6 g L-arginine+614 mg nitrate, 614 mg nitrate or placebo one hour before the test attempt, supplementation had no effect on arterial blood pressure (10). In another study, Yaman et al.[15], in their study conducted on football players playing in the university football team by giving them 6 g of L-arginine or placebo, reported a decrement in arterial blood pressure and an increase in

femoral artery diameter in the L-arginine reinforced group. On the other hand, Bailey et al.[5], in their study conducted on participants exercising recreatively, reported that 6 g L-arginine supplementation increased plasma NO synthase levels and lowered systolic blood pressure. The two tests conducted, seven days apart on trained male participants, indicated that arginine alpha-ketoglutarate (3700 mg) or placebo supplementation did not induce a significant change in blood pressure (16). In both studies (5,15), reporting an effect on blood pressure with arginine supplementation, the participants consisted of individuals who were not on high levels of training. In this study and the other aforementioned two studies (10,16), the reason for no change in blood pressure was due to the participants being trained and low dosages of L-arginine given.

There was no significant difference between arginine and placebo groups in RSAT fastest sprint, each RSAT sprint (except the 9th sprint), total sprint time, sprint decrement score. There was a significant difference between two groups in favour of arginine group in respect of only 9th sprint among 12 sprints during RSAT and the slowest sprint times. However, there was no significant difference in the other parameters (HR, blood pressure, total sprint time and sprint decrement score) between two groups that could support the cause of this difference. In comparison to arginine and placebo groups with regard to RSAT fastest sprint time, total sprint time and sprint decrement score, the supplementation of acute L-arginine in oral dosages of 0.15 g/kg/day had no effect on RSAT performance. Similarly, in the Wingate Anaerobic Test applied study conducted by Olek et al.[9] on moderately-trained healthy participants by administering 2 g single dosage of L-arginine 60 min prior to exercise, there were no changes in the performance improvement. Again, Sales et al.[8], in the study applied a test protocol with incremental intensity in cycling ergometer on trained volunteers, reported that 4.5 g arginine aspartate did not cause a difference in performance and had no effect of improving fatigue tolerance. On the other hand Yavuz et al.[7], in their study conducted on an incremental exercise protocol with male wrestlers in the cycling ergometer by administering a single dosage of 0.15g/kg/day L-arginine supplement, reported that there was no significant change in maximum heart rate, however the duration of fatigue was delayed in the arginine group. On the other hand, Hurst and et al.[6] stated that the use of relative dosage of L-arginine in male cyclists' cycling time-trial performance, and that the use of absolute dosages provided a reduction in the time of completion of the tests and an increase in the mean power output, and that the use of L-arginine in the relative dosage is more effective than the use of absolute dosages. Bailey et al.[5] also reported that the supplementation of 6 g L-arginine delayed fatigue in actively exercising individuals.

In the studies conducted with the trained participants, whether the uses of absolute or relative dosages of L-arginine supplementation had no effect on the development of performance. In the study conducted by Ermolao et al.[11], similar to our current study in terms of groups and test protocols used, the players were subjected to 11x20m RSAT with a recovery period of 20 seconds between each sprint and they were given 3 g arginine with carbohydrates, reported the supplementation did not cause any changes in RSAT performance.

When some studies with supplementation of chronic arginine was examined, the effect on physiological processes or exercise performances seemed different from the results obtained in acute supplementation. In studies in which chronic L-arginine supplementation was administered with different persons and at different dosages; changes were observed in muscle strength and mass (19), VO_2 max and increase in performance (20), increase in muscle strength and peak power (21) and prolongation of neuromuscular fatigue (22).

Studies that indicate that L-arginine supplementation has an effect on endothelial functions or physical performance are usually performed with individuals who are not trained highly or training at recreational level or administered with chronic L-arginine supplementation (23,24). Endothelial functions are associated with vascular health and exercise loads have NO bioactivity regulatory effect (24). In addition, the responses given to exercise loads by individuals with limited endothelial functions are more significant than those given by healthy sedentary/less-trained individuals, and that the metabolic responses shown by trained individuals through the supplementation of L-

arginine emerges more difficultly (according to the increasing training level) (24,25). In addition, the study participants' training levels, selected exercise/test protocol, supplementation dosage and the implementation of the supplementation whether acute or chronic is thought to affect the results of the study.

Conclusion

In this study, in which acute L-arginine supplementation to football players at a relative dosage of 0.15 g/kg/day, while a change was observed on the 9th sprint of 12 sprints in favour of the group administered L-arginine supplementation, no significant effect of supplementation has been observed on HR, blood pressure and RSAT performance. Considering that the football players in this study are trained individuals, the effect of oral administration of acute L-arginine on performance can be attributed to the fact that the metabolic responses in individuals with endothelial functions developed that are expected to occur are more difficult to detect than those with sedentary/recreational training.

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References

1. Vernec A, Stear SJ, Burke LM, Castell LM. A-Z of nutritional supplements: dietary supplements, sports nutrition foods and ergogenic aids for health and performance part 48. Br J Sports Med 2013; 47: 998-1000.
2. Castell LM, Burke LM, Stear SJ. BJSM reviews: A-Z of supplements: dietary supplements, sports nutrition foods and ergogenic aids for health and performance part 2. Br J Sports Med 2009; 43: 807-810.
3. Mason BC, Lavallee ME. Emerging supplements in sports. Sports Health 2012; 4: 142-146.
4. Tengan CH, Rodrigues GS, Godinho RO. Nitric oxide in skeletal muscle: role on mitochondrial biogenesis and function. International Journal of Molecular Sciences 2012; 13: 17160-17184.
5. Bailey SJ, Winyard PG, Vanhatalo A et al. Acute L-arginine supplementation reduces the O2 cost of moderate-intensity exercise and enhances high-intensity exercise tolerance. J Appl Physiol 2010; 109: 1394-1403.
6. Hurst HT, Sinclair JK, Beenham M. Influence of absolute versus relativ L- arginine dosage on 1 km and 16.1 km time trial performance in trained cyclists. Journal of Science and Cycling 2014; 3: 2-8.
7. Yavuz H, Turnagöl H, Demirel A. Pre-exercise arginine supplementation increases time to exhaustion in elite male wrestlers. Biology of Sport 2014; 31: 187-191.
8. Sales RP, Mine CEC, Franco AD et al. Effects of the acute arginine aspartate supplement on the muscular fatigue in trained volunteers. Rev Bras Med Esporte 2005; 11: 315e-318e.
9. Olek RA, Ziemann E, Grzywacz T et al. A single oral intake of arginine does not affect performance during repeated wingate anaerobic test. J Sports Med Phys Fitness 2010; 50: 52-6
10. Sandbakk SB, Sandbakk O, Peacock O et al. Effects of acute supplementation of L-arginine and nitrate on endurance and sprint performance in elite athletes. Nitric Oxide 2015; 48: 10-15.
11. Ermolao A, Zanotto T, Carraro N et al. Repeated sprint ability is not enhanced by caffeine, arginine, and branched-chain amino acids in modaretly trained soccer players. Journal of Exercise Rehabilitation 2017; 13: 56-61.
12. WHO (2008). Training course on child growth assesment WHO Child Growth Standards B Measuring a Child's Growth. Geneva.
13. Spencer M, Bishop D, Dawson, Goodman C. Physiological and metabolic responses of repeated sprint activities. Sports Med 2005; 35: 1025-1044.
14. Glaister M, Howatson G, Pattison JR, McInnes G. The reliability and validity of fatigue measures during multiple-sprint work: an issue revisited. J Strength Cond Res 2008; 22: 1597-1601.
15. Yaman H, Tiryaki-Sönmez G, Gürel K. Effects of oral L-arginine supplementation on vasodilation and VO2max in male soccer players. Biomedical Human Kinetics 2010; 2: 25-29.
16. Greer BK, Jones BT. Acute arginine supplementation fails to improve muscle endurance or affect blood pressure responses to resistance training. Journal of Strength and Conditioning Research 2011; 25: 1789-1794.
17. Forbes SC, Harber V, Bell GJ. The acute effects of L-arginine on hormonal and metabolic responses during submaximal exercise in trained cyclists. International Journal of Sport Nutrition and Exercise Metabolism 2013; 23: 369-377.
18. Liu T-H, Wu C-L, Chiang C-W, Lo Y-W, Tseng H-F, Chang C-K. No effect of short-term arginine supplemen-

tation on nitric oxide production, metabolism and performance in intermittent exercise in athletes. Journal of Nutritional Biochemistry 2009; 20: 462-468.
19. Angeli G, de Barros TL, de Barros DFL, Lima M. Investigation of the effects of oral supplementation of arginine in the increase of muscular strength and mass. Rev Bras Med Esporte 2013; 13: 112e-115e.
20. Pahlavani N, Entezari MH, Nasiri M et al. The effect of L-arginine supplementation on body composition and performance in male athletes: a double-blinded randomized clinical trial. Eur J Clin Nutr 2017; 71: 544-548.
21. Campbell B, Roberts M, Kerksick C et al. Pharmacokinetics, safety and effects on exercies performance of L-arginine alfa-ketoglutarat in trained adult men. Nutrition 2006; 22: 872-881.
22. Camic CL, Housh TJ, Zuniga JM et al. Effects of arginine-based supplements on the physical working capacity at the fatigue threshold. Journal of Strength and Conditioning Research 2010; 24: 1306-1312.
23. Alvares TS, Meirelles CM, Bhambhani YN, Paschoalin VMF, Gomes PSG. L-arginine is a potential ergogenic aid in healthy subjects. Sports Med 2011; 41: 233-248.
24. Bescos R, Sureda A, Tur JA, Pons A. The effect of nitric-oxide- related supplements on human performance. Sports Med 2012; 42: 99-117.
25. Maiorana A, O'Driscoll G, Taylor R, Green D. Execise and the nitric oxide vasodilator system. Sports Med 2003; 33: 1013-1035.

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Original article

Assessment of nutrition status of Turkish elite young male soccer players in the pre-competition period

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Summary. *Background*: Follow up of the nutrition consumption of young soccer players is of great importance not only for their sport performance, but also for the protection of health, physical growth and development. *Aim*: The objective of this study was to evaluate the nutritional sufficiency status of elite young male athletes in the pre-competition period. *Methods*: Three-day food consumption and anthropometric measurements of the elite young athletes in the pre-competition period were recorded for 26 male voluntary athletes from a pro-professional soccer team based in Ankara Turkey. *Results*: The mean height of the athletes were calculated as 175.2±6.8 cm, weight 67.3±5.9 kg, body mass index (BMI) as 21.9±1.3 kg/m^2 and body fat percentage 6.2±1.7. It was determined that the athletes received an average of 3225±692 kcal energy daily whereas their mean energy expenditures per day were 3322±240 kcal. The ratios of energy received from carbohydrate, protein and fats were 53.6%, 16.2%, and 30.2%, respectively. Carbohydrate and protein consumption/day/kg body weight were 6.3±1.7 g and 1.9±0.5 g, respectively. It was determined that vitamin D consumption was inadequate for 92.3% of the athletes and calcium consumption was inadequate for 50% of the athletes. On the other hand, all of the athletes consumed vitamins B_2, B_6 and B_{12} as well as phosphorus, iron and zinc above recommended quantities. *Conclusions*: Nutrient consumption of young athletes at the beginning of their professional life must be monitored by a qualified dietitian and each athlete must have an individual dietary plan. Nutrition education must be arranged and the athletes' families must be included in these trainings.

Key words: Soccer players, male, food consumption, anthropometry

Introduction

Soccer is a sports branch which includes physical performance components such as agility, speed, strength, power, and endurance and has the highest popularity in the world (1). According to FIFA (2006), although soccer is a team game, it is necessary for players to have individual nutrition strategies (2). It is very important to design nutrition plans for the young (adolescent) soccer players because it positively affects their performance parameters, physical growth and development and results in reduced risks of injury (3). The nutritional needs of young soccer players are assessed in a similar manner as adult athletes. However, since growth and development have not yet been completed in younger athletes; they are expected to have greater energy and nutrient requirements (4).

Although studies performed on young soccer players, considered as endurance athletes, reported inadequate energy and nutrient intake (5,6), number of studies conducted in Turkey is rather limited. This study was planned to determine the food consumption and nutritional status of young soccer players (over the age of 15) playing in the soccer team of a pro-professional sports club. Due to the limited number of studies regarding nutritional status of adolescent athletes, especially soccer players in Turkey, it may be considered that the findings of this study will be useful for the sports clubs in Turkey.

Methods

Sample: A total of 26 athletes regularly playing in Ankaragucu pro-professional soccer (PAF) team volunteered to participate in this study. Approval of the Ethics Committee of Clinical Investigations in Ankara was obtained for this study (Decision number 2009/11-107 dated 11.11.2009).

Study Protocol: This study was planned to be conducted over three consecutive days in the pre-competition period. Considering the possibility that food habits might change on weekends or on days when there was no workout, the study was planned so that one of the three days fell on a resting day for the athletes. At the beginning of the study, a questionnaire consisting of questions regarding the demographic and personal information was completed by the participants. Volunteers having other obstacles for participating in the study (physical ailment or incompatibility) were excluded. The same day anthropometric measurements of the athletes were taken and recorded. For evaluation of food consumption status, 3-day food consumption (nutrition intake) records were conducted.

Data Collection Tools: Data collection tools comprised of three-day food consumption record form, weighing scale, stadiometer (height-measuring scale) and calipers.

Three-day food consumption registration form: During the 3 day study, all the foods and beverages consumed by the athletes were recorded in the food consumption registration form.

Weighing scale: The body weights of the athletes were measured with an electronic weighing scale sensitive up to 0.1 kg (Tanita BC 418MA, Tokyo, Japan).

Height-measuring scale: The heights of the athletes were measured with a stadiometer (Seca 220, Germany) sensitive up to 1 cm.

Caliper: The skin fold thicknesses (triceps, biceps, suprailiac, abdomen, midaxillary, pectoral, subscapular, thigh, calf, and suprapatella) of the athletes were measured with a caliper (Holtain, UK).

Collection of Data / Processing Method
Anthropometric Measurements

The skin fold thicknesses (triceps, biceps, suprailiac, abdomen, midaxiller, pectoral, subscapular, thigh, calf and suprapatella) were measured with the help of a caliper to calculate body fat percentages of the athletes. Each measurement was repeated three times and averages were taken (7-9). The weight and height scale were used to collected weight and height measurement. The weight, height, and BMI of the athletes were compared with age appropriate reference values (10).

The body fat percentage calculation= ((4.95/ body density) – 4.5) × 100

Body density = 1.112 – (0.00043499 x sum of skinfolds) + (0.00000055 x square of the sum of skinfold sites) – (0.00028826 x age)

Food Consumption and Energy Expenditure Measurements

Nutrition intakes of the athletes were monitored by the researcher for 3 days by means of the food consumption recording form. The daily energy expenditures of the athletes were calculated by Harris-Benedict equation and Physical Activity Level (PAL) values (10,11).

Analysis of Data

Three Day Food Consumption

The venue of meal consumption by the participants (home or outside) was determined from the questionnaire. In order to determine the amount of nutrients present in each portion of the meal consumed by the participants eating at home, exact amounts of all ingredients used for cooking the meal was recorded. The "Food and Nutrition Photo Catalogue: Measurements and Quantities" reference book was used to determine the amount of nutrients consumed at meals (12). For determination of the amount of nutrients included in each portion of the meal consumed by the participants eating outside, a reference book titled "Mass Nutrition Standard Cookbook for Established Institutions" was used (13). Data were entered into the Nutrition Information System (BEBIS-5) program and the average energy and nutrient intakes of the athletes for 3 days were calculated. Daily dietary energy and nutrient consumption sufficiency status of the athletes were compared with Dietary Guidelines For Turkey (2004) indicating the recommended amounts according to age and gender (14). Nutrient intake was clas-

sified as inadequate, adequate, and excessive intake. Food intake is sufficient if it meets 67% of the recommended amount, and if it is more than 33% it is defined as excessive intake. During the study, nutritional support and supplements usage of the athletes were questioned. However, since none of the participants used any nutritional supplements as per records, these products were excluded from the data.

Statistical Analysis

SPSS (Statistical Package for the Social Sciences) package program (version 15) was utilized for statistical analyses of the obtained data from the questionnaire which comprised of questions regarding demographic characteristics and the body composition of the experimental population, their nutrient and fluid intake as well as use of nutritional support products. Means ($\bar{X}$)and standard deviations (SD) were calculated where applicable.

Results

The average age of the participants was 16±1.2 years. The athletes had been playing soccer for 7 years on average and had been taking soccer training 6±0.6 times a week, 110±14.6 minutes on average. None of the athletes were diagnosed with any diseases as per examination by a physician. No medications were being used by the athletes.

Anthropometry

The average height was 175.2±6.8 cm, the average body weight was 67.3±5.9 kg and average body mass index (BMI) was 21.9±1.3 kg/m^2. The weight, height, and BMI of the athletes were found to be within the required range according to their age. Total skin fold thicknesses were measured as 51.6±9.8 mm and average body fat percentage was calculated as 6.2±1.7% according to skin fold thickness measurements (Table 1).

Food consumptions

The mean and standard deviation values of three-day energy and macro nutrient intakes of the athletes are given in Tables 2. It was observed that the athletes receive an average of 3225±692 kcal energy per day and the energy expenditures were 3322±240 kcal. The ratios of energy received from carbohydrate, protein, and fat were 53.6%, 16.2%, and 30.2%, respectively. The carbohydrate and protein consumption per weight of the athletes were 6.3±1.7 g/kg/day and 1.9±0.5 g/kg/day, respectively.

Table 1. Mean ($\bar{X}$) and standard deviation (SD) values of anthropometric measurements of athletes

Anthropometric measurements	±SD
Height (cm)	175.2±6.8
Weight (kg)	67.3±5.9
BMI (kg/m^2)	21.9±1.3
Total skin fold thickness (mm)	51.6±9.8
Body fat percentage (%)	6.2±1.7

The mean ($\bar{X}$) and standard deviation (SD) of the daily dietary micronutrient intake values of the athletes are shown in Table 3.

Table 4 shows the nutritional sufficiency status of athletes in terms of micronutrient intakes in comparison to Dietary Guidelines For Turkey (2004) (14). It was determined that vitamin D consumption in 92.3% of the athletes and calcium consumption in 50% of the athletes were insufficient. All of the players consumed

Table 2. The average ($\bar{X}$) and standard deviation (SD) values of daily energy and macro nutrient intake values of the athletes

Energy and nutrient	±SD
Energy intake (kcal)	3225±692
Energy consumption (kcal)	3322±240
Carbohydrate (g)	418.2±90.3
Carbohydrate (g/kg/day)	6.3±1.7
Carbohydrate (%)*	53.6±6.5
Protein (g)	128.8±33.3
Protein (g/kg/day)	1.9±0.5
Protein (%)*	16.2±2.1
Total fat (g)	111.1±41.0
Total fat (%)*	30.2±6.4
Saturated fatty acids (%)*	14.1±4.1
Monounsaturated fatty acids (%)*	10.4±2.1
Polyunsaturated fatty acids (%)*	5.7±1.8
Cholesterol (mg)	397.0±168.5
Fiber (g)	25.6±7.0

* Daily received energy ratios

Table 3. Mean ($\bar{X}$) and standard deviation (SD) values of the micro-nutrient consumption values of the athletes

Vitamins / Minerals	±SD
A vitamin (µg RE)	1228.3±485.6
D vitamin (µg)	2.2±3.2
E vitamin (mg)	16.3±6.9
K vitamin (µg)	318.2±82.2
B_1 vitamin (mg)	1.3±0.3
B_2 vitamin (mg)	2.0±0.7
Niacin (mg)	21.9±7.6
B_6 vitamin (mg)	2.0±0.6
Folate (µg)	375.4±91.4
B_{12} vitamin (µg)	5.7±2.4
C vitamin (mg)	97.6±43.9
Calcium (mg)	1009.0±555.4
Magnesium (mg)	383.3±103.9
Phosphorus (mg)	1877.0±511.4
Iron (mg)	16.6±3.9
Zinc (mg)	17.6±4.6
Copper (mg)	2.5±0.7

Table 4. Assessment of sufficiency status of micro-nutrient consumption of the athletes according to Turkey-specific dietary guidelines

Micro-nutrient	**Insufficient**	**Sufficient**	**Over**
	n (%)	**n (%)**	**n (%)**
A vitamin (µg RE)	1(3.8)	14(53.8)	11(42.3)
D vitamin (µg)	24(92.3)	-	2(7.7)
E vitamin (mg)	3(11.5)	17(65.4)	6(23.1)
K vitamin (µg)	-	-	26(100)
B_1 vitamin (mg)	2(7.7)	19(73.1)	5(19.2)
B_2 vitamin (mg)	-	9(34.6)	17(65.4)
Niacin (mg)	1(3.8)	12(46.2)	13(50)
B_6 vitamin (mg)	-	10(38.5)	16(61.5)
Folate (µg)	2(7.7)	22(84.6)	2(7.7)
B_{12} vitamin (µg)	-	2(7.7)	24(92.3)
C vitamin (mg)	4(15.4)	12(46.2)	10(38.4)
Calcium (mg)	13(50)	12(46.2)	1(3.8)
Magnesium (mg)	2(7.7)	20(76.9)	4(15.4)
Phosphorus (mg)	-	9(34.6)	17(65.4)
Iron (mg)	-	8(30.8)	18(69.2)
Zinc (mg)	-	6(23.1)	20(76.9)
Copper (mg)	26(100)	-	-

vitamin B_2, vitamin B_6, vitamin B_{12}, phosphorus, iron, and zinc above the recommended quantities.

Discussion

Sufficient and balanced nutrition of the athletes is important for continuity of health, reduction of injuries, and sports performance. In particular, adequate and balanced nutrition of adolescent athletes, whose growth and development is not complete, is of great importance (4). However, the number of studies evaluating the nutritional status of athletes in the adolescence is very limited in the literature. This study which is planned and carried out in order to monitor nutrition and anthropometric measurement in elite male young athletes, exhibited inadequate nutritional status in terms of energy and some micro-nutrients as calcium and vitamin D. Considering that these group of athletes who will step into professionalism and carry the health and nutrition behaviors in their professional life, proper guidance and training in this area is of special significance.

Anthropometry

Soccer players show more homogeneous distribution in terms of body structure than other sport branches and are generally expected to be 178-183 cm in height and 68-78 kg in weight (15). Iglesias et al. (2008) conducted a study of 22 male soccer players aged 14-16 years. The athletes had a height of 178±0.9 cm, a weight of 62.8±8.7 kg and a BMI of 20.0±1.8 kg/m^2 (16). In this study, the height of the athletes was determined as 175.2±6.8 cm, weight 67.3±5.9 kg and BMI value 21.9±1.3 kg/m^2. The weight, height and BMI of the athletes were found to be in the required range according to their age.

The skin fold thicknesses obtained with the help of caliper from different regions of the body were used to evaluate the body composition of the athletes. It has been suggested that the sum of 7 skin fold thicknesses (abdominal, biceps, thigh, calf, subscapular, supraspinal and triceps) determined by International Society For Advances in Kinanthropometry (ISAK, 2009) to be between 30-60 mm in male athletes. In the study done by Iglesias et al. (2008), the sum of skin fold

thickness was found to be at 49.3±9 mm (16). In this study, the sum of the skin fold thickness of the athletes was found to be at 51.6±9.8 mm. Although, the athletes' skinfold thickness measurements were in the normal range, it was close to the upper accepted limit of 60 mm.

Body fat percentages of adolescent athletes were reported to be lower than those of non-athletes (17, 18). Rico-Sanz et al. (1998) found that body fat percentage of athletes with a mean age of 17.0±2.0 years was approximately 7.6±1.1% (19). In the study done by Iglesias et al. (2008), the body fat percentage was found to be at 9.0±1.6% (16). In this study, body fat percentage was calculated as 6.2±1.7% according to skin fold thickness measurements. The results of the measurements regarding the skin fold thickness of the athletes were found to be within the acceptable values according to the age group and the sports branch of the athletes.

Food Consumption / Nutrition Intake

Irrespective of their sports activity status, factors such as being in the adolescence phase, social circle, personal taste, body image etc. affect young people's food choices (20-22). Young athletes need special attention for the protection and development of their nutritional status. Studies have shown that athletes fail to meet their energy needs in general. Furthermore, looking at the nutrient distribution of energy especially in endurance athletes as soccer players, percentage of energy received from carbohydrates are unable to meet the daily nutritional recommendations (5,6).

A study of the nutritional status of adolescent athletes in Brazil found that the amount of protein consumed by athletes was above the ACSM (American College Sports Medicine) recommendation and that carbohydrate consumption was inadequate for female athletes (6).

In a study done by Garcin et al. (2009) on 26 runners, 12 sprinter and 25 handball players, it was determined that the athletes' energy intake was 452±456 kcal lower than their energy expenditure and that the athletes could not meet their daily energy needs (5). In this study, it was seen that athletes consumed an average of 3224.9±691.7 kcal energy and spent 3322±240.4 kcal energy per day. It was determined that only 42.3% (11 people) met the daily energy expenditure. It was found that the difference between energy expenditure and intake was 586.6±401.6 kcal on average for athletes who could not meet their energy expenditure. In the sports center where the research was conducted, the absence of a dietitian who could plan the menus of the athletes and could provide nutrition education, could be the cause of inadequate diet and healthy nutritional practices among the athletes. There is a need for more training and information about energy and carbohydrate intake especially in this group of young endurance athletes.

Exercise performance in endurance sports, such as soccer, depends on the maintenance of blood glucose during exercise and muscle glycogen renewal after exercise, and high carbohydrate diet is recommended due to the long exercise periods. According to ACSM (2016) (11); a high carbohydrate diet, of 6-10 g/kg/day per weight is recommended. A study evaluating the nutritional status of soccer players in different age groups (14, 15, 16.6, 20.9 years) showed that the athletes› nutrient consumptions were lower than recommended levels and in particular the carbohydrate consumptions (44.6%) were inadequate (23). Iglesias et al. (2008) study showed that the percentage of energy intake from carbohydrates was 46% and that no athlete could go above 55% (16).

In the study done by Garcin et al. (2009), it was determined that the percentage of the athletes receiving energy from carbohydrates was 51.3±3.0% and their consumption was below the recommendations (5). In this study, it was determined that they consumed 6.3±1.7 g/day of carbohydrate per kg weight and that they met their daily carbohydrate requirements at just the lower margin of the acceptable level. According to the ACSM (2016) recommendations, 30-60 g/h carbohydrate consumption during 1-2.5 hours of endurance exercise positively affect the sport performance. In this study, it was determined that the athletes did not consume carbohydrates during exercise.

Protein consumption is seen as a key nutrient for success in all sports (11). The daily protein requirement of the athlete varies according to endurance and power training. The recommended protein intake for endurance athletes is determined as 1.2-1.4 g/kg/day (24). In a study conducted by Iglesias et al. (2008) found that

the athletes› protein consumption was 1.8 g/kg/day and 15% of the energy was obtained from protein (16). In a study of protein consumption of 11 soccer players aged 15 years (25), it was found that daily protein consumption was 1.57 g/kg per weight and this amount provided a positive nitrogen balance. In this study, it was determined that the ratio of energy received from protein was 16.2% and the athletes consumed 1.9±0.5 g/day protein per weight. It was determined that the athletes' diets were well above the recommended protein consumption quantities for endurance athletes. The protein sources in the athletes' diets were high quality protein resources as cheese, meat, and eggs. Along with the thought that excessive protein intake will cause more muscle and power production, protein intake of the athletes has also increased as a result of a shift towards nutrients that are protein sources.

Fat, especially saturated fatty acids and cholesterol consumption are increasing due to the fact that athletes consume more protein and increase their meat consumption (26). In long-term exercises, the fats are used as fuel. The ACSM (2016) recommendation for fat consumption is 20-35% of energy supplied from fat. The studies show that the athletes› daily ratio of energy intake supplied from fat is over 30 (5,16).

In Iglesias et al. (2008) study, it was determined that the ratio of athletes› diets supplied by fat is 38% and that cholesterol intake was 343 mg/day (16). In a study conducted to determine the nutritional status of soccer players, it was found that the adolescent athletes whose average age was 15 years had a ratio of 29.1% energy intake from fat (25). In this study, it was determined that the athletes› energy intake from fat was 30.2±6.4% and the fatty acid distribution was as follows: saturated fat 14.1%, monounsaturated fatty acid 10.4% and polyunsaturated fatty acid 5.7%. Although it is suggested that the athletes› daily saturated fat consumption must be 8% of energy intake and the cholesterol intake must be less than 300 mg; in this study, saturated fat and cholesterol consumptions were 14.1% and 397.0±168.5 mg respectively. The reason behind unbalanced fatty acid distribution was due to the fact that athletes consumed a lot of eggs, cheese, and meat, but not fish.

Presently there is no recommendation about daily fiber consumption for athletes, but in Dietary Guidelines for Turkey (2004) (14), fiber consumption of approximately 25 g/day is recommended for this age group and gender. In a study done by Schenkel et al., it was determined that daily fiber consumption of male soccer players (average age at 15.3±31.2) was 14.4 ± 0.38 g (20). In this study, it was determined that the athletes› daily fiber consumption was 25.6 g, which was consistent with the fiber recommendation. It was thought that the athletes› daily consumption of vegetables-fruits (562.5±256.5 g), legumes (14.9±13.3 g), and oily seeds (4.1±16.2 g) were sufficient to meet their daily fiber needs.

Studies on the micro-nutrient intake of the athletes have shown that adolescent athletes do not sufficiently consume micronutrient contents such as calcium, magnesium, iron, zinc and vitamin D (20, 27-29). Garcin et al. (2009) conducted a study; it was found that athletes› vitamin D, vitamin E and magnesium consumptions were below the RDA for France (5). In a study done by Rico-Sanz et al. on elite soccer players, it was concluded that their calcium consumptions were inadequate after assessing their nutrition status (19). In this study, it was seen that 50% of the athletes consumed calcium inadequately when their micro nutrient requirements are compared with Dietary guidelines for Turkey (2004) values recommended fort his age group and gender.

While athletes are recommended to consume 450 g of milk and 30 g of cheese per day according to their ages and gender in Dietary Guidelines for Turkey (2004), it was determined in this study that athletes consumed 259.7±181.7 g of milk and 82.5 ± 105.3 g of cheese per day and therefore could not meet their daily calcium requirements. In the study, 92.3% of the athletes were found to have inadequate vitamin D in their diets. However, soccer being an open-air sport, they would benefit from sun rays which are the main source of vitamin D.

Conclusion

As a conclusion, the nutritional status of the athletes should be determined at regular intervals. Education of coaches, trainers, team workers, athletes, and athletes' families are an important part of the nutrition training protocol. The first aspect of nutrition education for athletes by sports dietitians must be the correct choice of nutrients in the diet. Even though

soccer players meet their energy needs, carbohydrate consumption and some micronutrient intakes are often inadequate.

The dietary intake studies for athletes should be done during their stay in the training camps. The weakness of this study is that the athletes remained at home during the study period and that some of the information provided by the participants was not observable and depended on the athlete's self-claim.

References

1. Veale JP, Pearce AJ, Koehn S, Carlson JS. Performance and anthropometric characteristics of prospective elite junior Australian soccerers: a case study in one junior team. J Sci Med Sport. 2008;11(2):227-30.
2. Nutrition for soccer: The FIFA/F-MARC Consensus Conference. J Sports Sci. 2006;24(7):663-4.
3. Petrie HJ, Stover EA, Horswill CA. (2004). Nutritional concerns for the child and adolescent competitor. Nutrition, 20(7-8):620-31.
4. Bar-Or O, Unnithan VB. Nutritional requirements of young soccer players. J Sports Sci. 1994;(12):S39-42.
5. Garcin M, Doussot L, Mille-Hamar L, Billat V. Athletes' dietary intake was closer to French RDA's than those of young sedentary counterparts. Nutr Res. 2009;29(10):736-42.
6. James FH, Wolinsky I, James MP, Elizabeth AN, Joseph FI, John ES. Nutritional profile of soccer athletes eating from a training table. Nutr Res. 1984;(7): 27-34.
7. Lohman TG, Roche AF, Martorell R. (Eds).Anthropometric Standardization Reference Manual, Kinetics Books, Champaign, Illinois, 1988.
8. Jackson AS, Pollock ML. Generalized equations for predicting body density of men. Br J Nutr. 1978;40(3):497-504.
9. Pollock ML, Jackson AS. Research progress in validation of clinical methods of assessing body composition. Med Sci Sports Exerc. 1984;16(6):606-15.
10. Baysal A. Diyet el kitabı. 5th editon. Ankara. Hatipoglu publisher, 2008.
11. Thomas DT, Erdman KA, Burke LM. American College of Sports Medicine Joint Position Statement. Nutrition and Athletic Performance. Med Sci Sports Exerc. 2016; 48(3):543-68.
12. Rakıcıoğlu N, Tek Acar N, Ayaz A, Pekcan G.Yemek ve Besin Fotoğraf Kataloğu. 3th edition. Ankara, Ata Ofset publisher, 2012.
13. Kutluay, T. Toplu beslenme Yapılan Kurumlar İçin Standart Yemek Tarifeleri. 2nd edition. Ankara, Hatiboğlu publisher, 1994.
14. Hacettepe University, Department of Nutiriton and Dietetics. Dietary Guidelines for Turkey. Ankara, 2004.
15. Godek SF, Bartolozzi AR, Burkholder R, Sugarman E, Peduzzi C. Sweat rates and fluid turnover in professional soccer players: a comparison of National Soccer League linemen and backs. J Athl Train. 2008; 43(2):184-9.
16. Iglesias-Gutiérrez E, García-Rovés PM, García A, Patterson AM. Food preferences do not influence adolescent high-level athletes' dietary intake. Appetite. 2008;50(2-3):536-43.
17. Croll JK, Neumark-Sztainer D, Story M, Wall M, Perry C, Harnack L. Adolescents involved in weight-related and power team sports have better eating patterns and nutrient intakes than non-sport-involved adolescents. J Am Diet Assoc. 2006;106(5):709-17.
18. Maksimović M, Ristić G, Maksimović J, Backović D, Vuković S, Ille T, Milović V. Relationship between physical activity and some parameters of nutritional state in adolescence. Srp Arh Celok Lek. 2009;137(1-2):58-62.
19. Rico-Sanz J, Frontera WR, Molé PA, Rivera MA, Rivera-Brown A, Meredith CN. Dietary and performance assessment of elite soccer players during a period of intense training. J Int Soc Sports Nutr. 1998;8(3):230-40.
20. Schenkel TC, Stockman NK, Brown JN, Duncan AM. Evaluation of energy, nutrient and dietary fiber intakes of adolescent males. J Am Coll Nutr. 2007;26(3):264-71.
21. Troiano RP, Flegal KM. Overweight children and adolescents: description, epidemiology, and demographics. Pediatrics. 1998;101:497-504.
22. Fox MK, Cole N. Nutrition and Health Characteristics of Low-Income Populations, USDA. 2004. Volume III, School-Age Children.
23. Ruiz F, Irazusta A, Gil S, Irazusta J, Casis L, Gil J. Nutritional intake in soccer players of different ages. J Sports Sci. 2005;23(3):235-42.
24. Bernardot D. Advanced sports Nutrition. Canada, Human kinetics, 2006.
25. Boisseau N, Le Creff C, Loyens M, Poortmans JR. Protein intake and nitrogen balance in male non-active adolescents and soccer players. Eur J Appl Physiol. 2002;88(3):288-93.
26. Ersoy G. Egzersiz ve spor performansı için beslenme. Ankara, Betik kitap, 2010.
27. Decarli B, Cavadini C, Grin J, Blondel-Lubrano A, Narring F, & Michaud P. Food and nutrient intakes in a group of 11to 16 year old Swiss teenagers. Int J Vitam Nutr Res. 2000;70:139-147.
28. Shatenstein, B., & Ghadirian, P. Nutrient patterns and Nutral adequacy among French-Canadian children in Montreal. J Am Coll Nutr. 1996;15:264-272.
29. Troiano RP, Briefel R, Carroll MD, Bialostosky K. Energy and fat intakes children and adolescents in the United states: data from the national health and Nutr examination surveys. Am J Clin Nutr. 2000;72:1343-1353.

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Original article

The effect of carbohydrate gel consumption on elite mountain bikers time to exhaustion and blood glucose metabolism

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Summary. *Study Objectives:* In athletes, additional carbohydrate (CHO) uptake is important during sub-maximal and high-intensity exercise and competitions where the cost of metabolism exceeds endogenous carbohydrate depots. The aim of this study was to investigate the effects of carbohydrate gel consumption on glucose concentration and exercise time to exhaustion of elite mountain bikers. *Methods:* 20 mountain bikers (22,5 ± 2,64 years; 67,4 ± 5,44 kg; 174,7 ± 4,59 cm) performed bicycle ergometer with increasing overload test at 48 hours intervals. The experimental group was given 42 g of carbohydrate gel 20 minutes before test and control group was not consumed carbohydrate gel. In addition, the glucose levels of the athletes were followed with blood samples taken before and after the tests. *Results:* The averages of glucose levels and exhaustion times were higher according to the carbohydrate gel consumption ($p<0.001$). The consumption of carbohydrate gel decreases the glucose levels by 4.48% and does not the consumption of carbohydrate gel decrease by 8.90%. The glucose levels of mountain bikers in the trials decrease by 4.48% and 8.90% according to carbohydrate gel consumption and not, respectively. The exhaustion times of carbohydrate gel consumed mountain bikers were higher (9.35%; 00:31 sec.). *Conclusion:* Mountain bikers consuming carbohydrate gels were found longer exercise times and higher blood glucose levels than those who did not consume carbohydrate gels.

Key words: carbohydrate gel consumption, exhaustion time, glucose, mountain biker

Introduction

In recent years, the ergogenic aids are commonly used to increase performance and achieve success by the athletes (1). Sportive performance improves with a balanced diet; unbalanced diet may affect negatively the performance (2). For this reason, supplement products have been one of the important issues that should be emphasized (3). Supplement products, having a wide range of products, are general vitamins, minerals, amino acid, essential fatty acid, various vegetables and their extracts (4). Carbohydrates used as nutrition support are the most significant component of energy metabolism during exercises (5). Additional carbohydrate intake is significant since sub-maximal and intermittent high intense exercises and metabolism energy consumption exceed the endogenous carbohydrate storage during competitions (6). It has been reported that carbohydrate (CHO) intake during exercises increases the performance of long exercises with limited endogenous carbohydrate (7). Recreational athletes and team sports players frequently consume carbohydrate-electrolyte solutions in order to raise blood glucose concentration.

Former studies showed that multiple transportable carbohydrates products caused low fatigue level and a positive increase in athletic performance (8-10). Recent studies have reported that carbohydrate gel consumption has become popular among athletes to eliminate loss of carbohydrate and dehydration during exercises (11-13). It was commonly shown in the literature that carbohydrate (CHO) based supplement

increases the endurance performance (14-17). Earnest et al. (12) examined the carbohydrate gel ingestion during simulated endurance (64 km) cycling time trial performance and their results showed a trend in time and wattage over the last 16 km of 64 km simulated time trial. In addition, Kingsley, Penas-Ruiz, Terry Russell, (18) stated in their study, conducted on recreational footballers, that carbohydrate-electrolyte gel increased sprint performance during intermittent high-intense exercises. It was also stated in another study that carbohydrate gel ingestion significantly raised intermittent endurance capacity of adolescent team players (19). Moreover, Campbell, Braun, Applegate, and Casazza, (20) examined the effectiveness of carbohydrate supplements (drink, gel and jellybeans) over water only on cyclists 80 min followed 10 km time trial performance. They reported that carbohydrate supplements in the form of a drink, gel, or sports beans during of approximately 2 hr with sufficient glycogen and in normal environmental conditions were effective in maintaining blood glucose levels and improving performance over water only.

The effects of carbohydrate gel consumption on blood levels and performance of different athletes in different branches have been researched in many studies. These studies have generally been conducted on recreational athletes (21) or young athletes training at early ages (19,13). Also, there are limited numbers of studies studying blood levels and performance components on the elite athletes. Although carbohydrate-based supplements are commonly used by cyclists, there is no study investigating the effects of carbohydrate-based supplementation on elite mountain bikers. In this context, the purpose of this study is to examine the effect of carbohydrate gel consumption on elite mountain biker's time to exhaustion and blood glucose concentration.

Method

Participants

20 elite (Turkish national team) mountain bikers ($\overline{X}_{age}$: 22,5±2,64 years; $\overline{X}_{height}$: 174,7±4,59 cm; $\overline{X}_{weight}$: 67,4±5,44 kg; $\overline{X}_{experience}$: 7,7±2,4 years) participated voluntarily in this research. Participants were informed about the purpose of the research, details of the tests, and the risks they may encounter. The participants were informed about the purpose of the study, details of the application and the risks that may cause due to the application and voluntary consent forms were signed by the athletes. Ethical approval was obtained from the Faculty of Medicine of Sakarya University with protocol number 16214662/050.01.04/71.

Data Collections Tools

Participants' heights were measured with Seca 213 (Germany) brand portable stadiometer with 1 mm sensitivity, and participants' weights were measured with Seca 808 (Germany) brand digital scale. Monark 839 E modal (Vansbro, Sweden) bicycle ergometer and computer software connected to the bicycle were used to measure endurance performance of participants. Polar M400 (Kempele, Finland) was used to follow the heart beats during tests. Participants' blood glucose levels were measured with Okmeter Optime OK-10H (Taiwan).

Observation of Participants' Diet and Activity

Participants were not performed physical activity 48 hours before and between the two test trials. The same diet programs (different food for every day) were applied to all athletes who perform a pre-competition preparation camp.

Collecting Data and Exhaustion Test Protocol

The cross-sectional study design was used to collect data from control and experimental group. Participants were randomly separated into two groups, 10 athletes in the control group and 10 athletes in the experimental group. Twenty minutes before the first trial, the experimental group was given carbohydrate gel (Multipower Multicarbo® 1 packet gel 40 g; 104 kcal, 26 g carbohydrate, 10 g sugar 350 mg salt) by a nutrition expert who was not involved in the research. Carbohydrate gel was not given to the control group. The resting heart rates of all athletes were determined before warm up and then all participants performed a standardized 15-min warm-up session. A graded exercises test with bicycle ergometer applied to determine bikers' time to exhaustion. The ergometer bike load was increased by 50 watts in every 2 minutes for the

Table 1. Comparison of glucose levels according to two-way repeated measures ANOVA of carbohydrate gel consumption

Variables	Glucose Levels (mg/dL)			Δ %	F	p
	Pre-test	Post-test	Total			
	$\bar{X}$ ± SD	$\bar{X}$ ± SD	$\bar{X}$ ± SD			
CHO +	98.00±2.62	93.80±3.16	95.90±2.89	4.48	12.911	0.001**
CHO –	96.70±2.68	88.80±3.22	92.75±2,95	8.90		
Total	97.35±2.69	91.30±4.04		Trial x Time F=37.972; p=0.001**		
	F=406.101; p=0.001**					

duration of the test. The test ended at athletes' exhaustion time and exhaustion time was recorded. At the end of every two minutes, heart rates (HR) of athletes were obtained via a HR monitor (Polar M400) during test protocol. Additionally, athletes' blood glucose levels were measured immediately after exercise. After 48 hours all athletes participated in the second trial and did the same study protocol as the first trial.

Statistical Analysis

SPSS 18 was used for data analysis. The descriptive statistics for heart rate during the tests were given as the mean and standard deviation. Differences in time to exhaustion between treatments were assessed using a paired t-test. Differences in glucose levels were analyzed using a 2X2 (treatment X time) repeated measures analysis of variance (ANOVA). The effects of carbohydrate gel consumption on exhaustion times and changes in Glucose levels were assessed by examining the carbohydrate gel consumption X trial interaction effect in 2-way repeated-measures ANOVA. Differences in percentages of glucose levels were calculated using the formula Δ% = ((Pretest - Posttest) / Pretest) × 100 (22). Significance was set at 0.05.

Findings

It was observed that the averages of glucose levels were statistically different according to measurement times (F=406.101; p=0.001). Accordingly, it was found that the total glucose levels decreased by a mean of 6.63% between the pre and post measurements. Moreover, it was also detected that the averages of glucose levels were statistically different according to the carbohydrate gel consumption (F=12.911; p<0.001). In addition, the interaction between the glucose levels of the carbohydrate gel consumption and measurement times was statistically significant (F=37.972; p=0.001). Accordingly, it was found that in exhaustion exercise, the consumption of carbohydrate gel decreases the glucose levels by 4.48% and does not the consumption of carbohydrate gel decrease by 8.90% (Table 1).

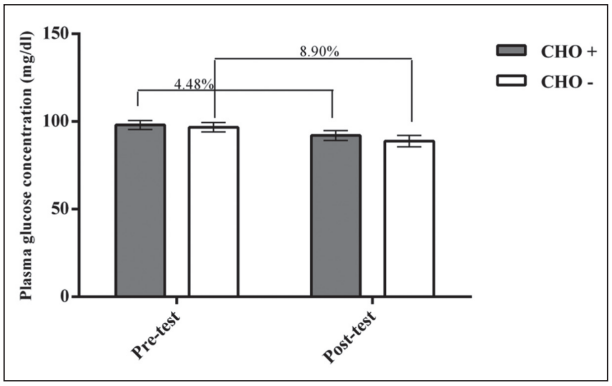

Figure 1. Comparison of glucose levels of mountain bikers according to carbohydrate gel consumption

It was observed that in the exhaustion times of mountain bikers were statistically different according to carbohydrate gel consumption (p<0,05; Table 2).

The heart rate of mountain bikers was increased linearly during the test period. According to these results, the average heart rate of mountain bikers who consumed carbohydrate gel was determined as 196.65±1.31 (bpm) at the end of the test.

Table 2. Comparison of time to exhaustion according to carbohydrate gel consumption

Variables	$\bar{X}$ ± SD (min)	t	p
CHO +	15:13±00:43	2,199	**0,040***
CHO -	14:42±00:38		

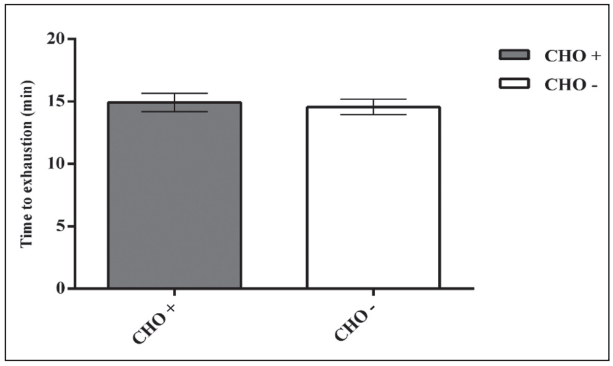

Figure 2. Comparison of the exhaustion times of mountain bikers according to carbohydrate gel consumption

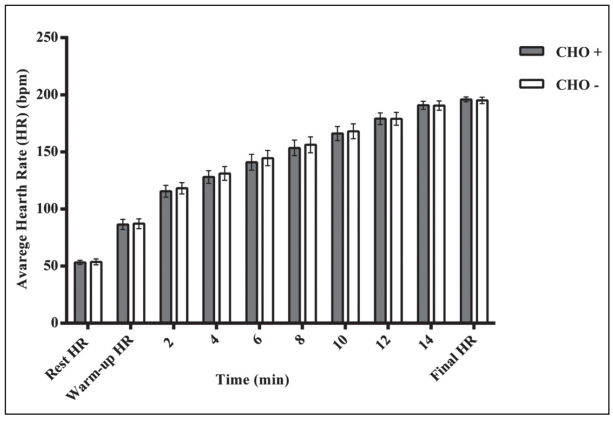

Figure 3. Comparison of average heart rate according to carbohydrate gel consumption in the exhaustion exercise

Discussion

The primary finding of this study was that the carbohydrate gel consumption increased the elite mountain bikers' blood glucose levels and exhaustion times during graded tests. When blood glucose concentration data was examined, it was determined that there were differences in total glucose levels of mountain bikers according to carbohydrate gel consumption. Additionally, the interaction between carbohydrate gel consumption and measuring time was significant. Athletes' blood glucose level decreased by 8.90% without CHO gel consumption; whereas, the blood glucose levels of the athletes who ingested CHO gel decreased by 4.48%.

Recent studies show that carbohydrate intake before and during exercises is important for maintaining blood glucose concentration and this situation is one of the important factors affecting performance (12). When the results of previous studies were examined, it was seen that carbohydrate gel causes an increase in blood glucose levels of athletes. However, the rate of increase in blood glucose levels greater than our study. For instance; Brooks, Bradley, Lane, and Hodgson, (23) reported that carbohydrate gel consumption produced a significantly greater increase in blood glucose levels against control treatment (1.6 mmol/l compared to 0.6 mmol/L). Similarly, Campbell et al. (20), examining different carbohydrate supplement products (jellybeans, sport drink and gel) in several studies, stated that blood glucose concentration of athletes, consuming supplement products were significantly higher from the athletes' glucose level who consume only water (5.7 ± 0.2 mmol/L for sports beans, 5.6 ± 0.2 mmol/L for sports drink, 5.7 ± 0.3 mmol/L for gel, and 4.6 ± 0.3 mmol/L for water). Nicholas et al. (24) reported that carbohydrate-electrolyte consumption (5 mL x kg^{-1}) and after every 15 min of exercise thereafter (2 mL x kg^{-1}) decreased muscle glycogen utilization levels during 90 min of intermittent high-intensity running by 22% (192.5 ± 26.3 mmol compare to control group 245.3 ± 22.9 mmol). These samples support the results of current research about blood glucose levels, but the level of increase in blood glucose or rates glucose utilization seems to be greater than the results of this study.

According to the second findings of the study, it was determined that exhaustion time of mountain bikers, consuming carbohydrate gel, was higher than those of bikers not using carbohydrate gel. Studies examining CHO gel ingestion show that exhaustion time of the athletes consume carbohydrate gel can be higher than the athletes do not consume CHO gel. Patterson and Gray (25) reported in their study, they examined effects of carbohydrate gel supplementation on intermittent high-intensity shuttle running, exhaustion time of the group, using carbohydrate gel, was 45% higher than the placebo group. Similarly, Phillips, Turner, Sanderson, and Sproule (19) stated in their study, applied to adolescent young athletes, exhaustion time of athletes using carbohydrate gel was 21% higher than the placebo group. In our study, there was a rate of 9.35% (00:31 seconds) difference between exhaustion times according to the carbohydrate gel

consumption. Moreover, any statistical difference was not found between athletes' heart rates using carbohydrate gel and not using. However, it was determined that mountain bikers using carbohydrate gel continued their exercises with higher heart rates towards the end of the exercise. Patterson and Gray (25) reported that carbohydrate gel consumption didn't change athletes' heart rates.

According to previous researches' results, it was determined that the effect of carbohydrate consumption on time to exhaustion and blood glucose concentration were higher than our results. Sample group in our study consists of athletes who race in international competitions, have similar training history, and condition levels. Sample group consists of recreational athletes in other studies. Low positive effects of carbohydrate consumption may result from the sample group. Hopkins has reported that the differences between the performances of the athletes competing at the international level may be very low (26).

Conclusion

In conclusion, it was determined that the exhaustion time of mountain bikers consuming carbohydrate gel was longer and the blood glucose level of the bikers was higher than bikers who didn't consume carbohydrate gel. According to this result, it can be said that carbohydrate gel used before exercises affects positively athletic performances by increasing blood glucose level, exhaustion time and by decreasing carbohydrate utilization.

References

1. Ünal, M. (2005). Sporcularda kreatin desteğ ve egzersiz performansı üzerine etkileri. Genel Tıp Dergisi, 15(1), 43–50.
2. Fox K. The Physiological Basis of Physical Education and Athletics. USA: Saunders College Publishing; 1988 p. 527.
3. Burke LM, Deakin V. (2006). Clinical Sports Nutrition. Sydney: McGrawHill; p. 188.
4. Tek NA, Pekcan G. Besin Destekleri Kullanılmalı mı? Hacettepe Üniversitesi, Sağlık Bilimleri Fakültesi, Beslenme ve Diyetetik Bölümü. Ankara: Klasmat Matbaacılık; 2008; p.1-8
5. Gollnick PD. Energy metabolism and prolonged exercise. InLamb, DR. &Murray, R, editors. Prespectives In Exercise Science and Sports Medicine Vol l: Prolonged Exercise. 2001; pp 1- 37.
6. Hargreaves M. Metabolic responses to carbohydrate ingestion: Effects on exercise performance. In D. R. Lamb & R. Murray (Eds.), Perspectives in exercise science and sports medicine. 1999; pp. 93–124. Carmel, IN: Cooper.
7. Luden ND, Saunders MJ, D'Lugos AC, Pataky MW, Baur DA, Vining CB, Schroer AB. Carbohydrate mouth rinsing enhances high intensity time trial performance following prolonged cycling. Nutrients. 2016; 8(9), 576.
8. Jeukendrup AE. Carbohydrate and exercise performance: the role of multiple transportable carbohydrates. Curr Opin Clin Nutr Metab Care. 2010; 13(4), 452-457.
9. Currell K, Jeukendrup A. Superior endurance performance with ingestion of multiple transportable carbohydrates. Med Sci Sports Exerc. 2008; 40(2), 275.
10. Jentjens RL, Achten J, Jeukendrup AE. High oxidation rates from combined carbohydrates ingested during exercise. Med Sci Sports Exerc. 2004; 36(9), 1551-1558.
11. Havemann L, Goedecke JH. Nutritional practices of male cyclists before and during an ultraendurance event. Int J Sport Nutr Exerc Metab. 2008; 18(6), 551-566.
12. Earnest, C. P., Lancaster, S. L., Rasmussen, C. J., Kerksick, C. M., Lucia, A., Greenwood, M. C., ... & Kreider, R. B. (2004). Low vs. high glycemic index carbohydrate gel ingestion during simulated 64-km cycling time trial performance. The Journal of Strength & Conditioning Research, 18(3), 466-472.
13. Harper LD, Briggs MA, McNamee G, West DJ, Kilduff LP, Stevenson E, Russell, M. Physiological and performance effects of carbohydrate gels consumed prior to the extra-time period of prolonged simulated soccer match-play. J Sci Med Sport. 2016; 19(6), 509-514.
14. Fritzsche RG, Switzer TW, Hodgkinson BJ, Lee SH, Martin JC, Coyle EF. Water and carbohydrate ingestion during prolonged exercise increase maximal neuromuscular power. J Appl Physiol. 2000; 88, 730–737
15. Ivy JL, Res PT, Sprague RC, Widzer MO. Effect of a carbohydrate-protein supplement on endurance performance during exercise of varying intensity. Int J Sport Nutr Exerc Metab. 2003; 13, 382–395
16. Murray R, Paul GL, Seifert JG, Eddy DE. Responses to varying rates of carbohydrate ingestion during exercise. Med Sci Sports Exerc. 1991; 23, 713–718
17. Saunders MJ, Luden ND, Herrick JE. Consumption of an oral carbohydrate-protein gel improves cycling endurance and prevents postexercise muscle damage. J Strength Cond Res. 2007; 21, 678–684
18. Kingsley M, Penas Ruiz C, Terry C, Russell M. Effects of carbohydrate-hydration strategies on glucose metabolism, sprint performance and hydration during a soccer match simulation in recreational players. J Sci Med Sport. 2014; 17(2), 239-243.
19. Phillips SM, Turner AP, Sanderson MF, Sproule J. Carbo-

hydrate gel ingestion significantly improves the intermittent endurance capacity, but not sprint performance, of adolescent team games players during a simulated team games protocol. Eur J Appl Physiol. 2012; 112(3), 1133-1141.
20. Campbell C, Prince D, Braun M, Applegate E, Casazza GA. Carbohydrate-supplement form and exercise performance. Int J Sport Nutr Exerc Metab. 2008; 18(2), 179-190.
21. Cooper R, Naclerio F, Allgrove J, Larumbe-Zabala E. Effects of a carbohydrate and caffeine gel on intermittent sprint performance in recreationally trained males. Eur J Sport Sci. 2014; 14(4), 353-361.
22. Isik O, Yildirim I, Ersoz Y, Koca HB, Dogan I, Ulutas E. Monitoring of pre-competition dehydration-induced skeletal muscle damage and inflammation levels among elite wrestlers. J Back Musculoskelet Rehabil. 2018; 31(3), 533-540.
23. Brooks D, Bradley, JL, Lane P, Hodgson R. Assessment of the effects of an isotonic carbohydrate gel on exercise performance. Med Sci Sport Exer. 2002; 34(5), S139.
24. Nicholas CW, Tsintzas K, Boobis L, Williams C. Carbohydrate-electrolyte ingestion during intermittent high-intensity running. Med Sci Sport Exer. 1999; 31(9), 1280-1286.
25. Patterson SD, Gray SC. Carbohydrate-gel supplementation and endurance performance during intermittent high-intensity shuttle running. Int J Sport Nutr Exerc Metab. 2007; 17(5), 445-455.
26. Hopkins, W. G. Measures of reliability in sports medicine and science. Sports Med. 2000, 30(1), 1-15.

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Original article

Is there any predictive equation to determine resting metabolic rate in ultra-endurance athletes?

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Summary. *Background/aims:* Only a few studies determined some equations to predict resting metabolic rate (RMR) in endurance athletes, however the validity in ultra-endurance athletes, such as triathletes and ultra-marathoners, had not been examined previously. The aim of this study was to assess the accuracy of commonly used RMR predictive equations (Harris-Benedict, Mifflin-St. Jeor, Cunningham, WHO/FAO/UNU (calculated by using body mass and height and body mass alone), Wang, and Sabounchi (Structure 4, 5, and 11) equations) comparing with measured RMR in ultra-endurance athletes. *Methods:* Male (n=15) and female (n=15) ultra-endurance athletes age 23 to 55 years from Ankyra Sports Club were included. The Bland-Altman plot was performed to determine mean bias and limits of agreement between measured and predicted RMRs. Linear regression analysis was used to determine the accuracy of each predictive equation by computing the standard error of estimate and root-mean-squared prediction error (RMSPE). *Results:* Mifflin-St. Jeor equation was found to be the best predictive equation with lowest RMSPE (275.85 kcal/day for men and 388.34 kcal/day for women) and mean difference (3.04±285.51 kcal/day for men and 185.57±353.10 for women) in ultra-endurance athletes. The Cunningham equation could be used in estimating RMR in male athletes (RMSPE, 310.77 kcal/day, the bias between measured vs. predicted RMR, 147.68±283.04 kcal/day). *Conclusions:* The Mifflin- St. Jeor and Cunningham equations for men and the Mifflin-St. Jeor equation in women could be used with caution in the absence of indirect calorimetry in ultra-endurance athletes. All other predictions significantly underestimated RMR for both sexes.

Key words: ultra-endurance athletes, resting metabolic rate, predictive equation, indirect calorimetry, energy metabolism

Introduction

Given the intensive and long periods of ultra-endurance events (>6 h/race), providing sufficient energy to maintain body mass and avoid performance decline plays an important role in achieving performance goals and maintaining health status, and the estimation of energy requirements is critical for ultra-endurance athletes to maintain their body hormesis and develop their race strategies (1).

The basic component of sports nutrition assessment is to estimate the energy expenditure and intake during the training and race. In case the energy cost of training or race exceeds energy intake, the athlete has a negative energy balance, and this leads to a decline in performance and may result in failure of achieving his/

her goals (2, 3). The Australian Institute of Sport determined resting metabolic rate (RMR) as an important tool for athletes, especially when they could not reach their performance targets in response to personal training interventions (1).

RMR is one of the largest components of total energy expenditure (approximately 60-80% for sedentary adults and 38-47% for elite endurance athletes) and could be measured by using direct or indirect calorimetry measurement (4). Although it is important, this technique is not commonly preferred in energy measurement of athletes because long measurement periods and expensive laboratory equipment are required to measure RMR and trained personnel are also needed to obtain accurate results. Therefore, several predictive equations are developed to offer an alternative low-cost method of RMR estimation (5, 6).

Predictive equations are developed based on sex, body height and weight, and fat-free mass (FFM), and each of these is validated from a different range of populations, ages, and body compositions (7). For example, Harris and Benedict (8) first developed an equation on 239 healthy adults (136 men, 103 women; mean body mass index for men, 21.4, and women, 24.4) and frequently used the equation to predict RMR. The RMR predictive equation of Mifflin et al. (9) was derived from 264 normal weight and 234 obese adult samples using body weight, height, and age. The World Health Organization (WHO)/Food and Agricultural Organization (FAO)/United Nations University (UNU) predictive equations (10) were developed using data from Schofield and James, including 2526 adults (2,279 men, 247 women; with 47% of Italian population) and using either body weight and height or body weight alone. Cunningham (11) used the variables of Harris and Benedict by adding 60 new trained adults, and developed an equation to predict RMR using lean body mass of the subjects, and revealed that the equation is more accurate in certain populations. Wang et al. (12) found a linear relationship between FFM and RMR and developed an equation using data from 6 different studies. Sabounchi et al. (13) used 47 population-specific predictive equations of RMR and developed the resulting "structures" based on different characteristics of target population to be more specific for different populations. The American College of Sports Medicine (ACSM) position statement recommended the use of the Cunningham or Harris-Benedict equation to obtain a reasonable estimate of RMR in athletes (4).

Because of differences in body composition, training/race load, or other endurance-related characteristics of ultra-endurance athletes, there remains a need for a valid predictive equation that could be used to estimate RMR of athletes. Thus, the aim of this study was to compare the RMR predicted according to gender by 9 RMR predictive equations with RMR measured by indirect calorimetry in ultra-endurance athletes to determine which one of these predictive equations is more suitable to use in the populations.

Materials and methods

Subjects

Fifteen male (triathletes, n=10; ultra-marathoners, n=5) (38.46±5.32 years; 178.27±7.36 cm; 73.01±7.38 kg; 63.36±6.39 kg of FFM; 13.16±3.89 body fat [BF%]) and female (triathletes, n=6; ultra-marathoners, n=9) (37.13±7.87 years; 162.67±3.72 cm; 56.46±4.07 kg; 45.31±2.78 kg of FFM; 19.64±3.14 BF%) ultra-endurance athletes participated in this study. The inclusion criteria were as follows: 1) participation in ultra-endurance races/events, 2) >15-18 h/week training for at least three years, and 3) no history of metabolic disorders. Athletes who are using any ergogenic aids or medications and have a history of metabolic or eating disorders were excluded from this study. The study was conducted at the Center of Athlete Training and Health Research of the Ministry of Youth and Sports between March 20, 2018 and April 25, 2018. Subjects were recruited from Ankyra Sports Club in Ankara. All athletes were informed about the study protocol and provided written informed consent form at the beginning of the study.

Data Collection

Anthropometric Measurements

Anthropometric measurements (body height, body mass, FFM, and fat mass [FM]) were performed while the athletes wearing underwear in a fasting state (12 h). Body mass, FM, and FFM were measured using multifrequency bioelectrical impedance analyzer (MF-

BIA) (TANITA MC-780, Japan; 0.1 kg accuracy), and height was measured while athletes were standing, head positioned in Frankfort horizontal plane, using a portable stadiometer (portable stadiometer, Holtain, London, United Kingdom; 0.1 cm accuracy).

Indirect Calorimetry

Indirect calorimetry was used with the reason it is a valid measurement to reach actual RMR value. All athletes completed an RMR test using indirect calorimetry (COSMED K5 metabolic cart; COSMED, Rome, Italy). The system was recalibrated after every 3 athletes.

RMR measurement was standardized according to the procedures in a systematic review conducted by Compher et al (5). All athletes were asked to visit the Exercise Performance Laboratory in a fasting (at least 5 h) and resting (24 h, without doing vigorous exercise the day before the test) state and refraining from caffeine (at least 4 h), cigarette (at least 2 h), and alcohol (at least 2 h) consumption. The RMR test was performed at 8:00 to 9:00 AM, and the athletes rested without falling asleep, lying in a supine position for 20 min, in a dusk, silent room with an ambient temperature of 20-25˚C. After the resting period, the test protocol was started while athletes were placed in a physically comfortable supine position and lasted for approximately 20 min. Data were recorded 5 min after the start of the test, and measurement was stopped after reaching a minimum of 5 min in steady-state conditions (achieving a 5-min period with ≤10% coefficient of variation for oxygen consumption [VO_2] and carbon dioxide production [VCO_2]).

Predictive Equations

RMRs for each participant were estimated using 9 commonly used standard predictive equations as presented in Table 1.

Statistical Analysis

Data were analyzed according to sex using SPSS version 23.0. Sample size was calculated using the formula ($n= (\ln(1-\gamma)/\ln(1-\pi))4$ based on Viechtbauer W et. al (14). We determined probability (π) of the study based on the results of similar studies (19, 25) and defined confidence interval (γ) as 0.95. Paired-sample t-test was used to compare measured and predicted RMRs. Linear regression analysis and Bland-Altman plot were used to determine the accuracy of each RMR predictive equation by comparing the indirect calorimetry measurement values and identify a proportional bias. The RMSPE was calculated to evaluate the accuracy of predicted RMR compared with the actual measured RMR for each athlete. Intraclass correlation coefficient was calculated to determine agreement between measured and predicted RMR. Data were represented as mean ± standard deviation (SD), and statistical significance was set at a P-value <0.05.

Results

The general information of the athletes is presented in Table 2. Table 3 represents the mean differences between measured and predicted RMR in ultra-endurance athletes. In men, values from all predictive equations with the exceptions of the Mifflin-St. Jeor (mean difference, 3.04±285.51 kcal) and Cunningham equations (mean difference, 147.68±283.04 kcal) were significantly different from the measured RMRs. The Harris Benedict, WHO/FAO/UNU (calculated with body mass and height), WHO/FAO/UNU (calculated with body mass alone), Wang, and all Sabounchi equations (Structures 4, 5, and 11) underestimated actual RMRs.

Values from all predictive equations with the exception of the Mifflin-St. Jeor equation (mean difference, 185.57±353.10 kcal) were significantly different from the measured RMRs in female ultra-endurance athletes. The Harris Benedict, Cunningham, WHO/FAO/UNU (calculated with body mass and height), WHO/FAO/UNU (calculated with body mass alone), Wang, and all Sabounchi equations (Structures 4, 5, and 11) prediction underestimated actual RMRs.

Table 4 presents the percentage of accuracy of each predictive equation according to the measured RMRs. Mifflin-St. Jeor and Cunningham equations in men (in 7 of 15 male athletes) and Mifflin-St. Jeor equation in women (in 8 of 15 female athletes) provided the most accurate RMR predictions.

Based on the regression analysis between measured and predicted RMRs for male ultra-endurance

Table 1. Resting metabolic rate predictive equations

No	Name	Equation
1	Harris-Benedict	Men: RMR[a] (kcal.d^{-1})= 66.47 +13.75*BM[b] (kg) + 5*H[c] (cm) - 6.76*A[d] (year)
		Women: RMR (kcal.d^{-1})= 655.1+9.56*BM (kg) + 1.85*H (cm)-4.66*A (year)
2	Mifflin-St. Jeor	Men: RMR (kcal.d^{-1})= 66.7+13.75*BM (kg)+5*H (cm)-4.92*A+5
		Women: RMR (kcal.d^{-1})= 66.7+13.75*BM (kg)+5*H (cm)-4.92*A-161
3	Cunningham	RMR[a] (kcal.d^{-1})= 500 + 22*FFM[e] (kg)
4	WHO/FAO/UNU[f]	BM (kg) and H (m):
		Men: age (year)
		8-30 RMR (kcal.d^{-1})= 15.4*BM (kg)-27*H (m)+717
		31-60 RMR (kcal.d^{-1})= 11.3*BM (kg) + 16*H (m)+901
		>60 RMR (kcal.d^{-1})= 8.8*BM (kg) +1.128*H (m)-1.071
		Women: age (year)
		18-30 RMR (kcal.d^{-1})= 13.3*BM (kg)+334*H (m)+35
		31-60 RMR (kcal.d^{-1})= 8.7*BM (kg) -25*H (m)+865
		>60 RMR (kcal.d^{-1})= 9.2*BM (kg) +637*H (m)-302
5	WHO/FAO/UNU[f]	BM (kg) alone:
		Men: age (year)
		18-30 RMR (kcal.d^{-1})= 15.3*BM (kg)+679
		31-60 RMR (kcal.d^{-1})= 11.3*BM (kg)+879
		>60 RMR (kcal.d^{-1})= 13.5*BM (kg)+487
		Women: age (year)
		18-30 RMR (kcal.d^{-1})= 14.7*BM (kg)+496
		31-60 RMR (kcal.d^{-1})= 8.7*BM (kg) +829
		>60 RMR (kcal.d^{-1})= 10.5*BM (kg) +596
6	Wang	RMR (kcal.d^{-1})= 24.6*FFM (kg) +175
7	Structure[h] 4	Men: RMR (kcal.d^{-1})= 361+21.1* FFM (kg) +4.77*FM[g] (kg)
		Women: RMR (kcal.d^{-1})= 360 + 21* FFM (kg) + 4.68* FM (kg)
8	Structure 5	Men: RMR (kcal.d^{-1})= 503 + 18.3*FFM (kg)
		Women: RMR (kcal.d^{-1})=473 + 20.1* FFM (kg)
9	Structure 11	Men: RMR (kcal.d^{-1})= 898-3.32*A + 14.3*FFM (kg) +6.46* FM (kg)
		Women: RMR (kcal.d^{-1})= 682-3.08*A+12.9*FFM (kg) +5.9* FM (kg)

[a]RMR, resting metabolic rate in kcal/day. [b]BM, body mass (kilograms). [c]H, height (all equations [except the WHO/FAO/UNU equation, which uses height in meters] use height in centimeters). [d]A, age (year). [e]FFM, fat-free mass (kilograms). [f]WHO/FAO/UNU, World Health Organization/Food and Agricultural Organization/United Nations University. [g]FM, fat mass (kilograms). [h]Population-specific meta-regression predictive equation developed by Sabounchi et al.

Table 2. Baseline characteristics of ultra-endurance athletes*

	Men (n=15)	Women (n=15)
Age (year)	38.46±5.32	37.13±7.87
Height (cm)	178.27±7.36	162.67±3.72**
Body mass (kg)	73.01±7.38	56.46±4.07**
Body fat (%)	13.16±3.89	19.64±3.14**
Fat-free mass (kg)	63.36±6.39	45.31±2.78**
Fat mass (kg)	9.65±2.99	11.15±2.29
Duration of training, hours/week	16.33±1.95	15.41±0.73
Maximum oxygen consumption (VO_2max), mL/min/kg	59.78±7.77	51.18±5.09**

** Mean ± standard deviation. **p<0.05*

athletes, the variance in predicted RMRs ranged from a standard error of estimate (SEE)=282.09 kcal/day from the WHO/FAO/UNU equation (calculated with H and BM) to a SEE= 293.20 kcal/day from the Harris Benedict equation, accounting for 18.5% and 11% of the variance in male ultra-endurance athletes, respectively. The Mifflin-St. Jeor equation yielded the lowest RMSPE value of 275.85 kcal and the highest intraclass correlation coefficient (ICC) of 0.76, which indicated that it has good reliability in estimating RMR for male ultra-endurance athletes, whereas the Structure 5 equation yielded the highest RMSPE value of 466.44 kcal.

Table 3. Comparison of measured and predicted RMRs in ultra-endurance athletes*

	Men (n=15)		Women (n=15)	
	RMR	**Mean difference**	**RMR**	**Mean difference**
RMR[a] measured	2041.60±301.03		1788.20±340.96	
Harris Benedict	1700.96±120.78	340.64±283.04**	1322.40±81.84	465.88±311.95**
Mifflin-St.Jeor	2038.56±125.56	3.04±285.51	1602.63±59.49	185.57±353.10
Cunningham	1893.92±140.61	147.68±283.04	1496.89±61.23	291.31±332.75**
WHO/FAO/UNU[b] *(calculated with BM[c] and H[d])*	1725.95±85.57	315.65±275.33**	1321.92±36.97	466.28±330.41**
WHO/FAO/UNU *(calculated with BM)*	1754.57±84.24	287.03±275.82**	1388.10±41.41	400.10±348.12**
Wang	1733.66±157.23	307.94±285.45**	1289.71±68.47	498.49±332.51**
Structure 4[e]	1743.94±137.31	297.66±279.10**	1363.75±62.30	424.45±331.06**
Structure 5[e]	1662.49±116.96	379.11±281.69**	1383.80±55.95	404.40±333.02**
Structure 11[e]	1738.70±89.06	302.90±277.89**	1158.41±29.79	629.79±334.65**

**Mean±standard deviation. **p<0.05. [a]RMR, resting metabolic rate in kcal/day. [b]WHO/FAO/UNU, World Health Organization/Food and Agricultural Organization/United Nations University. [c]BM, body mass (kilograms). [d]H, height (centimeters). [e]Population-specific meta-regression predictive equation developed by Sabounchi et al.*

Table 4. Percentage of ultra-endurance athletes whose RMR was accurate, overpredicted, or underpredicted as per predictive equation*

Equation	**Men (n=15)**			**Women (n=15)**		
	Accurate[a]	**Overpredicted[b]**	**Underpredicted[c]**	**Accurate[a]**	**Overpredicted[b]**	**Underpredicted[c]**
Harris Benedict	20.00	6.67	73.33	13.33	0.00	86.67
Mifflin-St.Jeor	46.67	26.66	26.66	53.33	13.33	33.34
Cunningham	46.67	33.34	20.00	20.00	13.33	66.67
WHO/FAO/UNU[d] *(calculated with BM[e] and f[g])*	20.00	6.66	73.34	13.33	0.00	86.67
WHO/FAO/UNU *(calculated with BM)*	20.00	6.66	73.34	26.66	0.00	73.34
Wang	26.67	6.66	66.7	13.33	0.00	86.67
Structure 4[g]	26.67	6.66	66.7	20.00	0.00	80.00
Structure 5[g]	13.34	6.66	80.00	26.66	0.00	73.34
Structure 11[g]	20.00	6.67	73.33	6.66	0.00	93.34

**For each equation, data are expressed as percent of the total sample. Each row sums to 100%. [a]Accurately predicted resting metabolic rate falls within ±10% of the value obtained from measured RMR. [b]Overpredicted resting metabolic rate is ≥10% of the value obtained from measured RMR. [c]Underpredicted resting metabolic rate is ≤ -10% of the value obtained from measured RMR. [d]WHO/FAO/UNU, World Health Organization/Food and Agricultural Organization/United Nations University. [e]BM, body mass (kilograms). [f]H, height(centimeters). [g]Population-specific meta-regression predictive equation developed by Sabounchi et al.*

According to the regression analysis of the bias (measured vs. predicted) for female ultra-endurance athletes, the variances ranged from a SEE= 303.12 kcal/day (Harris Benedict equation) to a SEE= 351.53 kcal/day (WHO/FAO/UNU equation [calculated with BM]), accounting for 26.6% and 1.3% of the variance, respectively. The Mifflin-St. Jeor equation presented the most accurate predictive equation in all used RMR predictions with the lowest RMSPE value of 388.34 kcal and had good reliability with ICC of 0.75, whereas the Structure 11 equation (with the highest RMSPE value [707.93 kcals], ICC of 0.11) had the worst performance in predicting the RMRs of female ultra-endurance athletes.

The results of the Bland-Altman plot analysis of each predictive equation for male and female ultra-endurance athletes are presented in Figures 1 and 2, respectively. A positive value indicates that the pre-

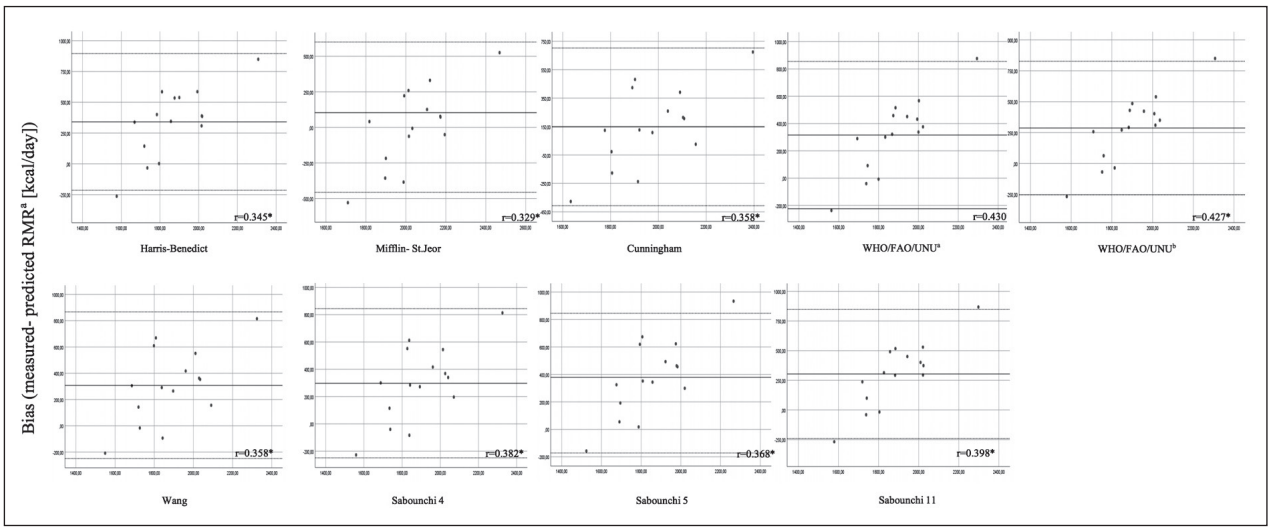

Figure 1. Solid line represents bias between measured and predicted RMR (kcal/day). Dashed lines represent ±1.96 SD of bias. [a]WHO/FAO/UNU, World Health Organization/Food and Agricultural Organization/United Nations University (calculated with body mass [kg] and height [m]). [b]WHO/FAO/UNU, World Health Organization/Food and Agricultural Organization/United Nations University (calculated with body mass alone [kg]). *p<0.05.

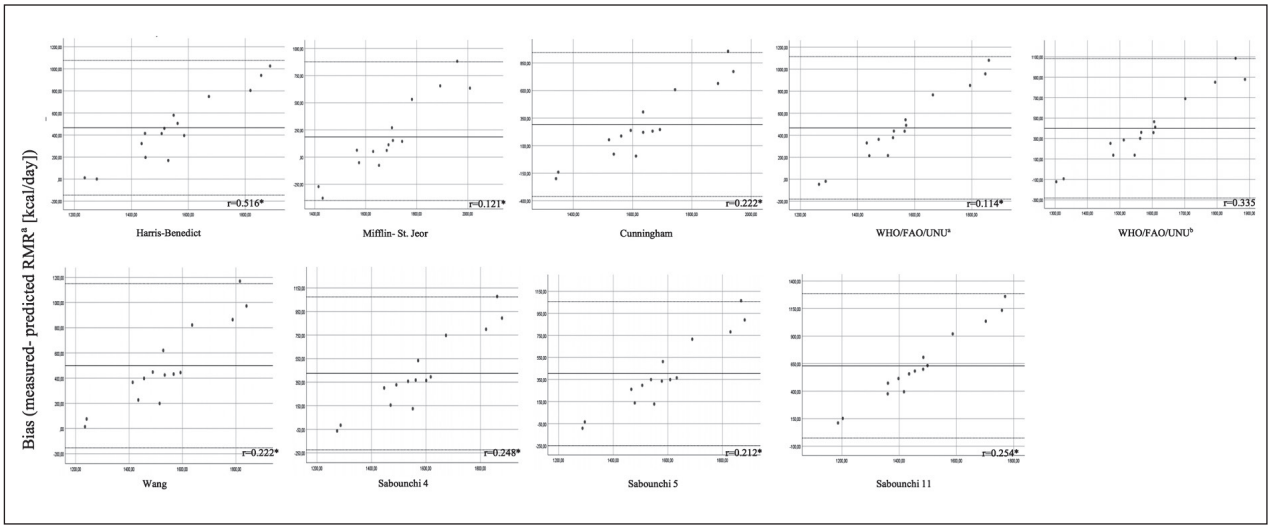

Figure 2. Solid line represents bias between measured and predicted RMR (kcal/day). Dashed lines represent ±1.96 SD of bias. [a]WHO/FAO/UNU, World Health Organization/Food and Agricultural Organization/United Nations University (calculated with body mass [kg] and height [m]). [b]WHO/FAO/UNU, World Health Organization/Food and Agricultural Organization/United Nations University (calculated with body mass [kg]). *p<0.05.

dicted RMR were greater than the measured RMR. All predictive equations were tested for each individual bias value near or exceeding the ±2 SD limits of agreement. The relationship between average and bias of measured and predicted RMRs were statistically significant for all predictive equations in men (Harris-Benedict, r=0.345; Mifflin-St. Jeor, r=0.329; Cunningham, r=0.358; WHO/FAO/UNU [calculating with BM and H]; r=0.430, WHO/FAO/UNU [calculating with BM alone], r=0.427; Wang, r=0.358; Structure 4, r=0.382; Structure 5, r=0.368; Structure 11, r=0.398), and significant for all predictive equation in women (Harris-Benedict, r=0.516; Mifflin-St. Jeor, r=0.121; Cunningham, r=0.222; WHO/FAO/UNU [calculat-

ing with BM and H]; r=0.114; WHO/FAO/UNU [calculating with BM alone], r=0.335; Wang, r=0.222; Structure 4, r=0.248; Structure 5, r=0.212; Structure 11, r=0.254).

Discussion

The physiology and training loads of men and women significantly differ from each other, therefore physiological evaluation criteria and formulations also differ according to sexes, for instance, an equation would be suitable for men while the same equation is not suitable for women. Therefore, the purposes of this study were to evaluate the accuracy of nine commonly used RMR predictive equations in a sample of ultra-endurance athletes and identify the differences in the accuracy of predictive equations between sexes.

In the last position stand on nutrition and physical activity conducted by the ACSM (4), sufficient energy intake for athletes was described as a cornerstone, and the use of the Cunningham and Harris-Benedict equations is recommended to predict RMR in athletic population. But, this recommendation was generalized for all athletic population, not specialized for any specific population. As all sports branches have required specific abilities and training conditions, the energy metabolism of athletes could be affected and changed according to sports type. Several studies emphasized that the Cunningham equation was recommended to be used for RMR prediction in athletes (15-17), while others reported that the equation underestimated the actual RMR (18, 19). The study found that Mifflin-St. Jeor (9) and Cunningham (11) equations for men and Mifflin-St. Jeor (9) equation for women had greater accuracy and predicted measured RMR within acceptable values in ultra-endurance athletes. The Harris-Benedict equation did not accurately predict measured RMR in both male (RMSPE, 436.81 kcal/day; R^2, 11.9%) and female (RMSPE, 554.86 kcal/day; R^2, 26.6%) ultra-endurance athletes in agreement with Jagim et al (16).

The Cunningham equation had good performance (RMSPE, 310.77 kcal/day; 44.5% of the variance; accuracy, 46.67% of the athletes, mean difference, 147.68±283.04 [$p>0.05$]) in male ultra-endurance athletes in this study, consistant with De Lorenzo et al (20). De Lorenzo et al (20) found that the Cunningham equation had the best performance in predicting RMR in 51 male athletes (12 judo, 22 water polo, 17 karate), accounting for 77% of the variance. However, even though the RMSPE of the Cunningham equation was low (433.82 kcal/day) compared with those of other equations except the Mifflin-St. Jeor equation and good based on the variance (68.4%) in female ultra-endurance athletes, the mean difference between measured and predicted values was statistically significant (291.31±332.75, $p<0.05$), and values were accurate in only 20.0% and underpredicted in 66.7% of the athletes in this study. As a result, the Cunningham equation accurately predicted RMR only in male ultra-endurance athletes.

It is known that pathophysiology of ultra-endurance athletes are complicated, and compared to all sports, even other endurance sports, could be defined as one of the most challenging conditions of training durations and race distances compared to all sports, even other endurance sports .These conditions could be reflected to their performance-related measurements like resting metabolic rate. Tthe Mifflin-St. Jeor equation was found to be the most accurate predictive equation in both male and female ultra-endurance athletes with the lowest RMSPE value of 275.85 and 388.34 kcal/day, predicted accurately in 46.6% and 53.3% of subjects, accounting for 52.4% and 88.7% of the variance in this study, respectively. Furthermore, the mean difference between measured and Mifflin-St. Jeor equation values was not significant for both sexes (3.04±285.51 kcal/day for men and 185.57±353.10 kcal/day for women). These results indicated that the Mifflin-St. Jeor equation could be used to predict RMR in both male and female ultra-endurance athletes. In contrast, Thompson and Manore (15) conducted a study on 37 endurance athletes (24 male, 13 female) and demonstrated that the Cunningham equation was the only predictive equation with accurate RMRs in both male and female endurance athletes, while the Mifflin-St. Jeor equation had underpredicted RMRs of endurance athletes. These results indicated that resting metabolic rate could be also varied between endurance and ultra-endurance athletes, therefore, this result should be considered in predicting the resting metabolic rate.

Although, in some cases, the mean difference between measured vs. predicted RMR was not statistically significant, the underestimation or overestimation of energy intake could be important and affect athletes' performance (21). The importance could be varied according to sex, types of sports (especially weight-dependent and weight-bearing sports), and periodization of training (season/off-season) (22). For instance, although the Mifflin-St. Jeor was found to be the best predicted RMR equation in women, the actual RMR is overestimated by 185 kcal/day, and it affects the total energy expenditure (TEE) by approximately 333-425.5 kcal/day as physical activity (PA) level (which was calculated as RMR*PA, PA coefficient is 1.6-2.4 in highly trained athletes) (10). The overestimation of total energy expenditure could have a negative impact on the nutrition program, which is regulated by total energy requirements, therefore more energy consumption might negatively affect sport performance (18).

Several factors had effects on RMR, especially in highly trained athletes. Although the Harris-Benedict equation (8) takes account of several body components such as body mass, height, and age, which have been proved to affect RMR, multiple studies investigated a significant relationship between FFM and measured RMR (11-13). Either FFM or both FM and FFM were utilized in predictive equations developed by Cunningham et al. (11), Wang et al (12), and Sabounchi et al (in Structures 4, 5, and 11) (13). These studies demonstrated that, since free fat is more active than adipose tissue and the best correlated component of RMR, FFM had more influence on energy requirements and could be used as a single predictor in estimating RMR (23-25). In contrast, Carlsohn et al. (18) conducted a study on 17 heavyweight endurance athletes and verified Cunningham and Harris-Benedict equations for use in the athletes and found that they had remarkable FFM (81.0±8 kg for men and 56.1±7.0 kg for women) and measured RMR (2675±526 kcal/day for men and 1577±253 kcal/day for women) and demonstrated that predictive equations underestimated the measured RMR in athletes with high FFM. Similarly, all equations that calculated RMR using FFM or both FFM and FM, with the exception of the Cunningham equations in men, underpredicted RMR in both male and female ultra-endurance athletes (underestimation percentage between 66.7% and 80% for men and between 66.67% and 93.34% for women) in the study. The difference between studies might be caused by study population characteristics, differences in measurements of FFM (via MF-BIA, DXA, or skinfold measurements), or differences in thermic effect of activity (TEA) that could be approximately 50% of TEE in elite endurance athletes (4).

The strengths of this study include actual measurement of RMR using the validated breath using the breath gas analyzer (COSMED K5 metabolic chart), and according to our knowledge, this is the first study investigating which predicted RMR equations could be used in both triathletes and ultra-marathoners. On the other hand, it should be emphasized that the sample size of the pilot study did not sufficient to generate a new predictive equation for ultra-endurance athletes, therefore our study provides the framework for future studies to generate a specific RMR equation for ultra-endurance athletes. Another limitation is that metabolic blood parameters such as thyroid hormones are not examined in this study. These factors, which may potentially have an effect on resting metabolic rate, could be determined in further studies.

In conclusion; the results of this study suggest that the Mifflin-St. Jeor and Cunningham equations for men and the Mifflin-St. Jeor equation for women remain the most accurate predictive equations in ultra-endurance athletes. Despite these findings, the bias measured vs. predicted RMR from 147.68±283.04 kcal/day (Cunningham) for men and 185.57±353 kcal/day (Mifflin-St. Jeor) for women could be considered when determining dietary requirements based on energy needs. Our study is a kind of pilot study and the findings are encouraging, and until future investigations validate or generate a new predictive RMR equation with a larger cohort of ultra-endurance athletes, the findings of this study could be used when predicting RMR in ultra-endurance athletes.

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References

1. Woods AL, Sharma AP, Garvican-Lewis LA, Saunders PU, Rice AJ, Thompson KG. Four weeks of classical altitude training increases resting metabolic rate in highly trained middle-distance runners. Int J Sport Nutr Exerc Metab 2017; 27(1):83-90.
2. Costa RJS, Swancott AJM, Gill S, et al. Compromised energy and macronutrient intake of ultra-endurance runners during a multi-stage ultra-marathon conducted in a hot ambient environment. Int J Sport Sci 2013;3(2):51-62.
3. Woods AL, Rice AJ, Garvican-Lewis LA, et al. The effects of intensified training on resting metabolic rate (RMR), body composition and performance in trained cyclists. PLoS One 2018;13(2):e0191644.
4. 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):501-28.
5. Compher C, Frankenfield D, Keim N, Roth-Yousey L. Best practice methods to apply to measurement of resting metabolic rate in adults: a systematic review. J Am Diet Assoc 2006;106(6):881-903.
6. Flack KD, Siders WA, Johnson L, Roemmich JN. Cross-validation of resting metabolic rate prediction equations. J Acad Nutr Diet 2016;116(9):1413-22.
7. Frankenfield D, Roth-Yousey L, Compher C. Comparison of predictive equations for resting metabolic rate in healthy nonobese and obese adults: a systematic review. J Am Diet Assoc 2005;105(5):775-89.
8. Harris JA, Benedict FG. A biometric study of human basal metabolism. Proc Natl Acad Sci U S A 1918;4(12):370-3.
9. Mifflin MD, St Jeor ST, Hill LA, Scott BJ, Daugherty SA, Koh YO. A new predictive equation for resting energy expenditure in healthy individuals. Am J Clin Nutr 1990;51(2):241-7.
10. Joint FAO/WHO/UNU Expert Consultation. Energy and protein requirements [Internet]. Geneva; 1985.
11. Cunningham JJ. Body composition as a determinant of energy expenditure: a synthetic review and a proposed general prediction equation. Am J Clin Nutr 1991;54(6):963-9.
12. Wang Z, Heshka S, Gallagher D, Boozer CN, Kotler DP, Heymsfield SB. Resting energy expenditure-fat-free mass relationship: new insights provided by body composition modeling. Am J Physiol Metab 2000;279(3):E539-45.
13. Sabounchi NS, Rahmandad H, Ammerman A. Best-fitting prediction equations for basal metabolic rate: informing obesity interventions in diverse populations. Int J Obes 2013;37(10):1364-70.
14. Viechtbauer W, Smits L, Kotz D, et al. A simple formula for the calculation of sample size in pilot studies. J Clin Epidemiol 2015;68(11):1375-9.
15. Thompson J, Manore MM. Predicted and measured resting metabolic rate of male and female endurance athletes. J Am Diet Assoc 1996;96(1):30-4.
16. Jagim AR, Camic CL, Kisiolek J, et al. Accuracy of resting metabolic rate prediction equations in athletes. J Strength Cond Res 2018;32(7):1875-81.
17. ten Haaf T, Weijs PJM. Resting energy expenditure prediction in recreational athletes of 18-35 years: confirmation of Cunningham equation and an improved weight-based alternative. PLoS One 2014;9(9): 11-21.
18. Carlsohn A, Scharhag-Rosenberger F, Cassel M, Mayer F. Resting metabolic rate in elite rowers and canoeists: difference between indirect calorimetry and prediction. Ann Nutr Metab 2011;58(3):239-44.
19. Joseph M, Gupta R Das, Prema L, Inbakumari M, Thomas N. Are predictive equations for estimating resting energy expenditure accurate in Asian Indian male weightlifters? Indian J Endocrinol Metab 2017;21(4):515-9.
20. De Lorenzo A, Bertini I, Candeloro N, Piccinelli R, Innocente I, Brancati A. A new predictive equation to calculate resting metabolic rate in athletes. J Sports Med Phys Fitness 1999;39(3):213-9.
21. Rodriguez NR, DiMarco NM, Langley S, American Dietetic Association, Dietitians of Canada, American College of Sports Medicine: Nutrition and Athletic Performance. Position of the American Dietetic Association, Dietitians of Canada, and the American College of Sports Medicine: Nutrition and athletic performance. J Am Diet Assoc 2009;109(3):509-27.
22. Burke L. Practical sports nutrition. Human Kinetics; 2007; 531-548.
23. Johnstone AM, Murison SD, Duncan JS, Rance KA, Speakman JR. Factors influencing variation in basal metabolic rate include fat-free mass, fat mass, age, and circulating thyroxine but not sex, circulating leptin, or triiodothyronine. Am J Clin Nutr 2005;82(5):941-8.
24. Müller MJ, Bosy-Westphal A, Kutzner D, Heller M. Metabolically active components of fat-free mass and resting energy expenditure in humans: recent lessons from imaging technologies. Obes Rev 2002;3(2):113-22.
25. Juzwiak CR, Winckler C, Joaquim DP, Silva A, de Mello MT. Comparison of measured and predictive values of basal metabolic rate in brazilian paralympic track and field athletes. Int J Sport Nutr Exerc Metab 2016; 26(4):330-7.

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The effect of healthy life approaches applied to families of children in preschool on obesity and healthy life behaviour

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Summary: *Aim:* The aim of this study was to determine the prevalence and associated factors of obesity and being overweight in 3-5 year-old children attending public kindergarten and develop healthy lifestyle behaviour by applying supportive healthy lifestyle approaches to families with overweight or obese children. *Method:* The study was composed of two parts: cross-sectional and interventional. The population for the cross-sectional part was 191 children aged between 3-5 and registered to kindergarten, along with and their families. The intervention group accounts for 23 mothers of overweight or obese children diagnosed in the cross-sectional part. Interventions contain counselling interviews, brochures, and letter deliveries showing the weights of their children. Data before and after the intervention were compared. *Results:* The prevalence of being overweight or obese was 27.2%. It was found out that being overweight or obese was associated with the children's sex (p=0.034), the mothers' educational status (p=0.043) as well as the children's breakfast habits (p=0.009). Following the intervention, positive results were acquired relating to the children's weight outcomes, their daily average TV, DVD watching duration, and parental behaviour regarding nutrition (p<0.05). *Conclusion:* It was shown that family-based approaches aiming at the development of healthy lifestyle behaviour in children are effective in preventing and treating obesity.

Key words: being overweight, obesity, preschool period, family-centered intervention

Introduction

Childhood obesity with a progressively increasing frequency in recent years is one of the most important reasons of chronic childhood disease in both developed and developing countries (1). Obesity is seen in every age group and mostly observed in the first years of life, between 5-6 years of age and puberty period due to rapid fat storage. It is known that obesity commencing before the age of 5 and after 15 has more hazardous effects up to adulthood (2). It is not possible to clarify the increase in childhood obesity seen worldwide with only alterations in the genetic structure. Therefore, it is approved that the role of environmental factors are of major priority in the formation of obesity (3).

Childhood obesity poses an important risk for adulthood obesity, on the other hand, it is also risky for many severe chronic diseases leading to early deaths and long term morbidity (1). Obesity in children also induces a substantial decrease in the quality of life, social exclusion, loss of self confidence, reduction in school success, avoidance of peer relations, and even depression and anxiety (4,5). Also, early childhood is particularly important in terms of positive life behaviour acquisition that will continue in the future. Therefore, protection from obesity should proceed from the perinatal period through the entire life span (6).

Families and especially mothers play a very important role in being an exemplar for their children in the pre-school period. Owing to cultural features in

our country, mothers are primarily responsible for the care of pre-school children. Accordingly, family support should be provided for the adoption of healthy lifestyles and effective attempts aiming at the prevention of obesity (7). While there are studies in many countries that indicate the effect of family-centered attempts intending for the acquisition of healthy lifestyle behaviour in pre-school children, there are no studies regarding the issue in question in Turkey (8–13).

The aim of this study is to determine the prevalence and associated factors of obesity and being overweight in 3-5 year-old children attending public kindergarten in 2014-2015 education period in Balçova, Izmir and develop healthy lifestyle behaviour by applying supportive healthy lifestyle approaches to the families of overweight or obese children and evaluate results of the approaches in question.

Methods

Subjects and setting

The study was composed of two parts. The first part was a cross-sectional study and the second was interventional. A total of 191 children aged 3-5 and registered to a public kindergarden in Balçova during the education term of 2014-2015, along with their families accounted for the population of the cross-sectional study. It was aimed to reach the whole population.

The interventional group was composed of 52 mothers of children aged between 3-5 who were either overweight or diagnosed as obese as a result of the cross-sectional research. No control group was involved in the study, the data before and after the intervention were compared in the same group. In overweight or obese children, one-sided decrease in BMI was predicted to be at medium-level and by taking effect-size as 0.50, it was estimated that at least 50 mothers with 80% power should be involved in the interventional group (14).

Ethics committee approval was obtained on the date of 24.07.2014 and 2014/25-15 numbered decision of Dokuz Eylul University Non-Invasive Clinical Research Ethics Committee.

Data collection

Cross-sectional part

Weight and height measurements of 191 children in kindergarden were carried out by a researcher in October and overweight and obese children were determined. In addition, data collection forms and informed consent forms were sent to the families prior to the intervention, and at first; mother, or father in the absence of mother, or in the absence of parents; the person responsible for the care of the child was asked to fill in the forms. Families who did not return the forms were called and the forms were sent again. In conclusion, data collection forms of 146 children were returned and the attainment rate was 76.4%.

Interventional part

The mothers of all overweight and obese children were called and they were informed about the interventional part of the research. As a consequence, 23 out of 52 mothers of overweight or obese children accepted to take part in the research. Every mother was interviewed 4 times in 6 months between November 2014 and April 2015. Throughout the interventions; 5 mothers withdrew from it willingly, hence the research was completed with 18 mothers (34.6%). Post-intervention data collection forms were also filled in by the mothers who took part in the last interviews in April. Weight and height measurements of all children were taken by the same researcher in the last week of April. The flow chart of the research is shown in Figure 1.

Variables of the study

Cross-sectional part

The dependent variable was the condition of a child being overweight or obese. Independent variables were age and sex of children, a chronic disease or condition of the child, mother's age, mother's BMI, parents' marital status, mother's working condition, educational level of parents, father's occupation, income perception, presence of an individual room for the child, having a personal computer/tablet pc, children's TV, DVD, and computer usage duration, nutri-

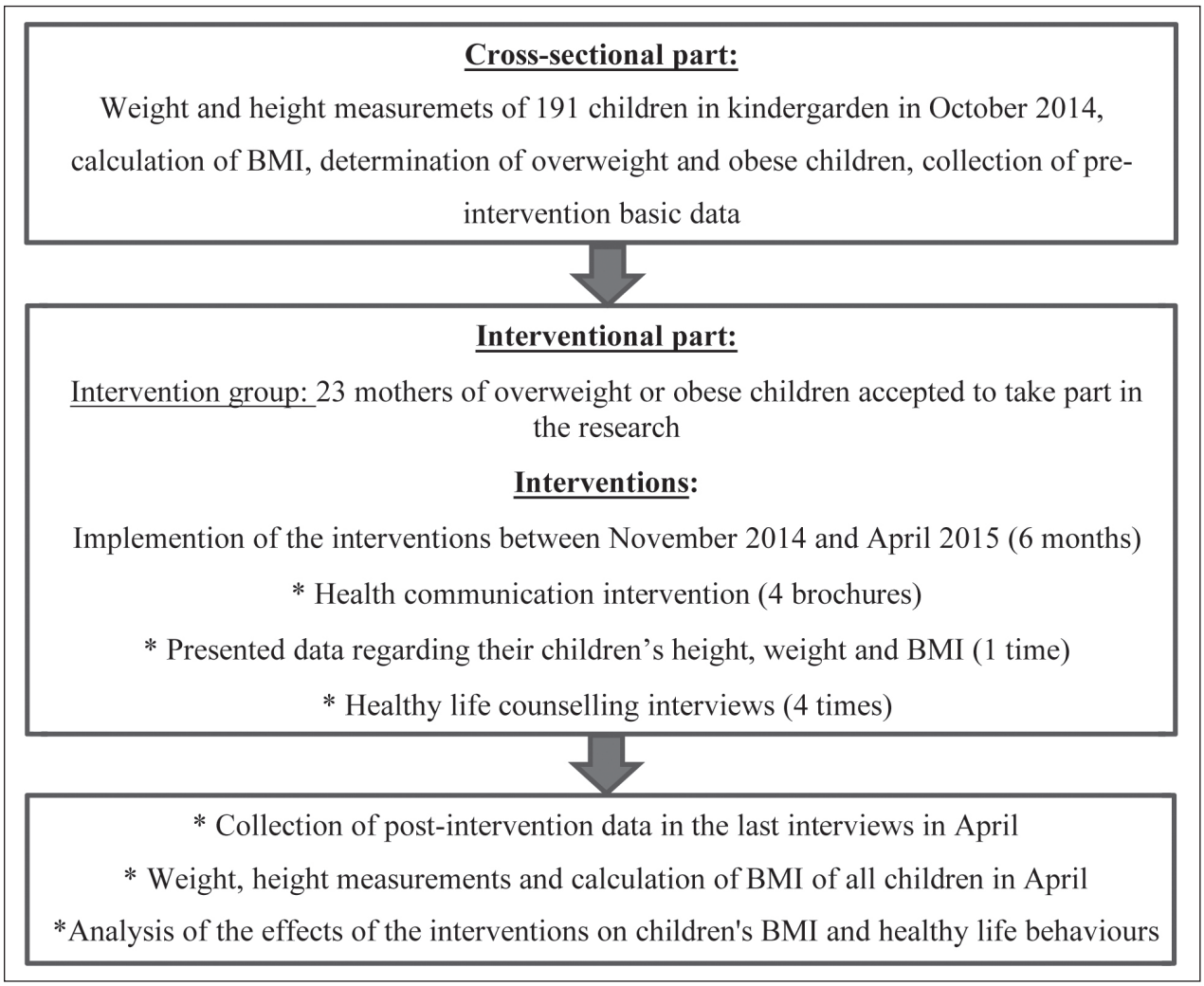

Figure 1. The flow chart of the research

tional behaviours of children, parents' behaviours related to; physical activity, screened devices, and nutrition.

Interventional part

Result variables were children's BMI percentile value and BMI z score, children's TV, DVD watching and computer using duration, their nutritional behaviours, and parents' behaviours related to physical activity, screened devices and nutrition.

Measures

Children's BMI percentile value, BMI z score and BMI of mothers

Weight and height measurements were obtained using standardised techniques before and after intervention. BMI percentile values and BMI z scores of weight and height measurements of children were calculated by using WHO Antro Version 3.2.2 programme; overweight and obese children were identified (15). While research findings were being presented, evaluations were carried out according to the BMI percentiles being utilized.

Weight and height values of mothers were registered according to their statements, and their BMI

(kg/m^2) was calculated. The BMI groups of mothers were determined based on WHO BMI categorization (16).

TV, DVD watching and computer usage durations of children

TV, DVD watching and computer usage durations of children were examined for 24 hours during weekdays and weekends before and after intervention. They were registered in the form of minutes. Duration spared for weekdays were multiplied by 5 and weekends by 2 and the obtained value was divided by 7. The acquired daily average TV, DVD watching and computer usage duration was thus determined.

Nutritional behaviours of children

Children's attitudes regarding having breakfast, lunch, dinner, the frequency of snacking and picky eating habits were collected.

Parental behaviours associated with healthy lifestyles

A total of 20 questions were compiled from the literature. Four of these questions were regarding the facilitation of physical activity of children. We examined families with various parental behaviours associated with physical activity, before and after intervention. Twelve of these questions were regarding parental behaviours concerning nutrition, while 4 questions probed parental behaviours related to screened devices (7,9,11–13).

Components of the Intervention

Health communication intervention

A total of four brochures promoting healthy diet and active life were prepared to be given at every interview. The aim of this intervention was to increase mothers' awareness of healthy life behaviours and confront the notion that fat children are healthy. Each brochure addressed a different issue: "What is obesity?", "Healthy eating and healthy diet examples", "Active life and exercise examples for little children" and "Monitoring sceen time". The focus was to create a parallel between the content of the brochures and the issue involved.

Disclosing children's height, weight and BMI

Letters dwelling on their children's percentiles were given to mothers, while discussing how weight, height and BMI measurements were going to be interpreted. The letters were presented to the mothers in the first interview and were evaluated together with the researcher.

Healthy life counselling interviews

Interviews were conducted with mothers, on a face-to-face basis, who agreed to participate in the study four times in total between November 2014 and April 2015 during 6 months. Solutions were recommended to mothers with regard to the problems determined specifically for their child, and established a set of targets to be completed prior to the next interview. In the first interview; recommendations were made on healthy diet and healthy diet preparation and healthy diet examples were presented. In the second interview; recommendations for active life and exercise for little children were provided. The third interview addressed time control issues for technological devices such as TVs, computers, tablet computers, and cell phones. In the last interview, an overall evaluation of the prior three interviews was carried out while respective feedback was recorded.

Statistical Analysis

For the cross-sectional study, chi-square and Fisher's exact tests were used for categorical variables in the comparison of overweight or obese and non-obese children. For continuous variables, independent-sample t test were utilized. Logistic regression analysis was carried out by forming a model with variables such as "child's frequency of having breakfast", "child's daily average TV, DVD watching duration" which were found significant in the univariate analyses. In addition, variables established as crucial in the literature such as

"child's sex", "mother's education level and employment status", "income perception" were also included.

In the interventional study; McNemar tests were used with the categorical variables to assess the amount of change that occurred before and after the intervention. For continuous variables, Wilcoxon signed rank test was utilized. In addition, results were re-evaluated by performing intention-to-treat analysis in order to minimize the influence of those who withdrew from the research.

Results

Cross-sectional part

Weight and height measurements of all children (n=191) in kindergarten were carried out, in order to establish the prevalence of being overweight and obese. Of the 191 families who were sent forms, 146 (76.4%) families responded, thus accounting for the sample size used in this study (n=146).There was no significant difference between the children of families responding to the questionnaire, as opposed to non-respondence in terms of the children's sex, age group and BMI group (p>0.05).

Regardless of percentile curves and z scores, the prevalence of being overweight or obese was 27.2% in total. In children whose data collection forms were returned (n=146), the prevalence of being overweight or obese was 29.5% in total regardless of their percentile curves and z score.

The average age of the children was 3.9 ± 0.8 with 56% of them being female. 87% of the children had a private room and one third of them had personal/tablet computers. Prevelance of chronic disease in the children were 4% (n=6). When families' characteristics were examined, more than one third of mothers and 80% of fathers were university graduates or had master/doctoral degrees. The percentage of mothers and fathers who were married and living together was 96%. The average age of mothers was 33.4 ±5.1, while 40% of them were employed. One in every three was overweight, obese or morbidly obese (Table 1).

The variables that were not associated to a large extent with the children being overweight or obese (p>0.05) included the children's sex, age, family features, and whether they had a private room, a computer/tablet computer, and chronic diseases.

In overweight or obese children, daily average TV, DVD watching duration was significantly higher than those without overweight or obese conditions (p=0.033), and this difference was due to a distinction in their weekday TV or DVD watching duration (p=0.029). There was no significant difference between

Table 1. Characteristics of the families

Characteristics (n=146)	**n**	**%**
Educational level of mother		
Elementary school	24	16.4
High school	67	45.9
University or master/doctorate degrees	55	37.7
Educational level of father		
Elementary school	30	20.6
High school	56	38.3
University or master/doctorate degrees	60	41.1
Marital status of parents (n=145)*		
Mothers and fathers were married and lived together	140	95.9
Mothers and fathers were divorced	6	4.1
Working condition of mother		
Employed	58	39.7
Unemployed	88	60.3
Father's job (n=145)*		
Unemployed	2	1.4
Blue-collar worker	49	33.8
White-collar worker	49	33.8
Artificer	42	28.9
Agricultural worker	2	1.4
Retired	1	0.7
Income perception		
Very good	1	0.7
Good	41	28.1
Middle	101	69.2
Bad	3	2.0
Mother's BMI group		
Weak	5	3.4
Normal	94	64.4
Overweight	37	25.3
Obese/ Morbidly obese	10	6.9

**One of the fathers was dead.*

overweight or obese children and those who were not, in terms of their daily average computer/tablet computer usage duration (p=0.805).

When nutritional behaviours were analyzed, the frequency of being overweight or obese was significantly low in children having daily breakfast, compared to those who were not (p=0.007). Having lunch, dinner, the frequency of snacking and picky eating habits were not associated with being overweight or obese (p>0.05).

Parental behaviours related to physical activity, screened devices and nutrition were not associated with being overweight or obese (p>0.05).

Logistic regression analysis was performed by forming a model with variables such as "child's frequency of having breakfast", "child's daily average TV, DVD watching duration" that were found significant in the univariate analyses. In addition, variables "child's sex", "mother's education level and employment status", "income perception" were found crucial according to the literature. The risk of being overweight or obese were 2.4 times more in boys compared to that of girls (p=0.034, 95% CI=1.1-5.3). It was discovered that the risk of being overweight or obese was 2.5 times more in children of mothers with high school or lower education level compared to those with university or master/doctorate degrees (p=0.043, 95% CI=1.1-6.1); the risk involved was 7.9 times higher in children with no breakfast habits or those with rare breakfast consumption (p=0.009, 95% CI=1.7-36.6) (Table 2).

Interventional part

18 mothers of 23 overweight or obese children agreed to participate in the interventional research. The characteristics of mothers who agreed to be involved (n=23) and who did not agree to take part in the interventional study (n=20) were compared. Obesity and unemployment frequency of mothers who agreed to join the research compared to those who did not participate were found significantly higher (respectively p=0.037, p=0.023).

Table 2. Risk factors associated with being overweight and obese in children

Characteristics (n=146)	**OR**	**%95 CI**	**p**
Sex			
Female	1.0		
Male	2.4	1.1-5.3	**0.034**
Educational level of mother			
University or master/doctorate degrees	1.0		
High school or lower education level	2.5	1.1-6.1	**0.043**
Working condition of mother			
Unemployed	1.0		
Employed	2.2	0.9-4.9	0.067
Income perception			
Very good, good	1.0		
Middle, bad	0.5	0.2-1.2	0.129
Children's frequency of having breakfast			
Every day	1.0		
Never or sometimes	7.9	1.7-36.6	**0.009**
Children's daily average TV, DVD watching duration	**1.0**	**0.9-1.0**	**0.163**
Constant: B= -2.460 p<0.001 Exp(B)=0.085			

Model: Sex, educational level of mother, working condition of mother, income perception, children's frequency of having breakfast, children's daily average TV, DVD watching duration

Table 3. Differences in children's measurements before and after the intervention

						Intention to treat			
		n	**Before**	**After**	**p***	**n**	**Before**	**After**	**p***
Weight of children	Mean ±SD	18	23.3±4.0	24.9±4.4	**0.001**	23	23.2±3.9	25.2±4.5	**0.001**
	Median	18	23.3	24.4		23	22.7	24.6	
Height of children	Mean ±SD	18	112.2±7.2	116.7±7.2	**0.001**	23	112.1±6.8	117.1±6.6	**0.001**
	Median	18	112.5	117.0		23	112.0	117.5	
BMI percentiles	Mean ±SD	18	94.3±4.7	91.4±9.8	**0.026**	23	94.2±4.8	90.9±10.1	**0.021**
	Median	18	96.5	95.1		23	96.3	95.1	
BMI z scores	Mean ±SD	18	1.9±0.9	1.8±1.0	0.061	23	1.9±0.9	1.8±1.1	0.127
	Median	18	1.8	1.7		23	1.8	1.7	

** Wilcoxon signed rank test was used.*

Of the children in the interventional group, 39% were girls, and 61% were boys. The average age of children was 3.9±0.6 and their median age was 4.0. Mean and median percentile values of children reduced significantly in the post-intervention, compared to values prior to intervention (p=0.026). When the results were re-assessed by performing intention-to-treat analysis, significance continued (p<0.05).

Children's daily TV, DVD watching duration decreased significantly in the post-intervention compared to that of pre-intervention (p=0.010), and this decrease was due to the reduction of watching duration over the weekend (p=0.002). When the results were re-assessed by performing intention-to-treat analysis, significance continued (respectively, p=0.016 and p=0.002) (Table 4).

No significant difference was determined in terms of the children's nutritional behaviours, and parental behaviours related to physical activity and screened device usage before and after the intervention (p>0.05). Among the nutritional behaviours of parents, 'giving permission to consume fast food (hamburger, pizza, fried potatoes etc)' and 'frequency of rewarding children with foods and beverages such as dessert, chocolate, coke' decreased significantly after intervention compared to pre-intervention (respectively, p=0.031 and p=0.016). When the results were re-evaluated by performing intention-to-treat analysis, significance continued (respectively, p=0.031 and p=0.016) (Table 5).

Discussion

Cross-sectional part

In all children (n=191) whose measurements were taken, the prevalence of being overweight or obese was identified as 27.2%. In boys, the risk of being overweight or obese was determined to ocur 2.4 times more frequently when compared to girls. Similar to the results of this research, Turkey Nutrition and Health Research established the prevalence of being overweight or obese as 26.4% in the 0-5 age group, boys being higher than girls (17). The higher frequency of obesity and being overweight in boys may be attributed to the differences in weight perception and intersexual care in society. In the local studies carried out in Turkey, the prevalence of being overweight or obese changed between 9-55% in pre-school children (18–29). Since the majority of these studies were conducted in very small groups, they would not be representative of the general Turkish population.

The risk of being overweight and obese was found to be 2.5 times higher in children of mothers with high school or lower education levels, compared to those with university or master/doctorate degrees. Contrary to the outcomes of the present research, obesity frequency was determined to increase in children under the age of 5, when mothers' education level and the families' income increases, according to Turkey Demographic and Health Survey (TDHS-2013) (30). In addition, global research indicates that the risk of being overweight or obese in pre-school children all over the world is more

Table 4. Differences in children's daily average TV, DVD watching and computer usage duration before and after the intervention

						Intention to treat			
		n	**Before**	**After**	**p***	**n**	**Before**	**After**	**p***
Children's daily average TV, DVD watching duration (min)	Mean±SD	18	168.2 **±72.**1	122.9 ±60.7	0.010	23	161.8 ±67.5	126.4 ±57.3	0.016
	Median	18	160.7	102.9		23	154.3	102.9	
Weekdays	Mean±SD	18	157.2 ±67.4	122.8 ±60.27	0.101	23	151.7 ±63.22	124.8 ±56.6	0.101
	Median	18	135.0	120.0		23	120.0	120.0	
Weekend	Mean±SD	18	195.6 ±114.2	123.3 ±81.6	0.002	23	186.9 ±105.8	130.4 ±78.7	0.002
	Median	18	195.0	120.0		23	180.0	120.0	
Children's daily average omputer usage duration (min)	Mean±SD	18	52.1 ±68.8	43.8 ±43.2	0.695	23	49.0 ±63.3	42.5 ±41.9	0.649
	Median	18	29.3	44.3		23	25.7	42.9	
Weekdays	Mean±SD	18	51.7 ±75.3	45.3 ±44.7	0.952	23	48.3 ±70.37	43.3 ±45.6	0.952
	Median	18	25.0	60.0		23	20.0	60.0	
Weekend	Mean±SD	18	53.3 ±62.0	40.0 ±46.9	0.282	23	50.8 ±60.1	40.4 ±48.2	0.282
	Median	18	40.0	30.0		23	20.0	30.0	

** Wilcoxon signed rank test was used.*

frequently seen in developed countries compared to developing ones (31). The discrepancy between global research and our present findings may be due to the nature of our sample with regards to income.

In the logistic regression analysis, the link between the children's average daily TV, DVD watching duration and them being overweight or obese lost its significance. In a study conducted by Demir, the frequency of being overweight or obese was determined to increase 2.2 times in children watching TV for a long duration, compared to those who were not (26). In international studies, spending time in front of devices such as TVs and computers was also indicated to have a correlation with being overweight and obese in pre-school children (32–34). Today, it has become prevalent to accept "spending time in front of a screen" as an indicator of a kind of sedentary life activity (35,36).

The risk of being overweight and obese was established to be 7.9 times more in children never or sometimes having breakfast, compared to those having breakfast regularly. International studies indicate that there was a relation between eating regularly and weight control. The risk of being overweight or obese was significantly higher especially in children missing breakfast, supporting the findings of this study (37–40). No significant relationship was found between parents' behaviours regarding physical activity, time spent with screened devices, dietary habits or the children being overweight and obese. Discrepancies between global findings and the present research may be a result of participants unable to evaluate their own behaviours objectively, which could give rise to high positive behaviour frequencies in all children. In this study, 76% of families think that they are a good role model for their children. Families' perception regarding their children's weight is mostly inadequate. This inability for objectivity may be preventing them to evaluate their own behaviours. In systematic reviews, the behaviours of families were closely related to children's healthy life behaviours and weight. It was

Table 5. Differences in parental behaviours related to nutrition before and after the intervention

	n=18					Intention to treat n=23				
	Before		After			Before		After		
	n	%	n	%	p*	n	%	n	%	p*
Parental behaviours related to nutrition										
How confident are you that you can offer enough fruit to your child?										
Not confident/No idea	7	38.9	4	22.2		8	34.8	5	21.7	
Confident/ Very confident	11	61.1	14	77.8	0.250	15	65.2	18	78.3	0.250
How confident are you that you can offer enough vegetable to your child?										
Not confident/No idea	7	38.9	4	22.2		10	43.5	7	30.4	
Confident/ Very confident	11	61.1	14	77.8	0.250	13	56.5	16	69.6	0.250
How confident are you that you can offer fat-free or low-fat foods to your child?										
Not confident/No idea	8	44.4	3	16.7		10	43.5	5	21.7	
Confident/ Very confident	10	55.6	15	83.3	0.125	13	56.5	18	78.3	0.125
How often do you offer fresh fruits and vegetables to your child at meals and for snacks?										
Less than once a day	7	38.9	5	27.8		11	47.8	9	39.1	
≥ 1 time / day	11	61.1	13	72.2	0.625	12	52.2	14	60.9	0.625
How often do you offer meat/fish/egg to your child at meals and for snacks?										
Less than once a day	13	72.2	11	61.1		18	78.3	16	69.6	
≥ 1 time / day	5	27.8	7	38.9	0.625	5	21.7	7	30.4	0.625
How often do you offer milk and milk products to your child at meals and for snacks?										
Less than once a day	5	27.8	4	22.2		7	30.4	6	26.1	
≥ 1 time / day	13	72.2	14	77.8	1.000	16	69.6	17	73.9	1.000
How often do you offer canned or frozen fruit/ vegetables to your child at meals and for snacks?										
Never	16	88.9	17	94.4		20	87.0	21	91.3	
≥ 1 time / week	2	11.1	1	5.6	1.000	3	13.0	2	8.7	1.000
How often do you give permission to consume fast food (e.g. hamburger, pizza, fried potatoes, etc) to your child?										
Never	9	50.0	15	83.3		13	56.5	19	82.6	
≥ 1 time / week	9	50.0	3	16.7	0.031	10	43.5	4	17.4	0.031
How often do you give permission to consume foods and beverages such as dessert, chips, chocolate, coke to your child?										
Never	2	11.1	5	27.8		2	8.7	5	21.7	
≥ 1 time / week	16	88.9	13	72.2	0.250	21	91.3	18	78.3	0.250
Do you reward your child with foods and beverages such as dessert, chips, chocolate, coke?										
Never	9	50.0	16	88.9		12	52.2	19	82.6	
≥ 1 time / week	9	50.0	2	11.1	0.016	11	47.8	4	17.4	0.016
How often do you eat your meals with all of your family?										
Never or Sometimes	7	38.9	9	50.0		9	39.1	11	47.8	
Every day	11	61.1	9	50.0	0.625	14	60.9	12	52.2	0.625
Do you think that you are a good role model for your children?										
Yes	11	61.1	14	77.8		16	69.6	19	82.6	
No	7	38.9	4	22.2	0.250	7	30.4	4	17.4	0.250

shown that interventions aimed at correcting family behaviours through education were effectively reducing BMI of children (40,41).

Interventional part

Obesity and unemployment frequencies were found significantly higher in mothers who agreed to participate in the study, compared to non-participant mothers. This difference may be the result of the obese mothers being more sensitive to the subject, and the unemployed mothers having the convenience to participate due to their lack of occupational time constraints.

While mean and median percentile values of children significantly decreased after intervention compared to pre-intervention, no significant reduction was determined in terms of z score mean and median. In a study carried out in 154 children and their families by Davidson et al., although there was no significant change in the z score of children after 6 months of family-centered intervention, obesity prevalence significantly reduced (13). In a family-centered interventional study performed with 85 children and their families by Bocca et al., z score means of children significantly decreased at the end of 16 weekends. The results were re-evaluated after 12 months, reduction was seen to be more distinct (8). In a systematic review where interventions aiming at preventing and treating obesity in children were analysed, family-centered and long-term follow-up programmes were indicated to be more effective (40).

Children's average daily TV, DVD watching duration decreased significantly after intervention, compared to that of pre-intervention and this decrease was due to the reduction in the weekend watching duration. According to implications obtained from interviews, the more children stay at home, the more they spend time in front of the TV. Therefore, one of the targets determined was to spend more time outdoors and actively during the weekends. Most of the families tried to implement this target and thus daily average TV, DVD watching duration was reduced significantly over the weekends. In a study carried out by Davidson et al., daily average TV watching duration decreased significantly at the end of 6 months (13). In Healthy Habits, Happy Homes study, there was no significant change in TV watching duration overall and weekdays, after 6 months of intervention. However, weekend TV watching duration significantly declined (42).

No significant difference was determined in any of the components of parental behaviour concerning physical activity before and after the intervention. In the interviews, it was established that the place of residency, the environmental planning, and the season are vital. Children living in housing complexes spend more time with activities such as riding bicycles and playing in playgrounds. Mothers also said that their financial situation was also important since many courses are so costly. In the literature, family-centered interventions have been implied to increase the frequency of physical activity, and thus account for good changes in the weight results (13,43).

In all components of parental behaviours associated with screened devices, no significant difference was identified in post-intervention when compared to pre-intervention. Although mothers stated that they limited the watching duration of TVs and computers, the rate of mothers who could set precise limitations was very low according to impressions obtained after the interviews. Differences in parental attitudes toward their children, and the inability of the parents to change their inappropriate behaviour, as well as family elders' different behaviours toward their children make obtaining positive behaviour difficult for children in general.

Of the parental behaviours regarding nutrition, the frequency with which permission was granted for fast-food consumption, as well as the frequency of rewarding children with beverages and foods such as desserts and chocolates by their mothers decreased significantly. In a study carried out by Daniels et al., the frequency of parents using foods as reward after the intervention decreased (44). During the whole intervention period, "reward concept" in terms of both nutrition and other life behaviours was particularly emphasized. It was stressed that no harmful food or behaviour should be used as a reward.

One of the most important limitations of this research was the small sample size. The fact that participant interest was very minimal, may have impacted data collection, which was largely subjective apart from the weight and height of children, which could have caused data collection bias. Only 23 mothers agreed to participate in the interventional research and 5 of them with-

drew from the study during intervention for a variety of reasons. Although appointments were given according to the mothers' schedules, there were too many cancellations. When interview rhythm was interrupted, and duration between two interviews increased, the implementation of targets was also observed to decline.

The powerful side of the research was that it was the first family-centered interventional research in Turkey aimed at development of healthy life behaviours for pre-school children. The research was novel in a sense that data collection, measurements and interventions were carried out by the same researcher, thus were standardized. Despite the restrictions, having positive healthy outcomes within such a small group and within such a short period of time, supports the idea that family-centered interventions were effective.

Conclusion

One in every three children in the research group was determined to be overweight or obese. It was found out that the risk of children being overweight or obese was associated with their sex, mother's educational status and children's breakfast habits. Following the intervention, positive results were acquired related to children's weight outcomes, their daily average TV, DVD watching duration, and some parental behaviours regarding nutrition in a short period of time. On the other hand, sustainability of these positive health results are important and merit further research and follow-up. Individual efforts do not suffice in maintaining the continuity of the research. More importantly, organizations which involve schools, families and primary healthcare institutions should be facilitated. In this context, nutrition-friendly school projects that include families should be promoted. In summary, prevention of childhood obesity should be identified as a serious social issue, and tackled at a level of domestic policy.

References

1. WHO. Set of recommendations on the marketing of foods and non-alcoholic beverages to children, 2010. http://apps.who.int/iris/bitstream/10665/44416/1/9789241500210_eng.pdf Access date: 20.07.2014.
2. Köksal G, Özel HG. Obesity in preschool period. Ministry of Health publication no:729. Ankara; 2012.
3. Repulic of Turkey Ministry of Health Turkish Public Health Instution. Healthy Nutrition and Active Life Program of Turkey, 2013-2017. Publication no: 773. Ankara; 2013.
4. WHO. Population-based approaches to childhood obesity prevention, 2012. http://www.who.int/dietphysicalactivity/childhood/WHO_new_childhoodobesity_PREVENTION_27nov_HR_PRINT_OK.pdf Access date: 20.07.2014.
5. Esin K, anlıer N. Psychosocial effects of childhood obesity. Journal of Nutrition and Dietetics. 2012;40(2):149–54.
6. De Onis M, Blössner M. Prevalence and trends of overweight among preschool children in developing countries. Am J Clin Nutr. 2000 Oct;72(4):1032–9.
7. Taveras EM, McDonald J, O'Brien A, Haines J, et al. Healthy Habits, Happy Homes: methods and baseline data of a randomized controlled trial to improve household routines for obesity prevention. Prev Med. 2012;55(5):418-26.
8. Bocca G, Corpeleijn E, Stolk RP, Sauer PJJ. Results of a multidisciplinary treatment program in 3-year-old to 5-year-old overweight or obese children: a randomized controlled clinical trial. Arch Pediatr Adolesc Med. 2012 Dec;166(12):1109–15.
9. Adams AK, LaRowe TL, Cronin KA, Prince RJ, Wubben DP, Parker T, et al. The Healthy Children, Strong Families intervention: design and community participation. J Prim Prev. 2012 Aug;33(4):175–85.
10. Ball GDC, Ambler KA, Keaschuk RA, Rosychuk RJ, Holt NL, Spence JC, et al. Parents as agents of change (PAC) in pediatric weight management: the protocol for the PAC randomized clinical trial. BMC Pediatr. 2012;12:114.
11. Duncanson K, Burrows T, Collins C. Study protocol of a parent-focused child feeding and dietary intake intervention: the feeding healthy food to kids randomised controlled trial. BMC Public Health. 2012;12:564.
12. Gerards SMPL, Dagnelie PC, Jansen MWJ, van der Goot LOHM, de Vries NK, Sanders MR, et al. Lifestyle Triple P: a parenting intervention for childhood obesity. BMC Public Health. 2012;12:267.
13. Davison KK, Jurkowski JM, Li K, Kranz S, Lawson HA. A childhood obesity intervention developed by families for families: results from a pilot study. Int J Behav Nutr Phys Act. 2013;10:3.
14. Portney LG, Watkins MP. Foundations of clinical research: applications to practice. 3rd ed. Upper Saddle River, N.J: Pearson/Prentice Hall; 2009.
15. WHO. Child growth standards. WHO Anthro (version 3.2.2, January 2011) and macros. http://www.who.int/childgrowth/software/en/ Access date: 30.08.2015.
16. WHO. Body mass index - BMI. http://www.euro.who.int/en/health-topics/disease-prevention/nutrition/a-healthy-lifestyle/body-mass-index-bmi Access date: 30.08.2015.
17. Repulic of Turkey Ministry of Health General Directorate of Health Research, Hacettepe University Faculty of Health Sciences Department of Nutrition and Dietetics, Ankara Numune

Training and Research Hospital. Turkey Nutrition and Health Survey-2010: Result report for assessment of nutritional status and habits. Publication no: 931. Ankara; 2014.
18. Eksen M, Eksen S, KarakuŞ A. Examination of blood pressure, weight, height, body mass index values and obesity and hypertension status in kindergarten students in Mugla central primary schools. International Journal of Human Sciences. 2004;1(1):1–9.
19. Erkan T, Yalvaç S, Erginöz E, ÇokuŞaŞF, Kutlu T. The evaluation of nutritional status of children by anthropometric measurements attending the day nursery of CerrahpaŞa Medical School. Turk Arch Ped. 2007;42(4):142–7.
20. Yalvaç S, Erkan T, Erginöz E, ÇokuŞaŞF, Kutlu T. The evaluation of nutritional status of children, by anthropometric measurements, living in day nursery of Bahçelievler. Turk Arch Ped. 2008;43(3):89–93.
21. Midyat L, AkŞit S, YaŞcı RV. Evaluation of nutritional status of 2- to 6-year-old children in Izmir. Bulletine of the Ege Pediatrics. 2008;15(3):161–72.
22. Akgün S, Bakar C, Kut A, Kınık ST. Obesity prevalence and the factors affecting obesity among children younger than five years of age admitted to the Pediatric Outpatient Clinics of Baskent University Hospital. Journal of Continuing Medical Education. 2006;15(4):60–6.
23. Yabancı N, imŞek I, ŞtanbulluoĞlu H, Bakır B. The prevalence of obesity and associated factors in a kindergarten in Ankara. TAF Prev Med Bull. 2009;8(5):397–404.
24. OĞuz , Derin DÖ. An investigation of some nutrition habits of 60-72 month-old children Elementary Education Online. 2013;12(2):498–511.
25. Önder ÖZ. Anthropometric evaluation of obesity in preschool children between 3-6 years [Master's Thesis]. [Ankara]: Ankara University Institute of Social Sciences, Department of Anthropology; 2011.
26. Demir . Relationship between overweight and resting/nutritional status of children aged 5 years in Konya province center [Master›s Thesis]. [Konya]: Selcuk University Institute of Health Sciences, Public Health Department; 2012.
27. Kerkez FŞ Tutal V, Akçınar F. Body Image Perceptions and Body Dissatisfaction Among Preschoolers. Hacettepe Journal of Sport Sciences. 2013;24(3):234–44..
28. Edem P. Obesity prevalence and risk factors in 3-6 years old children living in central districts of the metropolitan, Izmir [Specialization thesis]. [Ŝzmir]: Dokuz Eylül University Faculty of Medicine Department of Child Health and Diseases; 2013.
29. Muslu GK, Beytut D, Kahraman A, Yardımcı F, BaŞbakkal Z. Nutritional style of parents and examination of the effective factors. Turk Arch Ped. 2014;49(3):224–30.
30. Hacettepe University Institute of Population Studies. Turkey Demographic and Health Survey-2013. Ankara; 2014. http://www.hips.hacettepe.edu.tr/TNSA_2013_ana_rapor.pdf Access date: 20.08.2015.
31. de Onis M, Blössner M, Borghi E. Global prevalence and trends of overweight and obesity among preschool children. Am J Clin Nutr. 2010 Nov;92(5):1257–64.
32. Lumeng JC, Rahnama S, Appugliese D, Kaciroti N, Bradley RH. Television exposure and overweight risk in preschoolers. Arch Pediatr Adolesc Med. 2006 Apr;160(4):417–22.
33. Mendoza JA, Zimmerman FJ, Christakis DA. Television viewing, computer use, obesity, and adiposity in US preschool children. Int J Behav Nutr Phys Act. 2007;4:44.
34. Kimbro RT, Brooks-Gunn J, McLanahan S. Young children in urban areas: links among neighborhood characteristics, weight status, outdoor play, and television watching. Soc Sci Med 1982. 2011 Mar;72(5):668–76.
35. Berivanlı S, CeritoĞlu K, Bilgin Ç, Bayraktar F, Bulut H, VaizoĞlu SA, et al. Mothers' behaviours about viewing of television and TV ratings system. TAF Prev Med Bull. 2008;7(3):191–8..
36. Akçay D, Özcebe H. Evaluation of computer game playing habits of children at pre-school education levels and their families. J Child. 2012;12(2):66–71.
37. Dündar C, Öz H. Obesity-related factors in Turkish school children. The Scientific World Journal. 2012;2012:353485. doi:10.1100/2012/353485.
38. Geremia R, Cimadon HMS, de Souza WB, Pellanda LC. Childhood overweight and obesity in a region of Italian immigration in Southern Brazil: a cross-sectional study. Ital J Pediatr. 2015;41:28.
39. Brophy S, Cooksey R, Gravenor MB, Mistry R, Thomas N, Lyons RA, et al. Risk factors for childhood obesity at age 5: analysis of the millennium cohort study. BMC Public Health. 2009;9:467.
40. Ortega Anta RM, López-Solaber AM, Pérez-Farinós N. Associated factors of obesity in Spanish representative samples. Nutr Hosp. 2013 Sep;28 Suppl 5:56–62..
41. Upton P, Taylor C, Erol R, Upton D. Family-based childhood obesity interventions in the UK: a systematic review of published studies. Community Pract J Community Pract Health Visit Assoc. 2014 May;87(5):25–9.
42. Haines J, McDonald J, O'Brien A, Sherry B, Bottino CJ, Schmidt ME, et al. Healthy Habits, Happy Homes: randomized trial to improve household routines for obesity prevention among preschool-aged children. JAMA Pediatr. 2013 Nov;167(11):1072–9.
43. Gerards SMPL, Sleddens EFC, Dagnelie PC, de Vries NK, Kremers SPJ. Interventions addressing general parenting to prevent or treat childhood obesity. Int J Pediatr Obes IJPO Off J Int Assoc Study Obes. 2011 Jun;6(2-2):e28–45.
44. Daniels LA, Mallan KM, Nicholson JM, Battistutta D, Magarey A. Outcomes of an early feeding practices intervention to prevent childhood obesity. Pediatrics. 2013 Jul;132(1):e109–118.

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Original article

Is there any link between vitamin D and left atrial diameter in children?

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Summary. Vitamin D (vitD) deficiency has been found associated with rickets/osteomalacia, autoimmune diseases, cardiovascular system diseases, and infectious diseases. Growing evidence demonstrated that vitD has a key role in the renin-angiotensin-aldosterone system. Although there is a strong association between left atrial diameter and serum vitD level in adults, there is scarce data in children. Therefore, we aimed to assess the association between serum vitD level and left atrial diameter in children. We performed a prospective study with 97 children in routine control without any complaint. All patients were evaluated for the echocardiographic evaluation in the outpatients clinic, and the children were divided into two groups as vitamin D deficiency group (vitamin D<20ng/ml) and control group (vitamin D≥20ng/ml). Demographic data, echocardiographic parameters, and serum vitD level were analysed. A p-value of <0.05 was considered significant. The study population included 97 children, and the prevalence of vitD deficiency was found 46.3%. Although left atrial diameter was increased in children with vitD deficiency, there was no significant difference between the two groups with regard to left atrial diameters. Left atrial diameter was significantly associated with BMI z-score (r=0.301), age (r=0.307)(P<0.001), interventricular septum (r=0.209), weight (r=0.184), vitD (r=0.127). On multivariate analysis, BMI z-score was significantly associated with left atrial diameter. BMI z-score is independently associated with left atrial diameter. Although left atrial diameter was increased in patients with vitD deficiency, serum vitamin D level was not found an independently associated with the left atrial diameter.

Key words: vitamin D, left atrial diameter, Body Mass Index

Background

Left atrial (LA) diameter has important prognostic information in the adult population as well as in a variety of cardiovascular disease such as atrial fibrillation, stroke, congestive heart failure, myocardial infarction (1). Vitamin D (vitD) has established roles in calcium and bone metabolism. Vitamin D deficiency was found associated with increased risk of cardiovascular disease, hypertension, obesity, and increased LA diameter (2-12). Additionally, there is a strong association between obesity and LA diameter. In the same manner, the strong association between obesity and LA diameter in children (13,14).

Taking this into account, we aimed to evaluate the association between serum vitD level and LA diameter in children.

Methods

Patients

The study population included 45 children with vitamin D deficiency (<20ng/ml) and 42 children (control group ≥20ng/ml). All children were consecutively recruited from healthy child policlinics.

The medical records of 97 children were evaluated with regard to the association between serum vitD

levels and LA diameter. The epidemiological and demographic data including age, gender, weight, height, body mass index (BMI) were analysed. The exclusion criteria of this study were a history of diabetes, hypertension, inflammatory disease, malignancy, connective tissue disorders or skeletal dysplasia, more than mild valvular stenosis or regurgitation, cardiomyopathy and/or ventricular dysfunction.

Anthropometry

Height was measured with a standard wall-mounted stadiometer to the nearest centimetre, and weight was measured with calibrated electronic scales. BMI was calculated as weight (kg)/height (m)2. BMI z-score, and it's the corresponding percentile were determined by comparison with U.S growth charts from the Centers for Disease Control and Prevention (15).

Echocardiographic evaluation

Echocardiographic evaluations were performed with two-dimensional guided M-mode echocardiography obtained in the parasternal short and long-axis views, in accordance with the recommendations by the American Society of Echocardiography (16).

Left ventricular posterior wall (LVPW) thickness in diastole, interventricular septum (IVS) thickness were obtained by M-mode. Two-dimensional and colour Doppler imaging were routinely performed to diagnose valvular stenosis or regurgitation and other structural defects.

Laboratory studies

Levels of 25-hydroxy (OH) vitD was measured following a fasting period of eight hours. Serum 25-(OH) vitD levels were measured by chemiluminescence immunoassay using a Lıaıson analyser (DiaSorin Inc). VitD deficiency was defined as serum levels of 25-(OH) VitD < 20 ng/ml. The study protocol was approved by the local ethics committee.

Statistical analysis

Statistical analysis was performed with the SPSS version 20.0 for personal computers (Chicago, IL, USA). All continuous variables were tested for normality. Results were expressed as mean±standarddeviation (SD) or as the median. Student T-test or Mann–Whitney U test was used to compare continuous variables and chi-square test for categorical variables. Univariate logistic regression analyses were conducted to identify the independent predictors of left atrial diameter. Multiple linear regression was undertaken with LA diameter entered as dependent variables into separate models; independent variables were determined from the univariate analysis and entered in a step-wise fashion. A p value<0.05 was considered statistically significant in all the calculations.

Results

The study population included 97 children, and the prevalence of vitamin D deficiency was found 46.3%. The two groups were comparable for age, gender, height, left ventricular ejection fraction, systolic and diastolic blood pressure (Table1).

There was a significant difference between two groups with regard to weight, LVPW, IVS, LA diameter, and body mass index z-score (39.7±8.7 vs 30.8±9.1 p<0.001, 6,1±1.3 vs 5.1±0.9 p=0.004, 6.5±1.1 vs 5.1±1.1 p<0.001, 32.9±3.7 vs 27.7±2.9 p=0.002, 2.8±0.39 vs 1.4±0.21 p<0.001; respectively)(Table1).

The univariate regression analyses are shown

Table 1. Characteristics of patients

Patient Characteristics	Serum Vitamin D Level		P
	<20 ng/ml (45)	≥20ng/ml (42)	
Age (years)	8.9±3.1	9.1±2.8	0.531
Female gender, %	46	44	0.729
Weight (kg)	39.7±8.7	30.8±9.1	**<0.001**
Height (cm)	136.4±8.3	134.9±9.1	0.623
BMI z-score	2.8±0.39	1.4±0.21	**<0.001**
Left atrial diameter (mm)	32.9**±3.7**	27.7±2,9	**0.002**
LVPW (mm)	6.1±1.3	5.1±0.9	**0.004**
IVS (mm)	6.5**±1.1**	5.1**±1.1**	**<0.001**
Ejection fraction (%)	69.8**±5.7**	68.7**±4.9**	0.385
SBP (mmHg)	109**±9.4**	107**±8.9**	0.728
DBP (mmHg)	56.7**±5.8**	54.9**±5.1**	0.683

BMI; body mass index, LVPW; left ventricular posterior wall, IVS; interventricular septum, SBP; systolic blood pressure, DBP; diastolic blood pressure

in Table 2. LA diameter was significantly associated with BMI z-score (r=0.301, P<0.001), age (r=0.307, P<0.001), IVS (r=0.209, P<0.001), weight (r=0.184, P<0.001), vitD (r=0.127, P<0.001). On multivariate analysis, BMI z-score was significantly associated with left atrial diameter (Table 3).

Discussion

In our study, we found that BMI z-score is independently associated with the left atrial diameter in children. Although LA diameters were increased in patients with vitD deficiency, serum vitD level was not found an independently associated with the LA diameter.

LA enlargement is associated with an increased risk of cardiovascular diseases including atrial fibrillation, stroke, heart failure, and sudden cardiac death (1-12). Increased BMI is also associated with several cardiovascular diseases (17). In the Framingham Heart Study, BMI was found strongly correlate with LA diameter (18). Additionally, Pritchett et al. (19) demonstrated that BMI was significantly associated with LA diameter, after adjustment for age and gender.

Table 2. Univariate associations with left atrial diameter

Characteristics	Left atrial diameter	p
Age (years),	0.307	**<0.001**
BMI z-score	0.201	**<0.001**
Weight (kg)	0.184	**<0.001**
LVPW (mm)	0.138	0.428
IVS (mm)	0.313	**<0.001**
Vitamin D (ng/ml)	0.127	**<0.001**

BMI; body mass index, LVPW; left ventricular posterior wall, IVS; interventricular septum

Table 3. The relationship between left atrial diameter and age, vitamin D, IVS, and BMI z-score by multivariate analysis.

Predictor Variables	B(standardized coefficient)	p
Age (years)	0.562	**<0.001**
Vitamin D (ng/ml)	0.037	0.237
IVS (mm)	0.051	0.362
BMI z-score	0.462	**<0.001**

BMI; body mass index, IVS; interventricular septum

In the same manner, Daniels et al. (20) found that BMI was a significant predictor of LA diameter in multiple regression models that incorporated height, systolic blood pressure and left ventricular geometry. Hirschler et al. (21) demonstrated significant univariate correlations between the LA area and BMI, waist circumference, blood pressure and a measure of insulin resistance. In the stepwise multivariate analysis, BMI was not tested but in a model incorporating waist circumference, blood pressure, LV mass and HOMA–IR, waist circumference was the only significant independent predictor of LA size. Di Salvo et al. (22) have revealed that obese children had larger LA dimensions and lower atrial strain rate, suggesting an impaired atrial function in this group, in comparison with controls. In our study, we found that BMI z-score was found independently associated with LA diameter.

VitD regulates inflammatory responses and upregulates the expression of anti-inflammatory cytokines, such as interleukin-10, according to in vitro experiments. Also, vitD regulates the activity of the renin-angiotensin aldosterone system (RAAS). Activated RAAS can lead to oxidative stress and inflammation, both of which could culminate in AF. It is assumed that tissue angiotensin II may induce apoptosis of the cardiomyocytes and contribute to changes in atrial structure (23-25).

There is a strong association between vitD and LA diameters in adults. But there is scarce data about the association between vitD and LA diameters in children. In our study, although LA diameters were increased in patients with vitD deficiency, serum vitD level was not found an independently associated with the LA diameter.

Some limitations might be seen in the current study. Firstly, the small sample size. Secondly, we did not determine the parathyroid hormone and calcium levels of the study population.

In conclusion, consistent with previous studies, we found that BMI z-score is independently associated with the LA diameter in children. Although LA diameters were increased in patients with vitD deficiency, serum vitD level was not found an independently associated with the LA diameter. Further studies with a larger number of children are required to clarify the association between serum vitD level and LA diameter.

References

1. Lim TK, Dwivedi G, Hayat S, Majumdar S, Senior R. Independent value of left atrial volume index for the prediction of mortality in patients with suspected heart failure referred from the community. Heart 2009; 95: 1172–8.
2. Cerit L. Vitamin D as a Modifiable Risk Factor for Incident Heart Failure in Atrial Fibrillation. JACC Heart Fail. 2018; 6: 85-6.
3. Cerit L. Cardiac Syndrome X, Vitamin D, and Lipoprotein(a). Heart Lung Circ. 2017; 26: e104.
4. Cerit L. Vitamin D as a Predictor of Postoperative Atrial Fibrillation. Ann Thorac Surg. 2017; 104: 1433.
5. Cerit L, Duygu H. Association of pre-ablation level of vitamin D with atrial fibrillation recurrence after catheter ablation. Europace. 2017; 19: 1586.
6. Cerit L. Bermuda triangle; heart failure, atrial fibrillation, and vitamin D deficiency. J Cardiovasc Med (Hagerstown). 2017; 18: 121.
7. Cerit L. Prediction of thromboembolic events for patients with non-valvular atrial fibrillation; CHA2DS2-VASc-UA2-VitD. Int J Cardiol. 2017; 229: 13.
8. Cerit L. Heart failure and vitamin D receptor gene. Clin Nutr. 2017; 36 :313.
9. Cerit L, Kemal H, Gulsen K, Ozcem B, Cerit Z, Duygu H. Relationship between Vitamin D and the development of atrial fibrillation after on-pump coronary artery bypass graft surgery. Cardiovasc J Afr. 2017; 28: 104-7.
10. Cerit L. Postoperative atrial fibrillation and vitamin D. J Geriatr Cardiol. 2016; 13: 643-4.
11. Cerit L. Vitamin D3 supplementation: Response and predictors of vitamin D3 metabolites - A randomized controlled trial. Clin Nutr. 2016; 35: 1583. Cerit L. Genetic variation in vitamin D receptor gene (Fok1:rs2228570) is associated with risk of coronary artery disease. Biomarkers. 2017; 22: 387.
12. Iacobellis G, Ribaudo MC, Leto G, et al. Influence of excess fat on cardiac morphology and function: study in uncomplicated obesity. Obes Res 2002; 10: 767–73.
13. Chinali M, de Simone G, Roman MJ, et al. Cardiac markers of pre-clinical disease in adolescents with the metabolic syndrome: the strong heart study. J Am Coll Cardiol 2008; 52: 932–8.
14. Kuczmarski RJ, Ogden CL, Guo SS, et al. CDC Growth charts for the United States: methods and development. Vital Health Stat 2002; 11: 1–190.
15. Sahn DJ, DeMaria A, Kisslo J,Weyman A. Recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographic measurements. Circulation 1978; 58: 1072–83.
16. Echahidi N, Mohty D, Pibarot P, et al. Obesity and metabolic syndrome are independent risk factors for atrial fibrillation after coronary artery bypass graft surgery. Circulation 2007; 116: 213–9.
17. Vaziri SM, Larson MG, Lauer MS, Benjamin EJ, Levy D. Influence of blood pressure on left atrial size. The Framingham Heart Study. Hypertension 1995;25:1155–60.
18. Pritchett AM, Jacobsen SJ, Mahoney DW, Rodeheffer RJ, Bailey KR, Redfield MM. Left atrial volume as an index of left atrial size: a population-based study. J Am Coll Cardiol 2003; 41: 1036–43.
19. Daniels SR, Witt SA, Glascock B, Khoury PR, Kimball TR. Left atrial size in children with hypertension: the influence of obesity, blood pressure, and left ventricular mass. J Pediatr 2002; 141: 186–90.
20. Hirschler V, Acebo HLP, Fernandez GB, de Lujan Calcagno M, Gonzalez C, Jadzinsky M. Influence of obesity and insulin resistance on left atrial size in children. Pediatr Diabetes 2006; 7: 39–44.
21. Di Salvo G, Pacileo G, Del Giudice EM, et al. Atrial myocardial deformation properties in obese nonhypertensive children. J Am Soc Echocardiogr 2008; 21: 151–6.
22. Canning MO, Grotenhuis K, de Wit H, Ruwhof C, Drexhage HA. 1-alpha,25-Dihydroxyvitamin D3 (1,25(OH)(2)D(3)) hampers the maturation of fully active immature dendritic cells from monocytes. Eur J Endocrinol 2001; 145: 351–7.
23. Frustaci A, Chimenti C, Bellocci F, Morgante E, Russo MA, Maseri A. Histological substrate of atrial biopsies in patients with lone atrial fibrillation. Circulation 1997; 96: 1180–4.
24. Cardin S, Li D, Thorin-Trescases N, Leung TK, Thorin E, Nattel S. Evolution of the atrial fibrillation substrate in experimental congestive heart failure: angiotensin-dependent and -independent pathways. Cardiovasc Res 2003: 2; 315–25.

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Original article

Use of dietary supplements among physicians at a hospital in Turkey

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Summary. *Background and aim:* This research was carried out as a descriptive study in order to determine the status of dietary supplement and dietary habits of physicians working at Erciyes University Faculty of Medicine. *Methods:* A total of 370 people, 165 women, and 205 men, were included in the study. Data were collected by face-to-face interview using questionnaire forms. *Results:* The ratio of physicians using dietary supplements was 7.3%. Use of multivitamin-mineral supplements (51.9%) and daily dietary supplements (40.7%) constituted the majority. Maintaining good health was the primary reason for using the dietary supplement. It was found that dietary supplement use was more common in middle-aged physicians (51.9%) compared to younger physicians (37.0%) ($p < 0.05$). Those who used dietary supplement consumed more quantities of dairy products and vegetables and fruits compared to those not using the dietary supplement ($p< 0.05$). Consumption of legumes for 2-3 times a week was higher in participants using dietary supplements (66.7% vs. 44.6%, $p = 0.029$). In the group using dietary supplements, the daily consumption of dairy products and vegetables-fruits was higher than those who did not use dietary supplements ($p < 0.05$). *Conclusion:* As our knowledge, this is the first research determined the using of dietary supplements among physicians in Turkey. Although physicians using dietary supplement were found to have better dietary habits than those who did not use, neither user nor non-user physicians consumed food groups at the recommended frequency and amounts.

Key words: dietary supplement, dietary habits, physician

Introduction

Dietary supplements are products with predetermined daily doses in the form of capsules, tablets, pastilles, disposable powder packets, liquid ampoules, dropper bottles and other similar liquid or powder forms, containing nutrients such as vitamins, minerals, proteins, carbohydrates, fibers, fatty acids, amino acids or concentrates or extracts of other plants, substances of vegetable and animal origin, or bioactive substances with nutritional or physiological effects, used alone or in combination with each other to supplement current diet (1).

An adequate and balanced diet is the most appropriate approach to provide sufficient nutrients to our bodies. We can meet the energy and nutrient needs of our bodies in an adequate and balanced manner by following healthy nutrition recommendations (2). Turkey Dietary Guidelines (TDG) (3) contains appropriate healthy eating advice appropriate to the conditions of our country.

Dietary supplements are used to support vitamins, minerals and other nutrients that cannot be taken adequately by the daily diet. Dietary supplement plays an important role in the treatment of vitamin and mineral deficiencies (2). It is determined that the use of the dietary supplement is effective in preventing chronic diseases such as cardiovascular diseases, cancers, birth defects and infectious diseases (2, 4). However, high

doses of dietary supplements due to uncontrolled use may cause neurological disorders, gastrointestinal symptoms, liver toxicity, birth defects and drug interactions. It has been reported that high doses of β-carotene, vitamin E, and folate, in particular, have adverse effects (2, 5).

In the United States, it was found that the rate of dietary supplement usage has increased over the years (excluding pregnancies and infants under 1 year of age) and reached 49% over the whole population (6). In Australia, 43.2% of individuals over the age of 18 are using dietary supplements at least once every two weeks (7). In the Netherlands, it was found that 20% of individuals aged 19-91 were using multi-vitamin-mineral supplements every day (8). In Poland, the ratio of multi-vitamin mineral use the day before was found to be 31% for males and 40% for females between the ages of 20-74 (9). In Japan, the use of dietary supplements for one week or a year was 11.0% for males and 16.4% for females aged 45-74 years (10). Based on the results of the European Prospective Cancer and Nutrition Survey (EPIC) conducted in 10 European countries, including Denmark, France, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden and the United Kingdom, the highest use of food supplements within the last 24 hours in individuals aged 35-74 were found in Denmark (male: 51.0%, female: 65.8%), whereas the lowest use of food supplements was found in Greece (male: 2%, female: 6.7%) (11). Turkey Nutrition and Health Survey (TNHS) reported that 1.5% of individuals over the age of 12 years had used multivitamin-mineral supplements in the last seven days, the rate of dietary supplement use increased with age, and the most common dietary supplement was vitamin B12 (12).

Studying the use of dietary supplement by physicians is interesting for several reasons. 1-Health-related habits of physicians significantly affect counseling they provide to their patients (13). For this reason, the use of dietary supplements by physicians may affect the status of dietary supplement usage of their patients, therefore the society. 2- The use of dietary supplement has been found to be more prevalent in those with higher education levels (6, 14). Since the education level of physicians is high, the ratio of dietary supplement use among physicians is thought to be higher than the society in general (15). 3- There are data available on the use of dietary supplements by healthcare professionals and physicians (15-18). No study on the national or the local level that aimed to investigate the use of dietary supplements by physicians in Turkey. Two studies on different topics provided findings on the use of herbal products and the dietary habits of physicians (19,20). This research was carried out to determine the dietary supplement usage status and dietary habits of physicians working at Erciyes University Faculty of Medicine.

Materials and methods

Study design and sampling

The population of this study consisted of 762 physicians working at Erciyes University Faculty of Medicine in 2015. The results of the study conducted on female physicians were taken into consideration in determining the sample size (17). The rate of dietary supplement use among physicians was taken as 30% and the sample size was calculated as 363 with 95% confidence level ($\alpha = 0.05$) and 80% power ($\beta = 0.20$). A total of 370 individuals, 165 women, and 205 men agreed to participate in the study, and written consent was obtained from all participants through a voluntary consent form. Physicians who were pregnant or lactating were not included in the study. Approval was obtained from Erciyes University Medical Faculty Clinical Investigations Ethics Committee (Decision No: 2015/163) to carry out the research.

Data collection

Data was collected via face-to-face interviews with questionnaire including questions on certain socio-demographic characteristics and dietary habits, food frequency questionnaire in the last month, dietary supplement usage, reasons for using, frequency, duration and content of dietary supplement. In this study, the use of dietary supplement was determined on the basis of the definition given in the Communiqué of the Turkish Food Codex on Supplementary Foods in accordance with the declaration of the Republic of Turkey Ministry of Food, Agriculture and Livestock (1). Individuals receiving dietary supplements within

the last year were considered to be using regular or irregular a dietary supplement.

As the food frequency questionnaire was performed, consumption amounts were also questioned. The daily consumption quantities of the individuals who specified a quantity were calculated by to reference the BeBis software (21). For example, weekly total consumption of legumes was determined for an individual stating 50 g legume consumption three times a week (150 g), then this value was divided by seven and the daily consumption amount (21.4 g/day) was calculated.

While determining the status of the food consumption of individuals according to the recommended frequencies, TDG was used as a base. In this study, individuals regular consuming dairy products, eggs, bread, fruits and vegetables every day; red meat, chicken and fish 1-2 times a week; legumes 2-3 times a week; rice, pasta, bulgur and pastry etc. 3-4 times a week were accepted as consumption according to the recommended frequencies (3).

Physicians assessed their nutritional status by their own statements as good, moderate, or poor. Body weight and height were recorded by questioning the current measurements of the participants based on their statements. Using the weight and height values, body mass index (BMI) values were calculated using the formula [weight(kg)/height^2(m^2)].

Statistical analysis

SPSS 22.0 (Statistical Package for Social Sciences Statistics-SPSS) program was used to analyze data obtained in this study. Descriptive findings were expressed in numbers and percentages. Chi-square test was used to determine the difference between categorical variables. The Shapiro-Wilk test was used to determine whether the data showed normal distribution. Independent sample t test and Mann Whitney-U test were used to compare two independent groups. The significance level was accepted as $p < 0.05$.

Results

The median age of the physicians included in the study was 29.0 (min-max: 23.0-67.0) years, 55.4% of the participants were male, 57.0% were married, and the median duration of work was 4.0 (min-max: 1.0-43.0) years. The mean BMI was 26.2 ± 3.0 kg/m^2 for males and 22.4 ± 2.8 kg/m^2 for females ($p<0.001$). 11.6% of the physicians were smokers, and 15.1% were consuming alcohol.

The ratio of physicians using dietary supplement was 7.3% (n = 27). Among these physicians, 44.5% were using a dietary supplement for less than one year, 51.9% (n=14) were using multivitamin supplements, and 40.7% were using dietary supplement on a daily basis (Table 1). "Maintaining good health" was the primary reason for using a dietary supplement (Table 2). It was determined that dietary supplement usage was significantly higher in middle-aged physicians (51.9%) compared with younger physicians (37.0%) (p

Table 1. Physicians' use, type, duration, and frequency of dietary supplement

	n	%
Use of dietary supplement		
User	27	7.3
Non-User	343	92.7
Total	**370**	**100.0**
Duration of work		
Less than 1 year	12	44.5
1-10 years	11	40.7
More than 10 years	4	14.8
Type of supplement		
Multivitamins-minerals	13	48.2
Omega-3 fatty acid	7	25.9
Protein powder	2	7.4
Chia seed	1	3.7
Group B vitamins and iron	1	3.7
Omega-3 fatty acid and probiotics	1	3.7
Black cumin oil and ginger extract	1	3.7
Multivitamins-minerals, omega-3 fatty acid, protein powder	1	3.7
Frequency		
Every day	11	40.7
Once every two days	1	3.7
Once a week	5	18.5
Once every fifteen days	1	3.7
Once a month	2	7.4
In certain periods (irregular)	7	26.0
Total	**27**	**100.0**

Table 2. Physicians' reasons of dietary supplement use

Reasons *	n	%
Maintaining good health	11	40.7
Using as support after exercise	6	22.2
Prevention of fatigue feeling	5	18.5
Providing nutrients that the individual thinks is inadequate	5	18.5
Improving physical performance	5	18.5
Having a protective effect on cardiovascular diseases	4	14.8
Being therapeutic	1	3.7
Improving memory at advanced age	1	3.7
Support during fasting at Ramadan	1	3.7

*Multiple reasons were stated.

< 0.01). Among physicians using a dietary supplement, 77.8% were married, whereas this ratio was only 55.4% among physicians not using a dietary supplement (p < 0.05). Most of the physicians (52.2%) stated their nutritional status as 'good' (Table 3).

The rate of consuming dairy products and fruits-vegetables on a daily basis was higher among physicians using dietary supplement compared to non-users (p < 0.05). Similarly, the rate of consuming legumes 2-3 times a week was significantly higher among physicians using a dietary supplement (66.7% in users, vs. 44.6% in non-users, p < 0.05) (Table 4). The median daily consumption amount of dairy products (250 g) and fruits-vegetables (250 g) was significantly higher

Table 3. Comparison of dietary supplement use and some characteristics of physicians

	User	Non-User	Total		
Characteristics	**n (%)**	**n (%)**	**n (%)**	χ^2	*p*
Age* (years)					
20-34	10 (37.0)[a]	239 (69.9)	249 (67.3)		
35-50	14 (51.9)[b]	70 (20.5)	84 (22.7)	14.899	**0.001**
51-67	3 (11.1)	33 (9.6)	36 (10.0)		
Gender					
Male	16 (59.3)	189 (55.1)	205 (55.4)	0.175	0.828
Female	11 (40.7)	154 (44.9)	165 (44.6)		
Marital Status					
Married	21 (77.8)[a]	190 (55.4)[b]	211 (57.0)	5.12	**0.024**
Single	6 (22.2)	153 (44.6)	153 (44.6)		
Self-evaluation of nutritional status					
Good	14 (51.9)	179 (52.2)	193 (52.2)		
Moderate	11 (40.7)	127 (37.0)	138 (37.3)	4.234	0.833
Poor	2 (7.4)	37 (10.8)	39 (20.5)		
Chronic diseases					
Yes	7 (25.9)	43 (12.5)	50 (15.6)	3.839	0.073
No	20 (74.1)	300 (87.5)	320 (84.4)		
Number of meals					
Less than three	7 (25.9)	68 (19.8)	75 (20.2)		
Three	18 (66.7)	199 (58.0)	217 (58.6)	3.967	0.186
More than three	2 (7.4)	76 (22.2)	78 (21.2)		
Exercise					
Yes	19 (70.4)	191 (55.7)	210 (56.8)	2.199	0.200
No	8 (29.6)	152 (44.3)	160 (43.2)		
Total	**27 (100.0)**	**343 (100.0)**	**370 (100.0)**		

* Significant difference between indicated cells (a, b).

among physicians using dietary supplement compared to non-users (185.5g, 123g, respectively, p < 0.05) (Table 5).

Discussion

Not being able to reach any study in the literature that was conducted in Turkey on the use of dietary supplements by physicians shows the significance of our study. This is the first study conducted in Turkey with the opportunity of obtaining first-hand data about the use of dietary supplement among physicians. The rate of dietary supplement usage was among physicians at 7.3%, this ratio was still higher than the general population as revealed by TNHS results (multi vitamin-mineral 1.5%, omega-3 fatty acids 0.3%, iron 1.2%, and vitamin B12 2.4%) (12). In a study conducted on health workers in the United States (Nurses' Health Study and Health Professionals Follow-Up Study /NHS-HPFS), it was found that the rate of dietary supplement usage was about 65% in 1986, and this rate increased to 85% by 2006. It was shown that dietary supplement usage was higher in health workers compared to the general population, which was attributed to health workers'

Table 4. Comparison of dietary supplement use of physicians and consumption status of food groups at the suggested frequency

	User (n=27)	Non-User (n=343)		
Food/Food Groups	**Sayı (%)**	**Sayı (%)**	χ^2	*p*
Dairy products			4.645	**0.040**
Every day	19 (70.4)	167 (48.8)		
Red meat			0.002	1.000
1-2 times a week	6 (22.2)	75 (21.9)		
Chiken- fish			1.489	0.234
1-2 times a week	15 (55.6)	149 (43.4)		
Eggs			0.029	1.000
Every day	4 (14.8)	55 (16.0)		
Legumes			4.900	**0.029**
2-3 times a week	18 (66.7)	153 (44.6)		
Bread			0.531	0.548
Every day	13 (48.1)	190 (55.4)		
Rice, pasta, bulgur, pastry etc.			0.053	0.836
3-4 times a week	8 (29.6)	109 (31.8)		
Vegetables and fruits			4.520	**0.040**
Every day	19 (70.4)	168 (49.0)		

Table 5. Comparison of daily consumption amounts of some food groups and the use of dietary supplement by physicians*

Food/Food Groups	**User**	**Non-User**		
	Median (min-max)	**Median (min-max)**	*Z*	*p*
Dairy products (g)	(n=16) 250.0 (57.0-600.0)	(n=72) 185.5 (13.0-800.0)	0.185	**0.001**
Red meat (g)	(n=16) 85.5 (9.0-150.0)	(n=65) 86.0 (10.0-525.0)	0.908	0.853
Chicken and fish (g)	(n=14) 52.0 (10.0-114.0)	(n=64) 58.5 (5.0-550.0)	0.546	0.364
Eggs (g)	(n=15) 29.0 (2.0-50.0)	(n=61) 15.0 (1.0-300.0)	0.361	0.170
Legumes (g)	(n=14) 12.0 (7.0-50.0)	(n=52) 28.0 (0.3-143.0)	0.527	0.598
White bread (g)	(n=11) 50.0 (1.0-125.0)	(n=50) 53.5 (1.2-450.0)	1.373	0.585
Whole-grain bread (g)	(n=10) 37.5 (3.0-100.0)	(n=36) 25.0 (0.8-225.0)	1.209	0.718
Nuts-seeds (g)	(n=12) 15.0 (2.0-29.0)	(n=36) 9.0 (0.1-114.0)	3.323	0.226
Vegtables and fruits (g)	(n=16) 250.0 (110.0-450.0)	(n=52) 123.0 (28.0-500.0)	3.074	**0.002**

*Only those specifying amounts were taken into consideration.

knowledge of dietary supplements being higher than the general public (15). A study published in 1984, involving dietitians in the state of Washington, found that the usage rate of dietary supplements was approximately 60% (16). Again, in the United States, the rate of regular (47.3%) and occasional (17.0%) use of any dietary supplement among female physicians were high (17). In our study, the rate of dietary supplement usage was 6.7% among female physicians (n = 11 in 165 physicians), and 7.8% among males (p>0.05) (Table 3). These ratios were remarkably lower compared to the study conducted in the United States.

Among dietary supplements, the most commonly used are multi-vitamin minerals (6, 7, 11, 22, 23). In our study, among the dietary supplements users (n = 27), multivitamins and minerals were the most common (48.3%). In the United States, the use of multi-vitamins&minerals (regular and occasional) was found to be 84.5% in health workers (NHS-HPFS) and 49.8% among female physicians (15, 17). In our study, the ratio of multi-vitamins&minerals users (n = 14) was 3.8% among all physicians participating in the study (n = 370) (Table 1). It has been reported that low doses of multivitamin supplements may reduce the risk of cancer (particularly in malnourished individuals or men previously diagnosed with cancer) and age-related cataracts, and it has been reported that high dose of β carotene, folate, and vitamin E supplements may have harmful effects (5). Marine-derived omega-3 (n-3) fatty acids have beneficial effects on cardiovascular risk factors and for prevention of cardiovascular mortality and morbidity (24). The American Heart Association (AHA) has suggested that healthy adults consume two portions per week of preferably fatty fish (equivalent to 400-500 mg/day of n-3 fatty acid) (25). The American and Canadian Dietitians' Association suggests 500 mg/day of marine-origin n-3 fatty acid intake (26). AHA recommends intake of 1g/day n-3 fatty acids in patients with cardiovascular disease, and 2-4 g/day in patients with high triglyceride levels (> 500 mg/dl) under supervision of a physician (25). Cardio-protective benefits of marine-origin n-3 fatty acids can be partially explained by mechanisms such as antiarrhythmic effect, lowering triglycerides and blood pressure, reducing platelet aggregation, improving vascular functions and reducing inflammation (24). In the meta-analysis published in the JAMA Cardiology journal in 2018, it was concluded that the use of n-3 fatty acid supplements based on recommendations in individuals with risk of developing cardiovascular disease did not support the prevention of fatal coronary heart disease or any cardiovascular disease (27). In terms of diet-disease relationship, a more appropriate approach than focusing on a single food or nutritional item is to focus on all dietary components. In a Cochrane review examining the effect of the Mediterranean Diet on prevention of cardiovascular diseases; it was found that an increase in the components of the Mediterranean Diet in the daily diet will have positive effects on total cholesterol and LDL cholesterol levels. The Mediterranean Diet is a nutritional model where the main fat source is olive oil, with high consumption levels of plant foods (vegetables, fruits, legumes, cereals, oilseeds) and complex carbohydrates (wholegrain bread), moderate consumption levels of fish, eggs, poultry and dairy products (cheese, yogurt, etc.), low consumption levels of red meat and processed meat (fish, eggs, poultry and dairy products), and in which red wine is consumed at a low-moderate level with meals. It naturally contains important nutrients and phytochemicals (mono- and poly-unsaturated fatty acids, fiber, antioxidant vitamins and minerals, and polyphenols) and has a low saturated fat, trans fat and added sugar content (28). In our study, the ratio of n-3 fatty acid users alone (n = 7) or in combination with multiple food supplements (n = 2) was 2.4%. Among the physicians using dietary supplements (n = 27), n-3 fatty acids (33.3%) were most commonly used after multivitamin&minerals (Table-1). In the NHS-HPFS study, most commonly used dietary supplements were calcium, vitamins D, C, and E in women, and vitamin C, E and calcium in men, followed by fish oil, and the usage rate was 20.2% (15).

The lifestyles of individuals using dietary supplements are generally positive. For example; they do not smoke, consume alcohol or consume alcohol at moderate levels, exercise regularly, consume more vegetables and fruits. They are also more susceptible to nutrition-related messages (maintaining ideal body weight, eating micronutrients at an adequate level, consuming fruits and vegetables, etc.) (29). It was reported that individuals assessing their health condition as good use

more dietary supplement than those who assess their health condition as poor (22, 29). On the contrary, it was also found that individuals who assessed their health condition as poor used dietary supplements at a rate approximately twice that of individuals who assessed their health condition as good (23). According to the EPIC study, a dietary supplement was found to be more frequent in individuals reporting their health condition as good than those who reported a moderate/poor health condition, except for England (11). It was also found that supplement users had one or more illness than those who did not. In addition, it was reported that individuals with hypertension, cancer, and heart disease used less dietary supplement than those without these diseases (29). In our study, approximately half (52.2%) of the physicians rated their nutritional status as good. This was similar for physicians who use a dietary supplement (51.9%) and non-users (52.2%). Although the presence of chronic diseases (25.9%) and exercising (70.4%) was higher in physicians using dietary supplement than in non-users (12.5% and 55.7%, respectively), this difference was not significant ($p > 0.05$, Table 3). Individuals who use dietary supplements use these to maintain and improve health rather than preventing diseases (29). In our study, similarly, most of the physicians using food supplements (40.7%) reported that they used a dietary supplement to maintain good health.

A balanced and sufficient diet is the most appropriate approach to achieve sufficient intake of nutrients into our body. Dietary supplements are used to compensate for vitamins, minerals, and other nutritional substances that are not sufficiently supplied by a person's daily diet (2). As completely opposed to this case, it was reported that those who used dietary supplements had better diets than those who did not (18). In agreement with the literature, a significant result of our study was that the dietary habits of those who used dietary supplements had higher quality in comparison to those who did not use such supplements. Our findings that support this case are described below.

In our study, the daily consumption rates of dairy products and vegetables-fruits were higher in physicians using the dietary supplement ($p<0.05$). Similarly, the rate of consuming legumes 2-3 times a week was higher in physicians using the dietary supplement (66.7% compared to 44.6%)($p<0.05$) (Table 4). The median daily consumption amount of dairy products (250 g) and fruits-vegetables (250 g) was significantly higher among physicians using dietary supplement compared to non-users (185.5 g, 123 g, respectively) (Table 5). Turkey Dietary Guidelines recommends that adults should consume 3 servings of dairy products, and at least 5 servings (400 g) of vegetables and fruits daily, and 3 servings of legumes weekly. A medium size cup of milk or yogurt (240 mL) or 40-60 g of cheese, 8-10 tablespoons (130 g cooked) of legumes constitute a serving or portion (3). It is seen that physicians using dietary supplement consume higher levels of milk, vegetables, fruits and legume groups closer to the recommended frequency by TDG than those who do not use a dietary supplement. However, although daily consumption amounts of dairy products and vegetables and fruit are higher (250 g), these are still lower than the daily amounts recommended by TDG. 250 g dairy products may be considered as approximately 1 portion, and 250 g vegetables-fruits as approximately 1-2 portions. In this case, it can be said that physicians using dietary supplement consume 1 portion of dairy products and 1-2 portions of vegetable-fruits per day. In a study conducted on female physicians, it was found that daily vegetable consumption (3.4 servings) was higher in participants taking supplements regularly than occasional users (2.9 servings) and non-users (2.8 servings). It was also found that those who used regular dietary supplements consumed less fat than occasional users and non-users. It was also shown that vegetarians are more likely to use regular dietary supplements than non-vegetarians (17).

In the studies we have accessed, in general, the use of food supplements was found to be more prevalent among women, in advanced age, in individuals with higher education levels and income levels, non-smokers, those with higher physical activity, and those with low or normal BMI (18.5-24.9 kg/m^2) (6-10, 14, 19, 23). Among these studies, only one study conducted in the Netherlands found that the probability of using multi-vitamin minerals in individuals aged 19-34 years was higher than the age group of 65+ years. In the same study, the use of multi-vitamin&minerals was found to be more likely in single persons than in married (8). Regular use of dietary supplements also increases

among female physicians with age, whereas there is no significant relationship between marital status and dietary supplement (17). Consistent with the literature, in our study, middle-aged physicians (35-50 years) had a higher rate of dietary supplement use than younger participants (20-34 years). Usage rate was higher in married participants (77.8%) than single participants (55.4%). This suggests that a relationship with age exists since individuals are more likely to be married at advanced ages.

Conclusion

Although, as a result of our study, we obtained data on the rate of using dietary supplements among physicians (7.3%), the fact that the study was carried out at a single center may be misleading in terms of generalizing these data to the universe. Thus, the use of dietary supplements by physicians in Turkey should be determined by carrying out multi-center studies with larger samples. In addition to the use of dietary supplements, daily intake of energy and nutrients should be calculated by keeping records of food intake, and this should be compared to Dietary Reference Intakes (DRI).

References

1. Türk Gıda Kodeksi Takviye Edici Gıdalar Tebliği, T.C. Resmi Gazete, 16 Ağustos 2016, sayı: 28737. Available from: http://www.resmigazete.gov.tr/eskiler/2013/08/20130816-16.htm
2. Rautiainen S, Manson JE, Lichtenstein AH, Sesso HD. Dietary supplements and disease prevention- a global overview. Nat Rev Endocrinol. 2016; 12(7): 407-20
3. Türkiye Beslenme Rehberi "TÜBER 2015", "T.C. Sağlık Bakanlığı Yayın No: 1031, Ankara 2016.
4. Pekcan G, Acar Tek N. Besin Destekleri Kullanılmalı mı? T.C. Sağlık Bakanlığı Yayın No: 727, Ankara, 2008.
5. Hathcock JN, Vitamins and minerals: efficacy and safety Am J Clin Nutr 1997; 66(2): 427-37
6. Bailey RL, Gahche JJ, Lentino CV, et al. Dietary Supplement Use in the United States, 2003-2006. J. Nutr. 2011; 141(2): 261-66
7. O'Brien SK, Malacova E, Sherriff JL, Black LJ. The prevalence and predictors of dietary supplement use in the Australian population. Nutrients 2017: 9(10); 1154
8. de Jong N, Ocké MC, Branderhorst HA, Friele R. Demographic and lifestyle characteristics of functional food consumers and dietary supplement users. Br J Nutr. 2003; 89(2): 273-81
9. Waśkiewicz A, Sygnowska E, Broda G, Chwojnowska Z. The use of vitamin supplements among adults in Warsaw: Is there any nutritional benefit? Rocz Panstw Zakl Hig 2014; 65(2): 119-26
10. Ishihara J, Sobue T, Yamamoto S, Sasaki S, Tsugane S. Demographics, lifestyles, health characteristics, and dietary intake among dietary supplement user in Japan. Int J Epidemiol 2003; 32(4): 546-53
11. Skeie G, Braaten T, Hjartåker A, et al. Use of dietary supplements in the European Prospective Investigation into Cancer and Nutrition calibration study. Eur J Clin Nutr. 2009; 63 (Suppl 4): 226-38.
12. T.C. Sağlık Bakanlığı. Türkiye beslenme ve sağlık araştırması 2010. Beslenme durumu ve sağlık alışkanlıklarının değerlendirilmesi sonuç raporu. (Rapor No: SB-SAG-2014/0): Ankara. Sağlık Bakanlığı, Hacettepe *Üniversitesi*, Sağlık Bilimleri Fakültesi, Beslenme ve Diyetetik Bölümü, 2014
13. Frank E, Kunovichfrieze T. Physicians' prevention counseling behaviors: Current status and future directions. Prev Med 1995; 24(6): 543-5
14. Archer SL, Stamler J, Moag-Stahlberg A, et al. Association of dietary supplement use with specific micronutrient *ıntakes* among middle aged American men and women: The INTERMAP Study. J Am Diet Assoc 2005; 105(7): 1106-14
15. Kim HJ, Giovannucci E, Rosner B, Willett WC, Cho E. Longitudinal and secular trends in dietary supplement use: Nurses' health study and health professionals follow-up study, 1986-2006. J Acad Nutr Diet. 2014; 114(3): 436-43.
16. Worthington-Roberts B, Breskin M. Supplementation patterns of Washington state dietitians. J Am Diet Assoc 1984; 84(7): 795-800.
17. Frank E, Bendich A, Denniston M. Use of vitamin-mineral by female physicians in the United States. J Am Clin Nutr 2000; 72(4): 969-75.
18. Dickinson A, Boyon N, Shao A. Physicians and nurses use and recommend dietary supplements: report of a survey. Nutr J 2009; 8: 29
19. Yarar F (2014). Aile hekimleri ve aile hekimliği uzmanlarının kanserli hastalarda beslenme ve bitkisel ürün kullanımıyla ilgili yaklaşımları, bilgi ve deneyimlerinin değerlendirilmesi. Ondokuz Mayıs Üniversitesi Tıp Fakültesi Aile Hekimliği Anabilim Dalı, Uzmanlık Tezi, Samsun.
20. Fidan F (2016). Üniversite hastanesinde çalışan hekimlerin yeme tutum davranışı ve beden algısının değerlendirilmesi. Okan Üniversitesi Sağlık Bilimleri Enstitüsü, Yüksek Lisans Tezi, İstanbul.
21. BeBis: Nutrition Database software, İstanbul. Available from: http://bebis.com.tr/.
22. Datta M, Vitolins MZ. Food fortification and supplement use- are there health implications? Crit Rev Food Sci Nutr. 2016; 56(13): 2149-59
23. Knudsen VK, Rasmussen LB, Haraldsdóttir J, et al. Use of dietary supplements in Denmark is associated with health and former smoking. Public Health Nutr. 2002; 5(3): 463-8

24. Hill AM, Fleming JA, Kris-Etherton PM. The role of diet and dietary supplements in preventing and treating cardiovascular disease. Curr Opin Cardiol. *2009; 24(5):433*-41.
25. Lichtenstein AH, Appel LJ, Brands M, et al. Diet and lifestyle recommendations revision 2006, a scientific statement from the American Heart Association Nutrition Committee. Circulation 2006; 114(1): 82-96.
26. Kris-Etherton PM, Innis S, American Dietetic Association, Dietitians of Canada. Position of the American Dietetic Association and Dietitians of Canada: Dietary Fatty Acids. J Am Diet Assoc 2007; 107(9): 1599-611.
27. Aung T, Halsey J, Kromhout D, et al. Associations of omega-3 fatty acid supplement use with cardiovascular disease risks meta-analysis of 10 trials *involving* 77 917 *individuals*. JAMA Cardiol. 2018; 3(3): 225-34.
28. Mak TN, Louro Caldeira S. The role of nutrition in active and healthy ageing: for prevention and treatment of age-related diseases: evidence so far, 2014.
29. Greger JL. Dietary supplement use: consumer characteristics and interests. J Nutr. 2001; 131(4 Suppl): 1339-43.

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Original article

Do food labels affect Turkish consumers' nutritional choices and expectations?

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Summary. *Objective:* Food labels are tools that contribute to nutritional education significantly by providing accurate and clear information for societies. The aim of this study was to determine factors having an influence on individuals about reading of food labels, problems they encounter, and nutritional values they want to see on food labels. *Subjects and Methods:* This cross-sectional study was carried out with 800 individuals aged 20 to 64 years old. A questionnaire form created by the authors was used for data collection Independent Samples t-test and Chi-square (X^2) significance test were used for statistical analyses. The findings were evaluated at 95% confidence interval and $p<.05$ taken to indicate significance level. *Results:* According to results of the study all the participants read food labels. 66.5% of the participants in both genders read food labels because they thought this habit "contributes to healthy eating", and 55.1% of them thought "information on food labels does not adequately meet their incompetency expectations". The first two reasons for this incompetency were difficulty in finding production and expiry dates (26.3%) and tiny font size (18.3%). There were significant correlations between some food label symbols ("Gluten free", "Recyclable"), food label statements ("fiber" and "light"), and gender ($p<.05$). Expecting to see some food information on labels such as carbohydrates (sugar content), total fat, light, top vitamins, amount of fiber, calcium, sodium, potassium and iron content differed by gender ($p<.05$). *Conclusion:* In order to benefit from food labels, rearrangement in all aspects to ensure healthy food choices, and clearer labels may be effective.

Key words: food labelling, consumer choice, gender, symbols, expectations

Introduction

A label is a material that provides information about the content of a product and enhances the comprehension of this information by consumers (1). The purpose of labeling is to provide accurate information related to health, safety, and economic concerns, to protect consumers and producers from persuasive packaging and advertisements, and to promote equitable competition and product marketing. However, reading the information partaking on the food label while purchasing packaged food is an considerable parameter in terms of providing food safety (2). Food labels are the most basic and healthiest source providing information to the customers. It is important to ensure that the information on food labels should be accurate, clear and comprehensive to the consumers (3). Food labels contain the portion size, energy, and nutritional values of the given food (4,5). Consumers should be able to choose healthy food by reading nutrition facts on food labels during food purchase (6). Thus, the incidence of chronic diseases can be reduced, and management of the body weight can be controlled. In addition, some food labels include information about healthy eating (4,5). According to the literatüre there are correlations between reading food labels and high diet quality, low energy intake, increased consumption of fruit and vegetables, enhanced health outcomes and

other favorable activities (7-10). This study aims to determine factors affecting the food-label reading habits of consumers, problems they encounter while reading food labels and nutritional values they want to see on food labels.

Materials and Methods

Study Design

This cross-sectional study, was carried out with 800 individuals, 323 males (M:40.3%) and 477 females (F:59.7%), aged between 20 and 64 living in Ankara province which is the capital city of Turkey between December 2014 and May 2015. A questionnaire created by the authors was used to evaluate the sociodemographic and food label reading attributes. Pilot study of this questionnaire conducted with 50 volunteers to evaluate the validity of the items. Some items were reorganized based on the responses taken from the participants.

The first part of the questionnaire involved items examining general information about the participant (gender, age, marital status, education, employment, total number of family members, and diagnosis of a chronic disease), while the second part questioned the problems encountered during reading food labels, whether the symbols, phrases, and sample food labels were known, and other things that consumers wanted to see on food labels (Figure 1).

Research Ethics

This study was approved by the Ethical Committee of the Ankara University (179/1344/2014). The participants were informed about the purpose and the content of the study, and they were asked to sign a consent form.

Statistical Analysis

SPSS statistical software package was used in data analysis. The quantitative data were presented as mean and standard deviation values in the tables, while qualitative data were presented as numbers and percentages (%). The number "n" was considered for each choice while calculating percentages for items with multiple choices. For statistical analysis, Chi-square (X^2) significance test was used for non-parametric variables and Independent Samples t-test was used for parametric two independent groups. All data was split by gender. The findings were evaluated at 95% confidence interval and $p<.05$ determined as significance level.

Results

According to the results of the study, 40.3% (n:323) of the participants were male, 59.7% (n:477) were female, and 32.4% were married. Mean age of the participant was 30.1±11.0 years. Approximately half of them (44.7%) were aged between 20 and 24. High school and college graduation rate was 47.1% and 45.5%, respectively. There was significant difference found between age groups, education levels and marital status according to gender ($p<.05$). The number of people in the family was ≤4 with 65.1%. 18.4% of the participants had at least a chronic disease. Prevalance of the most common diseases were 23.1% for hypertension, 21.8% for cardiovascular diseases and 15.4% for diabetes.

All of the participants stated that they read food labels. Table 1 presents the factors that affect the participants for reading food label information. Accordingly, in both genders, the most given response (66.5%) was reading food labels because of "its contribution to healthy eating" (M:71.2%; F:63.2%), and the second popular response (11.1%) was "comparing

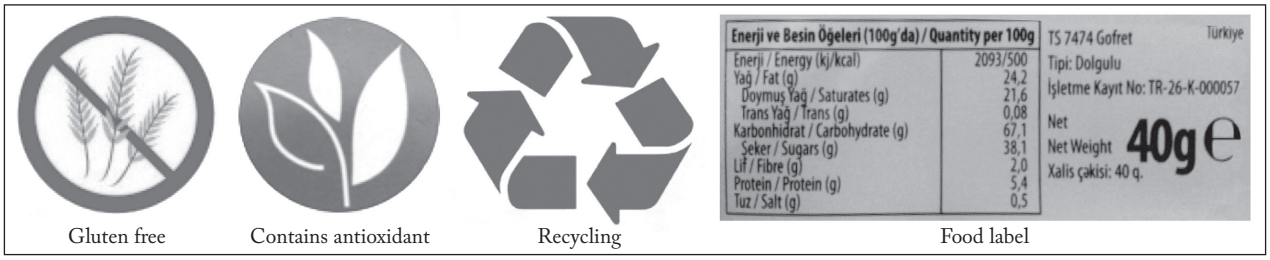

Figure 1: Symbols, selected sample food label

Table 1. The distribution of the most effective factors for reading food labels by gender, n (%)

Effective factors on reading food labels	Male	Female	Total	*p*
Going on a diet	15 (4.6)	29 (6.0)	44 (5.5)	
Maintaining body weight	12 (3.7)	45 (9.4)	57 (7.1)	
Having a chronic disease	8 (2.5)	10 (2.0)	18 (2.3)	
Contribution to healthy eating	230 (71.2)	301 (63.2)	531 (66.5)	***0.002***
Comparing two different products	39 (12.2)	50 (10.6)	89 (11.1)	
Contribution to nutritional awareness	19 (5.8)	42 (8.8)	61 (7.5)	

Table 2. Distribution of problems related to food label information by gender, n (%)

Problems related to food label information *	Male (n:168)	Female (n:273)	Total
Difficulty in finding production and expiry dates	51 (30.3)	65 (23.8)	116 (26.3)
Inconsistency about net amount and food label information	25 (14.8)	43 (15.7)	68 (15.4)
No price	15 (8.9)	19 (7.0)	34 (7.7)
Faint label print	20 (11.9)	29 (10.6)	49 (11.1)
Incomprehensive label language	21 (12.5)	41 (15.9)	62 (14.0)
Too small font size	26 (15.4)	55 (20.1)	81 (18.3)
Label information isn't highlighted	11 (6.5)	19 (7.0)	30 (6.8)
Label information is not written on the front side	10 (5.9)	14 (5.1)	24 (5.4)

**Participants choice more than one reason.*

similar products of two different brands" (M:12.2%; F:10.6%). However, the third effective factor was "maintaining body weight" in women (9.4%), whereas it was "contribution to nutritional awareness" in men (5.8%) (Table 1).

The participants (n 441) thought that the information on food labels did not adequately meet their expectations (55.1%) (M:52.0%; F:57.2%). Among the reasons for that were difficulty in finding the production and expiry dates (26.3%), too small font size (18.3%), inconsistency between net amount and food label information (15.4 %), and incomprehensive label language (14.0%) (Table 2).

Meaning of some of the symbols, icons and statements on the food label were asked to the participants (Table 3). According to Table 3, the rate of 5 correct answers in women and 2 in men was over 50.0%. On the other hand, frequency of incorrect responses was found to be high in two genders (M:85.8%; F:74.6%) when they were asked about the caloric density of a selected food label. While antioxidant symbol awareness was determined to be insignificant by gender ($p>0.05$), the difference between correct responses about other symbols, statements, and selected sample food label was found significant by gender ($p<.05$). The mean score of the questions asked in this section was 3.64±1.69 (M:3.19±1.66 points and F:3.94±1.65 points), and the scores classified by gender were statistically significant ($p<.05$).

Table 3. Correct responses to questioned symbols, selected sample food label, and phrases, n (%)

Symbols, selected sample food label, and phrases		True	False	*p*
Gluten free	M	141 (43.7)	182 (56.3)	0.000
	F	296 (62.1)	181 (37.9)	
Contains antioxidant	M	73 (22.6)	250 (77.4)	0.989
	F	108 (22.6)	369 (77.4)	
Recycling	M	263 (81.4)	60 (18.6)	0.000
	F	420 (88.1)	57 (11.9)	
Food label	M	46 (14.2)	277 (85.8)	0.000
	F	121 (25.4)	356 (74.6)	
Fiber	M	151 (46.7)	172 (53.3)	0.000
	F	323 (67.7)	154 (32.3)	
Light	M	149 (46.1)	174 (53.9)	0.000
	F	309 (64.8)	168 (35.2)	

M:Male, F:Female

Table 4 presents the distribution of nutrients of which the participants wanted to see on food labels by gender. Willingness to read carbohydrates (sugar content), total fat, light, salt and sodium, most common vitamins, fiber, calcium, potassium and iron content differed statistically by gender ($p<.05$), however the rate of others did not yield a significance (p>0.05). Moreover, though not statistically significant, the frequency of women who thought energy value, saturated fat, cholesterol, and protein content should always take part on food labels was determined to be higher than men.

Discussion

Adequate and balanced nutrition is important in increasing the life quality of individuals. As a part of that it is necessary to effectively use food label facts while choosing healthy foods. Thus, it is also possible to gain healthy eating habits. Nowadays, although consumers are aware of nutrient content of some foods, it is generally thought that the information on food labels of packaged foods is not always clear, consumers have somewhat difficulty in reading food labels, and that food labels can be confusing for consumers. For this reason, food labels should include clear and practical information about the product. Therefore, determining the factors which affect consumers' food label reading, also the problems encountered while reading food labels and the nutrients consumers would like to see on food labels was examined in this study.

As a result of the study, it was determined that all of the participants (n:800) were reading nutritional labels. In 65 of 120 studies conducted on consumers' reading habit of food labels, it was found that consumers read the information on food labels (10). In some of that studies based on individuals' self reports, consumers read food labels in detail (11-13). In other studies, the reading rate according to age group is evaluated and found that middle aged and young adults generally read food labels (10). The larger part of the participants in the study (83.9%) was made up of adults aged between 20 and 40, and most were high school and university graduates (92.6%). It was shown in most studies on food label reading that the habit of reading food labels was high in those with high educational levels (5,14-19). The high educational level in this study (with 92.6%) may have been effective in reading food labels. The high rates of food label reading in young and middle aged participants and in those with high educational levels can be explained by the fact that these variables are efficient on health awareness.

As the consumers' own statements are valid in such studies most of the time, the responses may also meet social expectations. For this reason, consumers are interested in food label information without fully

Table 4. The distribution of nutrients that the participants willing to see on food labels by gender, n(%)

Statements	Male			Female			p
	Always	Sometimes	Never	Always	Sometimes	Never	
Energy	280 (86.7)	31 (9.6)	12 (3.7)	434 (91.0)	30 (6.3)	13 (2.7)	0.152
Carbohydrate (sugar content)	238 (73.7)	72 (22.3)	13 (4.0)	395 (82.8)	65 (13.6)	17 (3.6)	0.005
Total fat	227 (70.3)	77 (23.8)	19 (5.9)	373 (78.2)	82 (17.2)	22 (4.6)	0.039
Saturated fat	216 (66.9)	77 (23.8)	30 (9.3)	341 (71.5)	104 (21.8)	32 (6.7)	0.273
Light	202 (62.5)	73 (22.6)	48 (14.9)	318 (66.7)	117 (24.5)	42 (8.8)	0.029
Cholesterol	213 (65.9)	61 (18.9)	49 (15.2)	329 (69.0)	93 (19.5)	55 (11.5)	0.323
Protein	215 (66.6)	62 (19.2)	46 (14.2)	342 (71.7)	88 (18.4)	47 (9.9)	0.137
Salt and Sodium	201 (62.2)	62 (19.2)	60 (18.6)	327 (68.6)	95 (19.9)	55 (11.5)	0.020
The most common vitamins	195 (60.4)	63 (19.5)	65 (20.1)	336 (70.4)	82 (17.2)	59 (12.4)	0.004
Fiber	164 (50.8)	77 (23.8)	82 (25.4)	299 (62.7)	110 (23.1)	68 (14.3)	0.000
Calcium content	188 (58.2)	61 (18.9)	74 (22.9)	338 (70.9)	78 (16.4)	61 (12.8)	0.000
Potassium content	183 (56.7)	68 (21.1)	72 (22.3)	318 (66.7)	88 (18.4)	71 (14.9)	0.008
Iron content	190 (58.8)	61 (18.9)	72 (22.3)	136 (70.4)	73 (15.3)	68 (14.3)	0.002

understanding the information on food labels. The most influential factor on reading food labels in this study was reported to be "contribution to healthy eating" (66.5%, Table 1). It was shown in some studies that only a general health statement on the food label induced an increase perception of the food as healthy (20-26). On the other hand, there are also studies showing that information on a label perceived as the risk of any illness had stronger effect in the choice of purchase (23,24).

In this study, the second important factor in food label reading of the participants was "comparing two similar products of different brands" (11.1%) (Table 1). Ares et al. (22) reported that brand name is the most significant factor on purchasing healthy food, and that there are two types of consumers in terms of purchasing. While the first group of customers take brand name and healthy content into consideration, the second group look for a certain brand and good taste. For this group, health benefits and price are less important than the other group of customers.

Another factor effective in reading food labels was statements related to chronic diseases (20). However, this study found that the least effective factor in reading food labels was statement of a chronic disease (2.3%) (Table 1). This may have been caused from the fact that the majority of the participants were young or middle aged adults, and that the rate of those who were diagnosed with a chronic disease was low with 18.4%. Saba et al. (24) determined that products with a general health statement were preferred less than products stating that they reduced the risk of any diseases. On the other hand, van Kleef et al. (23) found that health statements related to heart, cancer, and osteoporosis on food labels was more effective than other health statements such as reducing stress, and good for skin.

Making almost all the information on food labels more understandable (e.g. optimal font size and print color, statements, symbols, logos, and measurement units) is important for social nutritional education. More than half of the participants (n:441; 55.1%) in the study thought that the information on food labels did not meet their expectations (M:52.0%; F:57.2%). Accordingly, the primary reasons were found to be difficulty finding production and expiry dates (26.3%), very little font size (18.3%), inconsistency between the net amount of the product and the statement on the label (15.4%), incomprehensible label language (14.0%), location of the label on the package other than front face (5.4%), and unnoticeable print color (6.8%) (Table 2). Van Kleef & Dagevos (2015) reported that in particular, simplified nutrition labelling located on the front of packs has the potential to effectively inform consumers of the healthiness of food products and help prefer more conscious food choices (27).

Jacobs et al. (26) conducted study to investigate difficulties that consumers had while reading and understanding food label information. The results of the study was consistent with this study, as customers were disturbed by very small font size on food labels. Besler et al. (28) found in a similar study that food label information was incomprehensible and the label font was too small, also 24.9% of the consumers partly understood food label information, 19.6% understood nothing. By correcting these negative outcomes, it is possible to prevent meaning confusion in food label information, to increase food label reading, and to lead positive changes while choosing healthy food. In a literature review related to this topic carried out in America and Canada, it was revealed that consumers had several difficulties in understanding food label information both on the front and back of the food package. In that review, it was stated that the food label information on the front side of the food package should be written in a simpler language, and that a content table with simple and clear language should be included. It was also additionally stated that the terms used on the label should be presented with a simple language such as "high", "medium", and "low", the color should be noticeable, and that a traffic lamp-like system should be used to indicate the value of all nutrients in terms of health (29).

In addition to mandatory label information in our country, some symbols are also used on food labels. A great majority of community, consumers' interest in reading food labels and understanding statements and symbols is effective in their food label reading. Besler et al. (28) determined that the meaning of symbols and abbreviations on food labels were not understood by the customers. While there was no significant difference between knowing what "antioxidant" symbol meant by gender ($p>0.05$), there was a significant dif-

ference between had knowledge of other symbols by gender ($p<.05$) (Table 3). Many of the studies investigating whether food label information was read by consumers; was reported that women read food labels much higher than men (5,15,18,30-33). In accordance with other studies conducted on this subject, the rate of 5 correct answers in women and 2 in men was over 50.0% in this study. The mean score obtained from the statements in Table 3 was 3.64±1.69 (M:3.19±1.66 and F:3.94±1.65), and the scores obtained were statistically different by gender ($p<.05$).

"Recycling" symbol on the food label was known by the majority of the participants in the study. This may be due to increased awareness in the society as well as implementation of environmental protection policies Turkey. It's noteworthy, however, that the number of those who did not know such health-related symbols as "gluten free" (M:56.3%, F:37.9%) and "contains antioxidants" (M:77.4%, F:77.4%) was high (Table 3). This result may be due to the rate of those who had chronic diseases in the study was only 18.4%, and that individuals paid attention to health-related warnings on food labels when they or their family members had a chronic diseases (such as celiacs) investigated. Indeed, some studies showed that consumers investigated food label symbols more in the presence of a chronic disease (23,24). In another study carried out in Spain and Denmark, it was found that symbols could be misinterpreted due to cultural differences, however the interest in food labels can increase visually to a great extent (34). Bix L et al. (35) studied the effect of attention getting properties of food labels on the front side of food packages with eye contact duration in vitro and they found that prolonged examination of food label by faster but attractive eye contact. In addition, they determined that the reaction time given to food labels which use color-code system on the front side was faster. In this study, selected sample food label was shown to participants and they were asked how many calories it contains. As a result, the frequency of wrong response in both genders were high (M:85.8%, F:74.6%) and the mean difference found significant ($p<.05$).

Nowadays, food labeling is attracting more attention as a mechanism which is likely to put an impact on people's diets at the population level to provide a supporting remedy for high and growing levels of obesity and nutrition diseases (36-37). World Health Organization (WHO) and Food and Agriculture Organization (FAO) declared that in order to prevent common health problems, energy intake, total fat and trans fat, sugar and sodium should be reduced in the diet (38). Nutrition labels generally involves information about calories, serving size, and amounts and/or daily values of many macronutrients, vitamins, and minerals (e.g., carbohydrate, fats, calcium, Na) (39). For this reason, food labeling has become a part of dietary habits, physical activity and health strategies of WHO since 2004 (40). In the study, participants who wanted carbohydrate (sugar content), total fat, light, salt and sodium content, most common vitamins, fiber, calcium, potassium and iron content as label information were differed by ($p<.05$, Table 4). These results may be due to the increased awareness that amount of food intake for the treatment of increasing diseases associated with nutritional deficiencies (malnutrition, vitamin D deficiency, anemia, cancer, cardiovascular diseases, allergy, asthma, and obesity) (2).

Jacobs et al. (26) found that consumers read information about fat and cholesterol content in nutritional information of food labels. It was determined in a study carried out in Holland on this topic that "√" symbol, which meant "contains low energy, sugar, salt, saturated fat, and abundant fiber" and was used for body weight control, was known by 62.0% of consumers, especially more by women (68%) (41). Although not statistically significant, women wanted to see saturated fat, protein and energy content of a packaged food more than men (Table 4). In some studies conducted on the same subject, it was determined that willingness to examine the food label was related to customers' health, for instance a customer needs to low-fat food was interested in fat content on food label (7,42-43).

Conclusion

In conclusion, **c**onsumers living in Turkey are willing to read food labels. In this study, it was found that all of the participants were reading nutritional labels. Label readings of consumers with a high level of education and young and middle age groups were found

to be high. The most influential factor on reading food labels in this study was reported to be "contribution to healthy eating". More than half of the consumers stated that they could not meet the expectations of the information on the food labels. In addition to, findings reveal that there were significant correlations between some food label symbols, food label statements and gender. Expecting to see some food information on labels such as carbohydrate (sugar content), total fat, light, top vitamins, amount of fiber, calcium, sodium, potassium and iron content differed by gender.

Food labels are effective tools that help individuals make healthy food choices, and the information on the labels have an influence on the purchasing stage. Therefore it is recommended by the study that educational programs should be developed so that individuals can read food labels effectively, the symbols and statements on food labels can be taught via public spots, and that public awareness can be raised for reading and perceiving the food labels correctly.

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References

1. Stran KA, Knol LL. Determinants of food label use differ by sex. J Academy of Nutr and Diet 2013; 113: 673-79.
2. Türkiye'ye Özgü Besin ve Beslenme Rehberi. Besin Etiketleme ve Sağlık Beyanları, 2015. (Available at:http://www.bdb.hacettepe.edu.tr/TOBR_kitap.pdf) (02 January 2017, date last accessed).
3. İnan E, Ayaz A. Besin Etiketleri: Beslenme ve Sağlık Beyanları. Katkı Pediatri Dergisi Besin Güvenliği-II Özel Sayısı 2014; 36: 217-28.
4. Graham DJ, Laska MN. Nutrition label use partially mediates the relationship between attitude toward healthy eating and overall dietary quality among college students. J Acad Nutr And Diet 2012; 112: 414-18.
5. Satia JA, Galanko JA, Neuhouser ML. Food nutrition label use is associated with demographic, behavioral, and psychosocial factors and dietary intake among African Americans in North Carolina. J Am Diet Assoc 2005; 105: 392-02.
6. Ree M, Riediger N, Moghadasian MH. Factors affecting food selection in Canadian population. Eur J clin Nutr 2008; 62: 1255-62.
7. Lin CT, Lee JY, Yen ST. Do dietary intakes affect search for nutrient information on food labels? Soc Sci Med 2004; 59: 1955-67.
8. Temple JL, Johnson K, Recupero K, Suders H. Nutrition labels decrease energy intake in adults consuming lunch in the laboratory. J Am Diet Assoc 2010; 110: 1094-97.
9. Staser KW, Zollinger TW, Saywell RM Jr, Kunapareddy S, Gibson PJ, Caine VA. Dietary behaviors associated with fruit and vegetable consumption, Marion County, Indiana, 2005. Prev Chronic Dis 2011; 8: 1-9.
10. Campos S, Doxey J, Hammond D. Nutrition labels on pre-packaged foods: A systematic review. Public Health Nutr 2011; 14: 1496-506.
11. Cowburn G, Stockley L. Consumer understanding and use of nutrition labelling: a systematic review. Public Health Nutr 2005; 8: 21–8.
12. Grunert K. A review of European research on consumer response to nutrition information on food labels. J Public Health 2007; 15: 385–89.
13. Mhurchu CN, Gorton D. Nutrition labels and claims in New Zealand and Australia: a review of use and understanding. Aust N Z J Public Health 2007; 31: 105–12.
14. McArthur L, Chamberlain V, Howard AB. Behaviors, attitudes, and knowledge of low-income consumers regarding nutrition labels. J Health Care Poor Underserved 2001; 12: 415–28.
15. Worsley A. Consumers' personal values and sources of nutrition information. Ecol Food Nutr 2003; 42: 129–51.
16. Drichoutis AC, Lazaridis P, Nayga RM Jr. Who is looking for nutritional food labels? Wer sucht nach nahrwertangaben auf lebensmitteln?: Mais qui donc s' occupe du contenu nutritionnel sur les etiquettes? Eurochoices 2005; 4: 18–23.
17. Drichoutis AC, Lazaridis P, Nayga RM Jr. Nutrition knowledge and consumer use of nutritional food labels. Eur Rev Agric Econ 2005; 32: 93–18.
18. Jay M, Adams J, Herring SJ et al. A randomized trial of a brief multimedia intervention to improve comprehension of food labels. Prev Med 2009; 48: 25–31.
19. Hess S, Yanes M, Jourdan P, Edelstein S. Trans fat knowledge is related to education level and nutrition facts label use in health-conscious adults. Top Clin Nutr 2005; 20: 109–17.
20. Lewis JE, Arheart KL, LeBlanc WG, et al. Food label use and awareness of nutritional information and recommendations among persons with chronic disease. Am J Clin Nutr 2009; 90: 1351–7.
21. Lyly M, Roininen K, Honkapää K, Poutanen K, Lähteenmäki, L. Factors influencing consumers' willingness to use beverages and ready-to-eat frozen soups containing oat beta-glucan in Finland, France and Sweden. Food Qual Prefer 2007; 18: 242–55.
22. Ares G, Gime´nez A, Deliza R. Influence of three non-sensory factors on consumer choice of functional yogurts over regular ones. Food Q*ual Prefer* 2010; 21: 361–67.

23. van Kleef E, van Trijp HC, Luning P. Functional foods: health claim-food product compatibility and the impact of health claim framing on consumer evaluation. Appetite 2005; 44: 299–08.
24. Saba A, Vassallo M, Shepherd R et al. Countrywise differences in perception of health-related messages in cereal-based food products. Food Qual Prefer 2010; 21: 385–93.
25. Van Trijp HC, van der Lans IA. Consumer perceptions of nutrition and health claims. Appetite 2007; 48: 305–24.
26. Jacobs SA, de Beer H, Larney M. Adult consumers' understanding and use of information on food labels: a study among consumers living in the Potchefstroom and Klersdorp regions, South Africa. Public Health Nutr 2010; 14: 510-22.
27. van Kleef, E., & Dagevos, H. The growing role of front-of-pack nutrition profile labeling: A consumer perspective on key issues and controversies. Critical Reviews in Food Science and Nutrition, 2015; 55: 291e-303.
28. Besler HT, Buyuktuncer Z, Uyar MF. Consumer Understanding and Use of Food and Nutrition Labeling in Turkey. J Nutr Educ Behav 2012; 44: 584-91.
29. Temple NJ, Fraser J. Food labels: A critical assessment. Nutrition 2014; 30: 257-60.
30. Rasberry CN, Chaney BH, Housman JM, Miller PJ. Determinants of nutrition label use among college students. Am J Health Educ 2007; 38: 76–82.
31. Gorton D, Ni Mhurchu C, Chen M, Dixon R. Nutrition labels: a survey of use, understanding and preferences among ethnically diverse shoppers in New Zealand. Public Health Nutr 2008; 12: 1359–65.
32. Drichoutis AC, Lazaridis P, Nayga RM, Kapsokefalou M, Chryssochoidis G. A theoretical and empirical investigation of nutritional label use. Eur J Health Econ 2008; 9: 293–04.
33. Misra R. Knowledge, attitudes, and label use among college students. J Am Diet Assoc 2007; 107**:** 2130–34
34. Carillo E, Fiszman S, Lahteenmaki L, Varella P. Consumers' perception of symbols and health related label messages. A cross-cultural study. Food Research International 2014; 62: 653-61.
35. Bix L, Sundar RP, Bello NM, Peltier C, Weatherspoon LJ, Becker MW. To See or Not to See: Do Front of Pack Nutrition Labels Affect Attention to Overall Nutrition Information? PLoS ONE 2015; 10: E0139732. doi:10.1371/journal.pone.0139732.
36. Gregory D, Ballali S, Vogele C, et al. What is the value given by consumers to nutritional label information? Results from a large investigation in Europe. Journal of the American College of Nutrition 2015; 34: 120e-125.
37. Cecchini, M, Warin, L. Impact of food labelling systems on food choices and eating behaviours: A systematic review and meta-analysis of randomized studies. Obesity Reviews 2016; 17: 201e-210.
38. World Health Organization(WHO). "Diet, Nutrition and the Prevention of Chronic Diseases. Joint WHO/FAO Expert Consultation." WHO Technical Report Series no. 916, 2003. (Available at: http://science.report/pub/10901081.
39. Ollberding NJ, Wolf RL, Contento I. Food Label Use and Its Relation to Dietary Intake among US Adults. Journal of the American Dietetic Associatıon 2010; 110: 1233-1237.
40. World Health Organization (WHO). Global Strategy on Diet, Physical Activity and Health. World Health Organization, Geneva, 2004. (Available at: http://www.who.int/dietphysicalactivity/strategy/eb11344/strategy_english_web.pdf).
41. Vyth EL, Steenhuis HM Ingrid, Vlot JA, et al. Actual use of a front-of-pack nutrition logo in the supermarket: consumers' motives in food choice. Public Health Nutr 2010; 13: 1882-89.
42. Wandel M. Food labelling from a consumer perspective. Br Food J 1997; 99: 212–19.
43. Kreuter MW, Brennan LK, Scharff DP, Lukwago SN. Do nutrition label readers eat healthier diets? Behavioral correlates of adults' use of food labels. Am J Prev Med 1997; 13: 277–83.

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Original article

Do critically ill elderly patients show a different profile than younger patients in terms of prealbumin response to nutrition?

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Summary. Nutritional assessment is essential for the clinical nutrition practice.In critical care, prealbumin is still a useful marker for this purpose.Geriatric patient population is increasing in number in critical care and with accompanying comorbidities, they exert different treatment-response profiles.We analysed prealbumin response of critically ill elderly patients compared to younger patients. *Methods:* 1311 adult patients were included in the 5 years retrospective analysis in our tertiery medical-surgical ICU.Admission levels of prealbumin, albumin, C-reactive protein, as well as CRP/albumin and CRP/prealbumin ratios were compared between the young (<65 years) and elderly (≥65 years)groups of patients, and the relation with outcome was analysed . For the secondary part of the study, patients whose CRP levels persisting over 15mg/L were excluded and 704 patients whose inflammatory response subsided and who were equally fed were analysed for the prealbumin response in the following weeks. The difference between the admission and the outcome levels (died, discharged or the 28th day) of prealbumin were compared within and between the young and elderly groups and their subgroups(died or survived). *Results:* Prealbumin and albumin levels were significantly higher in young group compared to old group on admission. There were no difference in the admission prealbumin, albumin, CRP levels and CRP/albumin, CRP/prealbumin ratios in the died or survived subgroups of younger patients .Whereas in the older group, admission prealbumin, albumin levels were lower, CRP, CRP/albumin, CRP/prealbumin ratios were significantly higher in died subgroup in terms of the 28th day, and the hospital outcomes ($p<0,05$).Outcome levels of prealbumin were higher in both groups ($p<0,05$) but the younger group showed a more pronounced prealbumin response, although in comparison, the mean levels of increase were not statistically significant ($p>0,05$).In subgroup analysis, mean values of increase between the admission and outcome levels were higher in the survived subgroups ($p<0,05$). *Conclusion:* Under the similar protein intake (0,8-1gr/kg/day), geriatric patients showed a blunted response for prealbumin synthesis compared to younger patients.The failure in this response may be due to the chronic inflammatory state, comorbidities and age related physiological organ dysfunctions which make this nutritional marker even less reliable in this age group.

Key words: prealbumin, elderly, nutrition, critically ill

Introduction

As a result of global rising in the number of the aging population, increasing number of elderly patients are admitted to the intensive care units (ICU). These patients usually have multiple comorbidies presenting different pathophysiologic profiles as well as limited organ functions related with aging.

Along with the other physiologic limitation of the organ functions, nutrition is another important determinant of health in geriatric age group. Critically ill older patients has a high incidence of malnutrition and

sarcopenia that leads to increased morbidity and mortality compared to younger patients (1,2).

Critical illness is associated with hypermetabolism and marked protein catabolism (3,4). In the face of unabated protein catabolism patients can experience greater mortality, increased infections and worsened survival and that would be even more detrimental in older patients (5-8).

Therefore, it is important to appropriately identify who may be at risk for poorer clinical outcomes and who may benefit from an aggressive nutritional strategy, that makes assessment of nutritional status central to the clinical nutrition practice.

However, nutritional assessment is not easy in ICU patients.Andropometric measurements are generally unreliable, especially in older patients, due to the decreased ability to excreate water load, and prolonged overexpansion of extracellular water following resuscitation or sepsis (1,9).

Although the traditional serum protein markers (albumin, prealbumin, transferrin, retinol binding protein) are a reflection of the acute-phase response (increases in vascular permeability and reprioritization of hepatic protein synthesis) and may not accurately represent nutrition status in the ICU setting (10), as the other alternatives (calcitonin, C-reactive protein (CRP), interleukin-1, tumor necrosis factor (TNF), interleukin-6, and citrulline) are still investigational and measures like muscle mass ultrasound and computed tomography of skeletal muscles are not practical, an optimal assessment tool is still pending.

At present, the most useful biological marker of nutritional status still seems to be prealbumin.

Prealbumin is mainly synthetised and catabolised by the liver and excreated by the kidney and gastrointestinal tract with a half-life of 1.9 days (11). Although it is known that inflammation and critical illness render serum proteins including serum prealbumin concentration unreliable (12), during the recovery period of the acute illness where the inflammatory phase of the disease subsets, it still can be regarded as a marker of nutrition in critically ill patients.

In our retrospective analysis of critically ill patients, we aimed to compare the prealbumin responses of two different age groups. We planned to investigate if the older age group appropriately responds to the nutrition by increasing prealbumin levels when the amount of the calorie/protein intake is similar to the younger age group. We also compared two groups in terms of the prognostic value of nutritional indexes like CRP/albumin and CRP/prealbumin ratio.

Subjects and methods

The study is held in a university affliated teaching and research hospital. We performed a single center, retrospective observational study of the medical records of 21 bed ICU over a 5 -year period. Hospital management approval was obtained for the data collection from the hospital files. The nutritional assessments as well as the demographic and related parameters were recorded by the members of the nutrition team of the hospital; a dietitian and a nutrition nurse. They visited the patients admitted to ICU and followed up two times a week, but the prealbumin was checked once a week. The nutritional needs of the patients were generally estimated using a standardised amount of 25 kcal/kg/day. We analysed all patients admitted to our ICU, both diagnosed for surgical or medical reasons. As this is a retrospective study, if there was a missing data in this process, those patients were excluded.

Inclusion criterions:

Patients over 18 years old who stayed in ICU at least two weeks, and who has at least an admission and an outcome measurement of prealbumin/albumin/CRP levels.

Patients whose total weekly calorie intake after the first week was over 75% of the calculated needs.

Exclusion criterions:

For the prealbumin response, patients whose CRP levels over 15 mg/ L after the first week (Tto exclude the suppressive effect of inflammatory status on prealbumin production). Severe liver and kidney failure patients who need hemodialysis or hemodiafiltration. Patients who has known to have protein-losing nephropaties.

The patients who fulfilled the inclusion criterions were divided in two groups: patients between 18-64 years old and patients aged 65 years and over.

For the second part of the analysis, the groups were subdivided according to their outcome, dead or survived.

Data recordings and calculations:

Age, gender, body mass index (BMI), APACHE II, Nutritional Risk Screening (NRS 2002) scores (13), diagnosis on admission and comorbidities.

The amount of nutrients the patient needed and actually hand.

CRP, albumin, prealbumin levels, CRP/albumin and CRP/prealbumin ratios within the first 48 hours.

The last prealbumin, albumin and CRP levels on discharge, on death or on 28th day when the study ended; these values were taken as the level of outcome regardless of the lenght of stay in ICU (LOS ICU).

The difference between the prealbumin levels on admission and on outcome (Delta prealbumin levels)

Prealbumin, albumin and CRP results of patients who had the measurements in each consecutive week (n=113) were recorded until the patient discharged, died or up to the 28th day.

For the interpretation of the prealbumin levels, the hospitals laboratory reference value (prealbumin> 16 mg/dl; immunoturbimetric technique, Abbott lab.) was adjusted for the critically ill patient group: Low prealbumin<11 mg/dl; and normal prealbumin>11 mg/dl (14).

As the patients whose CRP levels were below 15mg/ L in the second week onwards were included in the study, and as the calorie-protein intakes were equal between younger and older groups, the prealbumin levels from the second week onwards were assumed to show the adequacy of, as well as the patients spesific response to the nutritional support.

Statistical analysis

Data were analysed using Statistical Package for Social (IBM SPSS Statistics). Whether or not the parameters were normally distributed was analysed with ShapiroWilks test. Student–t test was used for the comparison of the quantitative data and normally distributed variables as well as the complementery statistical methods (mean, SD, frequency). For the comparison of not normally distributed variables, Mann Whitney U test was used. For the comparison of normally distributed variables like admission-outcome data within the groups, Paired Samples t test was used. For the change of prealbumin in consecutive weeks, Repeated Measures Analysis of Variance (ANOVA) was used. For the comparison of the qualitative data, Chi square test and Yate's Correction for Continuity was used. The ROC curve was build to evaluate the cut-off points for outcome prealbumin levels. The correlation between the normally distributed parameters was assessed using Pearson Correlation Analysis, and the correlation between the not normally distributed parameters was evaluated using Spearman's rho Correlation Analysis.Two sided values of $p<0.05$ were considered as statistically significant.

Results

In the five years period a total of 6045 patients were admitted to the ICU. In those, 1311 were eligible for the study. After excluding the patients whose CRP levels were over 15 mg/dl from the second week onwards, 704 patients were included in the final analysis (211 patients <65years, 493 patients≥ 65 years old).
Patients characteristics were summarised in Table 1,Table 2, Table 3.
The ICU lenght of stay (<65 years:20.1±13.3 (15)days vs ≥ 65years:21.1±12.9 (17) days; p>0.05) and 28th days mortality rates (30.3% vs 35%; p>0.05) were not found to be significant between two groups, hospital mortality was higher in older patient group (37.4% vs 47.5% ; p<0.05)

Table 1. BMI, NRS, APACHE II and gender of the groups

	<65 years (n=211)≥65 years (n=493)		
	mean±SD (median)	**mean±SD (median)**	**p**
BMI	25.9±6.4 (25)	26.5±5,9 (25,8)	[1]0.072
NRS	4.74±1.2 (5)	5±0.9 (5)	[1]0.013*
APACHE II	22±7.2 (21)	22.6±7 (22)	[1]0.367
Gender n(%)			
Female	85 (40.3%)	256 (51.9%)	[2]0.005*
Male	126 (59.7%)	237 (48 %)	

*[1]Mann Whitney U; [2]Ki-kare; *p<0.05*

Prealbumin and albumin levels on admission were significantly higher in the young group compared to the old group (Table 4).

There were no differences in the admission prealbumin, albumin, CRP levels and CRP/albumin, CRP/prealbumin ratios in the died or survived subgroups of younger patients. Whereas all measured and calculated parameters were significantly different in the died subgroup compared to the survived subgroup of the older patents (Table 5), and the hospital mortality. Elderly patients who died had a longer ICU stay compared to both the survived ones and the subgroups of younger age group ($p<0.05$).

Significant increases on outcome levels of prealbumin were detected compared to admission values in each group ($p<0.05$). In comparison between the mean levels of delta prealbumin levels of younger and older patient groups, the amount of increases in both groups were found to be statistically similar ($p>0.05$) (Table 6).

Comparing the mean delta prealbumin levels of dead and survived subgroups, mean increases in the surviving patients in both groups were significant, whereas the patients with bad prognosis showed almost no increase in prealbumin levels compared to the ones who survive (<65 years: 0.56±6.76 (0)mg/dl vs 3.13±6.98 (2)mg/dl; ≥ 65 years: 0.5±4.99 (0)mg/dl vs 1.71±4.87 (1.6) mg/dl, p= 0.000) (Table 7).The prealbumin levels had a slightly increasing trend in both groups, but the level of increase per consecutive week was not statistically significant ($p>0.05$).

From the first week to the study end point that was the 28th day, the prealbumin levels were always higher in the younger age group and the differences with the elderly group were statistically significant ($p<0.05$) (Figure 1).

To define prealbumin cut-off value for the 28th day outcome in both groups, ROC curves were analysed (Figure 2 and 3).

Table 2. Diagnosis on admission

	<65years(n=211)	≥65 years(n=493)
Diagnosis on admision	**n (%)**	**n (%)**
Neurological Disease	13 (6.2%)	36 (7.3%)
Respiratory Disease	79 (37.4%)	243 (49.3%)
Cardiovascular Disease	19 (9%)	23 (4,7%)
Metabolic Disease	27 (12.8%)	122(24.7%)
Trama/Postoperative	73 (34.6%)	69 (14%)

*[1]Ki-kare test; *p<0.05*

Table 3. Number of comorbidities the patients have: Most of the patients in the elderly group had at least two or more comorbidities, whereas the majority had one comorbidity in younger group (Comorbidities: Hypertension, Diabetes Mellitus, Alzheimers Disease, Chronic Obstructive Airway Disease, Coronary Artery Disease, Cerebrovascular Disease, Chronic Renal Failure, Chronic Heart Failure, Malignities and Others)

Comorbidies (n)	**< 65years (%) n=211**	**≥ 65 years(%) n=493**
0	44 (20.8%)	18 (3.6%)
1	89 (42.1%)	96 (19.4%)
2	32 (15.1%)	154 (31.2%)
3	35 (16.5%)	134 (27.1%)
4	11 (5.2%)	72 (14.6%)
5	0	17 (3.4%)
6	0	2 (0.4%)

Table 4. Prealbumin and albumin values were significantly lower in elderly patients on admission

	<65 years	**≥65 years**	
	mean±SD	**mean±SD**	**p**
Prealbumin	12.58±6.66	9.86±4.69	[1]0.000*
CRP	11.27±8.18	10.39±7.36	[1]0.165
Albumin	2.6±0.7	2.5±0.68	[1]0.03*
CRP/Albumin (median)	4.9±4.5 (3.7)	4.6±3.8 (3.8)	[2]0.899
CRP/Prealbumin (median)	1.5±1.9 (0.86)	1.5±1.6 (0.99)	[2]0.234

*[1]Student t; [2] Mann Whitney U *p<0.05*

Discussion

The prevalence of malnutrition in older adults admitted to medical or surgical ICU reported from 25% up to 68% (15-20).

Regarding the elderly patient in the intensive care unit, the rate and the risks of the malnutrition combining the acute critical illness considerably increases the morbidity and mortality of the patients who already has limited organ functions as a result of the increasing age.That makes nutritional assessment of the geriatric ICU patients even more important.

Table 5. All measured parameters were significantly different in elderly group who died within 28 days

		28th day outcome		
		Died	Survived	p
		Mean±SD	Mean±SD	
<65 years	Prealbumin	12±7	12.8±6.49	[1]0.403
	CRP	11.77±7.9	11±8.3	[1]0.560
	Albumin	2.5±0.77	2.67±0.66	[1]0.204
	CRP/Albumin (median)	5.86±5.7 (4)	4.5±3.78 (3.4)	[2]0.142
	CRP/Prealbumin (median)	1.6±1.7 (1.2)	1.47±2 (0.8)	[2]0.184
≥65 years	Prealbumin	9±4.56	10.3±4.7	[1]0.007*
	CRP	11.68±7.67	9.7±7	[1]0.005*
	Albumin	2.3±0.57	2.6±0.71	[1]0.000*
	CRP/Albumin (median)	5.55±4.3 (4.7)	4.1±3.4 (3)	[2]0.000*
	CRP/Prealbumin (median)	1.86±2 (1.2)	1.34±1.4 (0.9)	[2]0.001*

*[1]Student t test; [2] Mann Whitney U Test; *p<0.05*

Table 6: Increase in outcome prealbumin levels were significant within groups, the delta prealbumin levels (median) were found to be similar between groups.

	<65 years	≥65 years	
Prealbumin(mg/dl)	**Mean±SD**	**Mean±SD**	**p**
Admission	12.58±6.66	9.86±4.69	[1]0.000*
Outcome	14.9±8.18	11.15±5.4	[1]0.000*
Delta prealbumin(median)	2.35±7 (1)	1.3±4.9 (1)	[2]0.184
Admission-outcome [3]p	0.000*	0.000*	

*[1]Student t test; [2] Mann Whitney U Test; [3]Paired Samples t test; *p<0.05*

Prealbumin still an acceptable nutritional marker in ICU, so it is important to define its use in detail for different patients and circumstances.

Related with aging, there are some physiological changes in the gastrointestinal tract like prolonged transit time, declined pancreatic secretions and insulin action (Insulin resistance) (21,22)

The composition of microbiota significantly corraletes with fraility, co-morbidity, nutritional status, and markers of inflammation in the elderly (23). The permeability appears to remain intact (24-25).Proteo-

Table 7. Surviving patients in both groups had increasing levels of prealbumin, whereas the patients with bad prognosis showed almost no increase

		28th day outcome		
		Died	Survived	p
Age	**Prealbumin(mg/dl)**	**Mean±SD**	**Mean±SD**	
<65 years	Admission	12±7	12.84±6.49	[1]0.403
	Outcome	12.56±7.8	15.96±8.15	[1]0.005*
	Delta prealbumin(median)	0.56±6.76 (0)	3.13±6.98 (2)	[2]0.010*
	Admission-Outcome[3]p	0.507	0.000*	
≥65 years	Admission	9±4.56	10.29±4.7	[1]0.007*
	Outcome	9.59±4.72	12±5.6	[1]0.000*
	Delta prealbumin(median)	0.5±4.99 (0)	1.7±4.87 (1.6)	[2]0.010*
	Admission-Outcome[3]p	0.194	0.000*	

*[1]Student t test; [2] Mann Whitney U Test; [3]Paired Samples t test; *p<0.05*

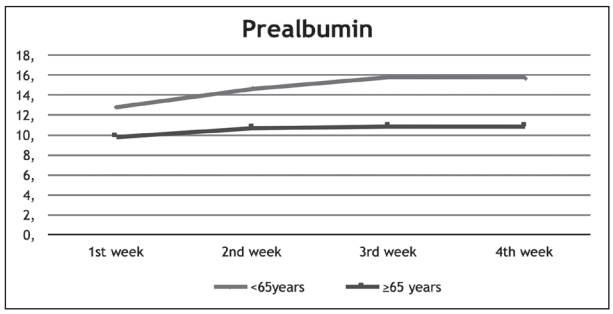

Figure 1: Prealbumin trends in consecutive weeks: Increase in the young group was more prominent than the elderly group

lytic activity in the small intestine appears to be sufficient to ensure a proper digestion of proteins. Absorption of the amino acids in the small intestine are not limited in the elderly but the peripheral availibility of amino acids could be strongly affected by an increased metabolic use of dietery amino acids in the gastrointestinal tract and the liver (26). The net balance of these effects of aging on plasma protein sythesis are not clearly understood.

Protein energy malnutrition leads to a reduction of both somatic proteins of fat-free mass and of visceral proteins.

Sergi et all studied on underweight elderly patients (BMI<20 kg/m2). Along with the low muscle mass, underweight subjects presented reduced mean values of albumin and other visceral proteins. He concluded that the underweight condition and low plasma proteins are both indexes of poor nutritional status enhanced in old people with sarcopenia (27).Our patients body mass indexes were similar, 18% patients in young and 20% patients in elderly group had BMI of <20%.But the admission levels of both albumin and prealbumin were lower in geriatric group.This is again the problem with the elderly and the criticaly ill, we did not know the actual fat and edema-free mass of the patients to confirm that the BMIs were actually true, especially in the geriatric group.

Although nutrition has been considered a very important factor regulating the albumin synthesis (28), in elderly, the presence of comorbid conditions has been considered as the most frequent cause of hypoalbuminemia (29). This is actually an unresolved issue, how much the relationship between hypoalbuminemia and mortality could depend either on malnutrition or on a condition of severe illness and comorbidities. Our elderly group has a high incidence of comorbidities (Table 2) The lower levels of albumin and prealbumin in this group when they accepted to ICU can be explained by malnutrition, sarcopenia, accompanying comorbidities and high inflammatory state associated with acute critical illness. The 28th day mortality and the lenght of stay in ICU were similar, but the long term hospital mortality was higher, this again undoubtly is the result

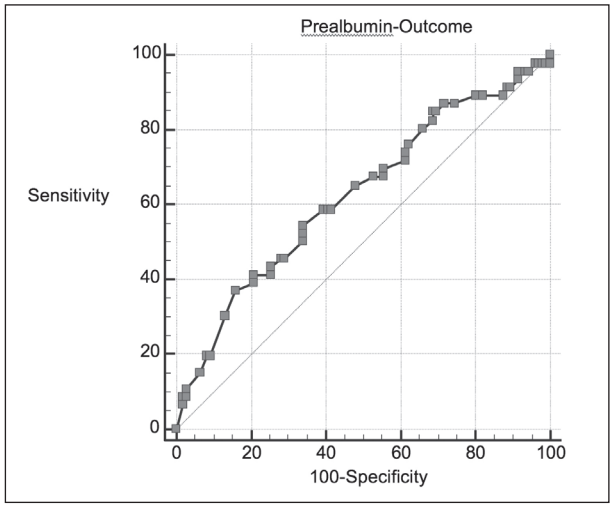

Figure 2: Prealbumin cut-off value for the 28th day outcome in group <65 years:The area under curve is calculated as 0.624, SD:0.05(p=0.015; p<0.05) .Prealbumin cut-off value for 28th day outcome is ≤8 mg/dl.(sensitivity:36.9%,specificity:: 83.9%)

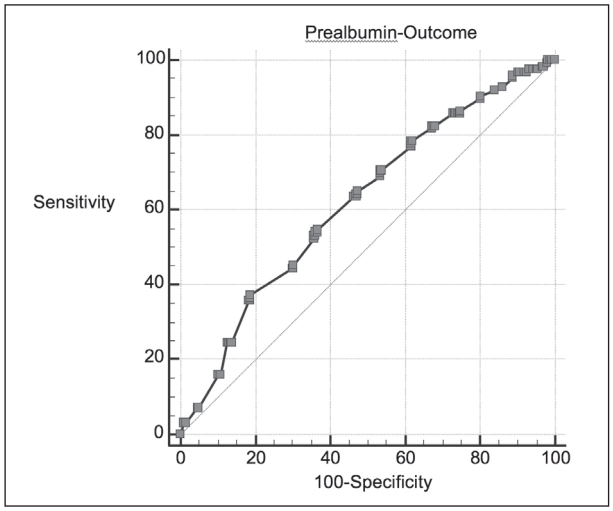

Figure 3: Prealbumin cut-off value for the 28th day outcome in group≥65 years:The area under curve is 0.619, SD:0.03(p=0.0005. p<0,05) and prealbumin cut-off value is ≤7.4 mg/dl for the old age group(sensitivity:37.3%, specificity: 81.3%)

of not recovering the whole complexity of the chronic inflammation and organ dysfunctions in the elderly.

In the mortality prediction, CRP/albumin and CRP/prealbumin ratios both were significant only in the geriatric group. The patients who died within the 28th days and after had higher CRP/albumin and CRP/prealbumin ratios together with lower albumin and prealbumin levels in the elderly group but not in the younger group. With these findings, it is possible to say that if malnutrition and high inflammation is together in an elderly patient, a worse outcome can be expected. Similarly, in critically ill trauma patients, the combination of low albumin level and increased age was found to be the most predictive of infection and mortality (30).

The prognostic and inflammatory nutritional index (PINI= Orosomucoid * C-reactive protein / albumin*prealbumin) was used to predict mortality in ICU patients (31,32). In the study of Gharsallah et all, this score was found to be correlated with the organ failure but not with the mortality. In their study, age again was the only significant parameter between the died and survived patients (32).

The positive relation with the CRP/albumin and CRP/prealbumin inflammation-based prognostic scores and morbidity-mortality is also shown in patients treated with parenteral nutrition. CRP/albumin is found to be relevant with mortality and major morbidities, with CRP/prealbumin, it is also significantly related with lenght of ICU stay and lenght of mechanical ventilation (33).

In a recent review on outcome prediction by nutrition indicators in the critically ill reported that 6 studies out of 12 found an association between improved outcome and serum prealbumin levels when measured in the following days of ICU stay (34). Another study reported that slightly low concentration of prealbumin (<16 mg/dl) at admission is associated with increased lenght of stay and mortality (35). It is also stressed that the estimate of initial value is needed to assess prognosis.

In their review, Delliere et al, proposed an algorithm to help clinicians as a reference on how to use prealbumin as a useful parameter (14). As the inflammatory process causes liver synthesis priority to favor inflammatory proteins such as C-reactive protein and alpha 2 macroglobulin at the expense of prealbumin, it is suggested that prealbumin should be interpreted only if the CRP level is below 15 mg/ L and there is no inflammatory syndrome.

For a patient accepted to ICU, CRP levels above this level is a common finding, but as the acute inflammatory state subsides, CRP levels is expected to decrease and the interpretation of prealbumin levels can become acceptable.We therefore have included all the suitable patients regardless of the CRP levels on admission, but after the first week, the ones with CRP values over 15mg/L were excluded.

According to the latest recommendations, the level of 11 mg/dl is suggested to be the treshold for the the good prognosis of the ICU patient and an increase of 4 mg /dl or more in prealbumin concentration per week reflects a metabolic switch to anabolism (14,36,37).

In our two groups of patients, the young group had a mean prealbumin value that is over the ICU treshold (12.6±6.66 mg/dl) but the geriatric group had lower levels (9.86±4.69 mg/dl) checked in the first 48 hours of admission.On outcome, both groups showed some increase in levels (14.9±8.18 mg/dl and 11.15±5.43 mg /dl). The differences in dead and survived subgroups were more remarkable. Similarly, the prealbumin increase in young and survived patients was reached almost the anabolic level, but the elderly and survived patients showed a modest increase, (3.13±6.98 (2) vs 1.7±4.87 (1.6), p= 0.000) (Table 6), that may suggest an inadequate response of the geriatric patient to nutrition.

We finally sub-analysed the patients who could be followed up for four weeks and who had prealbumin levels checked every consecutive week. The mean value of young patients on the 28th day was 15.77±6.6 mg/dl, the value in the elderly group could reach only 10.86±5 mg/dl (p=0,000).

The relationship between hepatic proteins like albumin and prealbumin and protein intake was assumed with the observation of the increasing levels after the acute illness subsets and when the patients nutritional intake improved, but determining the nutritional status may not be as easy as reviewing serum protein levels because it does not reflect the complexity of hepatic protein syhthesis, especially in the old (38).

Non-nutritional conditions as inflammatory states have an increased prevalance with advancing age, and these states may not always be clinically overt. Low values of albumin can be related to protein–vasting syndromes, hepatic disease or alterations of hydration state (28).

In a previous study, albumin synthesis was evaluated in young and elderly subjects who received an adequate protein intake (1.5 g /kg for 7 days) or a low protein intake (0.4 g/kg for 14 days). The fractional synthesis rate of the whole body albumin pool with adequate intake of protein was 4%/day in the young and 3.4%/day in the elderly. This fractional rate reduced significantly by giving the low-protein diet to the young subjects, but not reduced in the elderly. Serum albumin concentration was lower in the elderly at both levels of protein intake; the rate of albumin synthesis in the young, but not in the elderly was tought to be sensitive to changes in protein intake. It was suggested that albumin synthesis in the elderly had been controlled at a lower set point, which prevented its response to higher protein intakes (39).

We have choosen only the patients whose total calorie/protein intake was over 75% of the needs. Mean protein intake of both groups were calculated between 0.8-1 gr/kg/day.This amount is actually below the recommended limits of the critically ill patient group ; as the current clinical practice recommendations are to give patients with mild to moderate illness 0.8–1.2 g/kg protein per day, and to prescribe critically ill patients higher protein diets of 1.2–1.5 g/kg protein per day, that may go up to 2.5 g protein/kg/ day in the guidelines for critical illnesses (39-43).

However, it is currently being discussed that solutions have a nonprotein energy/nitrogen ratio too high to provide adequate 'protein' without overfeeding energy in critically ill patients. Protein prescription failed to meet most guidelines in >50% of ICU patients receiving full enteral nutrition, when constrained to not overfeed (45). Our study patients usually had standart formulas which do not adequately account for protein needs . Unless protein requirements are independently estimated and protein supplements are used, it is hard to meet the protein requirements without giving excess energy that may itself increase net protein catabolism (44,45).

Older patients have a higher prevalence of renal dysfunction and renal failure at admission to the ICU. This provides an additional challenge to providing adequate protein nutrition. If the patient is undergoing dialysis protein needs are increased (14). For those patients with moderately impaired renal function, the protein amount of 0.8 g/kg/ day is usually recommended in stable conditions, while during illness, it is recommended to increase protein intake to 1 g/kg/ day to meet the higher demand (46,47).

We excluded the patients with acute renal failure requiring renal replacement therapies. But the patients with mild to moderate degree of renal dysfunction who did not need dialysis were included. Regarding the geriatric patient, in the other hand, unless renal functions and the renal risk of the acute critical disease are closely monitorised, protein load may impose an extra renal risk. This may be a confounding factor for not to achieve the target level of anabolic state of these group of patients in ICU, also limiting the establishment of specific protein recommendations for the various stages of critical illness in geriatric patients.

In conclusion, under the similar energy/protein intake, geriatric patients showed a blunted response for prealbumin synthesis compared to younger patients. With the same APACHE II scores and similar BMIs, they had a higher risk of malnutrition, lower prealbumin-albumin levels and higher inflammatory indexes which may show worse prognosis.

On the other hand, when we focus on the non-survival subgroups of the both elderly and young patients, the low prealbumin-albumin levels and the finding of no increase in prealbumin values especially in these subgroups bring the discussion of these markers being indicators of morbidity and mortality, rather than being nutritional markers. In fact, this statement was currently expressed; according to the Academy of Nutrition's analysis, serum proteins such as albumin and prealbumin are not included as defining characteristics of malnutrition because evidence analysis shows that serum levels of these proteins do not change in response to changes in nutrient intake, they are more likely to have prognostic value like morbidity-mortality or recovery from acute and chronic disease (48-50).

Our study was a retrospective search of the five years recordings. The measurements were taken once

a week, not twice as recommended for a marker with short half life. In the light of the current suggestions on protein dosage for critically ill patients, our study may be repeated with a higher protein intake, with carefull follow up of the elderly group in terms of the renal functions.

References

1. Moisey LL, Mourtzakis M, Cotton BA, et al. Nutrition and Rehabilitation Investigators Consortium (NUTRIC), Skeletal muscle predicts ventilator-free days, and mortality in the elderly ICU patients. Crit. Care 2013, Sep 19;17(5):R206.
2. Du Y, Karvellas CJ, Baracos V, Williams DC,Williams DC, Khadaroo RG. Sarcopenia is a predictor of outcomes in very elderly patients undergoing emergency surgery. Surgery 2014;156(3):521-7.
3. Dickerson RN, Pitts SL, Maish GO, et al.A reappraisal of nitrogen requirements for patients with critical illness and trauma. J Trauma Acute Care Surgery 2012;73,549-57 (Cross Ref).
4. Monk DN, Plank LD, Franch-Arcas G, Finn PJ, Streat SJ, Hill GL. Sequential Changes in the metabolic response in critically injured patients during the first 25 days after blunt trauma. Ann Surg 1996;223,395-405 (Cross Ref).
5. Doig GS, Heighes PT, Simpson F, Sweetman EA. Early enteral nutrition reduces mortality in trauma patients requiring intensive care: A meta-analysis of randomised controlled trials. Injury 2011;42(1):50-6.
6. Rapp RP, Young B, Twyman D, et al. The favorable effect of early parenteral feeding on survival in head injured patients. J Neurosurg 1983;58,906-12 (Cross Ref).
7. Sacks GS, Brown RO, Teague D, Dickerson RN, Tolley EA, Kudsk KA., Early nutrition support modifies immune function in patients sustaining severe head injury.JPEN J. Parenter. Enter. Nutr. 1995;19,387-92 (Cross Ref).
8. Härtl R, Gerber LM, Ni Q, Ghajar J. Effect of early nutrition on deaths due to severe traumatic brain injury. J Neurosurg 2008;109(1):50-6.
9. Luckey AE, Parsa CJ., Fluid and electrolytes in the aged, Arch Surg 2003;138(10):1055-60 (Cross Ref).
10. Davis CJ, Sowa D, Keim KS, Kinnare K, Peterson S. The use of prealbumin and C-reactive protein for monitoring nutrition support in adultpatients receiving enteral nutrition in an urban medical center. JPEN J Parenter Enteral Nutr 2012;36(2):197-204.
11. Ingenbleek Y, Young V. Transthyretin (prealbumin) in health and disease: nutritional implications, Annu Rev Nutr 1994;14: 495-553 (Cross Ref).
12. Dennis RA, Johnson LE, Roberson PK, et al. Changes in prealbumin, nutrient intake, and systemic inflammation in the elderly recuperative care patients. J Am Geriatr Soc 2008;56(7):1270-5.
13. Kondrup J. Nutritional-risk scoring systems in the intensive care unit. Curr Opin Clin Nutr Metab Care 2014;17(2):177-82.
14. Dellière S, Cynober L. Is transthyretin a good marker of nutritional status? Clin Nutr 2017;36(2):364-70.
15. Sheean PM, Peterson SJ, Chen Y, Liu D, Lateef O, Braunschweig AC. Utilizing multiple methods to classify malnutrition among elderly patients admitted to the medical and surgical intensive care units (ICU), Clin Nutr 2013;32(5):752-7.
16. Tripathy S, Mishra JC. Assessing nutrition in the critically ill elderly patient: A comparison of two screening tools, Indian J Crit Care Med 2015; 19(9): 518-22.
17. Ülger Z, Halil M, Kalan I, et al. Comprehensive assessment of malnutrition risk and related factors in a large group of community-dwelling older adults, Clin Nutr 2010;29(4):507-11.
18. Kaiser MJ, Bauer JM, Rämsch C, et al. Frequency of malnutrition in older adults: a multinational perspective using the mini nutritional assesment, J Am Geriatr Soc 2010:58:1734-8.
19. Saka B, Kaya O, Ozturk GB, Erten N, Karan MA, Malnutrition in the elderly and its relationship with other geriatric syndromes, Clin Nutr 2010;29(6):745-8.
20. Norman K, Pichard C, Lochs H, Pirlich M. Prognostic impact of disease-related malnutrition. Clin Nutr 2008;27(1):5-15.
21. Didier R, Shahar DR, Gille D, et al. Understanding the gastrointestinl tract of the elderly to develop dietery solutions that prevent malnutrition. Oncotarget 2015;6(16):13858-98.
22. Basu R, Breda E, Oberg AL, et al. Mechanisms of the age-associated deterioration in glucose tolerance: contribution of alterations in insulin secretion, action, and clearence. Diabetes 2003;52(7):1738-48.
23. Claesson MJ, Jeffery IB, Conde S, et al. Gut microbiota compositioncorrelates with diet and health in the elderly. Nature 2012;488:178–84.
24. Saltzman JR, Kowdley KV, Perrone G, Russell RM.Changes in small-intestine permeability with ageing. J Am Geriatr Soc 1995;43:160-164. 58.
25. Valentini L, Ramminger S, Haas V, et al. Small intestinal permeability in older adults. Physiol Rep 2014;2(4):e00281.
26. Boirie Y, Gachon P, Beaufrère B,et al, Splanchnic and whole body leucine kinetics in young and elderly men. Am J Clin Nutr 1997;65:489-95.
27. Sergi G, Coin A, Enzi G, et al. Role of visceral proteins detecting malnutrition in the elderly, Eur J Clin Nutr 2006;60:203-9.
28. Marcus A, Rothschild MD, Murray O, Schreiber SS, Serum albumin. Hepatology 1988;8:385-401.
29. Friedman PJ, Caradoc-DaviesTH, Campbell AJ. Hypoalbuminemia in the elderlyis due to disease, not malnutrition, J Clin Exp Geront 1985;7:191-203.
30. Sung J, Bochicchio GV, Joshi M, et al. Admission serum albumin is predictive of outcome in critically ill trauma patients. Am Surg 2004;70(12):1099-102.

31. Bonnefoy M, Ayzac L, Ingenbleek Y, Kostka T, Boisson RC, Bienvenu J. Usefulness of the prognostic inflammatory and nutritional index(PINI) in hospitalized elderly patients. Internat. J Vit Nutr Res 1998;68,189-95.
32. Gharssallah, H Hajjej, Z, Naas I,et al. Assessment of Nutritional Status and Prognosis in Surgical Intensive Care Unit: The Prognostic and Inflammatory Nutritional Index (PINI). Int J Nutr Food Sci 2014;3:477-83. 10.11648/j.ijnfs.20140305.27.
33. Talaveron IJ, Badia-Tahull MB, Leiva-Badosa E. An inflammation based prognostic score score, the C-reactive protein/albumin ratio predicts the morbidity and mortality of patients on parenteral nutrition. Clin Nutr 2018;37(5):1575-83.
34. Ferrie S, Allman-Farinelli M. Commonly used 'nutrition' indicators do not predict outcome in critically ill: a systematic review. Nutr Cln Pract 2013;28:463-84.
35. Potter MA, Luxton G. Prealbumin measurement as a screening tool for protein calorie malnutrition in emergency hospital admissions: a pilot study. Clin Invest Med 1999;22:44-52.
36. Bernstein L. Measurement of visceral protein status in assessing protein and energy malnutrition: standart of care. Prealbumin in nutritional care consensus group. Nutrition 1995;11:169-71.
37. Ingenbleek Y, Young VR, Significance of transthyretin in protein metabolism. Clin Chem Lab Med 2002;40:1281-91.
38. Marcason W. Should albumin and prealbumin be used as indicators for malnutrition? Journal of the academy of nutrition and dietetics' knowledge center team 2017;7:1144.
39. Gersovitz M, Munro HN, Udall J, Young VR. Albumin synthesis in young and elderly subjects using a new stable isotope methodology: response to level of protein intake. Metabolism 1980;29(11):1075-86.
40. Taylor S, Dumont N, Clemente R, Allan K, Downer C, Mitchell A. Critical care: Meeting protein requirements without overfeeding energy, Clin Nutr ESPEN 2016;11:e55-e62.
41. McClave S, Taylor BE, Martindale RG et al. ASPEN. Board of directors and the American college of critical care medicine. guidelines for the provision and assessment of nutrition supporttherapy in the adult critically ill patient: Society of Critical Care Medicine (SCCM) and American Society for Parenteral and Enteral Nutrition (A.S.P.E.N.). J Parentr Enter Nutr 2009;33:277e317.
42. Singer P, Berger MM, Van den Berghe G et al. ESPEN guidelines on parenteral nutrition: intensive care. Clin Nutr 2009;28:387e400.
43. Ferrie S, Allman-Farinelli M, Daley M, Smith K. Protein Requirements in the Critically Ill: A Randomized Controlled Trial Using Parenteral Nutrition. JPEN J Parenter Enteral Nutr 2016;40(6):795-805.
44. Macias W, Alaka KJ, Murphy MH, Miller ME, Clark WR, Mueller BA, J Parentr Enter Nutr: Impact of thenutritional regimen on protein catabolism and nitrogen balance in patients with acute renal failure. JPEN J Parenter Enteral Nutr 1996;20(1):56-62.
45. Taylor S, Dumont N, Clemente R, Allan K, Downer C, Mitchell A, Critical care: Meeting protein requirements without overfeeding energy. Clin Nutr ESPEN 2016;11:e55-e62
46. Deer RR, Volpi E, et al. Protein Requirements in Critically Ill Older Adults. Nutrients 2018;10(3):378.
47. Deutz NEP, Bauer M, Barazzoni R, Biolo G, Boirie Y, Westphal A B, et al. ESPEN Expert Group, Protein intake and exercise for optimal muscle function with aging: Recommendations from the ESPEN Expert Group. Clin Nutr 2014;3(6):929-36.
48. Academy of Nutrition and Dietetics Evidence Analysis Library. Nutrition Screening (NSCR): Serum Proteins (2009). http://www.andeal.org/topic.cfm.menu=3584&cat=4302. Accessed April 12, 2017
49. Academy of Nutrition and Dietetics. Nutritional Care Manual. Malnutrition Coding.) 144942&ncm_toc_id=144942&ncm_heading=&. Accessed April 12, 2017
50. Academy of Nutrition and Dietetics, Pocket Guide to Nutrition Assessment.3rd ed. Chicago, IL: Academy of Nutrition and Dietetics 2016;104-6

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Original article

Effects of reproductive and sociodemographic factors on obesity in Turkish women: a pilot study

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Summary. *Background and aim:* Obesity has become a global epidemic. The current research aimed to determine sociodemographic and reproductive predictors of obesity among Turkish women. *Materials and methods:* Eligible subjects (n:833) were 40-64 years-old women living in Turkey. A questionnaire consisted of questions about sociodemographic and reproductive factors and the International Physical Activity Questionnaire were applied to participants by face to face interviews. Multivariate logistic regression examined the risk of being obese with a range of sociodemographic and reproductive factors. All analyses were performed with SPSS software (version 17.0; SPSS, Chicago, Ill., USA). *Results:* The mean BMI of women aged 51-64 years was 30.59±6.35 kg/m^2. After adjustments for all other variables, increased obesity risk remained significant in women who had two children, housewifes, minimum active ones, ex smokers and had less than high school education. For multiple regression analysis sociodemographic factors from the bivariate analyses were entered, controlling for menarch age, menopausal age, hormone RT, parity, number of stillbirth, abortion. There was significant association between family income, occupation, education and BMI. *Conclusion:* In summary these findings showed comparable patterns of association of sociodemographic and reproductive factors with obesity in Turkey. Specific healthy lifestyle counseling is important for decreasing obesity in childbearing age women.

Key words: women, obesity, reproductive factors, sociodemographics, body mass index

Introduction

The prevalence of obesity has been increasing worldwide and become a major public health problem in the World. The increasing number of obesity and overweight cases in developing countries, especially among women, requires serious attention because of its effects on the health care system and the quality of life. Obesity, which was detected among all socioeconomic and age groups in both developed and developing countries, may increase the risk of developing noncommunicable diseases (1).

Turkey has a population of about 76.7 million people with growing economy. In 2012, life expectancy at birth in Turkey was 74.6 years and increased by 3.5 years between 2000 and 2012. The prevalence and problem of overweight/obesity has increased significantly in the last 2 decades in both adults and children (2).

In 2011, more than one-in-five adults (22%) in Turkey was defined as being obese (based on actual measures of their height and weight). This rate remains lower than that in the United States (35% in 2012) and Mexico (32% also in 2012) (3).

Turkish Diabetes Epidemiology Study (TURDEP) I and II researches done in five cities and 15 provinces of Turkey. Between TURDEP-I (1997-1998) and TURDEP-II (2010) surveys, average age-

standardized BMI increased from 26.6 to 28.6 kg/m2 (six kg in women; seven kg in men) and average waist increased from 87.2 to 94.5 cm (six cm in women; seven cm in men) over 12 years in Turkey (4).

According to WHO report, adulthood obesity prevalence forecasts (2010-2030) predict that in 2020, 44% of men and 26% of women will be obese. By 2030, the model predicts that 51% of men and 25% of women will be obese (5).

Among Turkish adults, obesity is associated positively with age, female gender, hypertension, hyperlipidemia, diabetes, parity, smoking cessation, alcohol consumption, marital status, occupation, household income, and family history of obesity, diabetes and hypertension, and negatively with the level of education, current smoking, and physical activity (2, 6-8).

In a survey done in the Trabzon region, among women, a linear association was observed between parity (the number of live births) and the prevalence of obesity and also degree of obesity. In addition BMI were increased with parity (9).

Therefore, this study was conducted to determine the both demographic and reproductive risk factors of obesity among Turkish women.

Materials and Methods

Study participants

Eligible subjects for the study were women (n:833) who were 40-64 years-old and lived in Ankara, Turkey. These women were recruited for the study during the period from June to July 2015. This study was approved by Baskent University Institutional Review Board and written informed consent was taken from all subjects prior to study entry.

Questionnaire

A questionnaire which included age, marital and smoking status, household income, occupation, education, living arrangement, menarch and menauposal age, parity, hormone replacement therapy, number of stillbirth and abortion as sociodemographic and reproductive characteristics was administered by face to face interview method.

2.3 Physical Activity Level

Physical activity level was determined using the short form of the International Physical Activity Questionnaire (IPAQ). IPAQ was developed by a group of experts in the late 1990s by a multinational working group, supported by the WHO, in order to assess PA cross-nationally in adults aged 18-65 years. Four long (31-item) and four short (9-item) questionnaire versions have been designed, which can be self-administered or answered by telephone interview. The recall period used by all long and short IPAQ formats is either the last seven days or a "usual week". IPAQ instruments have been tested in both developed and developing countries and have demonstrated good reliability and acceptable validity properties, at least as good as other self-answered PAQs. The IPAQ committee suggests that the IPAQ-short, last seven days (last 7-d) version, is the format of preference for both national and internationally comparable prevalence studies. Following the published work by Craig et al.(10), the self-answered, last 7-d, IPAQ-short, has been very popular and many studies during the last six years have examined its reliability and validity properties. Turkish population reliability and validity of this questionnaire was determined by Öztürk in 2005(11). The participants physical activiy levels were categorized as 1)vigorous, 2)moderate intensity and 3)walking activities lasting for at least 10 minutes.

Body Mass Index (BMI)

Weight was measured to the nearest 0.1 kilogram using a balance-beam scale with subjects wearing light clothing and no shoes. Standing height was measured with a fixed stadiometer calibrated in centimeters. Body mass index (BMI) was calculated as the weight in kilogram (kg) divided by the square of the height in meters. According to the World Health Organization guidelines, BMI 18.5 to 25 kg/m^2 as normal and BMI ≥30 kg/m^2 as obese.

Statistical Analyses

The analysis of BMI data according to sociodemographic characteristics, smoking and reproductive variable are presented as mean±standard deviation (SD). The normality of the distribution of data was evaluated by the one sample Kolmogorov-Smirnov test.

Mean Differences between groups were determined using independent Student's t-test or Analysis of variance (ANOVA -F- test), as appropriate, followed by Tukey's post-hoc analysis. The univariate and multiple logistic regressions were performed to identify the effect of factors that are associated with obesity. The different logistic models (model 1 and model 2) were designed for reproductive and sociodemographic factors. The odds ratios (OR) are presented together with 95% CIs. Confidence intervals that do not include the value "1" are regarded as having statistical value. According to the given reference categories, CIs with values that include the value "1" are deemed not to be significant. The study data were analyzed using SPSS version 17.0 (SPSS, Chicago, IL, USA). Statistical significance was set at $p < 0.05$.

Results

The mean BMI of the Turkish women by some sociodemographic and reproductive characteristics are described in Table 1. The mean BMI was greater in women who had higher parity, abortus and stillbirth number, were housewives, older, had low education level and family income, both ex-smokers, non-smokers, actives and inactives. These differences were statistically significant ($p<0.05$). The mean BMI of women aged between 51-64 years and 40-50 years, were 30.5±6.35 kg/m^2 and 28.7±6.72 kg/m^2, respectively.

Table 2 shows the odds ratios (OR) for the association of obesity with some sociodemographic and reproductive factors. Menarch and menaupose age, received hormone replacement therapy, living arrangement and marrital status were not associated with obesity.

51-64 year-old Turkish women were more likely to be obese than 40-50 year-old [OR 1.88; 95% confidence intervals (CI) 1.42-2.49]. The risk of obesity increased with parity. Women who have more than 3 children were 8.8 times more likely to be obese compared with never childbirth women ($p<0.05$). Women who had at least one stillbirth or abortus had higher BMI besides 1.6 and 1.8 times more obesity risk than who do not stillbirth, respectively ($p<0.05$). It was found that obesity risk increased with higher income and education level. People with income level below the poverty threshold had increased risk of obesity [OR 2.20; 95% confidence intervals (CI) 1.61-3.01]. Housewives had 6.5 times higher obesity risk than working women. Women with less than high school education level had 10.5 times higher obesity risk compared with at least high school level. Both ex-smokers and smokers had almost 2.4 times higher obesity risk than non-smokers. Obesity risk was higher for inactive women than active ones [OR 2.41; 95% confidence intervals (CI) 1.81-3.22]. After adjustments for all other variables, increased obesity risk remained significant in women who had two children, housewifes, minimum active ones, ex smokers and had less than high school education ($p<0.05$).

Further adjustments were made for potentially confounding variables in two different models (Table 3). In the model 1 for multiple regression analysis sociodemographic factors from the bivariate analyses were entered, controlling for menarch age, menopausal age, hormone RT, parity, number of stillbirth, abortion. There was significant association between BMI and family income (OR 1.58; 95% CI 1.02-2.46), occupation (OR 5.46; 95% CI 3.47-8.59), education (OR 9.67; 95% CI 5.63-16.61).

In the model 2 reproductive factors from the bivariate analyses were entered, controlling for age, family income, marrital status, occupation, education, living arrangement. The model 2 showed a significant relationship between BMI and number of children [(parity: 2; OR 1.90; 95% CI 1.08-3.33 and ≥3; OR 2.57; 95% CI 1.35-4.90)] and abortion [(≥1 (OR 1.62; 95% CI 1.16-2.26)] (Table 3).

Discussion

Obesity is an increasing problem globally and it may cause many adverse effects on health. The prevalence of obesity in women has increased in the world. According to the National Health and Nutrition Examination Survey (NHANES), approximately 62% of American women greater than 20 years of age are overweight. If current trends continue, 58% of American adult women will be obese by 2030. In accordance with Turkish Adult Risk Factor Study (TEKHARF), the prevalance of obesity were 44.2% for women (12).

Table 1. Mean BMI* among Turkish women corresponding to selected risk factors

Risk factors	n	BMI (kg/m²)			t or F test	p value
		Min-Max	Mean	SD		
Age (years)						
40-50	545	18.40-54.30	28.77	6.72		
51-64	764	16.53-49.13	30.59	6.35	-3.95	0.000
Menarch age (years)						
≤13	772	16.53-54.30	29.81	6.61		
14	281	18.60-49.13	29.91	6.00		
15	158	18.78-46.33	29.61	6.81	0.07	0.978
≥16	98	18.02-43.29	30.03	7.51		
Menopausal age (years)						
≤45	275	18.70-46.40	30.58	6.67		
46-50	356	19.33-49.13	30.98	6.10	1.01	0.368
≥51	213	16.53-42.86	29.99	6.41		
Hormone RT*						
No	1157	16.53-54.30	29.72	6.57		
Yes	152	18.70-47.00	30.54	6.54	1.20	0.230
Parity						
0	146	18.02-45.35	26.82[a]	6.15		
1	196	16.53-43.28	26.43[a]	6.51		
2	589	18.60-49.13	29.76[b]	6.51	39.56	0.000
≥3	378	19.90-54.30	32.73[c]	5.41		
Number of stillbirth						
0	1164	16.53-54.30	29.59	6.52		
≥1	145	19.60-47.00	31.60	6.65	-2.86	0.004
Abortion						
0	667	18.02-46.33	28.71	6.39		
≥1	642	16.53-54.30	30.94	6.55	-4.97	0.000
Family income						
Above poverty line	882	18.02-54.30	28.93	6.43		
Poverty line	427	16.53-47.00	31.67	6.45	5.76	0.000
Marrital status						
Married	993	16.53-54.30	30.02	6.49		
Non-married	316	18.02-49.13	29.26	6.75	1.47	0.143
Occupation						
Employed	663	16.53-47.00	27.01	5.96		
Housewife	646	18.73-54.30	32.54	5.95	13.36	0.000
Education						
≥High-school	767	16.53-49.13	26.97	5.97		
<High school	542	18.60-54.30	33.33	5.49	15.97	0.000
Smoking status						
Non-smoker	764	18.02-46.40	30.30[a]	6.22		
Ex-smoker	190	16.53-49.13	31.06[a]	6.79	10.42	0.000
Smoker	355	18.40-54.30	28.18[b]	6.93		
Living arrangement						
Co-habitating	1218	16.53-54.30	29.82	6.57		
Alone	91	18.02-41.50	29.83	6.48	0.01	0.992
IPAQ*						
Active	45	19.60-47.01	31.09[a]	7.48		
Minimum Active	517	16.50-44.42	27.94[b]	6.23	23.26	0.000
Inactive	720	18.61-54.30	31.08[a]	6.40		

**BMI: Body Mass Index, RT: Replacement Therapy, IPAQ: International Physical Activity Questionnaire*
Values with different superscript letters are statistically significantly different (p<0.05).

Table 2. Univariate logistic regression of risk factors for obesity (BMI ≥ 30 kg/m²) in Turkish women

Risk factors	n	Unadjusted OR (95% CI)	p	Adjusted OR (95% CI)	p
Age (years)					
40-50	351	1.0§		1.0	
51-64	482	1.88 (1.42-2.49)	0.000*	0.82 (0.41-1.66)	0.595
Menarch age (years)					
≤13	493	1.0		1.0	
14	179	1.16 (0.81-1.65)	0.407	0.96 (0.52-1.78)	0.900
15	101	0.97 (0.63-1.51)	0.910	1.04 (0.48-2.25)	0.911
≥16	60	0.91 (0.52-1.56)	0.726	0.89 (0.34-2.35)	0.826
Menopausal age (years)					
≤45	182	1.0		1.0	
46-50	223	1.26 (0.83-1.92)	0.266	1.18 (0.68-2.05)	0.552
≥51	131	0.90 (0.56-1.43)	0.657	0.80 (0.42-1.52)	0.504
Hormone RT**					
No	727	1.0		1.0	
Yes	106	1.31 (0.85-2.01)	0.214	0.89 (0.46-1.72)	0.750
Parity					
0	100	1.0		1.0	
1	124	0.87 (0.50-1.52)	0.646	0.82 (0.32-2.10)	0.687
2	353	2.49 (1.57-3.94)	0.000*	2.43 (1.05-5.61)	0.037*
≥3	256	8.81 (5.21-14.86)	0.000*	2.16 (0.84-5.57)	0.109
Number of stillbirth					
0	735	1.0		1.0	
≥1	98	1.62 (1.03-2.56)	0.036*	0.95 (0.47-1.89)	0.887
Abortion					
0	417	1.0		1.0	
≥1	416	1.83 (1.38-2.43)	0.000*	1.62 (0.99-2.63)	0.052
Family income					
Above poverty line	560	1.0		1.0	
Poverty line	273	2.21 (1.61-3.01)	0.000*	0.81 (0.47-1.41)	0.470
Marrital status					
Married	615	1.0		1.0	
Non-married	218	0.76 (0.55-1.04)	0.083	1.42 (0.75-2.68)	0.271
Occupation					
Employed	409	1.0		1.0	
Housewife	423	6.56 (4.81-8.96)	0.000*	2.84 (1.61-5.01)	0.000*
Education					
≥High-school	459	1.0		1.0	
<High school	374	10.47 (7.37-14.86)	0.000*	4.95 (2.58-9.51)	0.000*
Smoking status					
Non-smoker	503	1.0		1.0	
Ex-smoker	105	2.43 (1.51-3.94)	0.000*	0.45 (0.25-0.81)	0.008*
Smoker	225	2.38 (1.73-3.29)	0.000*	1.29 (0.67-2.48)	0.431
Living arrangement					
Co-habitating	779	1.0		1.0	
Alone	54	0.98 (0.56-1.73)	0.965	1.16 (0.43-3.08)	0.765
IPAQ					
Active	32	1.0		1.0	
Minimum Active	316	1.72 (0.83-3.54)	0.145	5.50 (1.54-19.58)	0.008*
Inactive	468	0.69 (0.33-1.43)	0.312	1.97 (0.55-7.05)	0.297

*OR: Odds Ratio, CI: Confidence Interval, *p<0.05*, §Reference Group ***BMI: Body Mass Index, RT: Replacement Therapy, IPAQ: International Physical Activity Questionnaire*

Table 3. Multiple logistic regression coefficients of risk factors for obesity (BMI ≥ 30 kg/m²) in Turkish women

Risk factors	Adjusted OR	95% CI	p
Model 1			
Age (years)			
40-50	1.0§		
51-64	0.97	0.55-1.74	0.942
Family income			
Above poverty line	1.0		
Poverty line	1.58	1.02-2.46	0.040*
Marrital status			
Married	1.0		
Non-married	1.18	0.73-1.90	0.493
Occupation			
Employed	1.0		
Housewife	5.46	3.47-8.59	0.000*
Education			
≥ High-school	1.0		
< High school	9.67	5.63-16.61	0.000*
Living arrangement			
Co-habitating	1.0		
Alone	1.33	0.63-2.81	0.452
Model 2			
Menarch age (years)			
≤13	1.0		
14	0.88	0.58-1.34	0.567
15	0.71	0.41-1.22	0.222
≥16	0.59	0.30-1.16	0.129
Menopausal age (years)			
≤45	1.0		
46-50	1.12	0.68-1.84	0.652
≥51	0.88	0.49-1.56	0.669
Hormone RT			
No	1.0		
Yes	0.64	0.39-1.04	0.075
Parity			
0	1.0		
1	0.99	0.52-1.87	0.985
2	1.90	1.08-3.33	0.024*
≥3	2.57	1.35-4.90	0.004*
Number of stillbirth			
0	1.0		
≥1	1.10	0.63-1.89	0.733
Abortion			
0	1.0		
≥1	1.62	1.16-2.26	0.004*

*§ Reference Group; *p<0.05*
Model 1: adjusted for menarch age, menopausal age, hormone RT, parity, number of stillbirth, abortion
Model 2: adjusted for age, family income, marrital status, occupation, education, living arrangement

In the Turkish Epidemiology Survey of Diabetes, Hypertension, Obesity and Endocrine Disease (TURDEP-II) study which investigated the prevalence of obesity, 12-year obesity rise among women has been identified as 34% according to TURDEP 1(13). The average BMI of Turkish adults was 28.9±6.4 kg/m^2 as stated in Turkey Nutrition and Health Survey (14).

Women obesity is associated with increased risk of hypertension, metabolic syndrome, insulin resistance, dyslipidemia, systemic inflammation, cardiovascular disease, sleep apnea, polycystic ovarian syndrome, stroke, and mortality, various gender-spesific, colon and kidney cancers (15).

Multiple factors can account for obesity. Thus in this study the obesity risk factors for women was investigated and we found that some reproductive and sociodemographic factors, especially family income, parity, abortion, education contribute obesity among women aged 40-64 years in Turkey.

In the present study the mean BMI of 40-50 years and 51-64 years of women were 28.77±6.72 kg/m^2 and 30.59±6.35 kg/m^2, respectively (Table 1). 51-64 years old Turkish women were more likely to be obese than others and this group had 1.88 times higher obesity risk (95% CI 1.42-2.49) (Table 2). However this association was attenuated with adjustments in multiple logistic regression analyses ($p>0.05$) (Table 3). Weight gain increases with age for instance Wen et al. (7) found strongest association of age with obesity and obesity prevalance was highest among the women with 51-55 years of age.

The prevalence of overweight and obesity differs by marital status. The results of studies about marriage and weight gain are contradictory (16). A recent study indicated that non-married women were at increased risk overweight and obesity (17). While another study showed that entering marriage is associated with weight gain, particularly among women (18). In this study, non-married women had higher obesity risk than married women, but this risk wasn't statistically significant ($p>0.05$) (Table 2 and 3).

Education levels were strongly related to body weight in women. According to Turkey Nutrition and Health Survey (TNHS-2010), the average body weight of women decreased while education increased (14). Moreover the obesity prevalance was 18.4% in women with more than high school education whereas 54.2% in illiterate women. In a study conducted by Wardle et al. (19) stated that women with less education are at higher risk for obesity. A significant inverse association between educational level and BMI for women was found in the WHO MONICA Project in common with our study (20). After adjusted for reproductive factors (menarch age, menopausal age, hormone RT, parity, number of stillbirth, abortion), women who had less than high school education had 9.67 times higher obesity risk than who had at least high school education (Table 3).

In this study, the prevalence of obesity was conspicuously higher in housewife group. In the multiple logistic regression model adjusted for reproductive factors, housewifes had 5.46 times higher obesity risk than employed women ($p<0.05$). The occupational activity was thought to be the most potent factor protecting women against getting obese. Employed women might have more occupational walking activity (21). Housewives generally have less time to care about their health based on homemaking duties. Women's social participation has recently increased the number of working housewives, resulting in changes in dietary patterns. Changes in the dietary intake of housewives, along with decreases in physical activity, have led to the social problem of obesity (22).

Obesity rates are greater in high-income countries than middle and low income countries (23). According to Turkey Statistical Institute-Income and Living Conditions Survey, population below the poverty line was 15% of Turkish adults (24). Furthermore Erem et al. reported that there was a significant association between household income and prevalence of obesity in Trabzon, North side of Turkey (9).

The link between poverty and obesity may be complicated because of confounding factors especially physical activity status. In the previous study it is stated that low income women may be experiencing lower levels of physical activity (25). Thus we did adjustments for all other factors before investigated the association between income status and obesity, the relationship was not significant ($p>0.05$). However after adjustment for reproductive factors, women with low income (below the poverty line) had increased risk of obesity ($p<0.05$).

Sedentariness is associated with poor health, obesity, diabetes, other metabolic diseases, and premature death (5). In this research, according to IPAQ subgroups, minimum active and inactive women had higher obesity risk however after adjustment for other confounding factors minimum active women had significantly higher obesity risk than active ones.

The mechanism of weight gain after smoking cessation includes increased energy intake, decrease resting metabolic rate and physical activity, increased lipoprotein lipase activity (27). Consistent with the previous study (26), after adjustments for other confounding factors ex-smokers had significantly higher obesity risk than non-smokers in this study (Table 2).

Even adjusted for reproductive factors (menarch age, menopausal age, hormone RT, parity, number of stillbirth, abortion), income, occupation and education were associated with women obesity in the multivariate model (Table 3).

The average menopausal age of Turkish women was 46.4±1.9 years (27). Palacios et al. (28), observed the average menapausal age across the world: in Europe, it ranged from 50.1 to 52.8 years; in North America, it ranged from 50.5 to 51.4 years; in Latin America, it ranged from 43.8 to 53.0 years; and in Asia, it ranged from 42.1 to 49.5 years (29).

Menarche and menopausal age, hormone replacement therapy usage and number of stillbirth after adjusting for demographic factors (age, family income, marrital status, occupation, education, living arrangement) were not a strong predictor of obesity and were not significant in the multivariate model. However abortion and parity were strong predictors of obesity in this model (Table 3). Similar to our study, Bastian et al. (30) found that, in comparison to the reference group of no live births, increasing number of children were associated with higher rates of obesity in older women and that was independent of sociodemographic and other confounding factors.

There are some limitations of this study. The primary concern is the number of our study population may not be enough. Inclusion of more women in our study could make our data be true representative of the general population. The BMI is an usually valid method used for the evaluation of obesity. But the utility of BMI as an indicator of obesity may have limited our study's power to detect significant associations. Hence, BMI hasn't been found to be a reliable measure of obesity, it would be better if the body analyses of participants have examined.

In summary, these findings showed comparable patterns of association of sociodemographic and reproductive factors with obesity in Turkish women. Prevention of obesity among multiparous women may have a significant public health impact. Specific counseling such as promoting physical activity, healthy eating, and the maintenance of appropriate body weight is important for childbearing age women.

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References

1. Malik VS, Willett WC, Frank BH. Global obesity: trends, risk factors and policy implications. Nature Reviews Endocrinology 2013; 9: 13-27.
2. Erem C. Prevalence of Overweight and Obesity in Turkey. 1JC Metabolic&Endocrine 2015; 8: 38-41.
3. OECD Health Statistics 2014. How does Turkey compare? Available at: http://www.oecd.org/els/health-svstems/Briefmg-Note-TURKEY-2014.pdf (last accessed 10/07/2016).
4. Satman I, Omer B, Tutuncu Y, Kalaca S, Gedik S, Dinccag N, et al. Twelve-year trends in the prevalence and risk factors of diabetes and prediabetes in Turkish adults. Eur J Epidemiol 2013; 28: 169-80.
5. Nutrition, Physical Activity and Obesity Turkey. Available at: http://www.euro.who.int/ data/assets/pdf file/0017/243332/ Turkev-WHO-Country Profile.pdf?ua=l (last accessed 22/07/2016).
6. Maher CA, Mire E, Harrington DM, Staiano AE, Katzmarzyk PT. The independent and combined associations of physical activity and sedentary behavior with obesity in adults: NHANES 2003-06. Obesity 2013; 21: E730-E737.
7. Wen W, Gao YT, Shu XO, Yang G, Li H-L, Jin F, et al. Sociodemographic, behavioral, and reproductive factors associated with weight gain in Chinese women. Int J Obes Relat Metab Disord. 2003; 27: 933-40.
8. Lahmann PH, Lissner L, Gullberg B, Berglund G. Sociodemographic factors associated with long-term weight gain,

current body fatness and central adiposity in Swedish women. Int J Obes Relat Metab Disord. 2000; 24: 685-94.
9. Erem C, Arslan C, Hacihasanoglu A, Değer O, Topbas M, Ukinc K, et al. Prevalence of obesity and associated risk factors in a Turkish population (trabzon City, Turkey). Obesity Research 2004; 12: 1117-27.
10. Craig CL, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. Medicine and Science in Sports and Exercise 2003; 35: 1381-95.
11. Ozturk, M. Üniversitelerde eğitim-öğretim gören öğrencilerde uluslararası fiziksel aktivite anketinin geçerliliği ve güvenirliği ve fiziksel aktivite düzeylerinin belirlenmesi. Yüksek lisans tezi, Hacettepe Üniversitesi, Sağlık Bilimleri Enstitüsü, Ankara, 2005.
12. Altan O. The impact of obesity on cardiovascular diseases in Turkey. Turk Kardiyol Dem Ars. 2003; 31: 279-89.
13. Satman I, TURDEP-II Çalışma Grubu. TURDEP-II Sonuçlan. Available at: http://www.turkendokrin.org/files/file/TURDEP II 2011.pdf (last accessed 05/07/2016).
14. Pekcan AG, Samur G, Dikmen D, Kızıl M, Rakıcıoğlu N, Yıldız E, Gökmen-Özel H, Mercanlıgil SM, Ersoy G, Karaağaoğlu N, Aksoy B, Yılmaz D, Ilgaz F, Türkoğlu İ, Bilgiç P, Demir S, Ersoy N, Fisunoğlu M, Yalçın T, Tamer F, Coşkun Y, Türkyılmaz S, Güler S, Buzgan T, Besler HT. Population based study of obesity in Turkey: results of the Turkey Nutrition and Health Survey (TNHS)-2010. Progress in Nutrition. 2017; 19(3): 248-256 DOI: 10.23751/pn.v19i3.5607.
15. Azarbad L, Gonder-Frederick L. Obesity in women. Psychiatric Clinics of North America 2010; 33: 423-40.
16. Klos LA, Sobal J. Marital status and body weight, weight perception, and weight management among U.S. adults. Eating Behaviors 2013; 14: 500-7.
17. Grabauskas V, Petkeviciene J, Klumbiene J, Vaisvalavicius V. The prevalence of overweight and obesity in relation to social and behavioural factors (Lithuanian health behavior monitoring). Medicina 2003; 39: 1223-30.
18. Dinour L, Leung MM, Tripicchio G, Khan S, Yeh MC. The association between marital transitions, body mass index, and weight: A review of the literature. Journal of Obesity 2012; Article ID 294974, 1-16.
19. Wardle J, Waller J, Jarvis MJ. Sex differences in the association of socioeconomic status with obesity. Am J Public Health 2002; 92: 1299-304.
20. Molarius A, Seidell JC, Sans S, Tuomilehto J, Kuulasmaa K. Educational level, relative body weight, and changes in their association over 10 years: an international perspective from the WHO MONICA Project. Am J Public Health 2000; 90: 1260-8.
21. Ersoy C, Imamoglu S. Comparison of the obesity risk and related factors in employed and unemployed (housewife) premenopausal urban women. Diabetes Research and Clinical Practice 2006; 72: 190-6.
22. Chung KH, Shin KO, Yoon JA, Choi KS. Study on the obesity and nutrition status of housewives in Seoul and Kyunggi area. Nutrition Research and Practice 2011;5:140-9.
23. James WP. The epidemiology of obesity: the size of the problem. J Intern Med 2008; 263: 336-52.
24. Türkiye İstatistik Kurumu Gelir ve Yaşam Koşulları Araştırması 2014. Available at: http://www.tuik, gov.tr/PreHaberBul teni eri, do? i d=l 8633 (last accessed 11/06/2016).
25. Register CA, Williams DR. Wage effects of obesity among young workers. Soc Sci Q 1990; 71: 130-141.
26. Filozof C, Femandez-Pinilla MC, Fernandez-Cruz A. Smoking cessation and weight gain. Obesity Reviews 2004; 5: 95-103.
27. Vural ZT. Menopoz. Available at:http://istanbulsaglik.gov.tr/w/sb/per/belge/menopoz.pdf (last accessed 08/07/2016).
28. Palacios S, Henderson VW, Siseles N, Tan D, Villaseca P. Age of menopause and impact of climacteric symptoms by geographical region. Climacteric 2010; 13: 419-28.
29. Fonseca AMD, Bagnoli VR, Souza MA, Azevedo RS, Couto Junior EDB, Soares Junior JM, et al. Impact of age and body mass on the intensity of menopausal symptoms in 5968 Brazilian women, Gynecological Endocrinology 2013; 29: 116-8.
30. Bastian LA, West NA, Corcoran C, Munger RG. Number of children and the risk of obesity in older women. Preventive Medicine 2005; 40: 99-104.

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Original article

Fruit and vegetable consumption of last grade medical students and related factors

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Summary. *Objective:* This study aimed to determine consumption of fruit and vegetable (F&V) among last grade medical students who will have important roles in community nutrition and health. *Material methods:* This cross-sectional study was conducted on 246 last grade students of Erciyes University School of Medicine (Kayseri, Central Anatolian, Turkey) at 2014-2015 academic terms in June and July 2015.We examined gender, accommodation, marital status, family type, number of individuals living together, homeland, weight and socioeconomic status, number of meals, having regular snacks, eating away from home, water intake, tobacco and alcohol using, regular physical activity. Weight and height was measured and BMI was calculated. Main outcome measure was the intake of F&V by WHO recommendations. Percentage values were used for qualitative data. Comparisons between eating and health habits and adequacy of F&V intake were analyzed using chi-square test. *Results:* Only 38.6 % of students consumed adequate F&V. Rate of adequate F&V intake was higher in students who had regular snacks, ate away from home less than once a week, used no tobacco and lived not alone. *Conclusions and Implications:* The adequate consumption of F&V among last grade medical students' was low. Knowledge and behaviors of last grade medical students about F&V consumption must be improved.

Key words: Fruit, vegetable, medical students.

Introduction

Foods provides not only macro and micro nutrients needed for life but also other bioactive compounds such as antioxidants (carotenoids and vitamins C and E) which have beneficial effects for health promotion and disease prevention (1). The lack of antioxidants is a risk factor for cardiovascular diseases and malignant tumors (2). Consuming adequate amount of fruits and vegetables (F&V) protects overall health and is associated with a reduced risk of cardiovascular disease and certain cancers (3). F&V are the sources of fiber, vitamins, minerals, and phytochemicals. Vitamins, minerals and phytochemicals have important antioxidant and anti-inflammatory roles in metabolism (4).

As a result healthy nutrition behavior with increased intake of plant-based foods plays important roles in the prevention of chronic diseases, such as coronary heart disease, certain types of cancer, stroke and type II diabetes (5-8). World Health Organization (WHO) proposes eating at least 400 g, or five portions of fruits and vegetables per day to reduce the risk of chronic diseases (9).

Lock et al. (10) reported that increasing individual fruit and vegetable intake to up to 600 g per day could reduce the total worldwide burden of disease by

1.8 %, and reduce the burden of ischemic heart disease and ischemic stroke by 31 % and 19 % respectively. Moreover they estimated the potential reductions for stomach, esophageal, lung and colorectal cancer 19 %, 20 %, 12 % and 2 %, respectively.

Universities and particularly medical students have important roles in developing a healthy lifestyle and promoting nutrition education in the community (11-12). Having less knowledge about healthy nutrition could affect the students' general health conditions (12). On the other side, messages given by doctors in the care and management of chronic diseases related to nutrition are very important and last grade medical students are expected to do this after a short time. So they must be well-educated to give the right messages and to be the right roll-models in the community (12-13). To our literature review there is limited data on F&V intake of medical students (14). The aim of this study was to determine intake of F&V and related factors among the last grade medical students who will have important roles in community nutrition and health after a short time.

Material and Methods

Study Design and Sampling

This cross-sectional study was conducted on 246 last grade students of Erciyes University School of Medicine (Kayseri, Central Anatolian, Turkey) at 2014-2015 academic terms in June and July 2015. The target population of the study was 257 students attending the last grade and all target population was planned to be recruited to the study. But 11 students were excluded from the study because of absenteeism to school. For the study, ethical approval from Erciyes University Medical Faculty Ethics Committee (Erciyes University Ethical Committee approval no: 2015/362) and official permission from deanery of Erciyes University Medical Faculty were provided and the procedures followed were in accordance with the Helsinki Declaration. All of the participants provided informed consent.

Data Collection

The questionnaire consisting of 30 questions prepared by the researcher based on literature; 8 of the questions were about sociodemographic characteristics (age, gender, marital status, family type, number of individuals living together, homeland, accommodation and socioeconomic status) and 22 of them were about their dietary habits. The questionnaire was filled out by the students at the clinics. Body weight and height was measured with calibrated scale and stadiometers (Seca 769, Hamburg, Germany) in clinics. Based on body weight and height measurements BMI values calculated and classified as underweight (BMI <18,5 kg/m^2), normal weight (BMI 18,5-24,9 kg/m^2), overweight (BMI 25,0-29,9 kg/m^2) and obese (BMI ≥30,0 kg/m^2) according to World Health Organization (WHO) criteria (15). In addition, the amounts and kinds of F&V they consumed in 24 hours were asked to the students. The evaluation of F&V portions; a medium-sized fruit, or 3 or 4 of berries and 80 g vegetables has been recognized as one portion. The evaluation of adequacy F&V intake; 5 or more portions of F&V per day considered adequate, less than five portions considered inadequate (9).

Statistical Analyses

Data were analyzed using SPSS 16.0 (Statistical Package for the Social Sciences, SPSS Inc. Chicago, USA) software under the supervision of academicians from Erciyes University, Faculty of Medicine, Department of Biostatistics and Medical Informatics. Data was tested with Shapiro-Wilk test for normal distribution. Percentage values were used for sociodemographic characteristics. Comparisons between eating and health habits and adequacy of F&V intake were analyzed using the chi-square test. Values were considered significant at $P < .05$.

Results

Of the participating students 50.8 % were males, with mean age of 24.6 ± 1.7 years. Sociodemographic characteristics of the students are presented at Table 1.

Only 38.6 % of the students consumed adequate F&V while 7.7 % of them consumed neither fruit nor vegetable based in the last 24 hours before the questionnaire was performed. A quarter of the students (25.2 %) did not consume any fruit and 15.0 % of them

Table 1. Sociodemographic characteristics of the students.

Sociodemographic characteristics	n (246)	%
Gender		
Male	121	49.2
Female	125	50.8
Marital status		
Single	235	95.5
Married	11	4.5
Number of individuals living together		
Alone	23	4.1
2-3	29	46.3
>3	194	49.6
Homeland		
Marmara region	3	1.2
Aegean region	11	4.5
Mediterranean region	43	17.5
Central Anatolian region	131	53.3
Eastern Anatolian region	27	11.0
Southeastern Anatolian region	11	4.5
Black sea region	14	5.6
Foreign national	6	2.4
Accommodation		
Dormitory	14	5.7
Home	232	94.3
Self-reported economic status		
Poor	4	1.5
Middle	118	48.0
Good	124	50.5

did not consume any vegetable. The mean portions of F&V are shown at the Table 2.

49.1 % of the group was having regular snacks per day. Mostly preferred snack group was consisted of chocolate, biscuits, candies (78.5 %) and fruit preference rate was 19.1 %. Half of the students ate away from home at least once a week and while eating out mostly preferred meals were; fast-food (48.4 %) and kebab, pide (44.3 %). Rate of students consuming fewer than 3 meals a day were 17.5 %.

Table 2. The mean portions of F&V consumption

Features of F&V intake	Mean ± SD
Fruit consumption	1.67 ± 1.58
Vegetable consumption	2.71 ± 2.26
F&V consumption	4.10 ± 2.90
Variety of consumed fruit	1.40 ± 1.13
Variety of consumed vegetable	2.15 ± 1.60
Variety of consumed F&V	3.50 ± 2.21

The most known chronic diseases associated with inadequate F&V consumption among the students were cancer (29.6 %) and cardiovascular diseases (29.4 %). The mean BMI of the group was 23.08 ± 3.50 kg/m$^{2.}$ Most of the students (70.7 %) had normal weight.

The rates of inadequate F&V intake in males and females were similar (61.2 % and 61.6 % respectively). There was no significant relation between F&V intake and BMI categories. Likewise there was no significant relation between F&V intake and number of meals per day, water intake, alcohol using and regular physical activity. The rate of adequate F&V intake was significantly higher among the students having regular snacks per day and eating away from home at least once a week ($P < .05$). Also the rate of adequate F&V intake was significantly lower among tobacco user and living alone students ($P < .05$) (Table 3).

Table 4 summarizes declared barriers for F&V intake by the students. The remarkable barriers to F&V intake were "It is difficult to keep F&V" (45.5 %) and "It takes time to prepare vegetable meal" (45.5 %). We also analyzed the students' own opinions about their daily F&V intake (Table 5).

Discussions

The current study examined intake of F&V among the last grade medical students using a cross-sectional design and to the best of our knowledge this is the first study about the last grade medical students' F&V intake.

We found out the mean portion of F&V intake was 4.10 ± 2.90 per day among the last grade medical students. A cross-sectional study which recruited 18-25 years aged 960 female university students reported that the mean portion of F&V intake was 3.21 ± 2.65 of the students (16). Another study from Turkey which recruited seven universities' students stated that F&V intake per day was 3.67 ± 1.81 (17). As our study consisted only medical students, the mean portion of F&V intake was higher as expected.

We found out 38.6 % of the students consumed ≥ 5 F&V portions daily as recommended by WHO. In the USA 7.9 % of college and university students reported that they ≥ 5 F&V portions daily (18). A study

Table 3. Comparison of students some characteristics with their sufficient fruit and vegetable situation

	F&V Intake			
	Inadequate		Adequate	
	n	%	n	%
Gender				
Male	74	61.2	47	38.8
Female	77	61.6	48	38.4
X^2=0.005 *P*= .943				
Self-reported economic status				
Poor	2	50.0	2	50.0
Moderate	70	59.3	48	40.7
Good	79	63.7	45	36.3
X^2=0.713 *P*= .700				
Accommodation				
Dormitory	9	64.3	5	35.7
Home	142	61.2	90	38.8
X^2=0.053 *P*= .818				
Number of individuals living together				
Alone	19	82.6	4	17.4
2-3	14	48.3	15	51.7
>3	118	60.8	76	39.2
*X^2=6.499 P= .039**				
Weight status				
Underweight	8	57.1	6	42.9
Normal	107	62.2	67	37.8
Overweight	32	62.7	19	37.3
Obese	4	50.0	3	50.0
X^2=0.513 *P*= .916				
Number of meals per day				
<3	25	16.6	18	18.9
3	111	73.5	67	70.5
>3	15	9.9	10	10.5
X^2= 0.283 *P*= .868				
Having regular snacks per day				
Yes	66	54.5	55	45.5
No	85	68.0	40	32.0
X^2= 4.695 *P*= .036*				
Eating away from home				
At least once a week	84	68.3	39	31.7
Less frequently	67	54.5	56	45.5
X^2= 4.956 *P*= .026*				
Water intake				
Adequate (> 8 glasses)	122	64.2	68	35.8
Inadequate (< 8 glasses)	29	51.8	27	48.2
X^2=2.817 P= .118				
Tobacco using				
Yes	32	76.2	10	23.8
No	119	58.3	85	41.7
*X^2=4.685 P= .036**				
Alcohol using				
Yes	28	62.2	17	37.8
No	123	61.2	78	38.8
X^2=0.016 *P*= .898				
Regular physical activity				
Yes	14	51.9	13	48.1
No	137	62.6	82	37.4
X2=1.162 P= .281				
Homeland				
Aegean, Marmara and Mediterranean regions	36	63.2	21	36.8
Other regions	115	60.8	74	39.2
X^2=0.099 *P*= .877				

Table 4. Defined barriers to F&V intake

Barriers to F&V intake*	**n**	**%**
It is difficult to keep F&V	112	45.5
It takes time to prepare vegetable meal	112	45.5
Vegetable meals are not satisfying	97	39.4
It is difficult to obtain F&V	89	36.2
Others living with do not like vegetable meal	60	24.4
Do not like vegetables	47	19.1
F&V are expensive	33	13.4
Do not like fruit	24	9.8
Having indigestion problems when eat vegetable	11	4.5
Having indigestion problems when eat fruit	11	4.5

**Multiple choices*

Table 5. Students' opinions about their F&V intake

	F&V intake			
	Inadequate		Adequate	
	n	%	n	%
Students' opinions about their F&V intake				
Adequate	21	28.8	52	71.2
Inadequate	130	75.1	43	24.9
X^2=46.582 P ≤ .001*				
Students' opinions about their F&V intake in future				
Increase	109	71.2	44	28.8
Decrease	2	40	3	60
No chance	40	45.5	48	54.5
X^2=16.657 P ≤ .001*				
Total	**151**	**61.4**	**95**	**38.6**

designed at 7 universities at United Kingdom this rate was 14.0 % (19). In Germany only about 5.0 % of the first grade university students reported that their F&V intake were ≥ 5 portions (20). In another study aiming to compare the eating habits of 1st and 3rd grade medical school students, the first and third year students rates of having adequate F&V consumption were 16.5 % and 10.5 % respectively (21). The rate of adequate F&V consumption at our study was higher than the others'. This difference was probably due to the fact that our study group was educated about the subject.

Our study showed the rates of adequate F&V intake in males (38.4 %) and females (38.8 %) were similar (P > .05). Parallel results to our study from Brazil which recruited 5000 adults reported adequate F&V intake were 12.8 % (95 % CI: 11.0-13.9) in males, and 13.9 % (95 % CI: 13.0-15.0) in females (P < .05) (22).

Although there were opposite results (23), most of the studies in the literature showed females' F&V intake were more than males' F&V intake (17, 19). Research from seven universities in the UK showed that 16.5 % females and 11.3 % males ate ≥ 5 F&V portions daily (19). Also another study from Turkey reported that female university students were more likely to eat F&V (17). As our sample had nutrition education the gender differences could be eliminated.

Turkey consists of seven geographical regions with different cultural characteristics. Eating habits also differ among these geographical regions. Among Marmara, Aegean and Mediterranean regions vegetable consumption is more common (24). We compared the students coming from these three regions F&V consumption with the others. There was no significant difference between the fruit and vegetable consumption of the students from these three regions compared to the students from other regions.Top of Form

We found no significant relation between F&V intake and BMI categories. But a recent study in overweight individuals found that a two-fold increase in F&V intake for 16 weeks significantly decreased BMI (25). There were opposite results to our study in Serbian National Health Survey 2013 which recruits 12.461 adults reported that frequency of fruit consumption was significantly related to all BMI categories in men and to underweight in women, and the number of daily fruit portions was related only to underweight and overweight in women; vegetable consumption frequency was significantly associated with underweight and normal weight in men, while number of vegetable portions was significantly associated with normal weight in men (26). The majority of our study group had normal weight and this may have caused a limitation in assessing the relationship between F&V consumption and weight.

We found out the students who ate away from home less than once a week had significantly higher rate of adequate F&V intake ($P < .05$). Similar to our study; there was a negative association between frequency of FAFH (foods prepared away from home) and F&V intake. (43.1 % versus 54.0 % eating out 0-1 meal per week, respectively) (27) The Household Income and Labor Dynamics in Australia (HILDA) survey revealed time scarcity reduced to consume F&V, eat away from home more, and eat more discretionary calories like foods high in salt, sugar or fat (29). Eating out may be tempted for the students as it is easy, quick or delicious but it seems to be a serious barrier to intake F&V.

The current study found the remarkable barriers to F&V intake were "It is difficult to keep F&V" (45.5 %) and "It takes time to prepare vegetable meal" (45.5 %). Another study suggested that storage difficulties may impact consumption (28).

Snacks may be a chance for increasing F&V intake. Also the rate of adequate F&V portion was higher among the students who had regular snack intake. Although the current study interestingly showed the last grade medical students preferred unhealthy snacks.

At our study the most frequently reported snacks were chocolate, biscuits, candies and only 19 % of the students preferred fruit. Similar to our finding Ünüsan and colleagues (17) found the most frequently ate snacks among female adolescents as candy, soda, donuts, and cookies and only fewer than 25.0 % of adolescents reported the intake of nutritious snacks such as fruits, vegetables, juice, and low-fat milk .

We noticed no relation between economic status and F&V consumption. Studies have found numerous correlates inhibiting the intake of F&V, such as low economic status, inaccessibility to fresh F&V and lack of self-efficacy (28-32). This difference may be due to the fact that most of the students economic status were similar.

It is well known that tobacco using is negatively associated F&V intake (33). We also found out the rate of adequate F&V intake was significantly higher in the students who don't use tobacco consistent with the most of the studies in the literature. A study recruited 1543 adults reported that tobacco users consumed significantly fewer F&V than non-tobacco users (34). The same result from another study showed tobacco users significantly consumed more fast-food and white meat but less F&V and dairy product (35).

There was no difference between students' F&V consumption and their accommodation at the current study. A study reported that students who lived in fraternity or sorority houses had higher intake than did students living off campus (36). These results are similar to Brown and colleagues' study (37). In that study, students who resided in residence halls (and had pur-

chased a meal plan) consumed more F&V than those who lived off-campus. It is important to remember that at current study the proportion of students staying at dormitory was very small when comparing the data of the study with the others'.

The rate of adequate F&V intake was significantly lower among living alone students. At a review searched eight electronic databases it was suggested that, compared with persons who do not live alone, persons who live alone had a lower consumption of F&V (38). F&V consumption are low among the people living alone because probably they often prefer to eat away from home or prepared food.

The relationship between physical activity and fruit and vegetable consumption has been assessed in the belief that a person who has adopted one health-related behavior may have adopted the others. Although most of the studies in the literature show that low physical activity was associated with lower F&V intake (4, 39) we found out no relationship between regular physical activity and sufficient F&V intake. But, a cross-sectional study which reported different results to our study showed that higher F&V consumers significantly had high rates of regularly physical exercise (58.3 %).[4] Another similar results from a study which recruits 998 American Indians reported that factors associated eating ≥ 5 portions of F&V per day included having physical exercise ≥ 4 days in the past week ($P < .001$) (40). Last grade students have not had the opportunity to do regular physical activity because their daily lives are very intense and active.

We examined the students' opinions about their F&V intake; 13.9 % of the students who had inadequate portions of F&V were thinking their consumption was adequate and 26.5 % of them were not planning any changes about their F&V consumption. We concluded this result could arise from insufficient knowledge about adequate and balanced nutrition and disbelief to unchangeable practical issues and situational barriers.

Conclusion

Last grade medical students' eating habits are important for community health as well as their health because they will be role-models. The adequate consumption of F&V among last grade medical students' was low. Students did not have sufficient information about the relationship between chronic disease and nutrition. The nutritional education given to medical students in these regards should be improved.
The difficulty of storing F&V and preparing vegetables were the most defined barriers among F&V consumption. Snacks containing F&V can be offered for sale in places where students can easily access them on campus. Education should be given to keep students away from smoking because besides its' many adverse effects it also prevents consuming adequate F&V. CON

References

1. Liu RH. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. Am J Clin Nutr. 2003;78(3 Suppl):517-20.
2. World Health Organization. e-Library. http://www.who.int/topics/nutrition/en/ Acessed January 30, 2014.
3. Zhou G, Gan Y, Hamilton K, et al. The role of social support and self-efficacy for planning fruit and vegetable intake. J Nutr Educ Behav. 2017;49:100-06.
4. Alsunni AA, and Badar A. Fruit and vegetable intake and its determinants among Saudi university students. Journal of Taibah University Medical Sciences. 2015;10(2):201-07.
5. Willett WC. Balancing life-style and genomics research for disease prevention. Science 2002;296(5568):695-8.
6. Dauchet L, Amouyel P, Hercberg S, Dallongeville J. Fruit and vegetable intake and risk of coronary heart disease: A meta-analysis of cohort studies. J Nutr. 2006;136(10):2588-93.
7. Liu RH. Potential synergy of phytochemicals in cancer prevention: mechanism of action. J Nutr. 2004;134(12 Suppl):3479-85.
8. Boeing H, Bechthold A, Bub A, Ellinger S, Haller D, Kroke A, et al. Critical review: Vegetables and fruit in the prevention of chronic diseases. European Journal of Nutrition. 2012;51(6):637-63.
9. World Health Organization Technical Report Series 2003. Diet, nutrition and the prevention of chronic diseases: Report of a Joint WHO/FAO Expert Consultation. No. 916. Pp:23-25. http://apps.who.int/iris/bitstream/10665/42665/1/WHO_TRS_916.pdf Acessed February 10, 2014.
10. Lock K, Pomerleau J, Causer L, et al. The global burden of disease attributable to low intake of fruit and vegetables: Implications for the global strategy on diet. Bull World Health Organ. 2005;83(2):100-08.
11. Şahin H, Aykut M, Öztürk A, et al. Obesity prevalence and related factors among medical students in Kayseri. Erciyes

Medical Journal. 2015;37(2):51-8.
12. Curtis SM, and Willis MS. "Are you eating healthy?" Nutrition discourse in Midwestern clinics for the underserved. Patient Education and Counseling. 2016;99(10):1641-46.
13. Castillo M, Feinstein R, and Fisher M. Nutrition knowledge of medical graduates. J Adv Nutr Hum Metab. 2016;2:e1188. DOI: 10.14800/janhm.1188.
14. Terebessy A, Czeglédi E, Balla BC, et al. Medical students' health behaviour and self-reported mental health status by their country of origin: A cross-sectional study. BMC Psychiatry. 2016;16(1):171. DOI 10.1186/s12888-016-0884-8.
15. World Health Organization. Global Database on Body Mass Index. http://apps.who.int/bmi/index.jsp?introPage=intro_3.html Acessed February 12, 2014.
16. Al-Otaibi HH. Psychosocial factors associated with fruit and vegetable consumption among Saudi University students. J Food Nutr. 2014;1:1-10.
17. Ünüsan N. Fruit and vegetable intake among Turkish university students. Int J Vitam Nutr Res. 2004;74(5):341-48.
18. American College Health Association. American College Health Association National College Health Assessment Spring 2006 Reference Group Data Report (abridged). J Am Coll Health. 2007;55(4):195-206.
19. El Ansari W, Stock C, John J, Deeny P, Phillips C, Snelgrove S, Adetunji H, Hu X, Parke S, Stoate M, Mabhala A. Health promoting behaviours and lifestyle characteristics of students at seven universities in the UK. Central European Journal of Public Health. 2011 Dec 1;19(4):197.
20. Keller S, Maddock JE, Hannöver W, et al. Multiple health risk behaviors in German first year university students. Prev Med. 2008;46(3):189-95.
21. Skemiene L,Ustinaviciene R, Piesine L, et al. Peculiarities of medical students' nutrition. Medicina (Kaunas). 2007;43(2):145-52.
22. Jaime PC, and Monteiro CA. Fruit and vegetable intake by Brazilian adults, 2003. Cad. Saúde Pública. 2005;21(1)19-24.
23. Bertsias G, Linardakis M, Mammas I, et al. Fruit and vegetables consumption in relation to health and diet of medical students in Crete, Greece. Int J Vitam Nutr Res. 2005;75(2):107-17.
24. Food and Agriculture Organization of the United Nations http://www.fao.org/ag/agp/agpc/doc/counprof/Turkey/Turkey.htm Acessed 24.03.2017.
25. Järvi A, Karlström B, Vessby B, et al. Increased intake of fruits and vegetables in overweight subjects: Effects on body weight, body composition, metabolic risk factors and dietary intake. Br J Nutr. 2016;115(10):1760-68.
26. Maksimovic MZ, Gudelj Rakic JM, Vlajinac HD, et al. Relationship between health behaviour and body mass index in the Serbian adult population: Data from National Health Survey 2013. Int J Public Health. 2016;61(1):57–68.
27. Seguin RA, Aggarwal A, Vermeylen F, et al. Consumption Frequency of Foods Away from Home Linked with Higher Body Mass Index and Lower Fruit and Vegetable Intake among Adults: A Cross-Sectional Study. J Environ Public Health. 2016;2016:3074241. DOI: 10.1155/2016/3074241.
28. Dye CJ, and Cason KL. Perceptions of older, low-income women about increasing intake of fruits and vegetables. J Nutr Elder. 2005;25(1):21-41.
29. Venn D, and Strazdins L. Your money or your time? How both types of scarcity matter to physical activity and healthy eating. Soc Sci Med. 2017;172:98-106.
30. Kratt P, Reynolds K, and Shewchuk R. The role of availability as a moderator of family fruit and vegetable intake. Health Education and Behavior. 2000;27:471-82.
31. Siega-Riz A, and Popkin B. Dietary trends among low socioeconomic status women of childbearing age in the United States from 1977 to 1996: A comparison among ethnic groups. Journal of the American Medical Women's Association. 2001;56:44-8.
32. Pomerleau J, Lock K, Knai C, et al. Interventions designed to increase adult fruit and vegetable intake can be effective: A systematic review of the literature. J Nutr. 2005;135:2486-95.
33. Kvaavik E, Totland TH, Bastani N, et al. Do smoking and fruit and vegetable intake mediate the association between socio-economic status and plasma carotenoids? Eur J Public Health. 2014;24(4):685-90.
34. Palaniappan U, Starkey J, O'Loughlin J, et al. Fruit and vegetable intake is lower and saturated fat intake is higher among Canadians reporting smoking. J Nutr. 2001;131(7):1952-58.
35. Heydari G, Heidari F, Yousefifard M, et al. Smoking and diet in healthy adults: A cross-sectional study in Tehran, Iran, 2010. Iran J Public Health. 2014;43(4):485-91.
36. Adams TB, and Colner W. The association of multiple risk factors with fruit and vegetable intake among a nationwide sample of college students. Journal of American College Health. 2008;56;4:455-461.

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Original article

Impact of nutritional education on the nutrient intake of type 2 diabetes mellitus patients

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Summary. *Introduction:* Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder affecting the metabolism of carbohydrates and characterized by hyperglycemia, inadequate secretion of insulin, and/or peripheral insulin resistance. The most common method of T2DM treatment is still pharmacotherapy, due to its convenience. However, diet is one of the most important factors in the development and progression of this disease, and can make an important contribution to the treatment of T2DM. *Purpose:* The aim of the research was to evaluate the effects of nutritional education on the nutrient intake of T2DM patients. *Material and methods:* A total of 149 patients were enrolled (99 women and 59 men) aged 36-88, all diagnosed with T2DM or glucose metabolism disturbances. Nutrient intakes per subject were evaluated based on 24h dietary recall from the previous three days. Anthropometric measurements were also performed. *Results:* Intakes of various nutrients in the diet of T2DM, both under the care of a dietician and not, did not usually meet dietary recommendations. The diets of T2DM patients under the care of a dietician, despite significant dietary mistakes, provided significantly more nutrients with potential positive effects on carbohydrate and lipid metabolism: fiber, EPA, DHA and PUFA, minerals: K, Mg, Fe, Cu and Mn as well as vitamins and smaller amounts of the nutrients which are nutritional risk factors for the development and progression of T2DM: total amount of fat, SFA, Na and P. *Conclusion:* Nutritional education has a positive impact on the nutrient intake of T2DM patients. Routine dietitian care for people suffering from this disease should be recommended.

Key words: diabetes mellitus type 2, dietician, nutritional education, nutrient intake

Introduction

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder affecting the metabolism of carbohydrates and characterized by hyperglycemia, inadequate secretion of insulin and/or peripheral insulin resistance (1). Chronic hyperglycemia is associated with long-term damage, dysfunction and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels (2).

The amount of people with diabetes is constantly increasing. Globally, 425 million adults are living with diabetes, and the number is projected to rise to over 629 million by 2045 (3). 90-95% are patients who suffer from T2DM. Currently, approximately 2.4 million people with T2DM are treated in Poland, that is 5% of the total population (3). This epidemic has been attributed to urbanization and environmental transitions, including work pattern changes from heavy labor to sedentary occupations, increased computerization and mechanization, and improved transportation. Reduced physical activity and incorrect eating habits caused by drastic changes in food production, processing, distribution systems and increased accessibility of food with low nutritional value, lead to overweight and obesity, which contribute directly to the development

of insulin resistance and T2DM. The meta-analysis conducted by Abdullah (4) provided proof of a close association between being overweight and obesity and the progression of this disease.

Complete treatment of T2DM includes pharmacotherapy and complementary actions such as a well-balanced diet, physical activity and generally leading a healthy lifestyle. The most common method of T2DM treatment is still pharmacotherapy, due to its convenience. However, diet is one of the most important factors in the development and progression of this disease, and can make an important contribution to the treatment of T2DM and the maintenance of a healthy body weight (5, 6).

According to The Polish Diabetic Association (PDA), specialist diabetes hospital units should also contain a qualified dietitian, whose nutritional advice could have a significant impact on the eating habits and progression of the disease. The nutritional education of patients and their increased awareness of the role of a well-balanced diet on treatment could directly increase the amount of patients with a lower rate of disease progression (5, 7).

Epidemiological studies underline the beneficial role of nutritional education in reducing the development of T2DM. This is manifested by an improvement in biochemical markers, which are important in the assessment of the progression of the disease, and anthropometric parameters, which enable assessment of the nutritional status of patients (8-10). However, information regarding the association between nutritional education and nutrient intake are scarce (11, 12).

The aim of the research was to evaluate the effects of nutritional education on the nutrient intake of T2DM patients.

Subjects and Methods

The study participants were 149 patients (99 women and 59 men) with T2DM or peripheral insulin resistance, from 3 medical facilities in Lower Silesia. The study subjects were interviewed using an anonymous questionnaire, which consisted of questions concerning comprehensive socio-demographic details, physical activity, stimulants used (alcohol, cigarettes), dietary supplements and types of pharmacotherapy. Patients were also asked whether they were under the care of a dietician.

Nutrient intakes

The 24h dietary recall method was used to collect diet-related data from the previous three days. Nutrient intakes were calculated with DIETA 5.0 software (IŻŻ National Food and Nutrition Institute, Warsaw, Poland) and compared with the Estimated Average Requirements (EAR). Intake of β-carotene was compared with upper level (UL), intakes of Na, K, Ca, Mn, vitamin E and D were compared with adequate intake (AI), and the intake of α-linolenic acid (ALA) was compared with the Recommended Dietary Allowance(RDA) (5, 13).

Statistical Analysis

The data were analyzed using STATISTICA v. 12.0 software (StatSoft, Tulsa, OK, USA); p values of <0,05 were considered statistically significant. Most of the obtained results did not present the features of normal distribution, as was confirmed by the Shapiro-Wilk test. To evaluate the differences in distribution of dietary nutrient intakes between the educated (EG) and non-educated groups (N-EG), the Student t-test (for parametric data) and the U-Mann Whitney test (for nonparametric data) were performed. Differences in the percentages of EG and N-EG whose intake of nutrients was below the cut-off points were evaluated with the Chi^2 test. All parameters are presented as median and range. For all statistical procedures, the significance level was considered to be <0.05.

Results

Baseline characteristics of T2DM patients

The baseline demographic, anthropometric and dietetic characteristics of the T2DM patients who participated in the study are presented in Table 1.

Subjects (n=149) were distributed into EG (those who were under the care of a dietician) and N-EG (those who were not under the care of a dietician). The groups were sex-and age-matched. The majority of EG and N-EG had secondary education and lived in a big city.

Table 1. Baseline characteristics of T2DM patients

Variables	n	Educated group (EG)	n	Non-educated group (N-EG)
Gender; W/M; number	59	67.8/32.2	90	65.5/34.45
Age; years [median (min-max)]	59	62.0 (36.0-84.0)	90	68.0 (39.5-88.0)
Sociodemographic parameters				
Education: Primary/Secondary/Professional/University [%]	59	16.9/52.5/20.3/10.2	90	23.3/40/26.7/10
Place of residence: village/small town/big city [%]	59	**1.7/16.9/81.4**[a]	90	**20/16.7/63.3**[a]
Anthropometric parameters				
BMI [kg/m^2] [median (min-max)]	56	30.9 (21.1-49.2)	90	30.9 (20.9-48.1)
Normal weight/ overweight/ obesity [%]	56	12.5/33.9/53.6	90	7.7/33.3/58.9
Weight; kg; [median (min-max)]	58	84 (52-134)	90	80.5 (47-150)
WHR [median (min-max)]	46	0.96 (0.67-1.05)	64	0.96 (0.79-1.15)
AC; cm; [median (min-max)]	48	**33 (25.5-43)**[a]	64	**31.5 (25-40)**[a]
BFP; %; [median (min-max)]	44	37.8 (9.5-54.4)	64	36.35 (5.7-48)
Physical activity				
Low/medium/high [%]	59	**15.3/76.3/8.5**[a]	90	**36.7/58.9/4.4**[a]
Alcohol consumption				
Yes/No [%]	59	**45.8/54.2**[a]	90	**21.1/78.9**[a]
Frequency of alcohol consumption: regularly/ occasionally [%]	27	77.8/22.2	19	63.2/36.8
Wine/ /vodka/bear/champagne/liqueur/ cognac* [%]	27	23.7/20.3/13.5/6.8/3.4/3.4	19	36.8/52.6/15.8/5.3/10.5/10.5
Smoking cigarettes				
Yes/no [%]	59	10.2/89.8	90	13.3/86.7
Antihyperglycemic drugs				
Medications: yes/no [%]	59	91.5/8.5	90	96.7/3.3
Medications: metformin/sulphonylurea/insulin acarbose/ other* [%]	54	64.8/33.3/35.2/9.2/3.7	87	62.1/40.2/39.1/5.7/0
Dietary supplementation				
Dietary supplements: yes/no [%]	59	55.9/44.1	90	46.7/53.4
Taking medications and supplements simultaneously: yes/ no [%]	33	33.3/66.7	42	45.2/54.8
Reason for taking dietary supplements: dietician/ own choice/ physician/ disease/ commercials [%]	33	27.3/36.4/33.3/9.1/3		0/42.8/47.6/9.5/2.4

**Percentages do not add up to 100% due to the multiplicity of responses; a- statistically significant differences in the baseline characteristics between EG and N-EG: chi^2 test, p <0.05; W- women; M-man; BMI- body mass index (kg/m^2); WHR- waist-hip ratio; AC- arm circumference; BFP- body fat percentage*

The median BMI values of both groups were the same - 30.9, however we observed a slight tendency towards a higher percentage of overweight or obese patients in the N-EG group: 92% vs 87,5%. The median WHR in both groups was 0.96, however in N-EG we observed this parameter moving to a higher range. EG had a significantly higher AC than N-EG, moreover we found an unexpected bias towards a higher body fat percentage in this subgroup compared to N-EG. Most of the patients from both groups declared a medium level of physical activity, however EG was more active.

Significantly more EG patients answered the question about alcohol consumption positively than N-EG: 45.8% vs 21.1 %, respectively. Regular alcohol consumption (>1 portion/month) was declared by 78% of EG and 63% of N-EG. Almost 90% of EG and 87% of N-EG answered the questions about cigarette smoking negatively.

The majority of patients from both groups were under pharmacotherapy for T2DM: about 92% of EG and 97% of N-EG. The most commonly taken drug was metformin, followed by insulin in EG and sulphonylurea in N-EG.

Almost 56% of EG and 47% of N-EG took dietary supplements, and 33% of EG and 45% of N-EG took medication and supplements simultaneously. The most popular reasons for taking dietary supplements were as follows: their own choice and a physician's recommendation.

Nutrient intakes

The contribution of macronutrients to dietary energy provision is presented in Table 2. We did not observe statistical differences in total carbohydrates, saccharose, total fat, MUFA or PUFA contributions to energy provision between EG and N-EG, however there were significant differences in protein-energy, as well as SFA-energy, between the analyzed groups. N-EG provided a significantly higher amount of energy from protein when compared with the EG's diet: 85.6% vs 33.9% of respective groups with protein-energy >20%. Similarly, the N-EG diets provided a significantly higher amount of energy from SFA than those of the educated ones: 61.1% vs 42.2% of the respective groups, with SFA-energy >10%.

Table 2. Macronutrient contribution to energy provision in the diet

Components	**% of energy**	**% EG (n=59)**	**% N-EG (n=90)**	**p**
Total protein	<15	8,5	10	<0,001
	15-20	57,6	4,4	
	>20	33,9	85,6	
Total carbohydrates	<40	5,1	3,3	0,65
	40-50	32,2	38,9	
	>50	62,7	57,8	
Saccharose [g]	<10	84,7	81,1	0,57
	>10	15,3	18,9	
Total fat	<30	62,7	50	0,23
	30-35	23,7	26,7	
	>35	13,6	23,3	
SFA	<10	57,6	38,9	0,02
	>10	42,4	61,1	
MUFA	<10	40,7	38,9	0,59
	10-15	45,7	52,2	
	>15	13,6	8,9	
PUFA	<6	66,1	70	0,88
	6-10	28,8	25,6	
	>10	5,1	4,4	

SFA- saturated fatty acids; MUFA- monounsaturated fatty acids; PUFA- polyunsaturated fatty acids

Interestingly, a similarly high percentage of the groups took a high amount of energy from carbohydrates: in both groups the percentages of patients who took more than 50% of their energy from carbohydrates oscillated around 60%. A high percentage of patients, regardless of nutritional education, avoided high consumption of saccharose: more than 80% of both groups took less than 10% of their energy from this macronutrient. The majority of patients in EG (62,7%) and half of N-EG were supplied with total fat in amounts providing less than 30% of the energy consumed daily.

The contribution of MUFA and PUFA in energy provision was insufficient in both groups. Only about 14% of EG and 9% of N-EG consumed MUFAs in amounts providing more than 15% of energy. About one fourth of patients from both groups consumed the recommended amounts of PUFAs (6-10% of daily energy).

Particular nutrient intakes of T2DM patients in terms of their education by dieticians are presented in Table 3.

We did not observe statistical differences in energy, dietary fiber, ALA, cholesterol, Na, K, Ca, P, Mg, Fe, Zn, Cu, Mn, vitamin A (retinol and β-karoten), vitamin B group consumption or ratio of omega-6 to omega-3 between EG and N-EG, however there were significant differences in EPA, DHA, LC-PUFA, Mn, vitamin E, vitamin D, folic acid and vitamin C consumption between the analyzed groups. N-EG consumed significantly higher amounts of EPA, however EG consumed significantly higher amounts of DHA, LC-PUFA, Mn, vitamin E, vitamin D, vitamin C and folic acid.

Additionally we found a significantly lower percentage of EG than N-EG consuming Mg, Fe and vitamin C below EAR cut off points: and vitamin E below AI cut off points.

Discussion

This study focused on evaluation of the effects of nutritional education on the nutrient intake of T2DM

Table 3. Nutrient intake of T2DM patients

Components	EG (n=59)		N-EG (n=90)	
	Median (min-max)	% of EAR Median (min-max) % of patients with nutrient intake below the cut-off point	Median (min-max)	% of EAR Median (min-max) % of patients with nutrient intake below the cut-off point
Energy [kcal]	1068.6 (498.2-2892.2)	42.6 (13.1-85.1) 100.0	1150.4 (547.7-3283.0)	46.3 (22.6-131.3) 97.8
Dietary Fiber [g]	18.9 (10.3-36.7)	75.4 (41.3-147.0) 79.7	16.4 (3.4-42.8)	65.7 (13.8-171.2) 83.3
ALA C18:3 [g]	0.7 (0.2-3.1)	35.8 (12.0-153.6) 96.6	0.7 (0.2-25.0)	34.4 (10.5-1247.9) 94.4
EPA C20:5 [g]	**0,0[a] (0.0-0.5)**	- -	**0.0[a] (0.0-0.6)**	- -
DHA C22:6 [g]	**0.1[a] (0.0-1.3)**	- -	**0.0[a] (0.0-1.1)**	- -
LC-PUFA [g]	**0.1[a] (0.0-1.9)**	**48.6 (0.0-951.9)[b]** 62.7	**0.1[a] (0.0-1.7)**	**25.6 (0.0-846.4)[b]** 76.7
Cholesterol [mg]	174.4 (31.0-560.0)	58.1 (10.3-186.7) 11.9*	150.1 (21.8-893.1)	50.0 (7.3-297.7) 14.4*
omega-6/omega-3	6.1 (0.9-22.4)	-	6.6 (0.5-29.7)	-
Na [mg]	2578.8 (1244.9-10054.2)	192.8 (88.9-718.2) 94.9*	2901.0 (1048.7-7980.2)	221.2 (74.9-570.0) 97,8*
K [mg]	2493.1 (1355.9-4974.0)	71.2 (38.7-142.1) 86.4	2490.0 (958.8-5345.6)	71.1 (27.4-152.7) 93.3
Ca [mg]	344.1 (155.7-977.2)	26.5 (12.0-75.20) 100.0	347.0 (107.6-930.4)	27.1 (8.3-76.4) 100.0
P [mg]	875.8 (501.5-2000.4)	151.0 (86.5-344.9) 89.8*	901.6 (285.7-2555.0)	155.4 (49.3-440.5) 80,0*
Mg [mg]	252.2 (147.0-501.9)	88.6 (50.5-143.4) **64.4[#]**	240.3 (96.4-745.3)	77.9 (36.4-281.2) **78.91[#]**
Fe [mg]	9.0 (5.1-17.0)	149.6 (82.8-283.9) **10.2[#]**	8.1 (2.7-35.1)	134.0 (45.4-438.2) **24.4[#]**
Zn [mg]	7.6 (3.7-15.9)	102.3 (39.7-184.1) 40.7	7.8 (2.7-25.0)	96.4 (40.1-307.5) 55,6
Cu [mg]	1.0 (0.6-1.6)	136.8 (79.5-225.7) 22	0.9 (0.2-2.1)	123.9 (33.2-304.9) 26.7
Mn [mg]	5.5 (1.8-11.5)	**267.0 (79.3-640.7)[b]** 1.7	4.5 (1.3-15.1)	**195.8 (56.5-658.1)** 8.9
Vitamin A [µg, eq. retinolu]	680.5 (270.8-3417.3)	136.1 (43.0-683.5) 18.6	698.6 (222.6-7028.8)	129.0 (44.5-1405.8) 30.0
Retinol [µg]	219.4 (57.9-2935.4)	10.1 (2.0-195.7) 98.3	225.0 (31.0-5994.1)	12.1 (1.0-399.6) 97.8

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Table 3. Nutrient intake of T2DM patients

Components	EG (n=59)		N-EG (n=90)	
	Median (min-max)	% of EAR Median (min-max) % of patients with nutrient intake below the cut-off point	Median (min-max)	% of EAR Median (min-max) % of patients with nutrient intake below the cut-off point
β- karoten [µg]	2764.7 (593.3-13385.1)	39.5 (8.5-191.2) 91.5	2640.3 (367.4-9628.3)	37.7 (5.2-137.5) 94.4
Vitamin E [µg, eq. α-tokoferolu]	**6.3[a] (3.2-15.6)**	**73.6 (32.8-187.0)[b]** **78[#]**	**5.0[a] (1.5-22.7)**	**58.2 (18.3-226.5)[b]** **90.0[#]**
Vitamin D [µg]	**2.7[a] (0.3-14.5)**	**17.8 (2.2-96.4)[b]** 100.0	**2.0[a] (0.2-10.3)**	**13.3 (1.1-68.8)[b]** 100.0
Vitamin B_1 [mg]	0.9 (0.4-2.2)	94.5 (40.6-196.4) 57.6	0.8 (0.3-3.1)	87.2 (30.5-283.0) 68.9
Vitamin B_2 [mg]	1.0 (0.5-2.2)	105.5 (45.1-202.3) 42.4	1.0 (0.4-2.8)	103.8 (39.4-274.6) 45.6
Vitamin B_3 [mg]	14.4 (4.5-35.1)	123.7 (40.7-29.4) 32.2	12.6 (3.9-33.0)	109.5 (35.4-275.0) 41.1
Vitamin B_6 [mg]	1.4 (0.7-2.9)	108.2 (54.2-204.9) 42.4	1.4 (0.5-3.6)	104.1 (39.3-255.1) 42.2
Vitamin B_{12} [µg]	2.0 (0.5-9.0)	101.9 (23.8-450.4) 49.2	1.7 (0.2-17.8)	87.2 (10.5-888.2) 57.8
Folic acid [µg]	**206.9[a] (105.6-368.8)**	**64.6 (33.0-115.2)[b]** 89.8	**183.5[a] (66.1-402.1)**	**57.3 (20.6-125.7)[b]** 94.4
Vitamin C [mg]	**73.9[a] (17.3-213.0)**	**108.2 (23.1-355.0)[b]** **47.5[#]**	**52.4[a] (11.3-165.9)**	**80.3 (18.8-237.3)[b]** **64.4[#]**

ALA- αlinolenic acid; EPA- eicosapentaenoic acid; DHA- docosahexaenoic acid; *- value over EAR (% of participants); a- statistically significant differences in the supply of nutrients between educated and non-educated patients demonstrated by the U-Mann-Whitney test, $p < 0.05$; b - statistically significant differences in the meeting of recommendations between educated and non-educated patients demonstrated by the U test -Mann-Whitney, $p < 0.05$; #- statistically significant differences in the percentages of patients providing minerals in the diet below the recommendations between educated and non-educated patients

patients. In this study, almost 1/3 of the T2DM patients used the help of dieticians. Surprisingly, intakes of various nutrients in the diets of T2DM, both under the care of a dietician and not, usually did not meet dietary recommendations. However, the diets of EG, despite significant dietary mistakes, provided higher amounts of nutrients with potentially positive effects on carbohydrate and lipid metabolism: fiber, EPA, DHA, and PUFA, minerals: Mg, Fe, Cu and Mn as well as vitamins and smaller amounts of nutrients, which are nutritional risk factors for the development and progression of T2DM: total amount of fat, SFA, Na and P.

Świrska et al. (14) found that knowledge of basic dietary recommendations for diabetes among the majority of T2DM patients (64%) was unsatisfactory. The most significant deficit included lack of acquaintance with glycemic index and carbohydrate exchanges, thus making it impossible to use those recommendations in preparing everyday meals. Similar deficiencies in knowledge have also been demonstrated in other studies (15-17), which emphasises the need for specialist diabetes hospital units to include a qualified dietician.

Insufficient intakes of energy in both EG and N-EG could be related with being on a low-calorie diet. Overweight or obesity occurred in about 87% of EG and about 92% of N-EG. Current nutritional therapy recommendations from various organizations for diabetes management support intensive lifestyle interventions to achieve modest weight-loss and

weight-maintenance (18). In addition to the recommendations, a factor of major importance is the total calorie content of the diet, which should be adjusted to the patient's age, actual body weight, and level of physical activity, allowing a gradual but systematic body weight reduction. A reduction in the total energy intake (by 500–1000 kcal/day) should enable gradual but systematic body weight reduction (by about 0.5–1 kg/week) (5). The benefits of weight loss include improvements in glycemic control, risk factors for cardiovascular disease (CVD), quality of life, and other obesity-related coexisting illnesses (19). A meta-analysis of prospective cohort studies (20) suggested that the risk associated with a higher waist circumference is slightly stronger than the risk associated with a higher BMI. In clinical practice, it is important to monitor both BMI and waist circumference in the patients. In the presented study we monitored BMI and WHR in all patients. We observed a slight tendency to a higher percentage of overweight or obese patients (BMI >24.9 kg/m^2) and WHR moving to a higher range in N-EG.

The current nutrition recommendations for T2DM adults do not indicate the prescription of protein restriction. In most diabetic patients, similarly to the general population, proteins should provide 15–20% of total calorie intake (about 1–1.5 g/kg body weight/day). In the T2DM patients, and people with excessive body weight, it is important to maintain or increase protein intake (a low-calorie diet may contain 20–30% of protein) because an inadequate protein intake can cause lean muscle loss, and problems with enzyme production and antibodies (5, 18, 21, 22). The current recommendations for protein intake by T2DM patients with diabetic kidneys are differ between various organizations. According to The Polish Diabetic Association (15), in patients with chronic kidney disease protein intake should be about 0.8–1 g/kg body weight/day. The Canadian Diabetes Association (CDA) (21) also recommends considering the prescription of a protein restriction. Lopez et al. (23) evaluated the effect of a protein restriction diet on renal function and metabolic control in TD2M patients with or without nephropathy. All patients were randomly assigned to receive either a low protein diet (LPD) (0.6-0.8 g/kg per day) or a normal protein diet (NPD) (1.0-1.2 g/kg per day) for a period of 4 months. A moderated protein restriction diet improved renal function in T2DM patients and macroalbuminuria. HbA1c decreased significantly among microalbuminuric patients on both diets, and among macroalbuminuric patients who received NPD. In contrast to this observation, the American Diabetes Association (ADA) recommends against protein restriction (22). A meta-analysis conducted by Pan et al. (24) did not show beneficial renal effects from low-protein diets in the T2DM patients. The majority of N-EG diets provided energy from protein above the recommendations (above 20%). This could be related with their obesity, glycemic control or lack of knowledge about the possibility of the progression of kidney problems. Campos-Nonato performed a randomized clinical trial (25) about the effect of a High-Protein Diet (HPD) versus a Standard-Protein Diet (SPD) on weight loss and the biomarkers of metabolic syndrome. They found that the participants with a stronger adherence rate in the HPD group lost significantly more weight than adherent participants in the SPD group. Additionally, a meta-analysis of randomized controlled trials (RCT) suggested that various dietary patterns such as high-protein and also low-carbohydrate, low-GI and Mediterranean diets were effective in improving glycemic control and CVD risk factors in the T2DM patients (26). In clinical practice, it is important to help patients to lose weight and remind them about a well-balanced diet with proper macronutrient proportions, especially with a proper amount of protein.

According to the Polish Diabetic Association recommendations there is no need to limit animal protein intake, although substituting plant protein for animal protein may be beneficial in some patients (5). In the EPIC (27) (European Prospective Investigation Into Cancer and Nutrition) study, the long-term association between total, animal and plant protein intake and T2DM incidence was evaluated. The study demonstrated an association between high total and animal protein intake and a modest elevated risk of T2DM in a large cohort of European adults. It has also shown that the high intake of animal protein and its effect on the T2DM may be related to the supply of SFA which is found in the meat (27). In the presented study, N-EG diets provided a significantly higher amount of

energy from SFA than those of the educated group, which could indicate increased meat consumption by this group and therefore high animal protein provision in the diet.

The quality of fat is more important than total fat intake, and diets that favor plant-based fats over animal fats are more advantageous (18). According to the Polish Diabetic Association (5) recommendations for the general population, the average total fat intake should provide 30% to 35% of total calories. Saturated fats should provide less than 10% of the total calorie intake, and less than 7% of the total calorie intake in patients with serum LDL cholesterol level ≥ 100 mg/dL (≥ 2.6 mmol/L). Monounsaturated fats should provide 10–15% of the total calorie intake, and polyunsaturated fats should provide about 6–10% of the total calorie intake.

A significantly higher percentage of EG diets provided the proper amount of energy from SFA in comparison with N-EG. Western diets, which are rich in SFA, can cause insulin resistance, disturbances in secretion of insulin, and increased levels of circulating free fatty acids (FFAs). In addition, they contribute to β-cell failure in genetically predisposed individuals (28). Short-term high levels of FFAs in the blood inhibit glucose metabolism, which results in increased insulin secretion. The opposite effect is observed during long-lasting increases in FFA serum concentration, a situation in which pancreatic secretion of insulin is inhibited. FFAs cause beta-cell apoptosis and may thus contribute to progressive β-cell loss in T2DM. The mass of this organ's cells is reduced, and they lose their ability to compensate for insulin resistance (28, 29). The T2DM is associated with another long-term problem - coronary heart disease. According to the "Seven Country Study" (30), mortality due to coronary heart disease was shown to be positively correlated with mean cholesterol levels and average SFA consumption. To reduce serum LDL cholesterol levels, which coexist particularly in T2DM patients, low glycemic index carbohydrates and monounsaturated fats should be substituted for SFA (31).

More than half of all participants showed an insufficient intake of LC-PUFA. Dietary supplementation with EPA/DHA is recommended, especially for individuals with determined dietary deficiencies in LC-PUFAs (32). A significant amount of epidemiological evidence has found that increased n-3 PUFA consumption in intervention studies may alleviate metabolic and cardiovascular risk and progression of T2DM (33). LC-PUFAs affect lipid-carbohydrate metabolism and exhibit insulinotropic and anti-inflammatory activity through membrane receptors (34). They also inhibit the expression and activity of pro-inflammatory cytokines, and decrease the activity of the pro-inflammatory nuclear factor β (NF-β), which promotes the expression and increases the activity of many pro-inflammatory genes and molecules, e.g. cytokines and chemokines that induce insulin resistance (35). An increased intake of LC-PUFAs (EPA, DHA) also elicits an increase in skeletal muscle membrane fluidity, number of insulin receptors and insulin action (32, 36). Moreover, Chen et al. (37) performed a meta-analysis concerning the effects of omega-3 fatty acid supplementation on glucose control. They found that the ratio of EPA/DHA and early intervention with omega 3 fatty acids may have effects on glucose control and lipid levels, although no statistical significance was identified. On the other hand, the meta-analysis conducted by Wu et al. (38) showed that EPA +DHA from fish and seafood support neither major harm nor benefits regarding the development of diabetes, and suggest that ALA may be associated with a modestly lower risk of T2DM. In particular, replacing saturated fat with omega-6 PUFA was related to a lower risk of developing diabetes (39, 40).

T2DM patients are usually characterized by a lower serum concentration of Mg compared to normal subjects, as a result of an inadequate supply of this mineral from food and/or increased elimination from the body (41). However, the percentage of patients who showed insufficient intake of Mg in the EG was significantly lower compared to N-EG. The EG probably knew about the requirement to increase the intake of foods which are good sources of Mg. A proper supply of this nutrient from food or supplementation may have a beneficial effect on glycemic control in T2DM patients (42). Mg is actively involved in a number of metabolic reactions as an important co-factor, with a special emphasis on carbohydrate metabolism. The meta-analysis conducted by Mooren (43) suggested that reduced dietary Mg intake serves as a risk factor

for the incidence of both impaired glucose regulation and T2DM. Sinka et al. (44) demonstrated a negative correlation between serum levels of glucose and serum levels of Mg in diabetic subjects. Mg intake may be particularly beneficial in offsetting the risk of developing diabetes among those at high risk (45).

The percentage of patients who showed insufficient intake of Fe in the EG was significantly lower compared to N-EG. Both deficiency and excess Fe in the diet are dangerous. Insufficient Fe intake from the diet may lead to anemia, while excessive consumption (excessive dietary supplementation or medication intake) may lead to diarrhea, nausea and vomiting (13). Fe is increasingly recognized to influence glucose metabolism on multiple levels. Body Fe stores should be considered a potential target for therapy in subjects with T2DM, or those at risk for developing T2DM (46). Fe also seems to modulate β-cells, insulin secretion and thereby glucose homeostasis. In the pathogenesis of diabetes, an excessive intake of Fe generates reactive oxygen species (ROS) by participating in the Fenton chemistry, which can induce oxidative damage and apoptosis (47).

Mn is an activator of numerous enzymes involved in the synthesis of proteins, nucleic acids and fatty acids. Mn is also a part of the group of superoxide dismutase enzymes (MnSOD), which catalyse the superoxide anion dismutation into hydrogen peroxide and oxygen (48). In a cross-sectional survey, lower concentrations of Mn were reported in blood and scalp hair samples of type 2 diabetic patients compared to healthy controls, while Mn levels in urine were higher in T2DM patients, which can be evidence of increased elimination of Mn from their body (49). We can find in the EFSA report (50) that a low intake of Mn can cause disorders in lipid and carbohydrate metabolism (hypercholesterolemia and insulin resistance). EG provided significantly higher amounts of Mn than N-EG, which proved the beneficial effects of dietary consultation on patients' diets.

The Na intake of T2DM patients, both in the EG and N-EG, exceeded the recommendations established for this mineral. However the EG take in a slightly lower amount of sodium, but with no significant differences. Salt intake from all sources should not exceed 5 g per day and the current Na intake recommendation for diabetes management is < 2300 mg/d (5, 22). If reasonable, patients with hypertension may be advised more strict salt intake limitations according to the DASH diet principles (5). In one observational study, high Na intake was associated with increased mortality in T2DM people (51). Excess Na in the diet can significantly affect the blood pressure of patients, by increasing it, and thus increasing the risk of cardiovascular complications, which are particularly high in T2DM patients (13).

The majority of differences in dietary intakes between EG and N-EG concerned vitamins. Dietary deficiencies in vitamin D were shown in almost all the patients from both the EG and N-EG. However, comparing vitamin D intake between two groups shows that the dietary consultations had an impact on higher supplies of this vitamin, closer to the AI recommendations. In connection with insufficient dietary intake and the scarcity of sources of vitamin D, supplementation of vitamin D is widely recommended (5). Vitamin D plays an important role in the proper secretion and activity of insulin. Specific receptors for vitamin D are located on the surface of islet β cells, and binding vitamin D to them may enhance insulin secretion. Vitamin D is also known to be involved in maintaining the proper extracellular concentration of calcium ions and their flow into the pancreatic β cells. An increased concentration of calcium ions in the cytosol of β cells promotes the release of insulin (32). Kuchay et al. (52) assessed the relationship between the intake of vitamin D and the risk of progression of diabetes. It was found that supplementation of the diets of people with prediabetes with vitamin D can help to regulate carbohydrate metabolism. After a period of 12 months in the group supplementing with vitamin D there was significantly lowered fasting plasma glucose, 2h plasma glucose and HBA1c levels. Another study (53) showed a relationship between vitamin D deficiency and insulin resistance. However, the direct effect of deficiency of this vitamin on carbohydrate metabolism was not confirmed. The deficiency of vitamin D, which positively correlated with insulin resistance, was mostly found in people with obesity, which also promotes this disorder. In our groups problems also occur with overweight and obesity, which may additionally increase requirements for vitamin D.

In the present study, the EG consumed significantly larger amounts of vitamin E, and a significantly higher percentage of them met the EAR than N-EG. In view of the fact that vitamin E plays an important role in delaying the prevalence of diabetes and slows down its progression, it is necessary to supply an adequate amount of vitamin E. Baburao et al. (54) observed a decrease in postprandial glucose values and total cholesterol level and a reduction of increased blood pressure in the group of patients who take vitamin E supplementation compared to the control group. Shinde et al. (55) demonstrated a reduction in oxidative stress and an improvement in antioxidant enzyme activity and vascular endothelial function in T2DM patients treated with anti-diabetic agents supplemented with vitamin E, as compared with patients treated only with glucose-lowering drugs. In turn, vitamin E supplementation by T2DM patients can also help to reduce the risk of developing CVD by preventing the oxidation of low-density lipoproteins.

EG provided higher amounts of vitamin C, and therefore a lesser percentage of them did not meet the EAR of vitamin C, which proved the beneficial effects of dietary consultation on patients diets. EG probably consumed more vegetables and fruits than N-EG. Vitamin C plays the role of cofactor in many enzymatic and metabolic reactions, including the synthesis of collagen, the structure of the skin, bones and blood vessels. It is also an antioxidant, which reduces oxidative processes in the body (13). In the Darshike et al. study (53), it was demonstrated that the supplementation of T2DM patients with high doses of vitamin C - 2,000 mg / day for at least 90 days had a beneficial effect on the reduction of fasting glucose and HbA1c. Li et al. (56) examined the association between dietary vitamin C intake and T2DM. The study showed a significant negative correlation between dietary vitamin C intake and T2DM prevalence.

The EG diets provided significantly higher amounts of folic acid, which may confirm the beneficial effects of dietary intervention. Folates are responsible for proper cell division, functioning of the nervous system and hematopoietic system, and determining proper homocysteine metabolism. Disturbances in homocysteine metabolism are some of the key factors regulating the risk of CVD, therefore proper dietary folate has been proven to be necessary in alleviating the risk of CVD. (57). Folate deficiencies may increase during pharmacotherapy with metformin. About 60% of patients in both groups in the presented study took metformin, and it was the most popular drug among T2DM patients. In one of the studies, the importance of folate supplementation on diabetes parameters in obese men with T2DM using metformin was evaluated. The authors showed that supplementation with folic acid at the amount of 5mg /day may positively affect carbohydrate metabolism. Significant increases in folate and vitamin B12 concentrations and decreases in homocysteine, fasting glucose and HbA1C were observed in the above-mentioned study. Based on the above results, it can be concluded that supplementation with folic acid improves the parameters of diabetes and eliminates the negative effect of metformin on folate body content. In the above study folate supplementation has also been shown to improve glycemic control by reducing concentrations of glycosylated hemoglobin, fasting blood glucose, serum insulin and insulin resistance, as well as homocysteinemia in the T2DM patients (58).

Conclusion

In the present study, variations in nutrient supply in the diet of individual T2DM patients were demonstrated, both in the educated and non-educated groups. Despite this, the diet of educated patients was more balanced compared to the diet of non-educated patients. It can therefore be concluded that nutritional education has a positive impact on the nutrient intake of T2DM patients. Routine dietitian care for people suffering from this disease should be recommended.

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References

1. Korzeniowska K, Jabłecka A. Cukrzyca (część I). Farm Współ 2008; 1: 231 – 235
2. American Diabetes Associaton. Diagnosis and classification of diabetes mellitus. Diabetes Care 2014; 37: 81-90
3. International Diabetes Federation. IDF Diabetes Atlas. http://www.idf.org/diabetesatlas [access date: 20.03.2018]
4. Abdullah A, Peeters A, de Courten M, Stoelwinder J. The magnitude of association between overweight and obesity and the risk of diabetes: a meta-analysis of prospective cohort studies. Diabetes Res Clin Pract 2010; 89: 309-319
5. Polskie Towarzystwo Diabetologiczne. Zalecenia dotyczące postępowania u chorych na cukrzycę. Diab Klin 2017; 3: supl. A
6. Włodarek D, Głąbska D. Zwyczaje żywieniowe osób chorych na cukrzycę typu 2. Diabetol Prakt 2010; 11: 17-23
7. Baranik A, Ostrowska L. Praktyczne zalecenia dotyczące żywienia chorych z cukrzycą typu 2 i otyłością. Forum Zab Metabol 2011; 2: 222-230
8. Korzon-Burakowska A, Adamska K, Skuratowicz-Kubica A, Jaworska M, Świerblewska E, Kunicka K. Wpływ edukacji na parametry wyrównania cukrzycy i jakość życia chorych na cukrzycę typu 2 leczonych insuliną. Diabetol Prakt 2010; 11: 46-53
9. Komeda M, Adachi M, Shimizu T, et al. A 6-month nutritional education program for patients with diabetes mellitus is effective to improve HbA1C. Diabetes Res Clin Pract 2016; 120: 171
10. Sharifirad G, Entezari MH, Kamran A, Azadbakht L. The efectiveness of nutritional education on the knowledge of diabetis patients using the health belief model. J Res Med Sci 2009; 14: 1-16
11. Pimentel GD, Portero-McLellan KC, Oliveira EP. Long-term nutrition education reduces several risk factors for type 2 diabetes mellitus in Brazilians with impaired glucose tolerance. Nutr Res 2010; 30: 186-190
12. Breen C, Ryan M, Gibney MJ, O-Shea D. Diabetes-related nutrition knowledge and dietary intake among adults with type 2 diabetes. Br J Nutr 2015; 114: 439-447
13. Jarosz M (Red.). Normy żywienia dla populacji polskiej – nowelizacja. Instytut Żywności i Żywienia Warszawa 2017
14. Świrska J, Zwolak A, Dudzińska M, et.al. The knowledge of dietary rules among patients with diabetes. J. Educ, Health and Sport 2017; 7: 419-435
15. Hołyńska A, Kucharska A, Sińska B, Panczyk M. Poziom wiedzy żywieniowej a sposób żywienia chorych na cukrzycę leczonych insuliną. Pol Merkur Lekarski 2015; 39:292-296
16. Bronkowska M, Zatońska K, Orzeł D, Biernat J. Ocena wiedzy żywieniowej osób z cukrzycą typu 2 w świetle zaleceń dietetycznych, Bromatol Chem Toksykol 2013; 4: 520-530
17. Szypnicka M, Sińska A. Ocena wpływu wiedzy pacjentów o żywieniu na skuteczność leczenia dietetycznego w cukrzycy. Med Metab 2013; 18: 35-40
18. Ley SH, Hamdy O, Mohan V, Hu FB. Prevention and management of type 2 diabetes: dietary components and nutritional strategies. Lancet 2014; 7: 1999-2007
19. Zahorska – Markiewicz B. Otyłość i nadwaga a cukrzyca typu II. Forum Profilaktyki 2007; 1 http://www.pfp.edu.pl/download/forum8.pdf [access date: 20.03.2018]
20. Vazquez G, Duval S, Jacobs DR Jr, Silventoinen K. Comparison of body mass index, waist circumference, and waist /hip ratio in predicting incident diabetes: a meta-analysis. Epidemiol Rev 2007; 29: 115-128
21. Canadian Diabetes Association Clinical Practice Guidelines Expert Committee. Clinical Practice Guidelines: Nutrition Therapy. Can J Diabetes 2013; 37: 45-55
22. Evert AB, Boucher JL, Cypress M, et al. Nutrition therapy recommendations for the management of adults with diabetes. Diabetes Care 2014; 37: 120-143
23. López LV, Acosta JS, Robles VG, Tamayo TM, Limones CR. Effect of protein restriction diet on renal function and metabolic control in patients with type 2 diabetes: a randomized clinical trial. Nutr Hosp, 2008; 23: 141-147
24. Pan Y, Guo LL, Jin HM. Low-protein diet for diabetic nephropathy: a meta-analysis of randomized controlled trials. Am J Clin Nutr 2008; 88: 660-666
25. Campos-Nonato I, Hernandez L, Barguera S. Effect of a high-protein diet versus standard-protein diet on weight loss and biomarkers of metabolic syndrome: a randomized clinical trial. Obes Facts 2017; 10:238-251
26. Ajala O, English P, Pinkey J. Systematic review and meta-analysis of different dietary approaches to the management of type 2 diabetes. Am J Clin Nutr 2013; 97: 505-516
27. Nielen M, Feskens EJ, Mensink M, et al. Dietary protein intake and incidence of type 2 diabetes in Europe: the EPIC-InterAct Case-Cohort Study. Diabetes Care 2014; 37: 1854-1862
28. Rosołowska-Huszcz D. Działanie kwasów tłuszczowych za pośrednictwem receptorów błonowych a znaczenie tłuszczu w profilaktyce dietetycznej cukrzycy typu II, Diabetol Prakt 2011; 12: 42-51
29. Cnop M. Fatty acids and glucolipotoxicility in the pathogenesis of type 2 diabetes, Biochem Soc Trans 2008; 36: 348-352
30. Keys A. Coronary heart disease in seven countries. Circulation 1970; 41: 186-195
31. Szostak- Węgierek D, Kłosiewicz-Latoszek L, Szostak WB, et al. The role of dietary fats for preventing cardiovascular disease, Rocz Panstw Zakl Hig 2013; 64: 263-269
32. Zabłocka-Słowińska K, Dzielska E, Gryszkin I, Grajeta H. Dietary supplementation during diabetes therapy and the potential risk of interactions, Adv Clin Exp Med 2014; 23: 939-946
33. Nettlon JA, Katz R. n-3 long chain polyunsaturated fatty acids in type 2 diabetes: a review, J Am Diet Assoc 2005; 105: 428-440
34. Flachs P, Rossmeisl M, Bryhn M, Kopecky J. Cellular and molecular effects of n-3 polyunsaturated fatty acids on adipose tissue biology and metabolism. Clin Sci (Lond) 2009; 116: 1-16

35. Singer P, Shapiro H, Theila M, Anbar R, Singer J, Cohen J. Anti-inflammatory properties of omega-3 fatty acids in critical illness: novel mechanisms and an integrative perspective. Intensive Care Med 2008; 34: 1580-1592
36. Molendi-Coste O, Legry V, Leclercq IA. Why and how meet n-3 PUFA dietary recommendations? Gastroenterol Res Pract 2010; 2011: 1-11
37. Chen C, Yu X, Shao S. Effects of Omega-3 fatty acid supplementation on glucose control and lipid levels in type 2 diabetes : a meta-analysis. PLOS one 2015; 10: 1-14
38. Wu JH, Micha R, Imamura F, et al. Omega-3 fatty acids and incident type 2 diabetes : a systematic review and meta-analysis. Br J Nutr 2012; 107: 214-227
39. Salmerón J, Hu F, Manson JE, et al. Dietary fat intake and risk of type 2 diabetes in women. Am J Clin Nutr 2001; 73: 1019-1026
40. Hu FB, van Dam RM, Liu S. Diet and risk of Type II diabetes: the role of types of fat and carbohydrate. Diabetologia 2001; 44: 805-817
41. Walti MK, Zimmermann MB, Spinas GA. Low plasma magnesium in type 2 diabetes. Swiss Med Wkly 2003; 133: 289-292
42. Dong J, Xun P, He K. Magnesium intake and risk of type 2 diabetes. Diabetes Care 2011; 34: 2116-2122
43. Mooren FC. Magnesium and disturbances in carbohydrate metabolism. Diabates Obes Metab 2015; 17: 813-823
44. Satwika S, Sukanta S. Status of zinc and magnesium levels in type 2 diabetes mellitus and its relationship with glycemic status. Int J Diabetes Dev Ctries 2014; 34:220-223
45. Hruby A, Meigs JB, O'Donnell C, Paul F, Jacques PF,McKeown NM. Higher magnesium intake reduces risk of impaired glucose and insulin metabolism and progression from prediabetes to diabetes in middle-aged Americans. Diabetes Care 2014; 37: 419-427
46. Fernández-Real JM, McClain D, Manco M. Mechanisms linking glucose homeostasis and iron metabolism toward the onset and progression of type 2. Diabetes Care 2015; 38: 2169-2176
47. Hansen JB Moen IW, Mandrup-Poulsen T. Iron : the hard player in diabetes pathophysiology. Acta Physiol 2014; 210: 717-732
48. Wołonciej M, Milewska E, Roszkowska-Jakimiec W. Pierwiastki śladowe jako aktywatory enzymów antyoksydacyjnych. Postepy Hig Med. Dosw 2016; 70: 1483-1498
49. Kazi TG, Afridi HI, Kazi N, et al. Copper, chromium, manganese, iron, nickel, and zinc levels in biological samples of diabetes mellitus patients. Biol Trace Elem Res 2008; 122: 1-18
50. Scientific Opinion on Dietary Reference Values for manganese. EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) 2013; 11
51. Ekinci EI, Clarke S, Thomas MC, et al. Dietary salt intake and mortality in patients with type 2 diabetes. Diabetes Care 2011; 34: 703-709
52. Kuchay MS, Laway BA, Bashir MI, Wani AI, Misgar RA, Shah ZA. Effect of Vitamin D supplementation on glycemic parameters and progression of prediabetes to diabetes : A 1-year, open-label randomized study. Indian J Endocrinol Metab 2015; 19: 387-392
53. Darshika JCD, Girgis CM, Gunton JE. Effects of vitamins C and D in type 2 diabetes mellitus, Nutr Diet Suppl 2015; 7: 21-28
54. Baburao JA, Anand JV. Vitamin E, its beneficial role in diabetes mellitus (DM) and its complications. J Clin Diagn Res 2012; 6: 1624-1628
55. Shinde SN, Dhadke VN, Suryakar AN. Evaluation of oxidative stress in type 2 diabetes mellitus and follow-upalong with vitamin E supplementation. Ind J Clin Biochem 2011; 26: 74-77
56. Li X, Wang X, Wei J, Yang T. Relationship between dietary vitamin C and Type 2 diabetes. Zhong Nan Da Xue Xue Bao Yi Xue Ban 2015; 40: 1109-1114
57. Hayden MR, Tyagi SC. Homocysteine and reactive oxygen species in metabolic syndrome, type 2 diabetes mellitus, and atherosclerosis: the pleiotropic effects of folate supplementation. Nutr J 2004; 10: 3-4
58. Gargari BP, Aghamohammadi V, Aliasgharzadeh A. Effect of folic acid supplementation on biochemical indices in overweight and obese men with type 2 diabetes. Diabetes Res Clin Pract 2011; 94: 33-38

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Original article

Determination of health status perception and orthorexia nervosa tendencies of Turkish yoga practitioners: a cross-sectional descriptive study

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Summary. As part of the yoga the possibility that excessive attention paid by practitioners to diet and food quality is expected. This study was aimed to determine health status perception and orthorexia nervosa tendencies of yoga practitioners. This cross-sectional descriptive study was conducted at three yoga centers in Izmir. The population of the study consisted of 153 people registered in yoga centers between March and May 2017. The data were collected using a personal information form (11 questions), Perception of Health Status Scale and Orthorexia Nervosa (Orto-11) Scale. While perceived physical health was "good" for 68.0% of the participants, the mean perceived health score was 1.94±0.54. The mean score of Orto-11 Scale was 24.05±4.36. It was determined that the perceived health of those who were married and non-smoker were higher than the orthorexia tendencies of the individuals who were married and had the chronic illness ($p<.001$). More than half of yoga practitioners perceived their physical health as "good". It was determined that the perceived health status of those who were married and non-smokers were higher. It was found that the vast majority of yoga practitioners was at risk in terms of orthorexia. Some factors like marital status and the presence of chronic illness significantly affected orthorexia nervosa tendencies of yoga practitioners.

Key words: Orthorexia nervosa, yoga practitioners, health status, perception

Introduction

Nutritional intake is a physiological, sociological and psychological phenomenon (1). Orthorexia nervosa (ON) refers to a pathological obsession concerning healthy nutrition obsession or the desire to consume healthy food (2,3). The term "orthorexia" was first defined in a non-scientific yoga journal by Bratman (1997) with joining the Greek words 'orthos' (correct, appropriate) and 'orexia' (appetite). Even though orthorexia is similar to known eating disorders, consuming only "pure" and "healthy" foods are in the forefront in orthorexia rather than the desire to lose weight (2). Transformation of this desire to excessive effort mentally and behaviorally also resembles obsessive-compulsive disorder (4). It is stated that depression, anxiety, perfectionist tendencies and stress can accompany ON as in other eating disorders (5). While health promotion, treatment of a disease or loss of weight are the priorities in the basis of orthorexia, then this nutrition style starts to be the most important part in the lives of orthorectic individuals. Thus, they are having healthy eating obsession in order to protect and promote health (6). Obsessional behaviors related to orthorexia are related to the content of the food consumed rather than its' amount (7). In the orthorexia nervosa, the person controls everything he/she eats exaggeratedly. He/she examines the package of the products for hours and thinks hard exaggeratedly whether the product contains any carcinogenic substance, hormone, dye, and addictive substance (2,3). In this illness which can also lead to impaired social functioning, in-

dividuals maintain their lives on within the frame of strict rules about healthy eating (8,9). As a result of orthorexia, various behaviors based on insisting on eating only raw vegetables and eating food in a certain color are exhibited. Therefore, orthorexia nervosa does not only mean that the individual is obsessive about healthy eating but also uses certain cooking methods and eats only certain foods (10). Although DSM-V (Diagnostic and Statistical Manual of Mental Disorders-V, 2013 (DSM-V 2013) by American Psychiatric Association revised and expanded diagnostic categories, some eating disorders such as orthorexia were neglected (8,11). Although it is not formally recognized as a separate diagnostic category, ON is stated to have similarities and differences with other eating disorders having serious consequences. It is seen that ON can lead malnutrition or weight loss as in anorexia nervosa (AN), but unlike AN and bulimia nervosa, people are very much engaged in eating healthy and pure foods instead of the amount of consumed food and slim appearance (12). Indeed, even though there are currently no clinical guidelines for this purported disorder, ON is being considered as detrimental to human health, because it brings about excessive focus on food, health, behavior, etc (3,5).

While the prevalence of those showing orthorectic tendency ranged from 6.9% (4) to 57.6% in the general population (13), it was reported to range between 28-30% in sporters (14); to be 45.5% in physicians (15); 81.8% in opera artists (16) and 81.9% in dieticians (6). The groups having a high risk for orthorexia nervosa (ON) are women, adolescents, sport(wo)men, students having health education (7,9,15,17-24), yoga practitioners (5,26), primary care physicians (15,18) and performing artists (16) and different sociodemographic characteristics are reported in different studies. In terms of the culture within yoga activities, these activities often require self-discipline lifestyle modifications such as healthier eating habits (5). In the studies conducted with yoga practitioners, it has been reported that 55.2% of the participants and 33.1% of males have unhealthy weight control behaviors (26). Valera et al. (2014) reported that yoga teachers to avoid excessive reference to a healthy diet, which is natural component of yoga practice. The same study noted that the possibility that excessive attention paid by practitioners to diet and food quality as part of the yoga system (5). Yoga practitioners thought to have high awareness about healthy nutrition have strict rules about eating. In this respect, yoga practitioners can be thought to be at risk of ON. However, there has been no study about the issue in the literature.

From this point of view, this study was designed based on the following questions;

1. What are the levels of health status perceptions of yoga practitioners?

2. What are the levels of the orthorexia nervosa tendencies of yoga practitioners?

3. What are the factors associated with orthorexia nervosa tendencies of yoga practitioners?

The aim of the study was to determine the health status perceptions and orthorexia nervosa tendencies of yoga practitioners.

Methods

Setting

The study was conducted in three yoga centers in Karşıyaka town in Izmir city which is located in the western part of Turkey. All yoga centers (n:3) in Karşıyaka town included to the study.

Study design and participants

The study was designed as a cross-sectional descriptive type. The population of the study consisted of 153 people registered to yoga centers between March and May 2017. Non-probability sampling was used and the sample included 118 volunteers. The rate of participation was 77.1%.

Inclusion criterias of the study were;

1. Yoga users were defined by yoga use at least half-hour per week/ at least one month

2. Eligibility requirements were being at least 18 years of age

3. Accepting to participate in the study and giving verbal consent.

Approval from the scientific ethics committee of Ege University (date: 01.02.2017, protocol no: 04-2017) to collect the data, and the application permission from three yoga centers located in Izmir to carry out the study were obtained. Verbal informed consent

was obtained from all individual participants included in the study.

Instruments

Personal information form: The form prepared in accordance with the literature (27-29) by the researchers has a total of 11 questions about the individuals' personal characteristics (age, gender, occupation, marital status, smoking status, presence of a chronic disease, diet, status of being vegan, status of being vegetarian and BMI (height, weight)).

Perception of Health Status Scale: The original scale developed by Davis, Avery, and Donald (1978), the scale is adapted to the Turkish language by Esin and Erdogan (30). The scale has only one item, "How do you perceive your current health status?", which is answered by selecting "very good" (1 point), "good" (2 points), "poor" (3 points), or "very poor" (4 points). When assessing the scale, 4 is considered the lowest score, and 1 the highest. Cronbach alpha was 0.89. (30).

Orthorexia Nervosa (Orto-11) Scale: This scale was developed to determine the healthy eating obsessions in individuals. Its' original version, ORTO-15 scale, was developed by Donini et al. in Italy (4). Donini et al. developed the ORTO-15 scale by developing and changing the statements in the Orthorexia short form with 10 questions prepared by Bratman (3). Turkish adaptation of the scale was conducted by Arusoglu (8) and it was adapted as ORTO-11. The scale contains 11 items. Each expression is evaluated with a 4-point Likert-type rating. In the scale, the individuals were asked to state how often they feel themselves as described in the items by marking one of the options as "always," "frequently", "sometimes", and "never". When "1" point is given to the answers thought to be distinguishing for Orthorexia, "4" points are given to the responses showing normal eating behavior tendency. The cut-off value of ORTO-11 was reported to be 27 by Arusoğlu (31). Low scores obtained from the scale indicate that the risk of orthorexia nervosa or orthorectic tendency is increasing (8,10). The Cronbach's Alpha value of the scale was 0.82 (8,9) and the Cronbach's Alpha coefficient was found as 0.86 in this study. The participants' body mass index (BMI) was calculated based on their self-reported height and weight and categorized using the BMI groups defined by the World Health Organization (32).

Data analysis

In the assessment of data, Kolmogorov-Smirnov test was applied for normal distribution suitability in addition to the number, percentage, mean distributions from descriptive statistics. The test showed that the values of the scale were not normally distributed (Kolmogorov-Smirnov Z=2.63, p=0.034). In between-group comparison, Mann Whitney U test for two groups and Kruskal Wallis test for more than two groups from non-parametric tests were used. Statistical significance level was accepted as $p < 0.05$. Statistical analysis was performed using SPSS 22.0 (Statistical Program Social Sciences) packaged software.

Results

Profile of yoga practitioners

Of the yoga practitioners included in the study, the mean age was 30.48±9.19 years (range: 18-56), 92.4% were female, and 72% were single. 34.75% of the participants stated that they had a health-related profession. Of the participants, 89.8% stated that they were the non-smoker, 80.5% had no chronic diseases, 12.71% were in diet, 11% stated that they were vegetarian and 6.8% stated that they were vegan (Table 1). The body mass index of the yoga practitioners was 20.55±1.80 kg/m^2 in females and 24.3±1.59 in males, respectively (Table 2).

Perception of Health Status and ORTO-11 scores of yoga practitioners

Perceived physical health was "good" for 68.0% and "very good" for 32% of the participants. The mean perceived health score was 1.94±0.54 (minimum:1, maximum: 4). When the mean scores of the sample group from Orto-11 Scale were examined, the mean score was determined as 24.05±4.36 and 75.4% of the yoga practitioners (n:89) were found to be risky in terms of ON.

The mean perceived health status scores were 1.96±0.54 in females and 1.66±0.50 in males (Table 2). No statistical difference was found between the

variables of the mean perceived health status score and gender, BMI group, chronic disease, dieting, being vegetarian and vegan (p>0.05).

The distribution of Perception of Health Status Scale and ORTO-11 scores by some socio-demographic variables

When the characteristics and the health status perception mean scores of the participants were compared, it is found that the health status perception of those who were married (U=939.000; p<.001) and non-smoker (U=310.500, p<.001) were higher (Table 3).

Table 1. Socio-demographic variables of the yoga practitioners (n:118)

Variables	n	%
Gender		
Female	109	92.4
Male	9	7.6
Relationship		
Married	33	28.0
Single	85	72.0
Health-related profession		
Yes	41	34.75
No	77	65.25
Chronic diseases		
Yes	23	19.5
No	95	80.5
Smoker		
Yes	12	10.2
No	106	89.8
Doing diet		
Yes	15	12.71
No	103	87.29
Vegeterian		
Yes	13	11.0
No	105	89.0
Vegan		
Yes	8	6.8
No	110	93.2

ORTO-11 mean score of the participants was found as 24.05±4.36. Orto-11 mean score was 24.13± 4.34 for the female's and 23.04 ±4.82 for the male's. There was no statistical difference between Orto-11 mean score and the variables such as gender, BMI group, smoking, dieting, being vegetarian and vegan (p>0.05). When the participants' characteristics and Orto-11 mean scores were compared, it was determined that the orthorexia tendencies of those who were married (U=797.00; p<.001) and had chronic disease (U=570.50; p<.001) were higher (Table 3).

There was no correlation in between perception of health status and ORTO-11 (r=.156)

Discussion

The tendency of being healthy-healthism which is both a social and political movement ensures that individuals take responsibility to avoid risk factors detrimental to the health of individuals and turn to regular exercise and healthy eating behaviors to reach optimal health (33). Healthy eating today is one of the most important approaches for health promotion (15). The nutrition style of individuals varies according to many biological, psychological and sociocultural factors (10,14,18). When the literature is examined it is seen that the risk of orthorexia nervosa which is defined as the obsession of healthy eating. Orthorexia is more common particularly in models who are careful about being in a certain weight, dancers, yoga practitioners, sporters, healthcare professionals and especially in dietitians (5,6,14-16). This study was conducted to determine the health status perception and orthorexia nervosa tendencies of yoga practitioners.

Table 2. Antropometric values of the yoga practitioners (X ± SD)

Measures	Male (n:9)			Female (n:109)		
	X ± SD	Min	Max	X ± SD	Min	Max
Years	36.3±3.84	28.00	40.00	30.0±9.35	18.00	56.00
Weight (kg)	85.00±1.73	82.00	87.00	56.94±5.49	42.00	70.00
Height (cm)	1.87±0.61	1.75	1.93	1.66±0.06	1.48	1.78
*BMI (kg/m^2)	24.3±1.59	22.60	27.80	20.55±1.80	15.20	27.10

**BMI: Body mass index, SD:Stardard deviation*

The vast majority of the individuals participating in the study were female (92.4%) and aged between 18-56 years. Similarly, it was reported in previous studies that the yoga practitioners were predominantly female and aged between 21 and 44 years. Results of this study are similar to the literature (34-36). Notably, yoga use was higher among young adult women.

Yoga is described as a holistic system that unifies, harmonizes, and strengthens the mind, body, and spirit (37). It has been proposed that the popularity of yoga may be largely attributable to its psychophysiological effects, which attenuate the stress response and improve emotional stability and regulation, leading to a greater feeling of well-being and improving quality of life (38). According to the results of the study, variables like the practitioners' gender, occupation, BMI, presence of a chronic disease, dieting and being vegan/vegetarian did not affect their health status perceptions ($p \geq 0.05$), it was an important result that the health status perceptions of those who were married and non-smoker were higher than those who were not married and smoker. In another study, 46.3% of yoga users reported their health as "very good" or "excellent" (38.8%) (35). In the same study, yoga users agreed that yoga improved their health regardless of their gender (35). A study reported that yoga practitioners had the desire of having a better health status (34). The results of the present study confirm previous reports indicating that people improve their health and feel better by practicing yoga (25, 34).

Table 3. The distribution of Health Status Scale and ORTO-11 scores by some socio-demografic variables.

		HEALTH STATUS PERCEPTION			ORTO 11		
Variables	**n**	**Mean±SD**	**Test**	**p**	**Mean±SD**	**Test**	**p**
Gender							
Female	109	1.96±.54	U=366.000	0.116	24.13±4.34	U=392.00	.316
Male	9	1.66±.50			23.04±4.82		
Relationship							
Married	33	1.66±.59	U=939.000	**<0.001***	21.84±3.10	U=797.00	**.000***
Single	85	2.04±.48			24.90±4.50		
Health-related profession							
Yes	41	1.91±.33	U=421.00	0.265	24.35±1.31	U=361.60	.172
No	77	1.92±.74			24.61±0.64		
BMI Group							
<18.4	9	2.11±.78			22.55±6.12		
18.5-24.9	105	1.92±.53	χ^2=.996	0.608	23.96±4.21	χ^2=6.011	.050
25.0-29.9	6	2.00±-			27.83±1.83		
Smoker							
Yes	12	2.00±.49	U=310.500	**<0.000***	23.66±4.65	U=565.50	.528
No	106	1.41±.66			24.09±4.35		
Chronic diseases							
Yes	23	2.00±.42	U=1019.000	0.534	21.26±4.31	U=570.50	**.000***
No	95	1.92±.56			24.72±4.12		
Doing diet							
Yes	15	2±-	U=637.00	0.626	22.07±3.75	U=468.00	.064
No	103	1.93±.57			24.29±4.39		
Vegeterian							
Yes	13	1.92±.49	U=652.500	0.915	23.92±4.36	U=681.50	.993
No	105	1.94±.55			24.06±4.39		
Vegan							
Yes	8	2.12±.35	U=363.000	0.308	26.62±4.56	U=284.00	.094
No	110	1.93±.55			23.86±4.31		

*χ^2:Kruskal Wallis, U=Mann Whitney U test, * p<.001*
BMI: Body mass index, SD:Stardard deviation

The popularity of yoga is growing in western countries and also in Turkey (25). Sport for All Federation (39) in Turkey continues its activities with the slogan of "live with yoga, yoga is for all ages". It is stated in the literature that yoga practitioners are a high-risk group in terms of orthorexia tendency (13,25,40). Indeed, the first recorded reference to ON was in a lay publication, Yoga Journal (2). Valera et al. (2014) reported in their study that ON prevalence of yoga practitioners was 86% (5). Similar to the literature, three quarters (75%) of yoga practitioners were found to be ON risky in this study. Since healthy eating is a standard component of yoga practice in yoga practitioners, this is evaluated as an expected result (5). When examining the variables that can affect ON tendency of yoga practitioners, in the present study, it was found that ON tendencies were higher in married individuals than the single ones and in those who had chronic illness than those who did not. Similarly, it was stated in the dissertation study by Arusoğlu (2006) that married individuals had higher ON tendencies than the single ones (8). In another study, no relationship was found between orthorectic individuals and marital status (4). This result suggested that it was important to examine the marital status of individuals as one of the important factors affecting ON risk. It was stated in another study that university students who had chronic illness showed higher orthorectic tendencies than the university students who had no chronic illness (41). In another study, it was stated that the presence of chronic disease did not make any difference in terms of orthorexia tendency (8). In the literature, there are limited studies on how the presence of both marital status and chronic disease affects the orthorexia and the differences in the results of this study indicate the need for studies in larger sample groups for the relevant variables.

Strengths and limitations

While the results of this study are striking, it has some limitations. The lack of a control group and the usage of a self-reported health status as the only other dependent variable is the most important limitation in this study. The results of the study were based on self-reported data. Body mass index was calculated based on their self-reported weight and height. Anonymous internet surveys are at risk for recall bias and deception. Despite these limitations, the results of this study are compelling.

Conclusion

With ancient roots in India, yoga has evolved over two millennia from a discipline of mind and body for spiritual goals to a global practice aimed at maintaining physical health and psychological well-being. In this study, a vast majority of the yoga practitioners were women aged between 18-56 years. It was found that male and female yoga practitioners had a normal weight. More than half of the yoga practitioners perceived their physical health as "good" and the remaining ones perceived theirs as "very good". It was determined that the health status perceptions of individuals who were married and non-smoker were higher. The marital status and the presence of chronic illness significantly affected the orthorexia nervosa tendencies of yoga practitioners. It was also determined that individuals, especially who were married and had chronic illness had high ON risks. Several studies on large cohorts are needed to clarify the prevalence of ON and its pathophysiological consequences.

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There are no conflicts of interest.

References

1. Baysal A. General Nutrition. Hatipoğlu and Selvi Publication, Ankara, 2014;15.
2. Bratman S. Original Essay on Orthorexia. Retrieved from Orthorexia Nervosa, 1997;1-3. http://www.orthorexia.com/original-orthorexia-essay/.Accessed on 2 July, 2018.
3. Brytek-Matera A (2012) Orthorexia nervosa—an eating disorder, obsessive-compulsive disorder or disturbed eating habit? Arch Psychiatry Psychother 1:55–60.
4. Donini LM, Marsili D, Graziani MP, Imbriale M, et al. Orthorexia nervosa: a preliminary study with a proposal for diagnosis and an attempt to measure the dimension of the

phenomenon. Eat Weight Disord. 2004; 9151–7.
5. Valera JH, Ruiz PA, Valdespino BR, Visioli F. (2014). Prevalence of orthorexia nervosa among ashtanga yoga practitioners: a pilot study. Eating and Weight Disorders-Studies on Anorexia, Bulimia and Obesity. 2014; 19(4): 469-472.
6. Alvarenga MS, Martins MC, Sato KS, Vargas SV, Philippi ST, et al. Orthorexia nervosa behavior in a sample of Brazilian dietitians assessed by the Portuguese version of Orto-15. Eat Weight Disord. 2012; 17(1): e29-35.
7. Varga M, Thege BK, Dukay-Szabó S, Túry F, van Furth EF. When eating healthy is not healthy: orthorexia nervosa and its measurement with the Orto-15 in Hungary. BMC Psychiatry. 2014; 14(1): 59.
8. Arusoğlu G. The investigation of healthy eating fixation (Orthorexia) and the adaptation of the Orto-15 scale. Master Thesis, (Hacettepe University,Turkey), 2006.
9. Arusoğlu G, Kabakçi E, Köksal G, Merdol TK. Orthorexia nervosa and adaptation of Orto-11 into Turkish. Turkish Psychiatry J. 2008; 19: 283–91.
10. Gezer C, Kabaran S. The risk of orthorexia nervosa for female students studying nutrition and dietetics. SDU J Health Science Institute. 2013; 4(1): 14-22.
11. Duran S. The risk of orthorexia nervosa (healthy eating obsession) symptoms for health high school students and affecting factors. Pamukkale Med J. 2016; 9: 220–226.
12. Moroze RM, Dunn TM, Holland JC, Yager J, Weintraub P. Microthinking about micronutrients: a case of transition from obsessions about healthy eating to near-fatal "orthorexia nervosa" and proposed diagnostic criteria. Psychosomatics. 2015; 56(4): 397-403.
13. Ramacciotti C, Perrone P, Coli E, Burgalassi A, Conversano C, Massimetti G et al. Orthorexia nervosa in the general population: A preliminary screening using a self-administered questionnaire (Orto-15). Eat Weigh Disord. 2011; 16(2): 127–130.
14. Segura-Garcia C, Papaianni MC, Caglioti F, Procopio L, Nistico CG, Bombardiere L, et al. Orthorexia nervosa: a frequent eating disordered behavior in athletes. Eat Weight Disord. 2012; 17: e226–e233.
15. Bağci Bosi AT, Camur D, Güler C. Prevalence of orthorexia nervosa in resident medical doctors in the faculty of medicine (Ankara, Turkey). Appetite. 2007; 49: 661–6.
16. Aksoydan E, Camci N. Prevalence of orthorexia nervosa among Turkish performance artists. Eat Weight Disord. 2009; 14: 33–37.
17. Korinth A, Schiess S, Westenhoefer J. Eating behaviour and eating disorders in students of nutrition sciences. Public Health Nutr 2010; 13: 32–7.
18. Fidan T, Ertekin V, Işıkay S, Kırpınar I. Prevalence of orthorexia among medical students in Erzurum, Turkey. Compr Psychiatry 2010; 51: 49–54.
19. Ergin G. The prevalence of orthorexic behavior in health care workers and non-health care individuals. MSc Thesis (Başkent University,Turkey), 2014.
20. Arslantaş H, Adana F, Öğüt S, Ayakdaş D, Korkmaz A. Relationship between eating behaviors of nursing students and orthorexia nervosa (obsession with healthy eating): a cross-sectional study. J Psychiatric Nurs. 2017; 8(3): 137-144.
21. Brytek-Matera A, Donini LM, Krupa M, Poggiogalle E, Hay P. Orthorexia nervosa and self-attitudinal aspects of body image in female and male university students. J Eat Disord 2015; 3: 1–8.
22. Rudolph S. The connection between exercise addiction and orthorexia nervosa in German fitness sports. Eat Weigh Disord. 2017: 1-6.
23. Dalmaz M, Tekdemir Yurtdaş G. Prevalence of orthorexia nervosa symptoms among people who exercıse in gyms. Int Orthop Traumatology and Sports Med J. 2015; 4: 23-39.
24. Eriksson L, Baigi A, Marklund B, Lindgren EC. Social physique anxiety and sociocultural attitudes toward appearance impact on orthorexia test in fitness participants. Scand J Med Sci Sports 2008; 18: 389–394.
25. Erkin, Ö., Akçay, N. Self-Perceived Health Status and Yoga-Related Perceptions among Yoga Practitioner. Turkish Journal of Family Medicine and Primary Care, 2018;12(3), 193-199.
26. Neumark-Sztainer D, Eisenberg ME, Wall M, Loth KA. Yoga and pilates: Associations with body image and disordered-eating behaviors in a population-based sample of young adults. Int J Eat Disord. 2011; 44: 276–80.
27. Park CL, Braun T, Siegel T. Who practices yoga? A systematic review of demographic, health-related, and psychosocial factors associated with yoga practice. J Behavioral Med. 2015; 38(3): 460-471.
28. Park CL, Riley KE, Bedesin E, Stewart VM. Why practice yoga? Practitioners' motivations for adopting and maintaining yoga practice. J Health Psychology. 2016; 21(6): 887-896.
29. Gaiswinkler L, Unterrainer HF. The relationship between yoga involvement, mindfulness and psychological well-being. Complementary therapies in Med. 2016; 26: 123-127.
30. Esin MN. Identification and development of health behaviors of industrial workers. PhD Thesis, (İstanbul University, Turkey) 1999.
31. Çiçekoğlu P, Tunçay GY. A comparison of eating attitudes between vegans/vegetarians and nonvegans/nonvegetarians in terms of orthorexia nervosa. Arch Psychiatric Nursing. 2018; 32(2): 200-205.
32. World Health Organization. Body Mass Classification, 2018. http://apps.who.int/bmi/index.jsp?introPage=intro_3.html. Accessed on 3 July, 2018.
33. Oberle CD, Watkins RS, Burkot AJ. Orthorexic eating behaviors related to exercise addiction and internal motivations in a sample of university students, Eat Weight Disord-Studies on Anorexia, Bulimia and Obesity. 2018; 23(1): 67-74.
34. Saper HB, Eisenberg DM, Davis RB, Culpepper L, Phillips RS. Prevalence and patterns of adult yoga use in the United States: results of a national survey. Altern Ther Health Med. 2004; 10(2): 44-49.
35. Ross A, Friedmann E, Bevans M, Thomas S. National survey of yoga practitioners: mental and physical health bene-

fits. Complement Therapies in Med. 2013; 21(4): 313-323.
36. Telles S, Gupta RK, Yadav A, Pathak S, Balkrishna A. Hemisphere specific EEG related to alternate nostril yoga breathing. BMC Research Notes. 2017; 10(1): 306-310.
37. Khalsa SB. Yoga as a therapeutic intervention. Principles and practice of stress management. 2007; 3: 449-462.
38. Büssing A, Michalsen A, Khalsa SBS, Telles S, Sherman KJ. Effects of yoga on mental and physical health: a short summary of reviews. Evidence-Based Complement Altern Med. 2012: 1-8.
39. The Turkish Sport for All Federation. 2015. http://www.his.gov.tr/herkes-icin-spor.html. Accessed on 02 July, 2018.
40. Penaforte FR, Barroso SM, Araújo ME, Japur CC. Orthorexia nervosa in nutrition students: association with nutritional status, body satisfaction and coursed period. Jornal Brasileiro de Psiquiatria. 2018; 67(1): 18-24.
41. Tatarlar Ercen M. Investigation of the relationship between orthorexia nervosa levels, body ımage and obsessive compulsive disorder levels among university students. (MSc Thesis), (Near East University, Cyprus), 2015.

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Original article

Applying various criteria to assess the nutritional status among hospitalised patients aged 65 and over

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Summary. *Introduction:* Malnutrition commonly occurs among hospitalised patients. Should the condition be diagnosed early, it is possible to counteract development of negative effects associated with a weight loss and the systemic consequences of malnutrition. *Objective:* To determine the prevalence of abnormal nutritional status in patients aged 65 and over using various criteria for diagnosis, as well as an analysis of correlations between the criteria that were used. *Material and methods:* The study included 102 patients over the age of 65 years. Basic anthropometric measurements and a body composition analysis were performed. An abnormal nutritional status was diagnosed based on the results of the MNA-SF test, laboratory tests (serum albumin levels, total lymphocyte count - TLC) and the European Society for Clinical Nutrition and Metabolism's (ESPEN) 2015 criteria regarding nutritional status. *Results:* An abnormal nutritional status was found in 75% of the subjects. It was most frequently diagnosed based on the MNA-SF score (66%) and laboratory test results (53%), and most rarely based on a BMI measuring less than 18.5 kg/m^2 (5%). There was no statistically significant correlation between a BMI < 18.5 kg/m^2, the MNA-SF score and laboratory test results. The MNA-SF test score had the strongest correlation with results obtained using the ESPEN criteria which included a reduced fat-free mass index (FFMI) combined with an unexpected loss of body weight. *Conclusions:* Of the analysed criteria used to assess nutritional status, the MNA-SF screening tool and the laboratory test results had the highest sensitivity. In this age group, the ESPEN criteria including FFMI were the most useful, and the criterion based only on a BMI of less than 18.5 kg/m^2 was the least useful.

Key words: nutritional status, MNA-SF test, fat-free mass index (FFMI), laboratory test results

Introduction

Malnutrition among patients admitted to hospitals is usually underdiagnosed and untreated, and causes what is known as hospital malnutrition (1, 2). The condition is considered a separate disease entity, and is therefore is listed in the International Statistical Classification of Diseases and Related Health Problems under the "malnutrition" heading (E40 - E46) (3). Malnourished patients usually develop more common complications, which result in longer hospitalisation, longer recovery periods and even higher mortality compared to patients with a normal nutritional status. As with all diseases, malnutrition should be treated according to valid guidelines, and its early diagnosis should be considered a priority for medical staff (4-6).

The process of diagnosing malnutrition starts with a screening test, performed when patients are admitted to hospital. In Poland, according to the regulation of the Ministry of Health dated 15.09.2011 (amended 22.11.2013), the tools recommended for the routine evaluation of hospitalised patients' nutritional status are the NRS-2002 (Nutritional Risk Score-2002) and the SGA (Subjective Global Assessment) scales (7,8). For elderly patients, an alternative to these scales is the widely used MNA (Mini-Nutritional Assessment) scale. A shorter version of this tool (MNA-Short Form, MNA-SF® Nestlé Nutrition Institute)

(9) contains questions regarding reduced intake of food and loss of body weight during the previous 3 months; ability to move independently; presence of a serious somatic disease or severe psychological stress during the previous 3 months; assessment of cognitive impairment and/or depression; and current Body Mass Index (BMI). A total evaluation of nutritional status should be performed for each patient suspected to be malnourished or at risk of malnutrition. Apart from standard anthropometric measurements, this evaluation should include selected laboratory tests and a body composition analysis by the electrical bioimpedance method (10).

In 2015 the European Society for Clinical Nutrition and Metabolism (ESPEN) convened a group of experts to determine a minimum set of criteria to be used, irrespective of the related disease entity and its aetiology, to diagnose malnutrition, as well as to standardize international terminology. According to ESPEN, patients at risk of malnutrition should be initially identified using validated screening tools. Moreover, it was unanimously concluded that a BMI value below 18.5 kg/m^2 is not sufficient to diagnose malnutrition. However, when the patient's BMI is above this value, it is necessary to use one of two other equivalent methods to diagnose malnutrition. It is necessary to confirm both an unexpected loss of body weight and a low value for either BMI or fat-free mass index (FFMI). Such a loss of body weight is defined as a loss of more than 10% of body weight within an unspecified time frame, or more than 5% within 3 months. A reduced BMI is defined as less than 20 kg/m^2 and less than 22 kg/m^2 for young people and subjects over the age of 70 years, respectively. Meanwhile, a low value of fat-free mass index (FFMI) is defined as less than 15 kg/m^2 and less than 17 kg/m^2 for women and men, respectively (11).

Objective

The research aimed to determine the prevalence of abnormal nutritional status among patients over 65 years of age using various diagnostic criteria. An additional objective was to analyse correlations between the various criteria used to diagnose abnormal nutritional status.

Material and methods

The study included 102 patients (87 women and 15 men) of a geriatric unit in one of Warsaw's hospitals who were over the age of 65 years. The presence of a pacemaker was a criterion for exclusion from the study.

Examination included a short version of the MNA screening test (MNA-SF® Nestlé Nutrition Institute) (9, 12), patients who scored 12 points or greater were classified as subjects with a normal nutritional status; those with a lower score were classified as subjects with an abnormal nutritional status (8-11 points – risk of malnutrition; 0-7 points – malnutrition). In addition, body height was measured with a SECA stadiometer, and an analysis of body composition combined with body mass measurement was performed with a TANITA analyser. Based on the measurements obtained, BMI was calculated as the quotient of body weight in kilograms divided by the square of body height in metres; while fat-free mass index (FFMI) was calculated by dividing the fat-free mass in kilograms by the square of body height in metres. Additionally, laboratory test results for serum albumin levels and total lymphocyte count (TLC) per 1 mm^3 of blood, obtained during the hospitalisation, were used. The results obtained were interpreted on the basis of MNA-SF ® Nestlé Nutrition Institute test criteria (9), laboratory test standards, and the criteria for assessing nutritional status suggested by ESPEN in 2015 (Table 1). All measurements and evaluations were done by the same investigator.

The results were analysed with STATISTICA software, version 13.1. Elements of descriptive statistics were used, such as sample size tables, determination of distribution measures using measures of central tendency (arithmetic mean, median) and measures of variability (standard deviation, minimum, maximum). Moreover, the chi-square test for independence was used with appropriate modifications (corrections) depending on the predicted size samples in study groups (namely Pearson chi-square, chi-square with Yates correction, Fisher's exact test) to analyse correlations between the criteria used to diagnose abnormal nutritional status. A P value < 0.05 was assumed as the level of statistical significance. The C Pearson's contingency coefficient was used to assess the strength of correlations between variables analysed in the chi-square test.

Table 1. Criteria used to diagnose an abnormal nutritional status according to various criteria used.

Interpretation	Abnormal nutritional status
	MAIN CRITERIA
MNA-SF® Nestlé Nutrition Institute (9)	<12 points
ESPEN 1	BMI < 18.5 kg/m²
	or
	unexpected body weight loss (> 5% within the last 3 months or > 10% within an unspecified time frame)
	and
	BMI < 20 kg/m² for subjects < 70 yrs
	BMI < 22 kg/m² for subjects > 70 yrs
ESPEN 2	BMI < 18.5 kg/m²
	or
	unexpected body weight loss (> 5% within the last 3 months or > 10% within an unspecified time frame)
	and
	FFMI < 15 kg/m² for women
	FFMI < 17 kg/m² for men
Alb and/or TLC	serum albumin < 3.5 g/dL
	and/or
	total lymphocyte count (TLC) < 1500/mm³ of blood
	PARTIAL CRITERIA
ESPEN 1/2a	BMI < 18.5 kg/m²
ESPEN 1b	unexpected body weight loss (> 5% within the last 3 months or > 10% within an unspecified time frame)
	and
	BMI < 20 kg/m² for subjects < 70 yrs
	BMI < 22 kg/m² for subjects > 70 yrs
ESPEN 2b	unexpected body weight loss (> 5% within the last 3 months or > 10% within an unspecified time frame)
	and
	FFMI < 15 kg/m² for women FFMI <17 kg/m² for men

Results

The mean age of subjects was approximately 80 years, mean body weight was 69.3 ± 14.5 kg, body height 160.5 ± 7.3 cm, and BMI 26.8 ± 5.06 kg/m^2.

Table 2 presents laboratory test results, and tables 3 and 4 present results of the body composition analysis.

Of the 102 patients studied, 26 (25.5% of all participants) were classified as patients with normal nutritional status according to all accepted criteria. The remaining patients (n=76, 74.5%) were classified as patients with

Table 2 Laboratory test results of patients participating in the study (n=102)

	Mean ± SD	Median	Minimum	Maximum	Percentage of patients below the norm
Serum albumin levels (g/dL)	3.550 ± 0.427	3.600	2.500	4.600	34.3
Total lymphocyte count (TLC/ mm³ of blood)	1,890.8 ± 1,158.6	1,608.0	697.0	8,000.0	39.2

Table 3 Results of the anthropometric measurements and body composition analysis in women participating in the study (n= 87)

	Mean ± SD	Median	Minimun	Maximum	% of patients – below the norm	% of patients – above the norm
Body weight (kg)	67.45 ± 3.45	68.40	33.40	109.80	-	-
BMI* (kg/m2)	26.69 ± 5.02	26.22	15.25	41.33	29.8	32.2
Fat tissue (%)	32.92 ± 8.90	33.00	15.00	51.00	20.7	41.4
Fat-free mass index (FFMI) (kg/m^2)	3.07 ± 3.07	16.10	8.10	23.40	41.4	-
Body hydration (%)	48.50 ± 6.58	48.20	36.00	65.70	32.2	5.8

** reference values 24–29 kg/m² (11,13)*

Table 4 Results of the anthropometric measurements and body composition analysis in men participating in the study (n= 15)

	Mean ± SD	Median	Minimum	Maximum	% of patients – below the norm	% of patients – above the norm
Body weight (kg)	80.02 ± 6.02	81.30	60.60	96.80	-	-
BMI* (kg/m2)	27.53 ± 4.60	27.80	20.29	33.52	13.3	40.0
Fat tissue (%)	24.59 ± 6.42	23.50	15.70	37.10	0.0	46.7
Fat-free mass index (FFMI) (kg/m^2)	19.48 ± 2.36	55.30	15.11	22.42	20.0	-
Body hydration (%)	55.17 ± 5.53	20.21	44.90	65.70	20.0	6.7

** reference values 24–29 kg/m² (11,13)*

Table 5 Number of patients classified as subjects with an abnormal nutritional status depending on the criteria accepted.

	MNA-SF	ESPEN 1	ESPEN 2	Alb and/or TLC	ESPEN 1/2a	ESPEN 1b	ESPEN 2b
Number of patients (N)	67	17	34	54	5	16	34
Percentage of patients	66%	17%	33%	53%	5%	16%	33%

abnormal nutritional status based mainly on the MNA-SF malnutrition screening test (66%, including 40% of patients with risk of malnutrition and 26% of patients with malnutrition) and laboratory test results (Alb and/or TLC) (53%). However, the fewest patients were identified as malnourished, (5%), by a particular interpretation of ESPEN criteria which included only a body mass index of less than 18.5 kg/m^2 (ESPEN 1/2a) (Table 5).

Table 6 presents the results of a statistical analysis of correlations between the interpretations used to assess the nutritional status of the patients in the study. There was no statistically significant correlation between results of the BMI (ESPEN 1/2a) and the MNA-SF test, nor between BMI (ESPEN 1/2a) and laboratory tests (Alb and/or TLC). In the remaining cases, the studied correlations were statistically significant. The strongest correlation was observed between the ESPEN criteria: ESPEN 2 and ESPEN 2b (contingency coefficient ≈ 0.71), and ESPEN 1 and ESPEN 1b (contingency coefficient ≈ 0.69). Moreover, it was found that results from the MNA-SF test have the strongest predictive value for the results of ESPEN 2 and ESPEN 2b criteria (contingency coefficient ≈ 0.42). The weakest correlation was observed between laboratory test criteria (Alb and/or TLC) and ESPEN 1b (contingency coefficient ≈ 0.24), and between laboratory tests (Alb and/or TLC) and ESPEN 1 (contingency coefficient ≈ 0.25).

Of the 102 patients studied, 76 were classified as having an abnormal nutritional status according to any of the criteria. Taking this number of patients as 100%, 22.4% of the patients were classified with abnormal nutritional status by the MNA-SF test alone (according to which 17.1% patients were at risk of malnutrition, and 5.3% patients were classified as malnourished). A further 10.5% were classified according to laboratory test criteria alone (Alb and /or TLC) , and about 20% according to the MNA-SF test combined with laboratory tests criteria (Alb and/or TLC).

Table 6 Results of a statistical analysis of correlations between criteria used to assess the nutritional status of studied patients.

	Contingency coefficient	*P* value
MNA-SF vs ESPEN 1	0.3075624	< 0.01
MNA-SF vs ESPEN 2	0.4233308	< 0.0001
MNA-SF vs Alb and/or TLC	0.3667788	< 0.0001
MNA-SF vs ESPEN 1/2[a]	0.1619295	Ns
MNA-SF vs ESPEN 1b	0.2976232	< 0.01
MNA-SF vs ESPEN 2b	0.4233308	< 0.0001
ESPEN 1 vs ESPEN 2	0.4619431	< 0.0001
ESPEN 1 vs Alb and/or TLC	0.2548236	< 0.01
ESPEN 1 vs ESPEN 1/2a	0.4526787	< 0.0001
ESPEN 1 vs ESPEN 1b	0.6942101	< 0.0001
ESPEN 1 vs ESPEN 2b	0.4619431	< 0.0001
ESPEN 2 vs Alb and/or TLC	0.4166547	< 0.0001
ESPEN 2 vs ESPEN 1/2a	0.3057089	< 0.01
ESPEN 2 vs ESPEN 1b	0.4440715	< 0.0001
ESPEN 2 vs ESPEN 2b	0.7071068	< 0.0001
Alb and/or TLC vs ESPEN 1/2a	0.1221591	Ns
Alb and/or TLC vs ESPEN 1b	0.2376260	< 0.05
Alb and/or TLC vs ESPEN 2b	0.4166547	< 0.0001
ESPEN 1/2a vs ESPEN 1b	0.3725964	< 0.0001
ESPEN 1/2a vs ESPEN 2b	0.3057089	< 0.01
ESPEN 1b vs ESPEN 2b	0.4440715	< 0.0001

Ns – no statistical significance

Finally, 21% of the patients were classified as having an abnormal nutritional status based on all three criteria – MNA-SF, ESPEN 2 and Alb and/or TLC – and 15.8% of the patients were shown to have abnormal nutritional status by all of the criteria which were selected for this study (Figure 1).

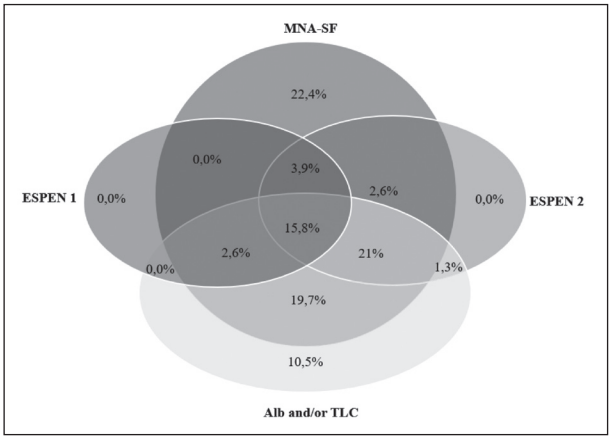

Figure 1 Percentages of patients classified as having an abnormal nutritional status according to the 4 main criteria.

Discussion

The average age of the patients studied was approximately 80 years, which is 'elderly' by the World Health Organisation (WHO) definition (14). Subjects of this age are often malnourished and it may be the result of chronic diseases common in this age group.

Moreover, malnutrition may be a result of insufficient energy supply and disorders associated with nutrient digestion and absorption (9,15). This is confirmed by the results of the authors' study - in the group of 102 patients studied, 76 or about 75% of the research participants, were classified as having an abnormal nutritional status. Similar results were obtained in a Portuguese study by *Antunes AC et al.* (16) using various methods to assess nutritional status: in a group of 201 hospitalised elderly patients, approximately 70% were shown to have an abnormal nutritional status. Meanwhile, studies in Ireland by O'Shea E. et al. (17) on a group of 606 patients aged over 70 years found that almost 60% had an abnormal nutritional status (45% were at risk of malnutrition and 18% were malnourished).

Analysing the results of the 7 interpretations studied (4 overall interpretations and 3 partial), it was concluded that the rate of studied patients classified as having an abnormal nutritional status (both at risk of malnutrition and malnourished) was the highest when the MNA-SF malnutrition screening tool (66%, n = 67) and the laboratory test results (Alb and/or TLC) (53%, n = 54) were used (Table 5). This may indicate that these methods are more sensitive for diagnosing abnormal nutritional status compared to the other interpretations analysed. The use of such criteria ensures a low risk of overlooking such disorders - a negative result is highly likely to indicate a normal nutritional status. However, it should be acknowledged that this would come at the cost of increasing the probability of obtaining a positive result in a subject with a normal nutritional status (a falsely positive result) (18). This may be confirmed by the results of the analysis per-

formed on the group of patients who were diagnosed with abnormal nutritional status based on any of the interpretations presented (n = 76). For 22.4% of the patients, this diagnosis was made based only on the results of the MNA-SF test – it was not confirmed by any other criteria. A similar situation may be observed for the interpretation of laboratory test results – 10.5% of the 76 patients analysed were classified as having an abnormal nutritional status based on this criterion, a classification not supported by the results of other criteria (Figure 1). However, in this case it may also show that biochemical indicators do not necessarily overlap with the other criteria used to diagnose malnutrition, and this might be due to other factors, mainly in connection with a disease.

The criterion which found the lowest percentage of patients with abnormal nutritional status (merely 5% of all respondents) was a BMI of less than 18.5 kg/m² (ESPEN 1/2a) (Table 5). This proves that this method is the least sensitive compared to the other criteria and has limited practical use for evaluating patients' nutritional status. This is also confirmed by the results which indicate no statistically significant correlation between the criteria described above (ESPEN 1/2a), the results of the MNA-SF test and laboratory test results (Alb and/or TLC). It should be also emphasised that all of the other correlations studied were statistically significant (Table 6).

Analysing only the diagnostic criteria suggested by the European Society for Clinical Nutrition and Metabolism (ESPEN) in 2015, it can be concluded that criteria based on the fat-free mass index (FFMI) (ESPEN 2 and ESPEN 2b interpretations) are more effective in detecting abnormal nutritional status than the results based on BMI (ESPEN 1, ESPEN 1b, ESPEN 1/2a interpretations). This is understandable considering the fact that the process of ageing is associated with a loss of muscle mass and an increase of fat tissue. Reduced muscle mass (sarcopenia) is often observed among elderly patients. *Roubenoff et al.* (19) noticed that these unfavourable changes in the body composition develop irrespective of changes in the body mass and, consequently, in the body mass index. Moreover, in elderly patients, sarcopenia very often coexists with obesity, leading to sarcopenic obesity. Whereas the incidence of sarcopenia is estimated at 13% of patients at the age of 60, this problem affects as many as 50% of patients at the age of 80 years old (20,21).

Three of the criteria for diagnosing abnormal nutritional status presented in this study, MNA-SF, Alb and/or TLC and ESPEN 2, are largely consistent (Figure 1). Moreover, correlations between these interpretations show a high contingency coefficient and this indicates a strong correlation (Table 8). Based on these results, it is concluded that such a set of criteria should be used widely to assess the nutritional status of patients over 65 years of age.

Conclusions

An abnormal nutritional status is common among hospitalised patients over the age of 65 years.

Of the nutritional status criteria analysed, the MNA-SF screening tool and laboratory test results seem to have the highest sensitivity.

For patients over 65 years of age, the results of the MNA-SF malnutrition screening tool have the strongest predictive value with regard to the results of a nutritional status assessment, including the reduced fat-free mass index (FFMI), combined with a confirmation of an unexpected loss of body weight (ESPEN 2 and ESPEN 2b).

The criterion based only on a BMI less than 18.5 kg/m^2 was the least useful for assessing nutritional status in patients in this age group, due to the unfavourable changes in body composition observed in elderly patients.

The set of criteria most effective for assessing nutritional status of patients over 65 years of includes the MNA-SF test, laboratory tests and a reduced FFMI, combined with confirmation of an unexpected loss of body weight.

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References

1. Correia M, Waitzberg D. The impact of malnutrition on morbidity, mortality, length of hospital stay and costs evaluated through a multivariate model analysis. Clin Nutr 2003; 22,3:235-239
2. Ostrowska J, Jeznach-Steinhagen A. Fight against malnutrition (FAM). Selected results of 2006 - 2012 Nutritionday survey in Poland. Rocz Panstw Zakl Hig 2016; 67(3): 291-300
3. International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10)-2015-WHO Version for 2015: http://apps.who.int./11.07.2017
4. Saunders J, Smith T, Stroud M. Malnutrition and undernutrition. Medicine 2011;391:45-50
5. Von Bokhorst-de van Schueren M, Soeters P, Allison S. Influence of malnutrition on function. Basics in Clinical Nutrition. Fourth Edition. Publishing House Galen 2011; 32-36
6. Kondrup J, Johansen N, Plum L et al. Incidence of nutritional risk and causes of inadequate nutrition care in hospitals. Clin Nutr 2002; 21: 461-468.
7. Rozporządzenie Ministra Zdrowia z dnia 15 września 2011 r. zmieniające rozporządzenie w sprawie świadczeń gwarantowanych z zakresu leczenia szpitalnego, Dz. U. 2011 nr 202 poz. 1191.
8. Jeznach-Steinhagen A, Ostrowska J, Czerwonogrodzka-Senczyna A. Evaluation of conducting a screening assessment of nutritional status of hospitalized patients. Presentation of main goals and objectives of the global health project "NutritionDay". Przegl Epidemiol 2016; 70(1):71-75
9. Nestle Nutrition Institute: Ocena stanu odżywienia Mini Nutritional Assessmen – MNA. Downloaded on 26.01.2018 r. (http://www.mna-elderly.com/forms/MNA_polish.pdf)
10. Wronka L, Sińska B, Wójcik Z. Przegląd metod oceny stanu odżywiania osób dorosłych. Żywienie Człowieka i Metabolizm 2010; 37:10-15
11. T. Cederholm, I Bosaeus, R Barazzoni, J et al. Diagnostic criteria for malnutrition – An ESPEN Consensus Statement. Clin Nutr 2015; 34(3), 335 – 340
12. Kaiser J, Bauer M, Ramsch C et al.: Validation of the Mini Nutritional Assessment short-form (MNA®-SF): A practical tool for identification of nutritional status. J Nutr Health Aging 2009; 13:782
13. Beck AM., Ovesen L.: At which body mass index and degree of weight loss should hospitalized elderly patients be considered at nutritional risk? Clin Nutr 1998;17:195-8
14. World Health Organization (WHO): Definition of an older or elderly person: http://www.who.int/healthinfo/survey/ageingdefnolder/en /08.07.2017.
15. Pertkiewicz M. Niedożywienie i jego następstwa. Postępy Żywienia Klinicznego, 2008; 2: 1–4
16. Antunes AC, Araújo DA, Veríssimo MT et al. Sarcopenia and hospitalisation costs in older adults: a cross-sectional study. Nutrition & Dietetics, 2017,74(1):46-50
17. O'Shea E Trawley S, Manning E et al. Malnutrition in Hospitalised Older Adults: A Multicentre Observational Study of Prevalence, Associations and Outcomes. J Nutr Health Aging 2017, 21(7):830-836
18. Altman D, Bland J. Diagnostic tests 2: Predictive values. BMJ 1994; 308: 1552
19. Roubenoff R. Sarcopenia: Effects on Body Composition and Function. The Journals of Gerontology Series A Biological Sciences and Medical Sciences, 2003, 58 (11): 1012-1017.
20. von Haehling S, Morley J, Anker S.: An overwiew of sarcopenia: facts and numbers on prevalence and clinical impact. J. Cachexia Sarcopenia Muscle 2010, 1(2), 129-133
21. Janssen I.: Evolution of sarcopenia research. Applied Physiology Nutrition and Metabolism, 2010, 35(5), 707-712

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Original article

Packaged ready-to-eat food consumption status of parents for their children and the factors that affect the consumption: Turkish case

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Summary. *Aim:* Consumption of packaged ready-to-eat food is increasing steadily worldwide. Indeed, relevant literature highlights that package food could affect children's growth and development negatively because of the additive ingredients. *Methods:* This descriptive research aims to investigate packaged ready-to-eat food consumption status of the parents who had under 18 years old children, and lived in a region where middle and low income families live, and the factors that affect ready-to-eat food consumption. Quantitative data were collected using a questionnaire that consisted of 31 questions. Number, percentage, mean, standard deviation, Chi square tests were used to present findings. *Results:* The findings showed that although most of the parents expressed that packaged foods were harmful to health, parents kept package foods in their houses and they had a store for the package foods that they called junk food drawer. Parents bought packaged foods for their children for various reasons because of children's desire, the effect of the trade of the product and the effect of the label of the food. Parents' age, gender, education and income status and also the number of children parents had affected buying packaged ready-to-eat food and gender, education and income, the number of children parents affect the reason why parents buy packaged ready-to-eat food and participants' age affected the features considered while buying the packaged ready-to-eat food affect the. *Conclusion:* Raising awareness among the parents regarding the negative effects of packaged ready-to-eat food is significant, and activities to raise awareness should be disseminated in all units of the society that are concerning children.

Key words: packaged ready food, consumption, parent, children, nursing

Introduction

Nutrition is very important in infancy and childhood periods when growth and development process is rapid (1). Rate of packaged ready-to-eat food consumption is increasing because of various reasons, including improvement in the economy, social and cultural changes, being easy for transportation and buying, colored package and good taste (2,3). It is notable that some packaged ready-to-eat foods are lack of protein, vitamin, mineral and fiber but also included some additive nutrients as high level refined sugar, white flour, trans fat, salt, food coloring and mono sodium glutamate which are lack of fiber and have high glycemic index (3,4). The relevant literature highlights that consumption of packaged ready-to-eat food affects mental health and could cause psychiatric stress and violence behavior (5).

Consumption of packaged ready-to-eat food is also common in our country, Turkey, like in various different countries all over the world (6).In Turkey, it is known that 64.0% of the children consumed fruit juices as additional food since the children are in their month after birth (7), 69,6% of school aged children

have junk food snacking habits and 7.5% of them were obese (7). Also, among the primary school children, 88% of the children consumed sugar-chocolate at least once or twice and more a week, 71.7% consumed crisps-potato, 40.4% consumed cola (8).

Given the alarming problem regarding the ready-to-eat food, there is an urgent need to raise awareness in the public worldwide. Transforming healthy nutrition to life style to reach expected quality of life during globalization process is significant (9). Thus, it is important to investigate parents' packaged ready-to-eat food consumption habits and disseminate information about the hazards of packaged ready-to-eat food for the healthy generations.

Methods

Aim and Design

This descriptive, cross-sectional study aimed to determine the ready-packaged food consumption of parents who had 18 aged and under children and the effective factors.

Sample and Setting

The data were collected using a questionnaire form in a middle and low-income level center of Ankara. The centers with income levels in Ankara city center were determined before the research started. The center district with low and middle income was selected randomly from between the 7 central districts determined. This center district was 524,222 total population (10). There was only a state hospital in the designated district. This hospital annual total outpatient population was 1.300.000 people approximately between January- December 2014.

Participant

The study were collected from 1,004 parents who had 18 aged and under children, accepted to participate. Firstly, adults over 18 years of age who were admitted to hospital for treatment were identified. Secondly, it was determined whether these adults had children under 18 years of age. Parents who were voluntary to participate in the research were identified. The study were finished with1,004 parents.

Data Collection

Data were collected between 01.09.2015 and 01.09.2016 using a questionnaire form. In the first part of the questionnaire, there were 31 questions, which aimed to obtain information regarding parents' socio-odemographic characteristics, children's size. In the second part of the questionnaire, parents were asked about the types of packaged ready-to-eat food consumed by their children, the frequency of consumption, reasons for buying ready-made food and the factors affecting them to buy. The underlying reason to use the questionnaire was the aim to reach as many parents as possible.

Analysis

For statistical analysis frequency, percentage, mean, standard deviation and chi-square tests were used. It is accepted meaningful when p value is under 0.05. Evaluation was done by IBM SPSS Statistics 21.0 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows Version21.0. Amornk, NY: IBM Corp.)

Ethical Approval

Prior to study, ethical permissions (Ankara Yildirim Beyazit University Ethical Committee, 28.08.2015/95) were taken. Participants signed a written consent form voluntarily after receiving sufficient explanation of the study purposes and procedure. The collected data were kept.

Results

Parents' median age was 33.91±7.78 years old, had 1,84±0.83 children and 2.4925±1.1100 in average. Most of the parents were women (69.7%), and they graduated from high school (46.9%), had got 2 children (41.5%), and a housewife (48.9%). Also, 67.1% of the children were students. Findings showed that 80.2% of the parents had a 'junk food drawer' in their houses, 99.3% of them bought packaged/junk foods for their children during shopping, 46.7% bought packaged/junk foods because their children wanted. Although most of the parents (77.3%) admitted that packaged/junk foods were harmful, 54.8% parents bought them because they were affected from the brand of the prod-

uct, %47.3 parents. In addition, 40.6% of the parents stated that their children consumed ready-packaged food every day, and 51.0% of them added that their children were affected from their friends the most to eat packaged/junk foods. 58.6% of the parents admitted that they allowed their children to eat packaged/junk foods after meals (Table 1).

Parents stated that their children consumed chocolate, ready fruit juice, crisp, biscuit, ready cake, wafer, fermented food as pepperoni/salami and packaged ice-cream twice a week, most frequently, opened ice-cream, packaged ice-cream and packaged sweets once a week and parents reported that their children did not consume fizzy beverages (Table 2).

A significant correlation was found out when parents' age, gender, education, having children and buying packaged/junk food, are compared with parents' gender, education, monthly income, number of children and reasons of buying packaged/junk food. Also, with parents' age, the features of packaged/junk foods that parents considered while buying ($p<0.05$, Table 3), there was a significant correlation.

Discussion

In Europe, as families' educational level increases, their high-energy food consumption increases. However, it is notable that families from all education level background consume high-energy food consumption, such as sugary-acid beverages, chips, sweet, and sugar, increases (11). Buying ready-packaged food was higher among women in this study. This finding is consistent with Yılmaz et al.,'s study which indicated that women are more effective in choosing junk food.[12] This study has shown that parents of every educational level and economic level tend to buy ready-packaged food. Younger age group and parents with high school graduation tend to take more of these kinds of foods. That parents who are young and female with two children affected ready-packaged food purchasing ($p<0.05$) (Table 3).

Most of the parents reported that packaged ready-to-eat food was harmful to health. However, most of the parents kept packaged ready-to-eat food in their

Table 1. Features belong to the status of reading ready packaged food

Features (N=1004)	n	%
Being a "Ready (Junk) Food Drawer"	806	80.2
Going with the child to the shopping	867	86.4
Buying ready packaged food for child during shopping	1003	99.3
Reason for buying ready packaged food		
Child's asking	468	46.7
Rewarding of child	222	22.1
Temporizing of child	234	23.3
Preventing child's emulating by seeing from other children	80	8.0
Thinking that it is harmful for health		
Yes	776	77.3
No	174	17.3
No idea	54	5.4
Features in chosing food*		
Trade of food	550	54.8
Cost of food	314	31.3
Child's desire	308	30.7
Being saturator of food	214	21.3
Advertisement of food	178	17.7
Taste of food	155	15.4
Package type	71	7.1
Previous experiences	67	6.7
Information that taken from family and friends	21	2.1
Frequency of consumption		
Every day	280	27.9
Once in a week	408	40.6
Twise and more in a week	247	24.6
None	69	6.9
Factors that were thought as effective on consumption*		
Friends	512	51.0
Television advertisement	475	47.3
Siblings	151	15.1
Parents (Mother/father)	94	9.4
Consumption times in day		
Before meals	117	11.7
After meals	588	58.6
Any time in day/when child wanted	266	26.5
None	33	3.3
Frequency of consumption		
Every day	280	27.9
Once in a week	408	40.6
Twise and more in a week	247	24.6
None	69	6.9

Table 2. Frequency of ready packaged food consumption according to types

FOOD TYPE (N=1004)	Frequency of Consumption							
	Once in a week		Twice or more in a week		Ever day		None	
	n	%	n	%	n	%	n	%
Chocolate	250	24.9	421	41.9	268	26.7	65	6.5
Fizzy drink	214	21.3	229	22.8	258	25.7	303	30.2
Ready fruit juice	276	27.5	367	36.6	201	20.0	160	15.9
Crips	285	28.3	320	31.9	194	19.3	205	20.4
Biscuit, ready cake, wafer	307	30,6	396	39.4	204	20.3	97	9.7
Fermented meat products (pepperoni/salami/sausage)	309	30.7	358	35.7	203	20.2	134	13.4
Open aci-cream	367	36.6	270	26.9	180	17.9	187	18.6
Packaged ice-cream	258	25.6	261	25.9	255	25.4	230	23.0
Packaged sweet	405	40.3	286	28.4	133	13.3	180	18.0

Table 3. Comparing sociodemographic features of parents and variables related to ready packaged food

Variables	Taking status (n=1004)				Reason of taking (n=1003)				Considered points during taking (n=986)			
	Yes		No		Child's desire		Initiative of parent		Belong to product		Belong to child/ parent	
	n	%	n	%	N	%	n	%	n	%	n	%
Age												
18-35 years	505	55.7	66	68.0	279	59.8	292	54.6	397	59.1	165	52.5
36-45 years	333	36.7	21	21.6	156	33.3	197	36.8	236	35.1	110	35.0
46-60 years	69	7.6	10	10.3	33	7.1	46	8.6	39	5.8	39	12.4
Analysis*	X^2: 8.808; **p: 0.012**				X^2: 2.734; p: 0.255				X'. 13.445; **p: 0.001**			
Gender												
Women	623	68.7	76	78.4	355	75.9	344	64.3	477	71.0	207	65.9
Man	284	31.3	21	21.6	113	24.1	191	35.7	195	29.0	107	34.1
Analysis*	X^2: 3.868; **p: 0.049**				X^2:15.781; **p: 0.000**				X^2:2.551; p: 0.126			
Education status												
Literate/primary	307	33.8	34	35.1	178	38.0	162	30.3	229	34.1	104	33.1
High school	436	48.1	33	34.0	200	42.7	269	50.3	309	46.0	154	49.0
University/ postgraduation	164	18.1	30	30.9	90	19.3	104	19.4	134	19.9	56	17.8
Analysis*	X^2:11.224**; p: 0.004**				X^2: 7.472; **p: 0.024**				X^2: 0.978; p: 0.613			
Income												
400-1500 Turkish Liras	167	18.4	20	20.6	85	18.2	102	19.1	120	17.9	65	20.7
1501-3000 Turkish Liras	594	66.3	65	67.0	292	62.4	366	68.4	443	65.9	201	65.3
3001-8000 Turkish Liras	146	16.1	12	12.4	91	19.4	67	12.5	109	16.2	48	15.3
Analysis*	X^2: 1.033; p: 0.597				X^2: 9.078; **p: 0.011**				X^2: 1.159; p: 0.560			
Number of children												
1 child	339	37.4	53	54.6	145	31.0	247	46.2	265	39.4	122	38.9
2 children	390	43.0	26	26.8	203	43.4	213	39.8	271	40.3	138	43.9
3 children	178	19.6	18	18.6	120	25.6	75	14.0	136	20.2	54	17.2
Analysis*	X^2:12.287; **p:0.002**				X^2:32.837; **p:0.000**				X^2:1.722; p:0.423			

*Pearson Chi Square test

house and they had a storage space called "Packaged ready-to-eat food drawer" in Turkey (Table 1). In other words, children had storage that children can reach the packaged ready-to-eat food whenever they wanted. Recently, Ministry of National Education in Turkey has introduced certain inhibitions on selling ready package food, such as fried, crisp, chocolate, wafer, sugar, cake and beverages with sweeting, at school canteens with an aim to keep the children away from these foods (13). However, not only the school environment but also the home environment seems to play an important role in the consumption of ready-to-eat foods. Even if the children can not reach the school, we think they can easily consume these ready-to-eat foods at their home. It is important to determine the prevalence of such practices by parents. Because determining the prevalence can assist to the identification of risks and the work done to gain the right consumption habits.

The main reasons why parents chose pre-packaged foods are the brand and price of the product and also the children's desire (Table 1). When the findings obtained in this study compared with Bal et al.,' findings (2006), their findings showed that 16.6% of the consumers preferred to buy the foods because they were influenced from the brand of the product (14) which is fewer than what is found in the represent research. The findings in the present study revealed that there was a significant difference among 18-35 age group of young parents who selected pre-packaged foods considering the characteristics of the product as shown in Table 3 ($p = 0.001$). Young parents were paying attention to the product feature in selecting ready-to-eat foods for their children. Consumption habits are shaped beginning from childhood stage. It is stated that shopping behaviors of parents could affect consumption behaviors of children (15).

In this study, the findings showed that the parents were shopping with their children and 90.3% of them bought ready-packaged food for their children while shopping (Table 1). Consumption habits are shaped from childhood. Parents' attitudes may have an effect on the children's consumption behavior towards the ready-packaged food. Some of the parents were aware of their own effect on their children, and they thought that their children were affected by themselves as mother and father while they were asking for these products (Table 1). Women mostly bought these foods because of their children's desire and parents who were graduated from high school and had 1501-3000 Turkish liras income and parents with only one child bought these foods because of their own initiative (Table 3). There was a significant difference among gender, education, income and number of children ($p<0.05$, Table 3) regarding reasons why parents bought ready-packaged food. We thought ready-packaged food intake rates together with the parents' children, were important.

In the present study, according to the parents, the two most important reasons why their children consumed ready-packaged food were their children's friends and TV adverts (Table 1). In the literature, there is a discussion that TV adverts promote the consumption of the packaged ready-to-eat food, and, with repeated messages, the adverts made the consumption of the packaged ready-to-eat food becomes as if they were normal to eat (16) although these foods increased the sugared food consumption among children (17). In Turkey, advertisements affected food shopping and mostly junk foods, such as chocolate, wafer, crisps, drinks and fizzy drinks were bought (9).

In Sweden, 24% of the pre-school children's daily energy is consumed from ready-to-eat foods, such as packaged ready-to-eat foods, sweet, cake, candy and crisps (18). However, in Turkey, children start to their additional nutrition with some foods as fizzy drinks in the 13th month, ready fruit juice in the 10th month and chocolate, crisps, wafer in the 11.7 month after birth (9). 42.3% of the 7-14 aged children consumed ready-packaged food at least once in a week (8). Although some parents stated that they did not allow their children to drink fizzy drinks, the findings showed that children consumed chocolate, fizzy drink, ready fruit juice, crisps, biscuit, cake and wafer at least once a week (Table 2). That sugared beverages and fruit juices have high fructose and starting to consume of these foods increases risk in term of health (19). Daily and weekly consumption of packaged ready-to-eat food is significantly associated with psychiatric stress in children (5). Parents are not aware of the potential risks, the frequency of ready-packaged food consumption was a considerable amount and even after meals, children consumed these types of foods mostly chocolate,

fizzy drinks, fruit juice, and packaged ice-cream after meals almost every day.

Packaged ready-to-eat foods which contain basic nutrients, such as fewer amounts of protein, vitamin, mineral, but more amounts of food additives, such as salt, sugar and fat (20) contain very different chemical components (21). It is known that oils as palm oil, salt, nitrite, nitrate, sodium and potassium salts that in the content of the packaged ready-to-eat foods could bring about cancer and cardiovascular diseases (22-26) and taking an excessive amount of monosodium glutamate could lead to a toxic and dependent effect (27). In the present study, the findings showed that 35.7% of the parents allow their children to consume processed foods, such as pepperoni, salami and sausage, twice and more in a week (Table 2). Although the hazards of the processed additives are known, it is notable that parents allowed their children to eat the processed foods.

As a result, the findings in the present study suggest that parents from all ages, education level and income status tend to consume ready foods for their children and most of the Turkish families also have ready foods. Parents think that their children's friends and television advertisements affect children's ready food consumption. The main aim of the child health nurse is to ensure that children and adolescents grow up in the community healthily in term of physical, emotional and social aspects (28). Given that nutrition has an important role in the children's growth and in childhood, inadequate nutrition in this period causes various health problems and may affect an individual's life negatively (1). Thus, child health nursing should inform the parents and children about possible dangers of prepackaged food and their consumption habits. In addition, activities should be organized to gain healthy eating habits. The reasons for the ready food consumption should be determined by more comprehensive research. While the trend of natural food consumption in the world is increasing, it should be investigated why ready food consumption in Turkey is high.

It is important to raise public awareness of the consequences of ready-foods consumption. For this purpose, regular multidisciplinary (health, education, media, etc) trainings and evaluation of the results of the trainings can be suggested.

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References

1. Erkan T, Yalvaç S, Erginöz E, Cokugrasli FC, Kutlu T. The evaluation of nutritional status of children, by anthropometric measurements, attending the day nursery of Cerrahpaşa Medical School *Turkish Archives of Pediatric*, 2007;42:142-147 http://dergipark.ulakbim.gov.tr/tpedar/article/view/5000002267
2. Yazicioglu I, Isin A, Koc B. The Reasons of University Students' Preference to Fast Food Products. *Journal of Tourism and Gastronomy Studies* 2013; 1(1): 36-41. http://www.jotags.org/Articles/2013_vol1_issue1/2013_vol1_issue1_article05.pdf
3. Ashakiran DR. Junk foods and their ımpact on health. *Journal of Krishna Institute of Medical Sciences University* 2012;1(2):7-15. http://www.jkimsu.com/jkimsu-vol1no2/jkimsu-vol1no2-RA-1-7-15.pdf
4. Jonhson S, Sahu R, Saxena P. Nutritional analysis of junk food. *Centre For Science And Environment* 2012; 41:3-24 http://www.cseindia.org/userfiles/Nutritional_Analysis_Junk_Food.pdf
5. Zahedi H, Kelishadi R, Heshmat R, Motlagh ME, Ranjbar SH, Ardalan G, Payab M, Chinian M, Asayesh H, Larijani B, Qorbani M. Association between junk food consumption and mental health in a national sample of Iranian children and adolescents: The CASPIAN-IV study. *Nutrition*,2014;30:1391–1397
6. School-Age Growth Monitoring of Children Project Research Report in Turkey, Ministry of Health, General Directorate of Primary Health Care Services, Hacettepe University Faculty of Health Sciences, Nutrition and Dietetics Department, Ministry of Education, Ministry of Health Publication No: 834, Ankara, 2011 http://beslenme.gov.tr/content/files/yayinlar/kitaplar/diger_kitaplar/tocbi_kitap.pdf
7. Savashan C, Oktay S, Aydogan U, Erdal M. Obesity frequency in school children and related risk factors. *Turkish Family Practice Journal* 2015;19 (1):2-9
8. Kutlu R, Civi S. The Assessment of Nutritional Habits and Body Mass Indexes of the Students Attending a Private Primary School. *Fırat Medical Journal*, 2009;14(1): 18-24. http://www.firattipdergisi.com/pdf/pdf_FTD_533.pdf
9. Turkey Nutrition and Health Research, 2010. Raport of Nutrition Status and Habits Evaluation .T.R. Ministry of Health, Hacettepe University,2010,1-636 http://www.sagem.gov.tr/TBSA_Beslenme_Yayini.pdf
10. Data of improvement levels belong to Ankara province center of Turkey Statistics Institute (TSI, 2016). (Data was sent in electronic environment by TSI)

11. Fernandez-Alvira JM, Mouratidou T, Bamman K, Hebestreit A, Barba G, Sieri S, Reisch L, Eiben G, Hadjigeorgiou C, Kovacs E, Huybrechts I, Moreno L. Parental education and frequency of food comsuption in European children: the IDEFICS study. *Public Health Nutrition*,2012;16(3):487-498. doi: 10.1017/S136898001200290X. Epub 2012 Jun 12
12. Yılmaz E, Oraman Y, .Inan IH. Determination of consumer behaviour dynamics relating to food products: "Trakya region example". *Journal of Tekirdag Agricultural Faculty* 2009;6(1):1-10.
13. Ministry of National Education. Occupational and Technical Education General Directorate. Cicular instruction in examination of food that will sell at school canteens and food units in terms of hygiene (2016). http://mevzuat.meb.gov.tr/html/kantingidahijyen/genelge.pdf date: 20.01.2017
14. Bal HSG, Göktolga ZG, Karkacıer O. The Examination of Consciousness Level of Consumers About Food Safety (A Case of Tokat Province). *Economy of Agriculture Journal* 2006;12(1):9 -18.
15. Gulerarslan A. Children as consumer and effects for family purchase decisions. *Journal of Selçuk Communication* 2011;6(4):126-137.
16. Dixon HG, Scully ML , Wakefield MA, White VM, Crawford DA. The effects of television advertisements for junk food attitudes and preferences. *Social Science& Medicine,* 2007;65:1311-1323.
17. Murray R, Battista M. Managing the risk of childhood overweight and obesity in primary care practice. *Curr Probl Pediatr Adolesc Health Care* 2009;39:146-65. DOI: 10.1016/j.cppeds.2009.03.002
18. Garemo M, Arvidsson-Lenner R, Strandvik B. Swedish pre-school children eat too much junk food and sucrose. Acta Pediatrica 2007;96: 266-272.
19. Isguzar Y, Akbulut G. High-fructose consumption and cancer**.** Şzmir Kâtip Çelebi University Journal of Health Sciences Faculty 2016;1(2):35-40.
20. Datar AB, Nicosia N. Junk food in schools and childhood obesity. *Journal of Policy Analysis and Management*, 2012;31(2): 312–337.
21. Maffini MV, Neltner TG, Vogel S. We are what we eat: Regulatory gaps in the United States that put our health at risk. *PLoS Biol* 2017; 15(12): e2003578. https://doi.org/10.1371/journal.pbio.2003578
22. Macit S, Sanlier NPalm oil and health. *Journal of Tourism and Gastronomy Studies*, 2014;2(1):13-20.
23. Yıldız E.*Cancer and nutrition*. Ankara: Klasmat Publication,2008. Available at: 20.02.2017 http://diyabet.gov.tr/content/files/yayinlar/kitaplar/hastaliklarda_beslenme/c5.pdf
24. Sezer C, Ogun M, Güven A.Determination of some chemical characteristics of salami and sausage. *Kafkas University Veterinary Faculty Journal*, 2013;19(1): 69-75.
25. Cakmak O, Isleyen A, Usca A. N-Nitroso Compounds and their Effects on Public Health.*TAF Preventive Medicine Bulletin*, 2009;8(6):521-528.
26. WHO. Guideline: Sodium intake for adults and children. Geneva, World Health Organization (WHO), 2012.
27. Rohmawati W, Istiananingsih Y, Nurdiana N, Barlianto W, Dwijayasa PM. Vitamin c-e and monosodium glutamateınduced ovarian toxicity. *Cukurova Medical Journal* 2014;39 (3):517-524.
28. Toruner EK, Buyukgonenc L. *Child health basic nursing approaches*,1th edition. Ankara, Göktug Publishing,2015:21-33

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Original article

Nutritional screening and the impact of malnutrition on poor postoperative outcomes in gynecological oncology patients

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Summary. *Background*: Sufficient nutrition effects the survival and life quality of gynecologic oncology patients. The prevalence of malnutrition among gynecological cancer patients at the time of their diagnosis is estimated to be 20%. The main aim of the study is to provide the care recommendations that can be applied to any gynecologic surgery clinic to reduce the incidence of malnutrition after surgery and to investigate the effects of malnutrition on the healing process of the patient. We aimed also to improve the nutritional status of inpatients and to increase the awareness of malnutrition in hospitals. *Methods:* Our study was a prospective study conducted with 403 patients, 334 of whom were oncologic, at the gynecology clinic of the University of Trakya between February 2017- January 2019. Nutritional characteristics were evaluated with NRS-2002 during the preoperative period. *Results:* The increase in the rate of complications was observed to increase with the risk of malnutrition. It was observed that oncology patients who were hospitalized and operated in gynecology services were at risk for malnutrition. Our study draws attention to the need for nutritional support and follow-up for those at risk of malnutrition. *Discussion:* To identify patients at risk for malnutrition and to intervene in their nutrition program can help to make significant progress in the patient's healing process. In our study, we observed that the increase in complication rate led to an increase in the tendency of malnutrition. The rate of gynecologic oncology patients who were at nutritional risk was not to be underestimated. Nutritional support plans of patients with preoperative malnutrition were required to reduce postoperative morbidity and improve long-term patient outcomes. It is therefore important that in gynecological cancer patients the nutritional risk is determined during their hospitalization and so that, trough treatment, malnutrition can be prevented.

Key words: nutrition, malnutrition, nutritional support, gynecological oncology, patient outcomes

Introduction

Inadequate nutrition is commonly associated with chronical conditions. These chronical conditions create an excessively demanding metabolic environment in which the organism's ability to retain its protein load becomes endangered. If this increase in demand is not supported through a rigorous diet or therapeutical sources, it will lead to gastrointestinal malabsorption, impaired immunological response, impaired plasma protein synthesis in the liver and ultimately consumption of the visceral protein load. The nutritional status of gynecological cancer patients should be assessed through a range of anthropometric measurements (weight loss, body mass index (BMI), immunological measurements (such as total lymphocyte count etc.)). The prevalence of malnutrition among gynecological cancer patients at the time of their diagnosis is estimated to be 20%. Sufficient nutrition effects the survival and life quality of gynecologic oncology patients (1, 2).

Malnutrition and patients care are topics that should be addressed at women's health services. Once the patients care has been assessed through a multidisciplinary approach it should be balanced at the

gynecological clinic. Four topics stand out regarding this matter; preparation for gynecological surgery, acknowledgement and prevention of nutritional risks, supplementations that will be needed to improve health condition after surgery and lastly prevention of possible complications by adequate nutritional management. Despite differences in quality of patient caretaking among different clinics, a fair level of standardization should me promoted.

Nutritional screening tests hold an important place in the evaluation of patients with malnutrition and malnutrition risk. Among these tests, NRS-2002 (NUTRITIONAL RISK SCREENING -2002) is a test that is widely used worldwide and is recommended by ESPEN (European Society for Clinical Nutrition and Metabolism) (3).

Many studies have shown that patients at risk for nutritional support benefitted greatly from nutritional supplements and the usage of NRS-2002 in the clinics has helped identify the patients who are more in need of nutritional support from those who are less. This valuable test is based on a large number of scientific studies, emphasizing its value and strength (3, 4).

The relationship between malnutrition and poor postoperative outcomes in surgical patients is well established in areas such as gastrointestinal, cardiovascular, orthopedic, neurosurgery and cardiovascular surgery. However, the literature specific to the gynecologic patient is limited. It is important to evaluate preoperative nutritional status and provide nutritional support or alternative treatment options when necessary.

In reproductive-age-women, hysterectomy is the second most common gynecological surgical procedure. Studies indicate that one in nine women will undergo hysterectomy sometime during their lifetime (5, 6). Because gynecological oncology operations are performed more in tertiary health centers, some studies show that the risk of malnutrition is higher therein (7, 8).

In the period leading to surgery, all health care nurses evaluated the nutritional risks in order to avoid malnutrition after surgery. The effect these risks had on complications and on the hospital stay was evaluated then in the post-surgical period.

The aim of our study is to determine the nutritional risk at the time of hospitalization, the nutritional support rate of patients at risk and the nutritional risk one week after hospitalization of gynecology patients being admitted at tertiary health centers. We aimed also to provide the nursing care recommendations that can be applied to any gynecologic surgery clinic to reduce the incidence of malnutrition after surgery and to investigate the effects of malnutrition on the healing process of the patient.

Methods

In our study, the data of 403 patients waiting to undergo surgery in Trakya University Medical Faculty Hospital Gynecology Clinic between February 2017 and January 2019 were investigated prospectively. The patients awaiting surgery for gynecological indications were evaluated during their first 48 hours of hospital stay. An informed consent was obtained from all volunteer patients. The patients' sociodemographic characteristics and risks of malnutrition were examined. Nutritional characteristics were evaluated with NRS-2002 during the preoperative period.

Pathological specimen of 334 of all patients showed malignant characteristics. Patients with kidney disease, heart failure, generalized edema, gastrointestinal system diseases were excluded from the study.

NRS 2002 is an accepted scale to determine the risk of malnutrition by ESPEN as well as by the Chinese Society of Parenteral and Enteral Nutrition (CSPEN). This is a screening test that assesses the adequacy of nutritional support, especially in patients hospitalized for any disease and it was developed not only for the elderly but for all hospitalized persons (young and old). It contains nutritional information and reflects the severity of the disease and increased nutritional requirements. It focuses on people who are in acute care and need nutritional support and is a screening test developed by randomized controlled trials (8). The necessity of nutritional support is based on the severity of the disease and the risk of malnutrition. It's a system that scores according to BMI, weight loss, nutrient intake and age (4). The fact that this system is based on many scientific data makes it even more valid. It is overall a screening test that assesses the adequacy of nutritional support, especially in cases of acute illness.

It is not always possible to obtain clear information about weight loss, BMI and recent nutrition over the past 3 months. In case of uncertainty, it is recommended and encouraged to accept the patient as being at risk. For patients who had a score of ≤3, but who were expected to be ≥3 in the near future (individuals undergoing major gyneco-oncologic surgery), a nutritional plan was made available to the attending physician and nurse and the patient was then referred to the dietitian. As recommended for other patients, the NRS-2002 was repeated one week later; at the hospital if the patient was still there and if not by telephone.

Ethical approval was obtained from Trakya University Medical Faculty Scientific Research Ethics Committee for our study (Decision number: 2017/49/21). Permission was also obtained from the board of the Faculty of Medicine Hospital.

SPSS 21.0 Package Program was used in all statistical analyzes. The normal distribution was evaluated by Shapiro-Wilk test. If two groups were compared, Mann-Whitney U test was used. One-way analysis of variance (Anova) was used when comparing three groups. Descriptive statistics for numerical variables were analyzed as mean, standard deviation or median and quarterly distribution ratio. Descriptive statistics for categorical variables were given as percentage and frequency. The significance level was determined as 5% in all statistical analyzes.

Results

A total of 403 planed and performed gynecologic operations of patients between 18 – 87 years-of-age were included in this study. The performed gynecological operation was suited to the matching indications. Patients were evaluated 24-36 hours before the operation. After the pathology results are examined 334 patients were found to malignant and 69 patients were found to have benign conditions. The mean age of patients with malignancy was 55.3; the mean age of the patients with benign pathology was 35.8.

Among the assessed benign etiologies, 22 patients had myoma uteri, 5 patients had pelvic inflammatory diseases, 17 patients had uro-gynecologic diseases, 14 patients had endometriosis and benign ovarian cysts and 11 patients had other benign gynecological pathologies. The patients with borderline and malignant pathology were included in the gynecological cancer group. 129 patients were operated for endometrial pa-

Table 1. NRS 2002 form used for the evaluation of patients

BASIC EVALUATION			
Impaired nutritional status	**Point**	**Severity of disease**	
Normal nutritional status	0 (None)	Normal nutritional requirements	0 (None)
Weight loss <5% in 3 months or food intake below 50–75% of normal requirement in preceding week	1 (Mild)	Hip fracture, chronic patients, in particular with acute complications: cirrhosis, COPD. Chronic hemodialysis, diabetes, oncology	1 (Mild)
Weight loss >5% in 2 months or BMI 18.5 – 20.5 + impaired general condition or food intake 25–60% of normal requirement in preceding week	2 (Moderate)	+ Major abdominal surgery, stroke, severe pneumonia, hematologic malignancy	2 (Moderate)
Weight loss >5% in 1 month (>15% in 3 months) or BMI <18.5 + impaired general condition or food intake 0-25% of normal requirement in preceding week	3 (Severe)	Head injury, bone marrow transplantation, Intensive care patients (APACHE >10).	3 (Severe)
TOTAL SCORE : A	+1 If age ≥70	**TOTAL SCORE : B**	
TOTAL NRS 2002: A + B			

*Original study1; NRS 2002 (Kondrup et al).

thologies and mesenchymal malignancies of the uterus, 108 for adnexal malignancies, 46 for cervical malignancies, and 51 for other malignancies (gestational trophoblastic diseases, etc.). After the gynecological operations, the results of the pathological results and the preoperative NRS 2002 results were examined.

403 patients, 334 of whom were oncologic, were included in the study. By using the NRS 2002 we prospectively evaluated the risk of malnutrition in patients in this tertiary gynecologic health center.

In our study we evaluated whether the BMI was below 20.5, whether there had been weight loss in the last 3 months, whether there was a decrease in dietary intake in the last week and whether there was an acute or chronic disease. If the answer to any of these questions was yes, then we switched to the scoring system. Scoring consisted of two parts: 'nutritional status' and 'disease severity' and was calculated by 4 separate scoring evaluations, 'no problem', 'mild', 'moderate' and 'severe'. Scoring 0-3 was used for each section. While assessing the nutrition section of the scoring, BMI, the percentage of recent weight loss and recent food intake was evaluated. Patients over seventy years of age also received 1 more point due to age. It was concluded that patients who a total score ≥ 3 were under nutritional risk.

Score 1 included patients with chronic diseases and hospitalized patients due to complications. These were fallen patients, but were able to get out of bed regularly. Protein requirements were increased, but were overcome by oral diet and support. Score 2 included patients who had become bedridden due to infection or a major abdominal surgery. Protein requirements were significantly increased and in some cases artificial feeding was required. In NRS 2002, score 3 consists of patients in need of intensive care with inotropic or ventilatory support. In most cases there is a markedly increased protein degradation and nitrogen loss, even with nutritional support. However, we did not have patients in this group. Only hospitalization was not counted by itself. That is, although a patient had been admitted, the severity of the disease could be scored as 0 (no risk).

In our study, the ratio of those who had no risk in the evaluation with NRS performed before the operation was found to be 31.01%. The ratio who had an intermediate risk from the NRS score were 39.7%, the ratio of those who were found to have three points therefore being at risk of severe malnutrition was 29.28%. Evaluation of postoperative complications with NRS was evaluated. The increase in the rate of complications was observed to increase with the increase in the risk of malnutrition (Table 2). A statistically significant correlation was found.

The duration of hospitalization and the differences in NRS results were examined in patients undergoing gynecologic surgery. Patients with an NRS score of 3 or more were found to have a longer hospitalization period than those with an NRS score of 0 (Table 3). The difference between them was statistically significant (p = 0.01).

In our study patients with gynecological malignancy the rate of NRS score 0 was 28.44%, while the rate of NRS score 1-2 was 39.22%, and those with NRS score ≥3 was 32.33%. It was observed that oncology patients who were hospitalized and operated in gynecology services were at risk for malnutrition. Other studies showed that nutritional risk groups in gynecologic cancers were evaluated with nutritional risk scales such as NRS 2002 and PG-SGA (Patient

Table 2. The relationship between the characteristics of malnutrition risks evaluated with NRS 2002 and complications in the gynecologic patients of our study and the comparison with the original study

NRS 2002 Score	Our study			*p*	Original study2*		
	All patients	Complications			All patients	Complications	
		None	Present			None	Present
Overall score	403 (100%)	365 (90.57%)	38 (9.42%)	<0.05	336 (100%)	299 (89.0%)	37 (11%)
NRS 0 score	125 (31.01%)	121 (96.80%)	4 (3.20%)	<0.001	78 (23.3%)	76 (% 97.4)	2 (2.6%)
NRS score 1–2	160 (39.70%)	150 (93.75%)	10 (6.25%)	<0.001	157 (46.7%)	145 (% 92.4)	12 (7.6%)
NRS score ≥3	118 (29.28%)	94 (79.66%)	24 (20.33%)	<0.001	101 (30.1%)	78 (77.2%)	23 (22.8%)

*Original study2: Harte et al.

Table 3. Evaluation of the relationship between mean hospital stay and NRS 2002 scores in our study and comparison with the original study

Mean hospital stay	Our study				Original study2*			
	All patients	Complications		*p*	All patients	Complications		*p*
		None	Present			None	Present	
Overall score	7.28	7.15	16.49	<0.001	7	6	16	*<0.001*
NRS 0 score	5.46	4.26	10.25	<0.001	4	3	4	*<0.001*
NRS score 1–2	9.54	8.09	13.90	<0.001	6	4	14	*<0.001*
NRS score ≥3	15.41	8.68	16.61	<0.001	10	8	19	*<0.001*

*Original study2: Harte et al.

Generated Subjective Global Assessment). Briefly, PG-SGA classifies patients as follows: Class A - well fed, Class B - moderately malnourished, Class C - seriously malnourished. With the risk scales of 1 to 3 according to the degree of risk, the risky group was found to reach up to 40% in gynecologic oncology cases (9-11). (Table 4). The association of leukocyte, hematocrit and platelet changes with NRS was not found to be significant.

In our study, the relationship between hemoglobin, hematocrit, leukocyte and platelet values was evaluated with NRS 2002 results. However, no significant correlation was found between the parameters investigated and the risk of malnutrition determined by NRS.

The use of NRS 2002 helped us identify patients who could benefit from nutritional support. In addition to facilitating the identification of patients with a risk of malnutrition, our study draws attention to the need for nutritional support and follow-up for those at risk of malnutrition.

Discussion

To identify patients at risk for malnutrition and to intervene in their nutrition program can help to make significant progress in the patient's healing process.

Some studies showed that up to 50% of hospitalized patients had a prevalence of malnutrition. In a multicenter study by using NRS 2002, 189 (15%) out of 1255 hospitalized patients in Western Europe were shown to be under risk of malnutrition. This multicenter study showed that patients with an NRS score of 3 or more had more complications, greater mortality, and longer hospitalization than patients with an NRS score <3. In our study, 29.28% of the patients had malnutrition prevalence. Studies have shown that there is a close relationship between malnutrition, increased complication rates, mortality, hospital stay and costs. Studies showing poor nutritional score in 24% of gynecological patients are similar to our study (12-14). In our study, according to the NRS score, 29.8% of the

Table 4. Comparison of other studies evaluating the nutritional risk prevalence in various gynecologic cancers with our study

Parameters	Hertlein et al. study	Rodrigues et al. study	Das et al. study	Hertlein et al. study
Diet scale used	NRS 2002	NRS 2002	PSG-SGA	PSG-SGA
Number of participants	334	272	146	60
Age range	18-87	28-97	-	13-74
Risk of malnutrition	Puan 0 = 28.44% Puan 1–2 = 39.22% Puan ≥3 = 32.33%	Puan 0 = % 27 Puan 1–2 = % 31 Puan ≥3 = % 42	Sınıf A = % 38 Sınıf B = % 47 Sınıf C = % 23	Sınıf A = % 12 Sınıf B = % 48 Sınıf C = % 40
Body mass index assessment	(+)	(+)	(+)	(+)
Evaluation of leukocyte and haematocrit	(+)	(-)	(-)	(-)

Abbreviations: PG-SGA: Patient-Generated Subjective Global Assessment
PG-SGA classifies patients in: Class A - well fed, Class B - moderately malnourished, Class C - seriously malnourished.
NRS-2002: Nutritional Risk Screening-2002

scanned surgical cohort was at risk of severe malnutrition (NRS> 3). Our results compared with the results of studies on nutritional risk assessment in gynecologic patients showed that prevention of malnutrition is an important issue in women undergoing gynecological surgery. Necessary attention should be given to nutritional assessment and the time required for patient care should be reserved.

The overall complication rate in patients who underwent surgery was 9.42%. In other studies it was found to be 17.6% and 11% (15). In our study, the risk of complication development in patients with an NRS score of 3 or higher was found to be 20.33%. This result was close to other studies which were determined as 22.8% and 30.6% (15, 16).

Unfortunately, there is no gold standard accepted worldwide for assessing the risk of malnutrition. Generally, it is determined that evaluation by a healthcare worker, anthropometric measurements and the NRS 2002 scale are valid reference methods (17). Although there is no gold standard for effectively defining nutritional status, well-fed patients have better results during the post operational period than patients with undernourished conditions and also recovered faster. Patients who are undernourished have a higher rate of complications, increased mortality, prolonged hospital stay and a higher total cost. Studies have shown that the NRS 2002 scale can be used to evaluate malnutrition in gynecologic patients. Studies have shown that nutritional problems occur often, and patients with 3 or higher NRS scores in the surgical cohort have a significant higher risk of malnutrition (30.1%). These data indicate that health care providers and nurses should take extra care of the patient's nutritional treatment before and after surgery (17, 18).

It is important that the members of the surgical team are aware of the risks in the nutritional area. Prevention of malnutrition after surgery requires responsibility for each member of the perioperative team. The individual roles of surgeons, nurses and the entire healthcare team should be defined. The nursing team plays an important role in ensuring the availability of medications arranged in treatment, organizing pre- and post-operative care, and ensuring that nutritional support to be followed during the patient's recovery process is monitored. Regulation of nutritional risk management and timing reduces surgical complications. In our study, we observed that the increase in complication rate led to an increase in the tendency of malnutrition. The results were similar to those of Hertlein et al. (11).

When the patients were malnourished (NRS score 3), the mean time was 10 days. In patients who developed complications, it became 16 days. The mean hospital stay was 19 days when the patients were malnourished and complications occurred (11). In our study, we saw that the average duration of hospital stay increased from 5.46 days to 15.41 as the risk of malnutrition increased. We observed that this period increased to 16.61 days in patients with complications and inadequate nutritional status. In another study, the mean duration of hospital stay was 7 days. Our study and the literature show that malnourished patients have a longer hospital stay than those with good nutritional status.

Studies have shown that in Australia and the United States, 20 to 50% of gynecological cancer patients are at least exposed to mild malnutrition (1, 19). In a study conducted in Germany only 22% of patients had normal nutritional status in gynecology. Studies in India and Brazil reported that 62% to 86% of gynecologic cancer patients presented with inadequate nutrition status. It is reported that malnutrition accounts for 20% of all cancer-related deaths (9, 10, 18). In our study, the rate of gynecologic oncology patients who were at nutritional risk was not to be underestimated. The percentage of patients with NRS score ≥3 was 32.33%. The high incidence of malnutrition in gynecological cancer patients is of concern. In gynecologic oncology patients, it was also seen that these results were consistent with studies reporting these estimates based on other malnutrition screening or assessment tools (Table 4).

Malnutrition in cancer patients is usually due to the inability to intake or absorbs enough nutrients. Surgery requires a period fasting of the patient and may lead to postoperative protein catabolism depending on the length of hunger. Loss of appetite further reduces dietary intake after surgery. In cases where the functional slowdown of the intestines is common, the deficiency of nutrients becomes a problem. Difficulties in gastric emptying increased bacterial growth and

gas may also affect food intake. Metabolic demand is another parameter that is known to increase as cancers grows and results in overuse of conserved proteins (17, 20). The combination of reduced nutritional intake, reduced nutritional absorption and increased metabolic demand may result in a negative nutritional balance and a reduced nutritional status.

Contemporary studies in Australia and the United States have shown that 20 to 53% of gynecologic cancer patients develop with at least mild malnutrition at the time of diagnosis. Malnutrition prevalence was reported to be higher in developing countries (62-88%) (9, 10). The British Association of Parenteral and Enteral Nutrition (BAPEN) listed a number of social and physical factors that increase the risk of malnutrition, including social isolation, poverty and cultural norms. Studies have investigated the nutritional parameters associated with postoperative complications in various types and stages of gynecologic cancers. In studies, hospitalization periods were used as an indirect indicator of hospital complication rates. Santoso et al. found that malnutrition was significantly associated with longer hospital stay, regardless of age, extent of disease, or primary tumor site (21).

For nurses, it is important to standardize preoperative nutritional support instructions and patient training materials. Preoperative feeding and care instructions should be provided to women who will undergo major gynecological surgery. Providing patient training materials and providing nutritional support shortens the length of hospital stay, makes patients' requests for pain treatment reasonable, and improves healing by providing more patient and family satisfaction. Patient anxiety and fear prevent the learning of nutritional information and useful recommendations (22). For this reason, when nutritional information is started early, during the treatment and care process, it allows effective relief for the patients' and families' concerns. It is mandatory to strengthen the patient's best learning forum, even though it is early. The patient education process should be strengthened by using various methods such as oral, written instruction pages, simulated representations.

Several studies have shown that low prealbumin or albumin levels are associated with a higher prevalence of postoperative complications in gynecological cancers (9, 16). As one of the limitations of our study, these parameters could not be evaluated from all patients that were admitted to the gynecology service. Additional parameters for malnutrition, such as albumin or bioimpedance measurements, may enhance the assessment of undernourished gynecological patients. Our study has shown that in gynecological patients and especially those with a risk of malignancy, awareness about the problem of malnutrition should be increased. Thus, the benefit provided to the patient during the care taking process increases and so does the healing quality. Healthcare providers should be able to recognize and in a short period of time provide nutritional support to patients assessed to be at nutritional risk, through easily applied and validated measurements.

Conclusions

In view of the results of our study, nutritional support plans of patients with preoperative malnutrition were required to reduce postoperative morbidity and improve long-term patient outcomes. However, the number of studies evaluating the value of preoperative nutritional support in surgical gynecologic cancer patients is limited in the literature. Therefore it is important that in gynecological and especially in gynecological cancer patients the nutritional risk is determined during their hospitalization and follow-up periods so that, through treatment, malnutrition can be prevented.

References

1. Laky B, Janda M, Bauer J, Vavra C, Cleghorn G, Obermair A. Malnutrition among gynaecological cancer patients. European journal of clinical nutrition. 2007;61(5):642.
2. Berek JS, Hacker NF, Hengst TC. Practical gynecologic oncology: Lippincott Williams & Wilkins; 2005.
3. Kondrup J, Rasmussen HH, Hamberg O, STANGA Z, Group AahEW. Nutritional risk screening (NRS 2002): a new method based on an analysis of controlled clinical trials. Clinical nutrition. 2003;22(3):321-36.
4. Kondrup J, Allison SP, Elia M, Vellas B, Plauth M. ESPEN guidelines for nutrition screening 2002. Clinical nutrition. 2003;22(4):415-21.

5. Wright JD, Herzog TJ, Tsui J, Ananth CV, Lewin SN, Lu Y-S, et al. Nationwide trends in the performance of inpatient hysterectomy in the United States. Obstetrics and gynecology. 2013;122(2 0 1):233.
6. Wu JM, Wechter ME, Geller EJ, Nguyen TV, Visco AG. Hysterectomy rates in the United States, 2003. Obstetrics & Gynecology. 2007;110(5):1091-5.
7. Burgos R, Sarto B, Elío I, Planas M, Forga M, Cantón A, et al. Prevalence of malnutrition and its etiological factors in hospitals. Nutricion hospitalaria. 2012;27(2).
8. Wang N, Dong Y, Huo T, Shao Y, Xing W, Li S. Nutritional risk, malnutrition and nutritional support among hospitalized patients in orthopedics/spinal surgery of a Hohhot teaching hospital. Asia Pacific journal of clinical nutrition. 2015.
9. Ushashree Das SP, Dave K, Bhansali R. Assessment of nutritional status of gynecological cancer cases in India and comparison of subjective and objective nutrition assessment parameters. South Asian journal of cancer. 2014;3(1):38.
10. Rodrigues CS, Lacerda MS, Chaves GV. Patient Generated Subjective Global Assessment as a prognosis tool in women with gynecologic cancer. Nutrition. 2015;31(11-12):1372-8.
11. Hertlein L, Kirschenhofer A, Fürst S, Beer D, Göß C, Lenhard M, et al. Malnutrition and clinical outcome in gynecologic patients. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2014;174:137-40.
12. Correia MIT, Campos ACL, Study EC. Prevalence of hospital malnutrition in Latin America:: The multicenter ELAN study. Nutrition. 2003;19(10):823-5.
13. Kuppinger D, Hartl WH, Bertok M, Hoffmann J, Cederbaum J, Küchenhoff H, et al. Nutritional screening for risk prediction in patients scheduled for abdominal operations. British Journal of Surgery. 2012;99(5):728-37.
14. Sorensen J, Kondrup J, Prokopowicz J, Schiesser M, Krähenbühl L, Meier R, et al. EuroOOPS: an international, multicentre study to implement nutritional risk screening and evaluate clinical outcome. Clinical Nutrition. 2008;27(3):340-9.
15. Obermair A, Simunovic M, Isenring L, Janda M. Nutrition interventions in patients with gynecological cancers requiring surgery. Gynecologic oncology. 2017;145(1):192-9.
16. Guaitoli PR, Jansma EP, de Vet HC. Nutrition screening tools: does one size fit all? A systematic review of screening tools for the hospital setting. Clinical Nutrition. 2014;33(1):39-58.
17. Kathiresan AS, Brookfield KF, Schuman SI, Lucci JA. Malnutrition as a predictor of poor postoperative outcomes in gynecologic cancer patients. Archives of gynecology and obstetrics. 2011;284(2):445-51.
18. Hertlein L, Zeder-Göß C, Fürst S, Bayer D, Trillsch F, Czogalla B, et al. Peri-operative oral immunonutrition in malnourished ovarian cancer patients assessed by the nutritional risk screening. Archives of gynecology and obstetrics. 2018;297(6):1533-8.
19. Gupta D, Lis CG, Vashi PG, Lammersfeld CA. Impact of improved nutritional status on survival in ovarian cancer. Supportive care in cancer. 2010;18(3):373-81.
20. Cantrell LA, Saks E, Grajales V, Duska L. Nutrition in Gynecologic Cancer. Current Obstetrics and Gynecology Reports. 2015;4(4):265-71.
21. Santoso JT, Canada T, Latson B, Aaaadi K, Lucci III JA, Coleman RL. Prognostic nutritional index in relation to hospital stay in women with gynecologic cancer. Obstetrics & Gynecology. 2000;95(6):844-6.
22. Brega AG, Freedman MA, LeBlanc WG, Barnard J, Mabachi NM, Cifuentes M, et al. Using the health literacy universal precautions toolkit to improve the quality of patient materials. Journal of health communication. 2015;20(sup2):69-76.

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Original article

The relationship between glomerular filtration rate, nutrition and activities of daily living in patients with chronic kidney disease receiving homecare

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Summary. *Introduction:* The aim of this research was to evaluate the relationship among glomerular filtration rate, nutrition and activities of daily living performance in Chronic Kidney Disease patients receiving homecare services. *Materials and Method:* We conducted a retrospective examination of the Sisli Hamidiye Etfal Training and Research Hospital's Homecare records for 2016. 345 patients were included. Glomerular filtration ratewas calculated using a simplified version of the Modification of Diet in Renal Disease. Mini-Nutritional Assessmen scores were used for nutrition. Barthel Index scores were used to identify activities of daily living. P values of ≤0.05 were considered statistically significant. *Results:* 225 women (65.2%) and 120 men (34.8%), were included. Mean value ofGlomerular filtration rate was 60.83 ± 17.10 ml/min/1.73 m2. Mini-Nutritional Assessmen test mean was 19.66 ± 4.97. Barthel Index of the study group was 30.39 ± 28.99. A statistically significant correlation was found between glomerular filtration rateand the Barthel Index(p = 0.022). When glomerular filtration ratedecreased, Barthel Index scores decreased. As Mini-Nutritional Assessmen scores decreased, glomerular filtration rate values also decreased (p = 0.029). Barthel Index and Mini-Nutritional Assessmen were also related (p = <0.001). *Conclusion:* In primary care, elderly individuals (especially those receiving homecare services) should undergo assessment of activities of daily living and nutritional status. Patients with Chronic Kidney Disease were at risk for malnutrition and dependence on activities of daily living.

Keywords: renal insufficiency, aged, home care services, malnutrition, Quality of Life, chronic disease

Introduction

Chronic kidney disease (CKD) is defined as kidney damage and/or decreased kidney function, as expressed by a minimum 3-month history – with or without a fall in glomerular filtration rate (GFR), regardless of cause (1). It frequently manifests as a complication of chronic diseases and considerably influences morbidity and mortality. In a study in Turkey, CKD was seen in 15.7% of patients who appliedfor any complaint (2). Evaluation of three years of Canadian homecare data revealed that the most frequent chronic illness was chronic renal disorder (3). Also many complications associate with CKD like anemia, hyperlipidemia, nutrition problems, osteodystrophy and cardiovascular risks.

Nutrition is a critical issue in CKD patients. In a study, the incidence and severity of malnutrition increased in relation to the degree of renal function loss and were predictive of one-year mortality (4). Many mechanisms were suspected to be the cause of malnutrition. In a study indicated that low acyl-ghrelin

levels, accompanied with high levels of TNF-α and IL-6, may be implicated in loss of appetite and poor nutritional status in CKD patients (5). In another study, as patients progress through the stages of CKD, nutritional requirements are altered and metabolism of protein, water, salt, potassium, and phosphorous are affected. These changes lead to ineffective energy generation despite adequate intake of protein, carbohydrate substrates; all were cause malnutrition(6).So identifying inadequate nutritional status in CKD patients is very important.

Malnutrition not only affected mortality but also showed that the ability to perform basic activities of daily living (ADLs) decreased(7). CKD can affect the ability to perform ADLs because of complications of the disease, such as anemia and hypoproteinemia.So determining of ADLs and nutrision status will be usefull for CKD patients to effect mortality and morbidity.

Therefore we can say that CKD patients have a risk for malnutrision and bad ADLs .CKD is more frequent in homecare patients because of the age group was old and had complicated more than one illnesses.

The specific aim of this research is to evaluate the relationship among GFR, nutrition and ADL performance in CKD patients receiving homecare services.

Materials and Methods

Sample

A retrospective evaluation of SisliHamidiyeEtfal Training and Research Hospital's Homecare records between 01.01.2016 – 31.12.2016 were used as data.

Exclusion Criteria:

- Patients with end-stage CKD,
- Patients who were unconscious or bedridden,
- Patients diagnosed as malnutrition secondary to intestinal inflammatory disease or operation
- Patients for whom inadequate information was available.

Inclusion Criteria:

- Patients who were CKD (except end Stage CKD),
- Patients who were not unconscious or bedridden,
- Patients who were not have any disease or operation that cause malnutrition,
- Patients who have the records of BI, MNA and GFR in files in the same visit.

Ethics Statement

Before collecting data, research approval was obtained from the Ethics Committee of SisliHamidiyeEtfal Training and Research Hospital (Date: 04.04.2017 / Number: 1492). The investigation conformed to the principles outlined in the Declaration of Helsinki. The researcher provided information from SisliEtfal Training and Research Hospital Homecare Unit.

Instruments

Data were collected from the files of SisliEtfal Training and Research Hospital Homecare Unit.
4 parts was recorded:
1-Demographic information:
Age, Gender were noticed.
2- Glomerular Filtration Rate (GFR):

GFR was calculated using a simplified version of the Modification of Diet in Renal Disease (MDRD) formula.GFR≥90 ml/min/1.73 m^2 and GFR<15 ml/min/1.73 m^2 were excluded from study.Patients were separated into two groups according to GFR;Patients whose GFR was between 60–89 ml/min/1.73 m^2 (CKD Stage 2) were classified as Group 1 and those whose GFR was <60 ml/min/1.73 m^2 (CKD Stages 3 and 4) were classified as Group 2.

3- Mini-Nutritional Assessment(MNA):

To identify the nutrition status, Mini-Nutritional Assessment (MNA) test was used. The MNA was first developed in 1991 and published in 1994 in Nutrition Reviews (8). It is an 18-item questionnaire that incorporates anthropometric measurements, dietary intake and global and self-assessment components.

We used the Mini-Nutritional Assessment (MNA) test as a screening tool for malnutrition. In MNA, Malnutrition Indicator Score cut-off points were as follows:

- **24–30 points:** normal nutritional status
- **17–23.5 points:** at risk of malnutrition
- **less than 17 points:** malnourished

4-Barthel Index (BI):

To identify ADLs, BarthelIndex(BI) was used. The Barthel Index has also been used to examine ADL

Table 1. Mean and median values of age, MNA Scores, GFR and Barthel Index regarding gender

	WOMEN	MEN	P
	Median (25%-75%)	Median (25%-75%)	
GFR	59 (46-72)	66(52,8-77.75)	0.001
Age	84(78-87.5)	82(73-88)	0.151
MNA	20.5(17.5-23.5)	20.5(16-23.5)	0.434
Barthel ındex	35(0-50)	30(0-50)	0.204

performance in geriatric patients since 1955. In 1955; the acute care hospitals in Maryland have used the Barthel Index to assess patients' ability to complete ADLs (9).

The index assesses the following ten items: presence or absence of fecal and urinary incontinence; help needed with grooming; toilet use; feeding; transfers (e.g. from chair to bed); walking; dressing; climbing stairs; and bathing. The Barthel Index scores are expressed as multiples with a range of 0 (completely dependent) to 100 (independent for basic ADLs). Higher scores were reflective of a higher degree of independence.

In the Barthel Index:

- **0-20 point:** totally dependent
- **21-60 point:** high-level dependent
- **61-90 point:** mid-level dependent
- **91-99 point:** low-level dependent
- **100 point:** totally independent

Take care of the patient's GFR, MNA and Barthel Index datas were from the same visits.

Data Analysis

To analyze the data obtained in the study, descriptive statistics (number and percentage distribution, means, standard deviations) were calculated first. Our study featured an abnormal distribution ($p < 0.001$) according to Shapiro–Wilk test. Consequently, the Mann–Whitney U-test was used to compare the two groups. Chi-square was used to explore goodness of fit.Spearman Correlation Analysis was performed to determine the level and direction of the relationship between the dependent and independent variables. Linear regression was performed to show the correlation is strong.P values of ≤0.05 were considered statistically significant.

Results

A total of 1105 patient visits were noted throughout the year. After exclution; total of 345 patients, 225 women (65.2%) and 120 men (34.8%), were included in our study sample. Age mean of the total sample was 81.47 ± 9.26 and mean value of GFR was 60.83 ± 17.10 ml/min/1.73 m^2. MNA test mean was 19.66 ± 4.97. Barthel Index of the study group was 30.39 ± 28.99.

The distribution of gender, GFR, MNA and BI values are displayed in Table 1 and Figure- 1. Age, MNA, the Barthel Index and gender were not related according to student t test ($p = 0,151$; 0.434; 0.204). Although women's age mean were older than men ; women's BI and MNA were better than those observed in men. Only men's GFR means was good from womens and there was a significantregression between gender and GFR(r^2=0,28; $p = 0.002$).This will not show only the relation between gender and GFR but also will show the relation between age and GFR. Smilarly with this according to our study there was a negative correlation and significantregression between GFR and age (r^2=0,20; $p = 0.009$).

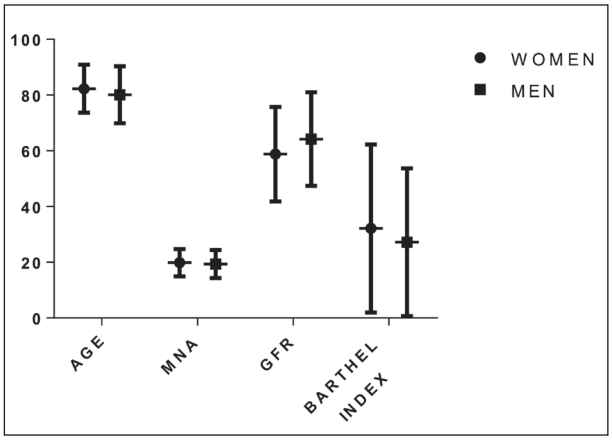

Figure 1. The distribution of gender and GFR, MNA, MNA-SF,BI values

According to MNA, 25.2% (n = 87) of the study group were malnourished and 52.8% (n = 182) were at risk of malnutrition. According to BI, 2.3% (n = 8) were totally independent; 2% (n = 7) were low-level dependent, 8.7% (n = 30) were mid-level dependent, 42.4% (n = 146) were high-level dependent; and 44.6% (n = 154) were totally dependent. Although MNA and BI increased with age, there was no relationship between age, MNA and Barthel Index scores (p = 0.506; 0.134).

A statistically significant correlation was found between GFR and the Barthel Index (p = 0.022). When GFR decreased, BI scores decreased (indicating increased dependence). As MNA scores decreased and nutrition worsened, GFR values also decreased(p = 0.029). BI and MNA were also related (p = <0.001)

Regarding GFR Groups, 182 (52.8%) patients were classified as Group 1 and 163 (47.2%) as Group 2. Group characteristics are displayed in Table 2. Age and GFR distrubutions were statistical significance (p = 0.004; p=0.00). Although nutrition and ADLs were worse in group GFR < 60 ml/min/1.73 m^2; there were no significant differences between two groups (p=0.212;0.067).

Discussion

According to the Turkish Statistical Institute (TSI), the elderly population was 6,651,503 in 2016, representing 8.3% of the entire population. Males comprised 43.9%, and females 56.1% (10). As exhibited in the general population, women comprised a greater percentage of our study cohort than men, which is expected given that life expectancies are greater for women than men.

A previous study in the USA revealed the prevalence of stage 1–4 CKD to be 13.1% (11). In Turkey, it was 15.7% (and observed mostly in women) (2). In our study, the relationship between age, gender and GFR was statistically significant and the female GFR were worse than those of the men (r2=0,28; p = 0.002) ; age was negatif related with GFR (r2=0,20; p = 0.009)and also women were older than men. This may be attributable to longer life expectancy in women, along with lower GFR as the complications of aging and chronic diseases manifest in the elderly. Conversely, younger women have an increased risk of urinary tract infections, a potential cause of CKD in adults and may cause this difference in elderly(12).

Progressive lowering of GFR can occur, independent of overt pathology in the elderly (13). In our study, GFR decreased as age increased.

In our study, women's nutrition was better than that of men but this did not rise to the level of statistical significance. In a 1999 study, having cooking skills enabled people to prepare meals, but may also have provided a degree of knowledge about ready-prepared meals. Traditionally, shopping for preparing and cooking food has been primarily the responsibility of women, with many older men never having mastered the art of cooking (14). Similarly, although the Barthel Index and gender were not related; women were more independent then men.

In a study performed in Ankara on elderly individuals, 5% were malnurished and 67% were at risk of malnutrition (15). A 2006 review of literature revealed

Table 2. The relations between gender, age, MNA, MNA-SF, BI and GFR groups

	Group 1 (GFR89-60 ml/min/1.73 m^2)		Group 2 (GFR < 60 ml/min/1.73 m2)		p
	N	%	N	%	
Gender					
Female	105	57.7	120	73.6	0.002
Male	77	42.3	43	26.4	
	Median		Median		P
Age	82(74-87)		85(78-90)		0.004
MNA	20.50(17-24)		20.25(16-23)		0.067
Barthel index	30(0-50)		30(0-50)		0.212
GFR	74.5(66-83)		47.6(38-54)		0.000

a similar pattern in out-patients and elderly individuals receiving home care (25 studies, n =3119 elderly), the prevalence of malnutrition was 9%and an additional 45% were at risk of malnutrition(16). Another study including 1,834 adults with predialysis CKD and protein-energy wasting found prevalences of 2.2%, 4.4%, 8.3%, 6.2%, 15.6% and 24.6% in CKD stages 1, 2, 3a, 3b, 4 and 5, respectively (17). In our study, 25.2% (n = 87) of our patients were malnourished and 52.8% (n = 182) were at risk of malnutrition. Our percentage is higher than other studies because the GFR of our study group was <90 ml/min/1.73 m^2 or low economic level of our study group.

There was a relation between GFR and MNA(p = 0.029). Nutrition is a very important consideration in CKD patients. Dietary metabolites are closely related to CKD progression (18), and CKD progression improves when nutrition improves (19). In our study, nutrition worsened with decreased GFR values. This may occur secondary to reduced protein and energy intake and lost protein with albuminurea in those with CKD. The degree to which appetite is lost is associated with decreases in GFR.

But when patients were separated in two groups according to GFR. MNA score was good in GRF 60–89 ml/min/1.73 m^2;however, this did not rise to the level of statistical significance. This may occur as a result of secondary (comorbid) diseases with CKD effects.

There were many studies about the relation between GFR and ADLs. In a study; GFR category and Barthel Index are independent risk markers for survival in older rehabilitation patients (20). This underscores the importance of observing and measuring ADLs in the elderly. In another study there was no relationship between the Barthel Index and the disability severity in CKD patients (21). This shows that there was no direct relation or there were some secondary things that effect the relation between GFR and BI. In our study, GFR and BI were related. But when we separated the patients in to two groups according to GFR level, there was no relationship between the BI and GFR groups. This may be because ADLs are multi-factorial and homecare patients frequently exhibit many other comorbid diseases, with CKD.

In a study said that BI can help patients at risk of malnutrition (22). In another; nutritional status, according to MNA, is related to ADL performance in geriatric patients (23). There was a strong relationship between MNA and the Barthel Index in our study too. Decreases in the MNA indicate increases in functional dependence.

Conclusions

Malnutrition is an important risk factor for CKD patients. MNA may help identify CKD patients who are malnourished or at risk of malnutrition. ADLs and GFR were related according to the Barthel Index. In elderly as the malnutrition increases also the GFR value increases and daily activities decrease. BI may be useful for determining the risks of poor nutrition and functional decline in CKD patients. In primary care, elderly individuals (especially those receiving homecare services) should undergo assessment of ADLs and nutritional status.

References

1. Levey AS, Coresh J, Balk E, Kausz AT, et al. National Kidney Foundation; Clinical Practice Guidelines for Chronic Kidney Disease:Evaluation, Classification, and Stratification; Ann Intern Med. 2003 Jul 15;139(2):137-47.PMID: 12859163
2. Suleymanlar G, Utas C, Arinsoy T, et al. A population-based survey of Chronic Renal Disease In Turkey--the CREDIT study. Nephrol Dial Transplant 2011;26:1862-71. PMID: 21051501
3. Donna M, Wilson DM, Corrine D, et al. Are Chronically Ill Patients High Users of Homecare Services in Canada? Am J Manag Care 2015;21(10):e552-9.PMID:26619056
4. Cano N. Malnutrition and chronic renal failure. Ann Med Interne (Paris) 2000;151:563-74.PMID: 11139657
5. Oner-IyidoganY, Gurdol F, Kocak H, et al. Appetite-Regulating Hormones In Chronic Kidney Disease Patients. J RenNutr 2011;21(4):316-21.PMID: 21193324
6. Thomas R, Kanso A, Sedor J.R. Chronic Kidney Disease and its Complications. Prim Care. 2008 Jun; 35(2): 329–vii. doi: 10.1016/j.pop.2008.01.008
7. SchraderE, Baumgartel C, Gueldenzoph H, et al. Nutritional status according to Mini Nutritional Assessment is related to functional status in geriatric patients--independent of health status.J Nutr Health Aging 2014;18(3):257-63.PMID: 24626752
8. Guigoz Y, Vellas B, Garry PJ.Assessing the nutritional status of the elderly: The Mini Nutritional Assessment as part of the geriatric evaluation.Nutr Rev. 1996 Jan;54(1 Pt 2):S59-65.PMID: 8919685

9. Mahoney FI and Barthel D. Functional evaluation: the Barthel Index. Md State Med J 1965;14:56-61.PMID: 14258950
10. Health Statistics 2015; Turkey Statictical Institute; 2016. http://www.tuik.gov.tr/PreTablo.do?alt_id=1095
11. Coresh J, Selvin E, Stevens LA, et al. Prevalence of chronic kidney disease in the United States. JAMA 2007;298:2038-47.PMID: 17986697
12. Food and Nutrition Guidelines for Healthy Older; Ministry of Health. New Zealand 2013https://www.health.govt.nz/system/files/documents/publications/food-nutrition-guidelines-healthy-older-people-background-paper-v2.pdf (Reached at 10.04.2017).
13. Yavuz D, Yavuz R, Sezer S. Nephrologic Problems in Old Age Period, Turkish Nephrology, Dialysis and Transplantation Journal2012;21(3):217-23 doi: 10.5262/tndt.2012.1003.03http://www.tndt.org/pdf/aip/761.pdf
14. Guidelines on Urological Infections, European Association of Urology, 2015.https://uroweb.org/wp-content/uploads/19-Urological-infections_LR2.pdf (Reached at 10.04.2017
15. Kucukerdonmez O, Navruz Varli S, Koksal E. Comparison of Nutritional Status in the Elderly According to Living Situations.J Nutr Health Aging 2017;21(1):25-30 PMID: 27999846
16. Guigoz Y. The Mini-Nutritional Assessment Review of the Literature - What does it tell us? J Nutr Health Aging 2006;10:466-87.PMID: 17183419
17. Hyun YY, Lee KB, Han SH, et al. Nutritional Status in Adults with Predialysis Chronic Kidney Disease: KNOW-CKD Study. J Korean Med Sci 2017;32(2):257-63 PMID: 28049236
18. Formiga F, Ferrer A, Cruzado JM, et al.Geriatric assessment and chronic kidney disease in the oldest old: The Octabaix study.Eur J Intern Med 2012;23:534-8PMID: 22863431
19. Kopple JD, Greene T, Chumlea WC, et al Relationship between nutritional status and the glomerular filtration rate: results from the MDRD study. Kidney Int 2000;57(4):1688-70PMID: 10760105
20. Doyle EM, Sloan JM, Goodbrand JA, et al.Association between kidney function, rehabilitation outcome, and survival in older patients discharged from inpatient rehabilitation. Am J Kidney Dis 2015;66(5):768-74PMID: 26048443
21. Vishnevski KA, Zemchenkov AIu, Korosteleva NIu, Smirnov AV.Use of the Charlson comorbidity index and the Barthel disability index in the integrated assessment of the sociomedical status of patients receiving continuous renal replacement therapy with hemodialysis. Ter Arkh.2015;87(6):62-7.PMID: 26281198
22. SerranoUrrea R,GarcíaMeseguer MJ,Relationships between nutritional screening and functional impairment in institutionalized Spanish older people.Maturitas 2014;78(4):323-8.PMID: 24953175
23. Schrader E, Baumgärtel C, Gueldenzoph H, et al.Nutritional status according to Mini Nutritional Assessment is related to functional status in geriatric patients--independent of health statusJ Nutr Health Aging 2014;18(3):257-63PMID: 24626752

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Original article

Comparison of nutritional screening parameters in oncology patients with malnutrition: handgrip strength as a reliable parameter

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Summary. *Aim:* To evaluate handgrip strength (HGS), phase angle (PA), serum albumin, nutritional risk screening (NRS) and quality of life (QoL) as markers for nutritional status and prognostic factors for survival in oncology patients with malnutrition and parenteral nutritional therapy. *Methods:* We conducted a prospective study with 36 patients between August 2013 and November 2015. HGS was measured using a hydraulic dynamometer, PA was calculated based on bioelectrical impedance tests. Serum albumin levels were measured and a nutritional risk screening (NRS 2002) was performed. The Short Form 12 (SF-12) questionnaire was used to assess QoL. PA was regarded as the gold standard for determining body composition and nutritional status. A 1-year follow-up was performed, and patient survival was evaluated. *Results:* HGS showed a significant correlation with PA in bioelectrical impedance analysis. Body mass index (BMI) was not correlated with HGS. Albumin and NRS showed poor specificity as compared to PA. QoL was not correlated with parameters for nutritional status. Overall survival was poor with a 1-year survival rate of 15%. *Conclusions:* Compared to serum albumin and BMI, HGS is a reliable tool for the assessment of nutritional status in oncology patients. When indication for parenteral nutrition is confirmed in cancer patients with malnutrition, overall patient survival is poor.

Key words: malnutrition, cancer, albumin, handgrip strength, phase angle

Introduction

Cancer is a global health challenge with growing incidence, morbidity, and mortality (1). Malnutrition occurs frequently in oncology patients due to effects of the disease and of disease therapies on patients' nutritional status (2). It is associated with adverse outcomes and decreased survival (3, 4) and impairs quality of life (QoL) (5, 6). Supportive nutritional therapy has been beneficial for outcomes and QoL of cancer patients (7-9). Thus, determining nutritional status in oncology patients is essential. Several tools have been described to pursue this objective. One of these is the bioelectrical impedance analysis (BIA) that is used for the determination of body composition and has previously been used for oncology patients (10, 11). A reliable parameter obtained during BIA measurements is the phase angle (PA), which is derived from changes in resistance and reactance as an alternating current passes through tissues causing a phase shift (12). PA is one of the most widely accepted parameters for nutritional status in cancer and other patients (13-15). Another more recently developed tool is handgrip strength (HGS) obtained by measuring static muscle strength with handgrip dynamometers. It was originally used for functional examination in hand surgery but has re-

cently been proposed for the evaluation of nutritional status by several authors (16-18). Low HGS is associated with malnutrition and cachexia (19, 20). HGS has also been evaluated as a prognostic tool in surgical and cancer patients and is applied in epidemiological studies (21, 22). Furthermore, some biochemical markers are used as parameters for nutritional status. Serum albumin levels have been used to evaluate the nutritional state of oncology patients, but also of non-oncology patients in some studies (23, 24). In clinical practice, it is still used for this purpose. Moreover, albumin is a prognostic marker for clinical outcomes, e.g. it predicts the risk for anastomotic leakage in colorectal surgery (25, 26). In addition to physiological and biochemical measurement methods, scoring systems have been developed to screen for nutritional risk. The nutritional risk screening (NRS 2002) was introduced to identify patients who are likely to benefit from nutritional support (27). It has been suggested to indicate malnutrition in head and neck cancer patients (28) and was found to help predict postoperative complications and prolonged hospital stay in gastric cancer (29).

Even though all these different tools are available, it still remains unclear which method is the best choice for which group of patients. In view of the increasing worldwide importance of oncology patients, for whom particular attention to nutritional status is of utmost importance – especially at advanced disease states – the objective of the present study was to compare the above-mentioned tools to assess the nutritional status of these patients. Therefore, the primary aim of this study was to evaluate HGS, PA, albumin, NRS and QoL as markers for nutritional status in cancer patients with parenteral nutritional therapy. Moreover, parameters were assessed for possible prognostic qualities as pertains to patient survival.

Patients and Methods

Ethics Approval

Ethics board approval was obtained from the Medical Ethics Commission II of the Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany (No. 2013-573N-MA). The study was performed according to the Declaration of Helsinki.

Patients

Eligible for this study were oncology patients with malnutrition and parenteral nutritional therapy treated at the Outpatient Nutrition Clinic at the University Hospital Mannheim between August 2013 and November 2015. The Outpatient Nutrition Clinic at our institution treats 60 oncological patients with parenteral nutritional support per year. All consecutive patients for whom there was no exclusion criterion and who were willing to consent to study participation were included. The inclusion of patients was complicated by the fact that oncological patients with parenteral nutrition therapy often present in advanced tumour stages. Exclusion criteria were: patients under 18 years of age, the presence of an implanted cardiac pacemaker and/or defibrillator and/or obvious impairments of measurement methods. Indication for parenteral nutrition had to be confirmed by the treating physicians and followed the criteria of the European Working Group on Sarcopenia in Older People (30). Patients of both sexes were included. Informed consent was obtained from all patients.

Technical measurements (BIA and HGS), NRS and QoL assessment were carried out on the same day. Blood samples for the determination of serum albumin were taken within 7 days before or after the day of the other measurements.

Patient survival was determined from review of medical records or from information obtained from the patients' treating physicians or family doctors.

Bioelectrical Impedance Analysis

After a period of physical rest for at least 30 minutes, BIA measurements were performed by applying four gel electrodes, with two detecting electrodes placed at the ulnar aspect of the wrist and the medial malleolus of the dominant body side, following standard protocols. Electrodes were connected to a multiple frequency BIA instrument (*Nutriguard-M*, Data Input GmbH, Frankfurt, Germany). Measurements were digitally recorded and calculations for body composition values were performed using the *Nutriguard Plus* Software Version 5.4. As cut-off values for PA indicating malnutrition, individual age and sex specific threshold values were used as provided by the manufacturer (Data Input GmbH, Frankfurt, Germany).

Static Muscle Strength

A Saehan *Hydraulic Hand Dynamometer SH5001* (Saehan Corporation, Changwon, South Korea) was used to determine HGS. Measurements were conducted first on the dominant, then on the non-dominant hand. For this investigation, patients sat in a comfortable position with a 90° angle at the elbow joint. Patients were asked to squeeze the dynamometer using their maximum strength. Measurement was repeated after a break of 30 seconds. In total, three measurements for each hand were recorded. The mean value of the three efforts was calculated and used for this study. This procedure is similar to previous investigations (31, 32).

Serum Albumin Levels

Serum albumin levels were determined via standard laboratory procedures from venous blood samples acquired from patients after inclusion in this study. Hypalbuminaemia indicating malnutrition was defined as a serum albumin level < 30 g/L.

Nutritional Risk Screening (NRS 2002)

Nutritional screening was performed using the Nutritional Risk Screening (NRS 2002) (27) following guidelines of the European Society of Parenteral and Enteral Nutrition (ESPEN) (33). Patients were regarded as nutritionally at-risk when an individual score ≥3 was observed.

Short Form 12 (SF-12) Questionnaire

Physical Health Composite Score (PCS) and Mental Health Composite Score (MCS) obtained from the Short Form Health Survey (SF-12) were used to assess QoL.

Statistical Analysis

Quantitative approximately normally-distributed parameters are presented by mean values and standard deviations; for skewed data, median and range are given. Qualitative data is described by its absolute and relative frequency. For approximately normally-distributed data, two sample t-tests have been used in order to compare the mean values of two groups. For skewed variables, Mann–Whitney U-tests were performed instead. For the comparison of qualitative parameters Fisher's exact tests were applied. Correlation values for interval and ratio variables were determined by calculating Pearson's correlation coefficient, and, for ordinal variables, a Spearman correlation coefficient was calculated. A logistic regression model was used to identify factors associated with an elevated risk of postoperative complications, expressed as the odds ratio (OR) (95% confidence interval (CI)). Furthermore, regression was used as a multiple statistical method to identify risk factors. For this technique, several factors were included in the model; the most important factors were selected by stepwise elimination. Survival curves were generated using the Kaplan–Meier method. All statistical tests were two-tailed, and the threshold for statistical significance was set to $P < 0.05$. All analyses were performed using the SAS software, release 9.3 (SAS Institute Inc., Cary, NC, USA).

Results

Between August 2013 and November 2015, 36 oncology patients were included in the study, 16 males (44.4%) and 20 females (55.6%). The mean age was 62.6 years. All included patients received parenteral nutrition for the treatment of malnutrition. The most frequent oncologic diagnosis was cancer of the gastrointestinal tract (GIT) (47.2%), including the upper-GIT *n*=13 (36.1%) and lower-GIT *n*=4 (11.1%). Other entities included cancer of the lung (16.7%), breast (8.3%), and prostate gland (5.6%) (Table 1).

Table 1. Patients' characteristics

Sex (n)	Male	16	(44.4%)
	Female	20	(55.6%)
Age (mean)		62.6	(30-87)
Type of primary cancer (n)			
	Gastrointestinal	17	(47.2%)
	Lung	6	(16.7%)
	Breast	3	(8.3%)
	Prostate	2	(5.6%)
	Other	8	(22.2%)
BMI (mean, kg/m2)		20.9	(15.6-32.0)
Albumin (serum, g/L)		24.8	(15.6-34.6)

Body Mass Index and Serum Albumin Levels

The mean BMI was 20.9 kg/m^2 (SD 4.2 kg/m^2; range 15.6-32.0 kg/m^2). The mean serum albumin level (*n*=30) was 24.8 g/L (SD 5.2 g/L; range 15.6-34.6 g/L) (Table 1). None of the patients in our study showed albumin levels within the reference range, all patients presented with hypoalbuminemia.

Handgrip Strength of the Dominant Hand

The mean value of the dominant hand was 21.6 kg for all patients (SD 8.9 kg; range 11.0-51.7 kg). The mean value for females was 16.2 kg (SD 4.5 kg; range 11.0-26.0 kg), the mean value for males was 28.1 kg (SD 8.9 kg; range 13.3-51.7 kg) (Table 2). These values were lower than the expected values of healthy individuals matched for sex and age.

Bioelectrical Impedance Analysis

The phase angle reflects the quality of the lean body mass. An average of 3.9° was observed. The mean lean body mass itself was 47.2 kg. These data and values for body cell mass, extracellular mass, body fat and body water are presented in Table 2.

NRS Score and Short Form 12

Both the median and the mode score in the Nutritional Risk Score was 5 points (Figure 1). The maximum score of 7 points was achieved by one patient, and the lowest score recorded was 2 points, which was observed in 3 patients. In the SF-12 questionnaire, the mean score of PCS was 29.5 points (SD 9.0; range 13.4-49.8) and the mean score of MCS was 36.1 points (SD 11.0; range 14.2-63.9).

Table 2. Results of handgrip measurement and bioelectrical impedance analysis

	unit	mean	SD
Handgrip strength dominant hand	kg	21.6	8.9
Phase angle	degree	3.9	0.9
Lean body mass	kg	47.2	11.5
Body cell mass	kg	18.9	4.8
Body cell mass of lean body mass	%	38.5	6.5
Extracellular mass	kg	29.2	8.4
Body fat	kg	10.8	10.4
Body fat	%	16.7	13.3
Body water	L	34.6	8.5

Handgrip Strength as a Reliable Marker for Nutritional Status

In univariate analysis, body cell mass (r=0.61, p < 0.0001), body water (r=0.41, p = 0,0141), PA (r=0.39, p=0,0182), lean body mass (r=0.36, p=0.0334), and the ratio of body cell mass on lean body mass (r=0.34, p=0,0398) showed significant correlation with handgrip strength. However, when the influence of gender (female versus male) was considered (p<0.0001) and multiple regression analysis was performed, only the factors gender (p<0,0001), PA (p=0.0168), and ratio of body cell mass on lean body mass (p=0.0172) were independent factors with significant influence on HGS (Table 3). No statistically significant impact on HGS was discovered for serum albumin levels (r=0.01, p=0.9492), BMI (r=-0.04, p=0.8183) or patient age (r=-0.27, p=0.1166).

Serum Albumin Levels Compared to Screening Parameters for Nutritional Status

Univariate analysis showed no significant correlation of serum albumin levels with the other parameters (*n*=30). When analysis of contingency tables was

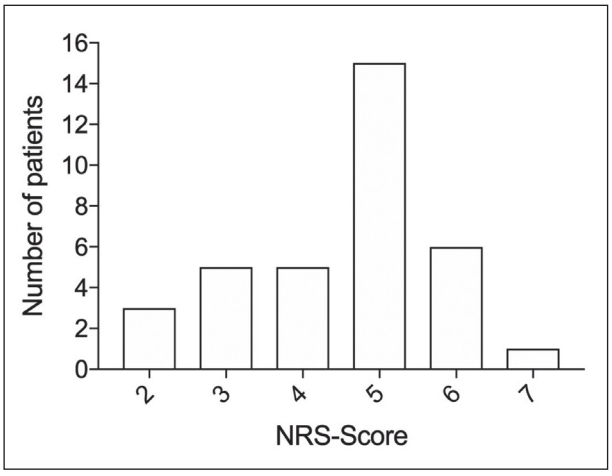

Figure 1. Distribution of NRS-Scores

Table 3. Independent factors influencing handgrip strength (dominant hand)

	p-value
Sex	< 0,0001
Phase angle	0,0168
BCM/LBM	0,0172

performed for albumin and PA (PA set as the gold standard measurement for body composition), a sensitivity of 83% (serum albumin levels < 30 g/L) and a specificity of 33% (serum albumin levels great than or equal to 30 g/L) was calculated in comparison to PA. Reference ranges for PA in this analysis were matched for age and gender; no values above the reference range were observed.

Nutritional Risk Score Compared to Screening Parameters for Nutritional Status

Statistical analysis revealed a negative correlation of NRS-scores with BMI (r=-0.46, p=0,0062), body fat (kg) (r=-0.45, p=0.0071), and body fat percent (r=-0.35, p=0.0405). This is not surprising since BMI is included in NRS screening. However, contingency table analysis of NRS regarding PA as the gold standard measurement for body composition showed a high sensitivity of 93% (NRS scores >=3) but a low specificity of only 17% (NRS scores < 3) for NRS predicting PA. Reference ranges for PA in this analysis were matched for age and gender; no values above the reference range were observed.

Results of Short Form 12 Questionnaire in Nutritional Screening

Results of the SF-12 questionnaire showed a significant correlation of the PCS with serum albumin levels (r=0.42, p=0.0288). Beyond that, no further significant correlation was found for PCS or MCS with the other parameters. Quality of life assessment did not correlate with PA and HGS measurements and was not significantly related to overall patient survival.

Survival Analysis and One-Year Follow-up

Survival analysis was performed for patients who completed a 1-year follow-up, or, for deceased patients. One patient committed suicide and was therefore excluded from the analysis. Loss of follow-up occurred in 2 cases. In total, 33 of 36 patients in this study (92%) were included in the Kaplan-Meier analysis (Figure 2). Median survival was 62 days with a 1-year survival-rate of 15%. A rapid decrease in survival occurred in the early observation period.

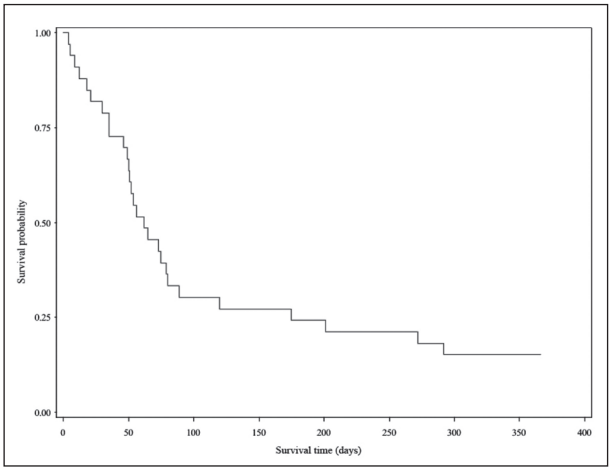

Figure 2. Kaplan-Meier analysis of survival

Discussion

In this prospective clinical study, we examined several surrogate parameters for malnutrition in a group of 36 cancer patients who received parenteral nutritional therapy for malnutrition between August 2013 and November 2015. Furthermore, patient survival was evaluated.

Main finding was that HGS is, compared to other parameters such as BMI and serum albumin, a reliable parameter for nutritional status in oncology patients with malnutrition. Moreover, the 1-year survival rate of only 15% indicates that confirmed indication for parenteral nutritional support in oncology patients comes along with poor overall survival.

HGS has been reported to predict survival in advanced cancer patients in some studies (34), but other studies failed to reproduce these findings (11). For BIA measurements and PA, which was regarded as the gold standard for body composition in our study, prognostic value in different clinical settings, including oncology and surgery, has been described (35, 36). Both PA and HGS can be used to predict post-interventional body composition in bariatric patients (32). HGS is lower in patients with malnutrition and colorectal cancer before tumor resection (10).

In our study, we found a significant correlation of HGS with PA, indicating that HGS may be a reliable parameter for the assessment of nutritional status in oncology patients. This is particularly relevant, since HGS measurement is suitable for clinical practice,

compared to alternative methods, because HGS is very simple to obtain, non-invasive, and inexpensive.

In clinical practice, albumin is used to determine patient nutritional status, e.g. when parenteral nutrition is applied in the course of "preoperative patient optimization" prior to surgical procedures low serum albumin levels are measured. However, albumin is not a sole nutritional marker because it is influenced by various conditions such as impaired liver function, volume status and inflammatory states including cancer (37). It is a negative acute phase protein (38), and oral protein intake does not influence serum albumin levels in some studies (39). Nevertheless, ESPEN guidelines for enteral nutrition consider albumin levels for the identification of patients nutritionally at risk. Our data do not indicate that albumin is a reliable marker for malnutrition. However, it can be used complementary since it is non-invasive, easy to obtain and cost-effective. All individuals in our study showed hypoalbuminemia, a fact that likely explains why our data did not demonstrate a significant prognostic effect of albumin levels on overall survival, as was demonstrated by other authors (40).

The NRS 2002 system was introduced to identify patients who are likely to benefit from nutritional support (22) and was shown to be a reliable tool in this regard (23). In our study, we found high sensitivity, but poor specificity of NRS, predicting PA. A possible explanation for this is the small number of patients with NRS scores < 3, and PA values within the reference range which limits statistical power. Some authors found data indicating that the NRS system was able to predict more postoperative complications and longer hospital stays in oncology patients (24). Contrary to this, in other studies, NRS scores >= 3 were associated with postoperative complications but did not turn out to be independent predictors when multivariable analysis was performed (38). Other data suggest that nutritional support based upon NRS 2002 screening might even result in over-nutrition (23). Our study failed to demonstrate prognostic significance of NRS as relates to survival. Since specificity compared to PA was low, other parameters might turn out to be more reliable for nutritional screening.

Patients in our study showed poor overall survival with a median survival of 62 days. The limited survival was not surprising since oncology patients with confirmed indication for parenteral nutrition are likely to be in poor general condition, have significant comorbidities, and present in advanced tumour stages. In other studies, survival ranges from 4 to 9 months for advanced pancreatic cancer (41) and is about 12 months for metastatic gastric cancer (42) and adenocarcinoma of the oesophagus (43). Moreover, this explains the small number of patients in our study, because patients with end-stage oncological diseases are generally hard to include in clinical trials.

Our data shows that oncology patients who require parenteral nutritional support are exposed to considerable health risks including reduced survival. Further investigation is needed to determine whether this can be improved by earlier nutritional screening and intervention. In this context, HGS and PA measurements seem to be the most useful, reliable, and easy-to-implement tools.

The validity of our study is limited due to the small number of patients and the heterogeneity of the study population. For these reasons, detailed statistical analysis of tumour stages and their entities could not be performed.

Conclusions

HGS is a reliable tool for the assessment of nutritional status in oncology patients. In this respect, BMI, NRS and QoL assessment seem to be less useful. Serum albumin is a prognostic tool in various clinical conditions, but alone, it is not particularly suitable for the diagnosis of malnutrition. The prognosis of oncology patients with indication for parenteral nutritional therapy is poor, which is reflected in low survival rates.

Conflict of interest

All authors have read and understood the policy on declaration of interests. The research reported in this study was supported by *B. Braun Melsungen AG*, Melsungen, Germany, which develops products related to the research being reported. The terms of this arrangement have been reviewed and approved by the Department of Surgery, University Hospital Mannheim, Faculty of the University of Heidelberg, in accordance with its policy on objectivity in research. The authors of this study have no further professional affiliation with *B. Braun Melsungen AG*.

References

1. J. Ferlay, I. Soerjomataram, R. Dikshit, S. Eser, C. Mathers, M. Rebelo, D. M. Parkin, D. Forman, and F. Bray, "Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012," *Int. J. Cancer*, vol. 136, no. 5, pp. E359–E386, 2015.
2. J. Arends, V. Baracos, H. Bertz, F. Bozzetti, P. C. Calder, N. E. P. Deutz, N. Erickson, A. Laviano, M. P. Lisanti, D. N. Lobo, D. C. McMillan, M. Muscaritoli, J. Ockenga, M. Pirlich, F. Strasser, M. de van der Schueren, A. Van Gossum, P. Vaupel, and A. Weimann, "ESPEN expert group recommendations for action against cancer-related malnutrition.," *Clin. Nutr.*, vol. 36, no. 5, pp. 1187–1196, Oct. 2017.
3. J. A. Virizuela, M. Camblor-Álvarez, L. M. Luengo-Pérez, E. Grande, J. Álvarez-Hernández, M. J. Sendrós-Madroño, P. Jiménez-Fonseca, M. Cervera-Peris, and M. J. Ocón-Bretón, "Nutritional support and parenteral nutrition in cancer patients: an expert consensus report.," *Clin. Transl. Oncol.*, Oct. 2017.
4. M. Muscaritoli, S. Lucia, A. Farcomeni, V. Lorusso, V. Saracino, C. Barone, F. Plastino, S. Gori, R. Magarotto, G. Carteni, B. Chiurazzi, I. Pavese, L. Marchetti, V. Zagonel, E. Bergo, G. Tonini, M. Imperatori, C. Iacono, L. Maiorana, C. Pinto, D. Rubino, L. Cavanna, R. Di Cicilia, T. Gamucci, S. Quadrini, S. Palazzo, S. Minardi, M. Merlano, G. Colucci, P. Marchetti, and PreMiO Study Group, "Prevalence of malnutrition in patients at first medical oncology visit: the PreMiO study.," *Oncotarget*, vol. 8, no. 45, pp. 79884–79896, Oct. 2017.
5. H.-S. Lim, G.-S. Cho, Y.-H. Park, and S.-K. Kim, "Comparison of Quality of Life and Nutritional Status in Gastric Cancer Patients Undergoing Gastrectomies.," *Clin. Nutr. Res.*, vol. 4, no. 3, pp. 153–9, Jul. 2015.
6. J. Polański, B. Jankowska-Polańska, I. Uchmanowicz, M. Chabowski, D. Janczak, G. Mazur, and J. Rosińczuk, "Malnutrition and Quality of Life in Patients with Non-Small-Cell Lung Cancer.," *Adv. Exp. Med. Biol.*, vol. 1021, pp. 15–26, 2017.
7. P. Ravasco, I. Monteiro-Grillo, P. M. Vidal, and M. E. Camilo, "Dietary counseling improves patient outcomes: a prospective, randomized, controlled trial in colorectal cancer patients undergoing radiotherapy.," *J. Clin. Oncol.*, vol. 23, no. 7, pp. 1431–8, Mar. 2005.
8. P. Ravasco, I. Monteiro-Grillo, and M. Camilo, "Individualized nutrition intervention is of major benefit to colorectal cancer patients: long-term follow-up of a randomized controlled trial of nutritional therapy.," *Am. J. Clin. Nutr.*, vol. 96, no. 6, pp. 1346–53, Dec. 2012.
9. F. D. Ottery, "Supportive nutrition to prevent cachexia and improve quality of life.," *Semin. Oncol.*, vol. 22, no. 2 Suppl 3, pp. 98–111, Apr. 1995.
10. S. T. Burden, J. Hill, J. L. Shaffer, and C. Todd, "Nutritional status of preoperative colorectal cancer patients," *J. Hum. Nutr. Diet.*, vol. 23, no. 4, pp. 402–407, 2010.
11. J. Härter, S. P. Orlandi, and M. C. Gonzalez, "Nutritional and functional factors as prognostic of surgical cancer patients," *Support. Care Cancer*, vol. 25, no. 8, pp. 2525–2530, 2017.
12. M. C. G. Barbosa-Silva, A. J. D. Barros, J. Wang, S. B. Heymsfield, and R. N. Pierson, "Bioelectrical impedance analysis: population reference values for phase angle by age and sex.," *Am. J. Clin. Nutr.*, vol. 82, no. 1, pp. 49–52, Jul. 2005.
13. M. K. Kilic, M. C. Kizilarslanoglu, G. Arik, B. Bolayir, O. Kara, H. Dogan Varan, F. Sumer, M. E. Kuyumcu, M. Halil, and Z. Ulger, "Association of Bioelectrical Impedance Analysis-Derived Phase Angle and Sarcopenia in Older Adults.," *Nutr. Clin. Pract.*, vol. 32, no. 1, pp. 103–109, Feb. 2017.
14. I. C. R. Cardoso, M. A. Aredes, and G. V Chaves, "Applicability of the direct parameters of bioelectrical impedance in assessing nutritional status and surgical complications of women with gynecological cancer.," *Eur. J. Clin. Nutr.*, vol. 71, no. 11, pp. 1278–1284, Nov. 2017.
15. D. Ringaitiene, D. Gineityte, V. Vicka, A. Sabestinaite, A. Klimasauskas, E. Gaveliene, K. Rucinskas, J. Ivaska, and J. Sipylaite, "Concordance of the new ESPEN criteria with low phase angle in defining early stages of malnutrition in cardiac surgery.," *Clin. Nutr.*, Aug. 2017.
16. A. W. Knudsen, A. Naver, K. Bisgaard, I. Nordgaard-Lassen, U. Becker, A. Krag, and F. Slinde, "Nutrition impact symptoms, handgrip strength and nutritional risk in hospitalized patients with gastroenterological and liver diseases.," *Scand. J. Gastroenterol.*, vol. 50, no. 10, pp. 1191–8, 2015.
17. B. Yardimci, A. Sumnu, I. Kaya, M. Gursu, Z. Aydin, S. Karadag, S. Uzun, E. Tatli, S. Ozturk, E. Cetinus, and R. Kazancioglu, "Is handgrip strength and key pinch measurement related with biochemical parameters of nutrition in peritoneal dialysis patients?," *Pakistan J. Med. Sci.*, vol. 31, no. 4, pp. 941–5.
18. C. Silva, T. F. Amaral, D. Silva, B. M. P. M. Oliveira, and A. Guerra, "Handgrip strength and nutrition status in hospitalized pediatric patients.," *Nutr. Clin. Pract.*, vol. 29, no. 3, pp. 380–5, Jun. 2014.
19. T. Cederholm, C. Jägrén, and K. Hellström, "Nutritional status and performance capacity in internal medical patients.," *Clin. Nutr.*, vol. 12, no. 1, pp. 8–14, Feb. 1993.
20. G. A. Ozorio, K. Barão, and N. M. Forones, "Cachexia Stage, Patient-Generated Subjective Global Assessment, Phase Angle, and Handgrip Strength in Patients with Gastrointestinal Cancer.," *Nutr. Cancer*, vol. 69, no. 5, pp. 772–779, Jul. 2017.
21. A. Oksuzyan, P. Demakakos, M. Shkolnikova, M. Thinggaard, J. W. Vaupel, K. Christensen, and V. M. Shkolnikov, "Handgrip strength and its prognostic value for mortality in Moscow, Denmark, and England.," *PLoS One*, vol. 12, no. 9, p. e0182684, 2017.
22. S. F. Maurício, J. Xiao, C. M. Prado, M. C. Gonzalez, and M. I. T. D. Correia, "Different nutritional assessment tools as predictors of postoperative complications in patients undergoing colorectal cancer resection.," *Clin. Nutr.*, Sep. 2017.

23. A. V Stone, A. Jinnah, B. J. Wells, H. Atkinson, A. N. Miller, W. M. Futrell, K. Lenoir, and C. L. Emory, "Nutritional markers may identify patients with greater risk of re-admission after geriatric hip fractures.," *Int. Orthop.*, Oct. 2017.
24. E. U. Cidon, "Nutritional Status After Total Gastrectomy for Gastric Cancer.," *World J. Oncol.*, vol. 1, no. 2, pp. 87–90, Apr. 2010.
25. M. Frasson, P. Granero-Castro, J. L. Ramos Rodríguez, B. Flor-Lorente, M. Braithwaite, E. Martí Martínez, J. A. Álvarez Pérez, A. Codina Cazador, A. Espí, E. Garcia-Granero, and ANACO Study Group, "Risk factors for anastomotic leak and postoperative morbidity and mortality after elective right colectomy for cancer: results from a prospective, multicentric study of 1102 patients.," *Int. J. Colorectal Dis.*, vol. 31, no. 1, pp. 105–14, Jan. 2016.
26. M. Parthasarathy, M. Greensmith, D. Bowers, and T. Groot-Wassink, "Risk factors for anastomotic leakage after colorectal resection: a retrospective analysis of 17 518 patients.," *Colorectal Dis.*, vol. 19, no. 3, pp. 288–298, Mar. 2017.
27. J. Kondrup, H. H. Rasmussen, O. Hamberg, Z. Stanga, and Ad Hoc ESPEN Working Group, "Nutritional risk screening (NRS 2002): a new method based on an analysis of controlled clinical trials.," *Clin. Nutr.*, vol. 22, no. 3, pp. 321–36, Jun. 2003.
28. H. Orell-Kotikangas, P. Österlund, K. Saarilahti, P. Ravasco, U. Schwab, and A. A. Mäkitie, "NRS-2002 for pre-treatment nutritional risk screening and nutritional status assessment in head and neck cancer patients.," *Support. Care Cancer*, vol. 23, no. 6, pp. 1495–502, Jun. 2015.
29. W. Guo, G. Ou, X. Li, J. Huang, J. Liu, and H. Wei, "Screening of the nutritional risk of patients with gastric carcinoma before operation by NRS 2002 and its relationship with postoperative results.," *J. Gastroenterol. Hepatol.*, vol. 25, no. 4, pp. 800–3, Apr. 2010.
30. A. J. Cruz-Jentoft, J. P. Baeyens, J. M. Bauer, Y. Boirie, T. Cederholm, F. Landi, F. C. Martin, J.-P. Michel, Y. Rolland, S. M. Schneider, E. Topinková, M. Vandewoude, M. Zamboni, and European Working Group on Sarcopenia in Older People, "Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People.," *Age Ageing*, vol. 39, no. 4, pp. 412–23, Jul. 2010.
31. M. B. Budziareck, R. R. Pureza Duarte, and M. C. G. Barbosa-Silva, "Reference values and determinants for handgrip strength in healthy subjects.," *Clin. Nutr.*, vol. 27, no. 3, pp. 357–62, Jun. 2008.
32. M. Otto, S. Kautt, M. Kremer, P. Kienle, S. Post, and T. Hasenberg, "Handgrip Strength as a Predictor for Post Bariatric Body Composition," *Obes. Surg.*, vol. 24, no. 12, pp. 2082–2088, 2014.
33. J. Kondrup, S. P. Allison, M. Elia, B. Vellas, M. Plauth, and E. S. of P. and E. N. (ESPEN) Educational and Clinical Practice Committee, "ESPEN guidelines for nutrition screening 2002.," *Clin. Nutr.*, vol. 22, no. 4, pp. 415–21, Aug. 2003.
34. R. D. Kilgour, a Vigano, B. Trutschnigg, E. Lucar, M. Borod, and J. a Morais, "Handgrip strength predicts survival and is associated with markers of clinical and functional outcomes in advanced cancer patients.," *Support. Care Cancer*, vol. 21, no. 12, pp. 3261–70, Dec. 2013.
35. K. Norman, N. Stobäus, M. Pirlich, and A. Bosy-Westphal, "Bioelectrical phase angle and impedance vector analysis--clinical relevance and applicability of impedance parameters.," *Clin. Nutr.*, vol. 31, no. 6, pp. 854–61, Dec. 2012.
36. L. Genton, K. Norman, A. Spoerri, C. Pichard, V. L. Karsegard, F. R. Herrmann, and C. E. Graf, "Bioimpedance-Derived Phase Angle and Mortality Among Older People.," *Rejuvenation Res.*, vol. 20, no. 2, pp. 118–124, Apr. 2017.
37. S. Bharadwaj, S. Ginoya, P. Tandon, T. D. Gohel, J. Guirguis, H. Vallabh, A. Jevenn, and I. Hanouneh, "Malnutrition: laboratory markers vs nutritional assessment.," *Gastroenterol. Rep.*, vol. 4, no. 4, pp. 272–280, Nov. 2016.
38. J. P. Doweiko and D. J. Nompleggi, "The role of albumin in human physiology and pathophysiology, Part III: Albumin and disease states.," *JPEN. J. Parenter. Enteral Nutr.*, vol. 15, no. 4, pp. 476–83.
39. C. A. Raguso, Y. M. Dupertuis, and C. Pichard, "The role of visceral proteins in the nutritional assessment of intensive care unit patients.," *Curr. Opin. Clin. Nutr. Metab. Care*, vol. 6, no. 2, pp. 211–6, Mar. 2003.
40. H. Djaladat, H. M. Bruins, G. Miranda, J. Cai, E. C. Skinner, and S. Daneshmand, "The association of preoperative serum albumin level and American Society of Anesthesiologists (ASA) score on early complications and survival of patients undergoing radical cystectomy for urothelial bladder cancer.," *BJU Int.*, vol. 113, no. 6, pp. 887–93, Jun. 2014.
41. S. C. Lau and W. Y. Cheung, "Evolving treatment landscape for early and advanced pancreatic cancer.," *World J. Gastrointest. Oncol.*, vol. 9, no. 7, pp. 281–292, Jul. 2017.
42. E. Van Cutsem, X. Sagaert, B. Topal, K. Haustermans, and H. Prenen, "Gastric cancer.," *Lancet (London, England)*, vol. 388, no. 10060, pp. 2654–2664, 2016.
43. J. H. Rubenstein and N. J. Shaheen, "Epidemiology, Diagnosis, and Management of Esophageal Adenocarcinoma.," *Gastroenterology*, vol. 149, no. 2, p. 302–17.e1, Aug. 2015.

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Original article

Prediction of high salt and low potassium intake behavior from urinary sodium and potassium excretion in Japan

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Summary. Salt reduction policies have been implemented worldwide and increased potassium intake is also recommended. However, simultaneous investigations of dietary behavior and sodium and/or potassium intake estimated by urinary excretion are sparse. This study aimed to predict high salt and low potassium intake based on dietary behaviors in Japan. The study was comprised of 2627 participants aged 20 to 74 years (Men%: 50%) living in Niigata City, Japan. Participants completed a questionnaire about dietary behaviors potentially relevant to salt and potassium intake. A casual urine specimen for estimating dietary salt and potassium intake per day was collected the same day. The relationship of estimated dietary salt and potassium intake to questionnaire responses was examined using analysis of covariance for age, gender, and body mass index. Twenty-eight items on the questionnaire revealed an association with dietary salt and/or potassium intake and were divided into 4 categories: related to both high salt and low potassium intake, only high salt intake, only low potassium intake, and both high potassium and high salt intake. Identified were 28 dietary behaviors that enabled the prediction of high salt and low potassium intake behavior and provided information for encouraging decreased salt intake and increased potassium intake among Japanese.

Key words: salt intake, potassium intake, dietary behavior, casual urine specimen, Japanese

Introduction

Reduction of dietary salt intake is encouraged throughout the world to prevent hypertension, which is quantitatively the most important risk factor for cardiovascular disease (1): systolic blood pressure above 140 mmHg causes 51% of stroke deaths and 45% of ischemic heart disease deaths (2). Hypertension is more common than other major risk factors, including cigarette smoking, high blood glucose, and dyslipidemia (2). Many countries have salt reduction policies with various goals set for daily salt intake (<5.8 g/d in the United States (3), 6 g/day in United Kingdom (4), and 8.0 g/day in men and 7.0 g/day in women in Japan (5)). Several countries reported the relationships of salt intake to dietary behavior using information from urinary sodium excretion (6-8).

In addition to salt reduction, increasing potassium intake is recommended, such as ≥3510 mg/day by the World Health Organization (9), since supplementation with potassium produces vasodilatation and increases the urinary excretion of sodium chloride (10). To more effectively prevent hypertension, it is necessary to implement nutritional policies and education that would include both reduction of salt intake and increased potassium intake. There has not been evi-

dence of the value of intakes of salt and potassium estimated by urinary excretion. However, there have been several previous randomized controlled trials of the effect of adjustments in both intake of salt and potassium on blood pressure (11).

To provide effective nutrition policies and evaluation systems in preventing hypertension, it is necessary to assess dietary behavior that affects both high salt and low potassium intake and either salt or potassium intake because multiple nutrients are usually ingested with diets that include salt and potassium (12, 13). Intake of food and nutrition is affected by the food environment, such as whether food consumed is from a fast food restaurant, a full-service restaurant, or is homemade (14). Thus, we aimed this study to predict dietary behavior related to high salt and low potassium intake from a simultaneous sampling of urine sodium and potassium excretion among Japan participants.

Methods

The study was comprised of 2627 participants, aged 20 to 74 years, living in Niigata City, Japan, led by a working group in Niigata prefecture that provided data analysis and made policy recommendations to create a nutrition policy and evaluation system. The activities involving the study participants were carried out in November 2015 and 2016. Participants were selected in all districts of Niigata City, including Kita, Akiha, Minami, Higashi, Chuo, Konan, Nishi, and Nishikan. Recruitment of participants was done by a stratified random sampling method to recruit nearly equal numbers of participants into each of the six strata defined by gender (men and women) and age groups (20-39 years, 40-59 years, and 60-74 years) for each of the 8 districts. Call for entry for participation in this survey was made by mail to 8560 people. Of these, 2627 participants (30.7%) were examined at the public health center nearest to his/her residence. The participants consisted of 1302 men and 1325 women (Men%: 49.6%), and number of participants aged 20-39 years, 40-59 years, and 60-74 years were 822, 948, and 857, respectively. Within one day, participants answered a questionnaire on dietary habits, provided a casual urine specimen on the day the questionnaire was collected using a urine receptacle while alone. They also underwent other assessments on the same day. The questionnaire on dietary habits and the urine receptacle were sent by mail at the same time. The protocol for the study was performed in accordance with the Declaration of Helsinki and the Ethical Guidelines for Medical and Health Research Involving Human Subjects of the Japanese Ministry of Health Labor and Welfare, and was approved by the ethics committee of the University of Niigata prefecture (#1406). After providing written informed consent, participants were enrolled in this study.

A casual urine specimen for estimating dietary salt and dietary potassium intake per day among participants was collected from each participant. All specimens were refrigerated at 4°C for 24 hours and frozen at -20°C within 7 days and analyzed at a clinical laboratory center in Niigata City. Na and K studies were conducted by emission flame photometry and Cr by the Jaffé method. Estimated sodium excretion from a casual urine specimen was validated with that of 24-h urinary (24HU) excretion. The obtained formulas for 24-h urinary sodium excretion (24HUNaV) and potassium (24HUKV) excretion were reported as follows (15): (i) PRCr (mg/day) = -2.04 x age + 14.89 x weight (kg) + 16.14 x height (cm) -2244.45; (ii) estimated 24HUNaV (mEq/day) = 21.98 x $XNa^{0.392}$; and (iii) estimated 24HUKV (mEq/day) = 7.59 x $XK^{0.431}$; where PRCr = predicted value of 24HUCr, SUNa = Na concentration in the spot voided urine, SUK = K concentration in the spot voided urine, SUCr = creatinine concentration in the spot voided urine, and XNa (or XK) = SUNa (or SUK)/SUCr x PRCr. These were reported to be useful formulas for estimating population mean levels of 1-day Na and K excretion ($r = 0.54$, $p < 0.01$, and $r = 0.56$, $p < 0.01$, respectively). Estimated 24HUNaV and 24HUKV were calculated to reflect dietary salt and potassium intake, respectively, by the following formula according to their atomic weights: dietary salt intake (g/day) = 24HUNaV (mEq/day)/1000 ÷ 23.3× 58.4, and dietary potassium intake (mg/day) = 24HUKV (mEq /day) ÷ 39.1.

All participants completed a questionnaire on dietary behavior potentially relevant to salt and/or potassium intake. This questionnaire was comprised of 36 questions developed from an empirical perspective

gained from interviews with registered dietitians associated with public administrations or medical services. Construction of the questionnaire and possible responses are shown in Table 2. The questionnaire was based on the frequency of healthy eating behavior (No. 1-5), intake of salty food (No. 6-15), and potassium-rich food (No. 7-9, 15, and 16), as well as behavior related to salt restriction (No. 17-20, 32, 33, and 36) and increased potassium intake (No. 17, 18, 21, 23-30, and 36). The questionnaire also elicited information on the participant's knowledge related to salt restriction (No. 34 and 35) and increased potassium intake (No. 22, 31, and 35). Height and weight of all participants were measured by trained healthcare professionals including doctors, nurses, and dietitians. Accuracy of the entire instrument was provided based on the Japanese Measurement Act.

Participants' characteristics were described as mean ± SD or percentages. Differences in salt and potassium intake between the major characteristics according to gender, age, and body mass index (BMI), household composition, and smoking status were examined by t-tests or linear regression analysis. Multiple linear regression analyses were used for examining the relationships of salt and potassium intake to each answer to the questionnaire regarding dietary behavior potentially relevant to salt and/or potassium intake. Data were then examined after adjustment for gender, age (20-39, 40-59, 60-70 years), BMI (<18.5, 18.5-24.9, ≥ 25.0 kg/m^2), household composition (1 person, 2 persons from 1 generation, 2 generations, >2 generations), and smoking status (Yes, Quit, Never). The associations were presented as linear regression coefficients (B) and p-values. All p-values are two-sided, and the significance level is 0.05. All statistical analyses were carried out using SPSS Statistics 23 (IBM, New York, NY, USA).

Results

Characteristics of participants

Daily salt and potassium intake according to the characteristics of the 2627 participants are shown in Table 1. Participants' mean age was 49.1 years, mean BMI was 22.6 kg/m^2, and 49.6% were men. Mean daily salt intake was 10.0 and 9.5 g in men and women, respectively (p<0.001). Participants with higher salt intake included significantly more men and those of an older age, with a high BMI, and who had quit

Table 1. Characteristics of participants who completed a questionnaire on dietary habits and who underwent spot collection of urine.

		Salt intake (g/day)						Potassium intake (g/day)				
		N	Mean	±	SD	B	p	Mean	±	SD	B	p
Gender	Men	1302	10.0	±	2.4	-	< 0.001	1644.1	±	365.1	-	0.287
	Women	1325	9.5	±	2.2			1659.4	±	374.5		
Age group	20-39 years	822	9.4	±	2.3			1545.4	±	376.7		
	40-59 years	948	10.0	±	2.3	0.2	< 0.001	1651.9	±	358.6	104.2	< 0.001
	60-74 years	857	9.9	±	2.2			1753.9	±	346.4		
Household composition	1 person	195	9.9	±	2.5			1674.9	±	389.8		
	2 persons from 1 generation	545	9.9	±	2.3	-0.1	0.22	1723.2	±	363.0	-31.5	< 0.001
	2 generations	1408	9.7	±	2.3			1625.7	±	364.6		
	>2 generations	479	9.8	±	2.3			1638.2	±	374.7		
BMI	<18.5 kg/m^2	229	8.9	±	2.3			1504.5	±	379.0		
	18.5-24.9 kg/m^2	1872	9.6	±	2.2	0.2	< 0.001	1651.0	±	368.0	95.6	< 0.001
	≥ 25.0 kg/m^2	526	10.6	±	2.3			1718.9	±	354.2		
Smoking	Yes	467	9.9	±	2.4			1603.6	±	356.0		
	Quit	514	10.1	±	2.2	-0.2	0.004	1678.1	±	332.9	19.9	0.032
	Never	1646	9.6	±	2.3			1657.3	±	383.5		

Table 2. Dietary salt intake and responses to the questionnaire on dietary habits potentially relevant to salt and/or potassium intakes

			Sodium intake			
No. Questionnaire items	**Answer**	**n**	**Mean**	**[95% CI]**	**B**	**p**
1. Frequency of alcohol consumption	Daily	524	10.1	[9.8-10.3]		
	5-6 times/week	212	9.8	[9.5-10.1]		
	3-4 times/week	211	9.9	[9.6-10.2]		
	1-2 times/week	257	9.6	[9.4-9.9]	-0.1	0.001
	1-3 times/month	260	9.7	[9.5-10.0]		
	Rarely	560	9.8	[9.6-10.0]		
	Never	603	9.5	[9.3-9.7]		
2. No. meals/day consisting of a staple food, main dish, and side dishes	3 times/day	737	9.6	[9.4-9.8]		
	2 times/day	1027	9.8	[9.7-9.9]	0.1	0.033
	1 time/day	863	9.9	[9.7-10.0]		
3. Makes oneself gorge on a meal	Usually	1345	9.9	[9.8-10.0]		
	Sometimes	958	9.7	[9.6-9.8]	-0.2	< 0.001
	Really	324	9.4	[9.1-9.6]		
4. Frequency of ≥2 different staples foods	≥ 3 times/week	269	10.1	[9.8-10.4]		
	1-2 times/week	753	10.1	[9.9-10.2]	-0.3	< 0.001
	< 1 time/week	1605	9.6	[9.5-9.7]		
5. Consumption of 1-dish meals	≥ 3 times/week	62	10.3	[9.8-10.9]		
	1-2 times/week	1444	9.8	[9.7-9.9]	-0.1	0.108
	< 1 time/week	1121	9.7	[9.6-9.8]		
6. Consumption of noodles	≥ 3 times/week	408	10.1	[9.9-10.3]		
	1-2 times/week	1586	9.8	[9.7-9.9]	-0.4	< 0.001
	< 1 time/week	633	9.4	[9.2-9.6]		
7. Quantity of soup consumed when eating noodles	Total volume	518	10.3	[10.1-10.5]		
	1/2	795	9.9	[9.7-10.1]	-0.4	< 0.001
	1/3	698	9.8	[9.6-9.9]		
	very little	616	9.1	[9.0-9.3]		
8. No. servings of soups	≥ 3 servings/day	143	10.0	[9.6-10.4]		
	2 servings/day	761	10.0	[9.9-10.2]	-0.2	< 0.001
	1 serving/day	1346	9.7	[9.6-9.8]		
	< 1 serving/day	377	9.5	[9.3-9.7]		
9. No. servings of simmered dishes	≥ 4 servings/day	135	9.7	[9.4-10.1]		
	2-3 servings/day	833	9.8	[9.7-10.0]	0.0	0.406
	1 serving/day	1212	9.8	[9.6-9.9]		
	< 1 serving/day	447	9.7	[9.5-9.9]		
10. Consumption of salt-cured fish eggs	≥ 1 times/day	173	10.1	[9.7-10.4]		
	1 time/2 days	431	10.2	[10.0-10.4]	-0.3	< 0.001
	< 1 time/2 days	2023	9.6	[9.5-9.7]		
11. Consumption of salted fish	≥ 1 times/day	254	10.0	[9.7-10.2]		
	1 time/2 days	972	9.9	[9.7-10.0]	-0.2	0.008
	< 1 time/2 days	1401	9.7	[9.5-9.8]		
12. Consumption of processed meat products	≥ 1 time/day	404	9.9	[9.6-10.1]		
	1 time/2 days	1456	9.8	[9.6-9.9]	0.0	0.534
	< 1 times/2 days	767	9.8	[9.6-9.9]		

(Continued)

Table 2. (continued) Dietary salt intake and responses to the questionnaire on dietary habits potentially relevant to salt and/or potassium intakes

		Sodium intake				
No. Questionnaire items	**Answer**	**n**	**Mean**	**[95% CI]**	**B**	**p**
13.Consumption of fish paste products (e.g. Kamaboko, chikuwa)	≥ 1 time/day	112	9.9	[9.5-10.3]		
	1 time/2 days	1144	9.8	[9.7-10.0]	-0.1	0.141
	< 1 time/2 days	1371	9.7	[9.6-9.8]		
14. Consumption of salty snacks	≥ 1 times/day	653	9.9	[9.7-10.0]		
	1 time/2 days	862	9.7	[9.6-9.9]	-0.1	0.195
	< 1 time/2 days	1112	9.7	[9.6-9.9]		
15. Consumption of pickled vegetables	≥ 1 times/day	433	10.2	[9.9-10.4]		
	1 times/2 days	1082	9.8	[9.7-9.9]	-0.3	< 0.001
	< 1 times/2 days	1112	9.6	[9.4-9.7]		
16. No. vegetable side dishes	≥ 5 servings/day	324	9.6	[9.3-9.8]		
	3-4 servings/day	1022	9.7	[9.6-9.9]	0.1	0.041
	< 2 serving/day	1281	9.8	[9.7-10.0]		
17. Frequency of eating out	≥ 4 times/week	103	9.9	[9.4-10.3]		
	2-3 times/week	598	9.8	[9.6-10.0]	-0.1	0.538
	<2 times/week	1926	9.8	[9.7-9.9]		
18. Frequency of using delicatessen food	≥ 4 times/week	422	9.9	[9.7-10.1]		
	2-3 times/week	1043	9.8	[9.7-9.9]	-0.1	0.035
	<2 times/week	1162	9.7	[9.5-9.8]		
19. Attempts use of dashi flavor, broth, the stock and the natural taste of food	trying actively	514	9.7	[9.5-9.8]		
	trying a little	715	10.0	[9.8-10.1]	0.0	0.837
	not trying much	830	9.7	[9.5-9.8]		
	not trying at all	568	9.7	[9.6-9.9]		
20. Prefers strong-tasting meals	Yes	980	10.0	[9.9-10.2]	-0.4	< 0.001
	No	1647	9.6	[9.5-9.7]		
21 Prefers eating vegetables	Prefers	1587	9.8	[9.7-9.9]		
	Prefers relatively	752	9.7	[9.5-9.9]	0.0	0.695
	Prefers a little	263	9.8	[9.5-10.1]		
	Does not prefer	25	9.6	[8.8-10.5]		
22. Ability to cook vegetable side dishes	Being able	1366	9.8	[9.6-9.9]		
	Being relatively able	648	9.7	[9.5-9.9]	0.0	0.568
	Being relatively unable	307	9.9	[9.6-10.2]		
	Being unable	306	9.8	[9.5-10.1]		
Importance of the following points for buying and eating vegetables:						
23. freshness	Very important	1400	9.7	[9.6-9.9]		
	Quite important	1101	9.8	[9.7-9.9]	0.0	0.911
	Not so important	93	9.8	[9.3-10.2]		
	Not at all important	33	9.4	[8.6-10.2]		
24. Seasonal foods	Very important	749	9.7	[9.5-9.9]		
	Quite important	1376	9.8	[9.7-9.9]	0.1	0.128
	Not so important	401	9.8	[9.6-10.0]		
	Not at all important	101	10.1	[9.7-10.6]		
25. Reasonable prices	Very important	1465	9.8	[9.7-9.9]		
	Quite important	1005	9.7	[9.6-9.9]	-0.1	0.136
	Not so important	110	9.6	[9.2-10.0]		
	Not at all important	47	9.5	[8.8-10.1]		

Table 2. (continued) Dietary salt intake and responses to the questionnaire on dietary habits potentially relevant to salt and/or potassium intakes

			Sodium intake			
No. Questionnaire items	**Answer**	**n**	**Mean**	**[95% CI]**	**B**	**p**
26. Local products	Very important	442	9.6	[9.4-9.8]		
	Quite important	1007	9.7	[9.6-9.9]	0.1	0.175
	Not so important	799	9.9	[9.8-10.1]		
	Not at all important	379	9.7	[9.5-9.9]		
27. Safety	Very important	1329	9.8	[9.7-9.9]		
	Quite important	1033	9.7	[9.6-9.8]	0.0	0.904
	Not so important	217	10.1	[9.8-10.4]		
	Not at all important	48	9.5	[8.8-10.1]		
28. Preference of oneself and one's family	Very important	1072	9.8	[9.6-9.9]		
	Quite important	1337	9.8	[9.6-9.9]	0.0	0.943
	Not so important	166	9.8	[9.4-10.1]		
	Not at all important	52	9.7	[9.1-10.3]		
29. Time and effort for cooking	Very important	435	9.7	[9.5-9.9]		
	Quite important	1271	9.8	[9.7-9.9]	0.0	0.854
	Not so important	697	9.8	[9.6-9.9]		
	Not at all important	224	9.6	[9.4-9.9]		
30. Health effects	Very important	804	9.7	[9.6-9.9]		
	Quite important	1350	9.8	[9.6-9.9]	0.0	0.474
	Not so important	389	9.9	[9.6-10.1]		
	Not at all important	84	9.8	[9.3-10.2]		
31. Number of vegetable side dishes per day considered for health	Don't know	142	9.6	[9.2-10.0]		
	1-2 servings/day	250	10.1	[9.8-10.4]		
	3-4 servings/day	1155	9.8	[9.6-9.9]	-0.1	0.213
	5-6 servings/day	810	9.7	[9.6-9.9]		
	≥ 7 servings/day	270	9.6	[9.3-9.9]		
32. Trying to reduce salt intake	Trying actively	261	9.3	[9.1-9.6]		
	Trying a little	1049	9.7	[9.5-9.8]	0.2	< 0.001
	Not trying much	1051	9.9	[9.8-10.1]		
	Not trying at all	266	10.0	[9.7-10.2]		
33. Knowing the amount of salt intake per day desirable for health	Yes	1238	9.7	[9.6-9.8]	-0.2	0.060
	No	1389	9.8	[9.7-10.0]		
34. Recognizing one's salt intake as moderate	A little	86	9.0	[8.5-9.4]		
	Relatively small	212	9.6	[9.3-9.9]		
	Moderate	873	9.6	[9.4-9.7]	0.3	< 0.001
	Relatively great	1250	9.9	[9.8-10.0]		
	Very great	206	10.3	[10.0-10.6]		
35. Knowing that potassium is highly involved in vegetables and fruits and promotes urinary sodium excretion	Yes	1170	9.6	[9.5-9.8]	0.2	0.015
	No	1457	9.9	[9.7-10.0]		
36. Checks nutrition facts when buying food and eating out	Checks actively	200	9.5	[9.2-9.9]		
	Checks a little	787	9.8	[9.6-9.9]		
	Not checks much	671	9.7	[9.6-9.9]	0.1	0.201
	Not checks at all	918	9.8	[9.7-10.0]		
	Don't know	51	9.7	[9.1-10.3]		

Analyses were conducted by analysis of covariance for gender, age group, household composition, BMI (<18.5, 18.5-24.9, ≥ 25.0), and smoking status.

smoking (p<0.001, <0.001, <0.001, and 0.004, respectively). There were no significant trends in household composition among values for salt intake. As to daily potassium intake, mean consumption by men and women were 1644.1 mg and 1659.4 mg, respectively (p= 0.287). Potassium intake differed significantly according to gender, age group, household composition, and smoking status.

Dietary salt intake and responses to the questionnaire

The association between dietary salt intake according to responses to the questionnaire on dietary behaviors potentially relevant to urinary values for salt and/or potassium intake is shown in Table 2. Sixteen of the 36 items on the questionnaire showed relationships with high salt intake per day, and 15 of these 16 items were questions relevant to healthy eating behavior, salty food intake, and behavior and knowledge related to salt restriction. Those 15 were high frequency of alcohol consumption (p = 0.001), small number of meals consisting of a staple food, main dish, and side dishes per day (p= 0.033), high frequency of making oneself gorge on a meal (p <0.001), high frequency of having ≥2 different staple foods per meal (p <0.001), high frequency of having noodles (p < 0.001), large quantity of soup consumed when eating noodles (p <0.001), large number of soups per day (p <0.001), high frequency of having salt-cured fish eggs per day (p <0.001), high frequency of having salted fish per day (p =0.008), high frequency of having pickled vegetables per day (p <0.001), high frequency of using delicatessen food (p =0.035), preference for strong-tasting meals (p <0.001), not trying to reduce salt intake (p <0.001), estimating one's own salt intake as more than moderate (p <0.001), and not knowing that vegetables and fruits have high levels of potassium, which and promotes urinary sodium excretion (p = 0.015). The remaining 1 item was a question about potassium-rich food intake, and a small number of vegetable side dishes per day was significantly associated with high dietary salt intake (p =0.041).

Potassium intake and responses to the questionnaire

As for low potassium intake, an association was shown for potassium intake in 23 of the 36 items (Table 3). These 23 items included not only questions related to healthy eating behavior, potassium-rich food intake, and behavior and knowledge related to increased potassium intake but also those related to salty food intake and knowledge and behavior related to salt restriction. These items included low frequency of alcohol consumption (p <0.001), small number of meals consisting of a staple food, main dish, and side dishes per day (p <0.001), low frequency of making oneself gorge on a meal (p =0.016), and high frequency of having ≥2 different staple foods per meal (p <0.001) as healthy eating behavior. A small quantity of soup consumed when eating noodles (p =0.015), small number of soups per day (p <0.001), small number of simmered dishes per day (p <0.001), low frequency of having pickled vegetables per day (p = 0.035), and a small number of vegetable side dishes per day (p <0.001) indicated intake of potassium-rich food. Five of these 6 items were also items relevant to salty food intake. As to behavior and knowledge relevant to increased potassium intake, the following apply: high frequency of using delicatessen food (p =0.006), not preferring to eat vegetables (p <0.001), being unable to cook vegetable side dishes (p = 0.001), not considering freshness important when buying and eating vegetables (p = 0.001), seasonal foods (p = 0.005), local products (p = 0.008), safety (p = 0.006), and health effects (p <0.001), not knowing that a small number of vegetable side dishes per day is important for health (p = 0.001), not knowing that potassium is highly involved in vegetables and fruits and promotes urinary sodium excretion (p <0.001), and not checking nutrition facts when buying food and eating out (p <0.001). Not trying to use dashi flavor, broth, stock, and the natural taste of food (p <0.001), not trying to reduce salt consumption (p = 0.001), not knowing the amount of desirable salt intake per day for health, and self evaluation of one's salt intake as less than moderate (p = 0.004) were items involving behavior and knowledge related not to increased potassium intake but to restriction of salt intake.

Summary of questionnaire items of dietary habits related to both dietary salt and potassium intake

Table 4 summarizes the questionnaire items for dietary habits related to dietary salt and potassium intakes. A total of 28 of 36 items were related to dietary

Table 3. Dietary potassium intake and responses to the questionnaire on dietary habits potentially relevant to salt and/or potassium intakes

No. Questionnaire items	Answer	n	Mean	[95%CI]	B	p
1. Frequency of alcohol consumption	Daily	524	1694.4	[1662.1-1726.7]		
	5-6 times/week	212	1696.5	[1648.5-1744.6]		
	3-4 times/week	211	1742.8	[1694.8-1790.9]	-16.7	< 0.001
	1-2 times/week	257	1627.9	[1584.3-1671.4]		
	1-3 times/month	260	1645.1	[1601.3-1688.9]		
	Rarely	560	1625.5	[1595.7-1655.3]		
	Never	603	1604.8	[1576.0-1633.7]		
2. No. meals/day consisting of a staple food, main dish, and side dishes	3 times/day	737	1685.5	[1659.3-1711.7]		
	2 times/day	1027	1666.8	[1645.0-1688.6]	-40.7	< 0.001
	1 time/day	863	1605.3	[1581.1-1629.5]		
3. Makes oneself gorge on a meal	Usually	1345	1665.0	[1645.5-1684.4]		
	Sometimes	958	1649.0	[1626.3-1671.7]	-25.5	0.016
	Really	324	1605.8	[1565.5-1646.0]		
4. Frequency of ≥2 different staple foods	≥ 3 times/week	269	1651.0	[1608.0-1694.0]		
	1-2 times/week	753	1644.4	[1618.6-1670.2]	5.0	0.638
	< 1 time/week	1605	1655.5	[1637.8-1673.1]		
5. Consumption of 1-dish meals	≥ 3 times/week	62	1714.1	[1624.6-1803.5]		
	1-2 times/week	1444	1646.3	[1627.8-1664.9]	0.4	0.978
	< 1 time/week	1121	1655.5	[1634.3-1676.6]		
6. Consumption of noodles	≥ 3 times/week	408	1657.4	[1622.2-1692.5]		
	1-2 times/week	1586	1653.0	[1635.4-1670.7]	-6.3	0.585
	< 1 time/week	633	1645.3	[1617.0-1673.5]		
7. Quantity of soup consumed when eating noodles	Total volume	518	1659.7	[1627.8-1691.7]		
	1/2	795	1676.8	[1651.8-1701.7]	-17.3	0.015
	1/3	698	1648.9	[1622.3-1675.6]		
	very little	616	1616.4	[1587.4-1645.4]		
8. No. servings of soups	≥ 3 servings/day	143	1694.7	[1635.8-1753.7]		
	2 servings/day	761	1687.2	[1661.7-1712.6]	-34.6	< 0.001
	1 serving/day	1346	1637.7	[1618.6-1656.8]		
	< 1 serving/day	377	1614.6	[1578.4-1650.9]		
9. No. servings of simmered dishes	≥ 4 servings/day	135	1722.2	[1661.1-1783.3]		
	2-3 servings/day	833	1683.4	[1658.6-1708.1]	-37.4	< 0.001
	1 serving/day	1212	1636.2	[1616.0-1656.4]		
	< 1 serving/day	447	1614.3	[1580.3-1648.2]		
10. Consumption of salt-cured fish	≥ 1 times/day	173	1625.2	[1571.6-1678.8]		
	1 time/2 days	431	1652.3	[1618.2-1686.5]	10.2	0.403
	< 1 time/2 days	2023	1654.0	[1638.4-1669.6]		
11. Consumption of salted fish eggs	≥ 1 times/day	254	1659.4	[1615.4-1703.3]		
	1 time/2 days	972	1672.0	[1649.2-1694.8]	-19.8	0.065
	< 1 time/2 days	1401	1636.5	[1617.5-1655.5]		
12. Consumption of processed meat products	≥ 1 time/day	404	1631.3	[1596.1-1666.4]		
	1 time/2 days	1456	1655.6	[1637.2-1674.0]	10.0	0.359
	< 1 times/2 days	767	1655.6	[1630.0-1681.2]		
13. Consumption of fish paste products (eg. Kamaboko, chikuwa)	≥ 1 time/day	112	1596.0	[1529.8-1662.2]		
	1 time/2 days	1144	1667.9	[1647.1-1688.7]	-6.4	0.598
	< 1 time/2 days	1371	1643.0	[1624.0-1662.0]		
14. Consumption of salty snacks	≥ 1 times/day	653	1646.4	[1618.9-1673.9]		
	1 time/2 days	862	1663.9	[1640.0-1687.8]	-2.0	0.824
	< 1 time/2 days	1112	1645.6	[1624.5-1666.8]		

(Continued)

Table 3. (continued) Dietary potassium intake and responses to the questionnaire on dietary habits potentially relevant to salt and/or potassium intakes

No. Questionnaire items	Answer	n	Mean	[95%CI]	B	p
15. Consumption of pickled vegetables	≥ 1 times/day	433	1671.7	[1637.1-1706.3]		
	1 times/2 days	1082	1662.8	[1641.4-1684.2]	-21.1	0.039
	< 1 times/2 days	1112	1633.4	[1611.8-1655.1]		
16. No. vegetable side dishes	≥ 5 servings/day	324	1752.0	[1712.6-1791.3]		
	3-4 servings/day	1022	1666.8	[1644.7-1688.8]	-64.0	< 0.001
	< 2 serving/day	1281	1614.6	[1594.6-1634.6]		
17. Frequency of eating out	≥ 4 times/week	103	1622.7	[1552.8-1692.5]		
	2-3 times/week	598	1650.1	[1621.0-1679.2]	9.3	0.486
	<2 times/week	1926	1653.9	[1637.8-1670.0]		
18. Frequency of using delicatessen food	≥ 4 times/week	422	1625.3	[1590.9-1659.6]		
	2-3 times/week	1043	1638.3	[1616.7-1660.0]	26.5	0.006
	<2 times/week	1162	1673.6	[1653.0-1694.2]		
19. Attempts use of dashi flavor, broth, the stock and the natural taste of food	trying actively	514	1694.2	[1663.2-1725.3]		
	trying a little	715	1677.7	[1651.5-1703.9]	-28.8	< 0.001
	not trying much	830	1626.2	[1601.9-1650.6]		
	not trying at all	568	1618.3	[1588.7-1648.0]		
20. Prefers strong-tasting meals	Yes	980	1633.8	[1610.9-1656.6]	28.8	0.054
	No	1647	1662.6	[1645.1-1680.1]		
21 Prefers eating vegetables	Prefers	1587	1688.7	[1670.9-1706.4]		
	Prefers relatively	752	1603.0	[1577.5-1628.6]	-60.6	< 0.001
	Prefers a little	263	1571.7	[1527.7-1615.7]		
	Does not prefer	25	1625.8	[1486.1-1765.5]		
22. Ability to cook vegetable side dishes	Being able	1366	1680.0	[1660.3-1699.7]		
	Being relatively able	648	1621.2	[1593.7-1648.7]	-25.9	0.001
	Being relatively unable	307	1641.2	[1600.3-1682.1]		
	Being unable	306	1601.7	[1559.5-1643.9]		
Importance of the following points for buying and eating vegetables:						
23. freshness	Very important	1400	1663.7	[1644.9-1682.4]		
	Quite important	1101	1651.3	[1630.2-1672.4]	-37.0	0.001
	Not so important	93	1545.0	[1472.2-1617.8]		
	Not at all important	33	1469.6	[1347.6-1591.6]		
24. Seasonal foods	Very important	749	1676.3	[1650.7-1702.0]		
	Quite important	1376	1653.3	[1634.4-1672.2]	-26.0	0.005
	Not so important	401	1605.0	[1569.7-1640.4]		
	Not at all important	101	1636.2	[1565.9-1706.5]		
25. Reasonable prices	Very important	1465	1649.3	[1630.8-1667.8]		
	Quite important	1005	1660.3	[1637.9-1682.7]	-3.9	0.713
	Not so important	110	1638.3	[1571.4-1705.2]		
	Not at all important	47	1582.3	[1479.7-1684.9]		
26. Local products	Very important	442	1665.3	[1631.6-1698.9]		
	Quite important	1007	1673.2	[1651.0-1695.3]	-20.6	0.008
	Not so important	799	1635.0	[1610.1-1659.9]		
	Not at all important	379	1615.0	[1578.4-1651.5]		
27. Safety	Very important	1329	1662.4	[1643.1-1681.6]		
	Quite important	1033	1651.3	[1629.5-1673.0]	-26.9	0.006
	Not so important	217	1630.6	[1582.6-1678.5]		
	Not at all important	48	1469.4	[1368.3-1570.6]		

Table 3. (continued) Dietary potassium intake and responses to the questionnaire on dietary habits potentially relevant to salt and/or potassium intakes

No. Questionnaire items	Answer	n	Mean	[95%CI]	B	p
28. Preference of oneself and one's family	Very important	1072	1643.7	[1622.2-1665.2]		
	Quite important	1337	1667.1	[1648.0-1686.3]	-6.8	0.513
	Not so important	166	1612.1	[1557.7-1666.5]		
	Not at all important	52	1553.3	[1456.0-1650.7]		
29. Time and effort for cooking	Very important	435	1626.6	[1592.9-1660.4]		
	Quite important	1271	1665.5	[1645.9-1685.2]	-0.1	0.988
	Not so important	697	1649.3	[1622.7-1676.0]		
	Not at all important	224	1630.8	[1583.9-1677.7]		
30. Health effects	Very important	804	1676.8	[1652.0-1701.5]		
	Quite important	1350	1657.6	[1638.6-1676.6]	-38.4	< 0.001
	Not so important	389	1606.2	[1570.4-1642.1]		
	Not at all important	84	1531.7	[1455.1-1608.3]		
31. Number of vegetable side dishes	Don't know	142	1629.2	[1570.3-1688.1]		
per day considered for health	1-2 servings/day	250	1628.6	[1584.3-1673.0]	23.5	0.001
	3-4 servings/day	1155	1634.6	[1613.9-1655.3]		
	5-6 servings/day	810	1666.9	[1642.1-1691.7]		
	≥ 7 servings/day	270	1713.9	[1671.1-1756.7]		
32. Trying to reduce salt intake	Trying actively	261	1677.2	[1633.1-1721.2]		
	Trying a little	1049	1680.1	[1658.1-1702.0]	-31.4	0.001
	Not trying much	1051	1628.2	[1606.3-1650.0]		
	Not trying at all	266	1609.2	[1565.5-1652.9]		
33. Knowing the amount of salt intake	Yes	1238	1682.7	[1662.6-1702.7]	58.3	< 0.001
per day desirable for health	No	1389	1624.4	[1605.5-1643.3]		
34. Recognizing one's salt intake	A little	86	1517.0	[1441.0-1593.1]		
as moderate	Relatively small	212	1625.3	[1577.1-1673.4]	23.6	0.004
	Moderate	873	1654.8	[1631.1-1678.6]		
	Relatively great	1250	1660.2	[1640.4-1680.0]		
	Very great	206	1671.9	[1622.6-1721.1]		
35. Knowing that potassium is highly involved in	Yes	1170	1688.1	[1667.4-1708.7]	-65.3	< 0.001
vegetables and fruits and promotes urinary sodium excretion	No	1457	1622.7	[1604.3-1641.2]		
36. Checks nutrition facts	Checks actively	200	1670.4	[1620.6-1720.1]		
when buying food and eating out	Checks a little	787	1701.6	[1676.3-1726.9]	-34.0	< 0.001
	Not checks much	671	1651.8	[1624.8-1678.9]		
	Not checks at all	918	1608.3	[1584.8-1631.8]		
	Don't know	51	1594.7	[1496.5-1693.0]		

Analyses were conducted by analysis of covariance for gender, age group, household composition, BMI (<18.5, 18.5-24.9, ≥ 25.0), and smoking status.

salt and/or potassium intakes and were divided into 4 categories as related to both high salt and low potassium intake, only to high salt intake, only to low potassium intake, and to both high potassium and high salt intake. However, 11 of the 28 items were inconsistent with registered dietitians' initial expectations regarding the relationship to salt and/or potassium intake.

Five question items involved relationships with both high salt and low potassium intake; of these 5 items there was 1 item that was expected to indicate healthy eating behavior (No. 2), 1 item expected to indicate behavior for both salt restriction and increased potassium intake (No. 18), 1 item expected to indicate knowledge on both salt restriction and increased potassium intake (No. 35), 1 item expected to be only related to knowledge on salt restriction (No. 32), and

Table 4. Summary of questionnaire items of dietary habits related with dietary salt and potassium intakes

No. Questionnaire items	Type of question
Items related to both high salt and low potassium intakes	
2. No. meals/day consisting of a staple food, main dish, and side dishes	HE
16. Small number of vegetable side dishes	PI
18. High frequency of using delicatessen food	SB, PB
32. Trying to reduce salt intake	SB
35. Knowing that potassium is highly involved in vegetables and fruits and promotes urinary sodium excretion	SK, PK
Items related with only high salt intake	
4. Frequency of having ≥2 different staple foods	HE
6. Consumption of noodles	SI
10. Consumption of salt-cured fish eggs	SI
11. Consumption of salted fish	SI
20. Prefers strong-tasting meals	SB
Items related with only low potassium intake	
9. No. servings of simmered dishes	SI, PI
19. Attempts use of dashi flavor, broth, the stock and the natural taste of food	SB
21. Prefers eating vegetables	PB
22. Ability to cook vegetable side dishes	PK
Not considering the following points to be important for buying and eating vegetables:	
23. Freshness	PB
24. Seasonal foods	PB
26. Local products	PB
27. Safety	PB
30. Health effects	PB
31. No. vegetable side dishes per day considered for health	PK
33. Knowing the amount of salt intake per day desirable for health	SB
36. Checks nutrition facts when buying food and eating out	SB, PB
Items related with both high potassium and high salt intake	
1. Frequency of alcohol consumption	HE
3. Makes oneself gorge on a meal	HE
7. Quantity of soup consumed when eating noodles	SI
8. No. servings of soups	SI, PI
15. Consumption of pickled vegetables	SI
34. Recognizing one's salt intake as moderate	SK

Abbreviations: HE, healthy eating behavior; SI, salty food intake; PI, potassium-rich food intake, SB, behavior for salt restriction; PB, behavior for increased potassium intake; SK, knowledge for salt restriction; PK, knowledge for increased potassium intake.

1 item expected to be only related to intake of potassium-rich food (No. 16). Five items involved only high salt intake and these items were anticipated to be about healthy eating behavior (No. 4), salty food intake (No.6, 10, and 11), and behavior for salt restriction (No. 20). There were 12 items only related to low potassium intake: 6 of the 12 items were the questions expected to indicate behavior for increased

potassium intake (No. 21, 23, 24, 26, 27, and 30), 2 were expected to indicate knowledge about increased potassium intake (No. 22 and 31), 2 were expected to indicate behavior regarding salt restriction (No. 19 and 33), 1 was expected to indicate intake of both salty and potassium-rich food (No. 9), and 1 was expected to indicate behavior about both salt restriction and increased potassium intake (No. 36). Six items related to both high potassium and high salt intake were found. Of these, 2 items were anticipated to involve healthy eating behavior (No. 1 and 3), 2 items anticipated salty food intake (No. 7 and 15), 1 item was anticipated to involve intake of both salty and potassium-rich food (No. 8), 1 item was about potassium-rich food intake (No. 32), and 1 item was anticipated to indicate knowledge about salt restriction (No. 15).

Discussion

Dietary salt reduction and increasing potassium intake are encouraged worldwide for reduction of blood pressure to decrease the incidence of cardiovascular disease such as stroke and coronary heart disease. These challenges are especially important for Japan as Japan has the highest dietary salt intake among developed countries (16). However, simultaneous investigations of salt and/or potassium intake and dietary behavior relating to high salt and low potassium intake are sparse. Our current study addressed this issue with the cooperation of Niigata city, Japan. Our findings can contribute to developing evidence-based education on reducing salt intake and increasing potassium intake for Japanese people.

Our results showed that a total of 28 of 36 items on the questionnaire developed from an empirical perspective gained from registered dietitians associated with public administrations or medical services were related to dietary salt and/or potassium intake. Given that the majority of items were related to salt and/or potassium intake, it was reasonable to assume that the questionnaire generally followed the lines of questionnaires used in previous dietary education to reduce salt intake. However, results that were inconsistent with registered dietitians' initial expectations regarding the relationship with salt and/or potassium intake were obtained for 11 of the 28 items. Because of this, attention should be paid when providing information based on these items to participants in community salt reduction interventions so as not to mislead them. However, the majority of the questionnaire items were relevant to salt and/or potassium intake.

Among the five questionnaire items categorized as "Items related to both high salt and low potassium intakes", "Small number of vegetable side dishes per day" (item No. 16) and "Not trying to reduce salt intake" (item No. 32) had initially been expected to be related to "potassium-rich food intake" and "behavior for salt restriction", respectively. Regarding item No. 16, a previous report demonstrated that individuals who consumed vegetables infrequently and in small quantities had a low level of health awareness, including awareness of salt reduction (17), suggesting that low vegetable consumption and associated unhealthy eating habits in terms of salt intake may have led to low potassium intake as well as high salt intake. Similarly, individuals who do not engage in reducing salt intake tend to eat vegetables less frequently and in small quantities (17). This may explain the relationship of item No. 32 with both high salt and low potassium intake. In summary, individuals who report eating a small number of vegetable side dishes and those who report not trying to reduce salt intake are likely to adopt other unhealthy eating habits in terms of salt and potassium intakes, highlighting the importance of encouraging this group of individuals to acquire the knowledge and skills to reduce salt intake in their daily lives through educational interventions.

All five items in the "Items related to only high salt intake" category had been expected by registered dietitians experienced in community nutrition to be related to healthy eating behaviors or salt intake. This result may support the validity of their expectations and indicates that nutritional strategies focusing on these items should continue to be implemented in the future.

Among the 12 items categorized as "Items related to only low potassium intake", "Small number of simmered dishes per day" (item No. 9) had initially been believed to be related to "Behavior for both salt restriction and increased potassium intake", while "Not trying to use dashi flavor, broth, stock and the natural taste of

food" (item No. 19) and "Not knowing the amount of desirable salt intake per day for health" (item No. 33) were initially believed to be related to "Behavior for salt restriction only", and "Not checking nutrition facts when buying food and eating out" (item No. 36) had been believed to be related to "Behavior for both salt restriction and increased potassium intake". Regarding item No. 9, since simmered dishes are often prepared with vegetables and traditionally high-salt seasonings, such as soy sauce and *miso* (18), we hypothesized when designing the questionnaire that the consumption of simmered dishes would increase both potassium and salt intakes. However, it was found that the consumption of simmered dishes was significantly related only to potassium intake in this questionnaire. This may be because the use of those seasoning products has decreased in the last 20 years, as reported in the National Health and Nutrition Survey in Japan (19-20), and that foreign food cultures, including the use of Western seasonings and spices with a relatively low salt content, have become popular (21). In the present study, a significant relationship with the consumption of simmered dishes was only observed for potassium intake, a parameter associated with vegetable consumption. With respect to item No. 19, stocks and broths were reported to enhance the perceived taste and flavor of low-salt diets because of their umami substance contents (22). On that basis, we hypothesized that the effective use of stocks and broths in meals would reduce salt intake. However, this study failed to detect a significant relationship between not making good use of stocks, broths, or the natural flavors of foods and salt intake. Thus, it is recommended that ways to make stocks and broths in participants' homes be assessed, and that appropriate methods for their preparation be promoted through cooking classes and other activities. A possible reason for the significant relationship with high potassium intake is that stocks and broths are often used in soups and simmered dishes, which are commonly prepared with vegetables (18). Thus, individuals who do not use stocks and broths are likely to have a low-vegetable, i.e., low-potassium, diet.

Knowing appropriate healthy levels of daily salt intake (item No. 33) would logically be expected to be related to salt intake, and referring to food labels when purchasing food or making eating-out decisions (item No. 36) to salt and potassium intake. However, these items were found to be related only to potassium intake. The results suggest that individuals who recognize appropriate levels of daily salt intake may have a high level of awareness about healthy diets, such as those rich in vegetables (17), but may fail to take action to reduce salt intake. Taken together, these results, particularly those for items No. 19, 33, and 36, demonstrate that Japanese people have not yet fully adopted effective food preparation and food purchasing skills needed to successfully reduce salt intake in a sustained manner. To address this issue, future efforts should focus not only on continuously raising awareness and knowledge of salt intake among the population, but also on providing long-term educational interventions, such as cooking classes and role-playing activities simulating food purchasing situations through a population-based approach.

Six items were identified as "Items related to high potassium and high salt intake at the same time". With respect to "Frequency of alcohol consumption" (item No. 1), given that alcohol consumption itself is a risk factor for hypertension (23), and that salt intake has a more direct impact on blood pressure elevation than potassium intake (10), it is still reasonable to recommend reducing alcohol consumption. Regarding frequency of "Making oneself gorge on a meal" (item No. 3), it is assumed that both salt and potassium intakes would increase with increases in food intake. Similarly, as for "Quantity of soup consumed when eating noodles" (item No. 7) and "Number of soups per day" (item No. 8), the use of salt and high-salt seasonings in noodle soup (the soup in soup-based noodle dishes) and soup dishes (18), and leaching of nutrients, including potassium, from ingredients into the soup in these dishes (24) may be responsible for the concomitant increase in salt and potassium intakes. In terms of "Frequency of having pickled vegetables per day" (item No. 15), pickled vegetables can be a source of both potassium and salt, due to the potassium content of their major ingredients (24). Since the prevention of hypertension is more strongly affected by salt than by potassium intake (10), it is still necessary to promote recommended eating practices, including not eating until feeling completely full, limiting the consumption of noodle soup and soup dishes, and reducing the

consumption of pickles. As for "considering one's own salt intake as more than moderate" (item No. 34), individuals who are already aware of their high salt intake should be encouraged to translate this awareness into action, with a key focus on reducing salt intake, which is more effective in lowering blood pressure than increasing potassium intake.

The present study had several limitations. First, the participants were all residents of Niigata city in Japan. It has been reported that many countries have regional differences in dietary habits and nutritional intake including salt and potassium intakes (25-27). Additional research in other prefectures is of importance for nationwide salt reduction policies. Second, this study included only participants aged 20 years or older. A proper lifestyle, which includes proper dietary habits, is the basis for healthy status in childhood and tends to persist throughout life (28, 29). Further studies involving minors are required in the future. Another limitation is that estimated dietary salt and potassium intakes were obtained from a casual urine specimen. However, estimated sodium and potassium excretions from a casual urine specimen were validated with that of 24-h urinary excretion with significant correlations ($r = 0.54$, $p < 0.01$, and $r = 0.56$, $p < 0.01$, respectively.) (15) and its use has been reported in a number of epidemiological studies (30, 31).

Conclusion

In conclusion, we clarified that 28 out of the 36 dietary behaviors on the questionnaire gained from an empirical perspective of registered dietitians related to dietary behavior were associated with dietary salt and potassium intake in cooperation with Niigata city in Japan. Additionally, these items were composed of 4 categories: items related with both high salt and low potassium intakes, only high salt intake, only low potassium intake, and both high potassium and high salt intake. On the other hand, some items were inconsistent with registered dietitians' initial expectations regarding the relationship with salt and/or potassium intake. These findings would contribute to developing evidence based on the value of reducing salt intake and increasing potassium intake for Japanese people. Based on our current findings, education in regions and/or individuals according to specific dietary habits and development of an effective program related to high salt intake and low potassium intake are needed for successful prevention of hypertension including dietary salt reduction and increasing potassium intake.

References

1. Laslett LJ, Alagona P Jr, Clark BA 3rd et al. The worldwide environment of cardiovascular disease: prevalence, diagnosis, therapy, and policy issues: a report from the American College of Cardiology. J Am Coll Cardiol 2012; 60: S1-49.
2. World Health Organization. Global health risks: Mortality and burden of disease attributable to selected major risks. World Health Organization, Geneva 2009.
3. Health and Human Services Dept. and Agriculture Dept. Dietary Guidelines for Americans, 2015-2020. Health and Human Services Dept. and Agriculture Dept, Washington, D. C., 2015.
4. Public Health England. SACN Salt and Health report. The Stationery Office, Norwich, 2003.
5. Ministry of Health, Labour and Welfare, Japan. Overview of Dietary Reference Intakes for Japanese (2015). http://www.mhlw.go.jp/file/06-Seisakujouhou-10900000-Kenkoukyoku/Overview.pdf
6. Charlton KE, Steyn K, Levitt NS, Jonathan D, Zulu JV, Nel JH. Development and validation of a short questionnaire to assess sodium intake. Public Health Nutr 2008; 11: 83-94.
7. Lee JY, Cho DS, Kim HJ. The effect of salt usage behavior on sodium intake and excretion among Korean women. Nutr Res Pract 2012; 6: 232-37.
8. Horikawa C, Murayama N, Tochikura E et al. Developing a questionnaire for prediction of high salt intake based on salt usage behavior: Analysis from dietary habits and urinary sodium excretion in Japan. Food Nutr Sci 2017; 7: 1-18.
9. World Health Organization. Guideline: Potassium intake for adults and children. World Health Organization, Geneva, 2009.
10. Haddy FJ, Vanhoutte PM, Feletou M. Role of potassium in regulating blood flow and blood pressure. Am J Physiol Regul Integr Comp Physiol 2006; 290: R?546-52.
11. Aaron KJ, Sanders PW. Role of dietary salt and potassium intake in cardiovascular health and disease: a review of the evidence. Mayo Clin Proc 2013; 88: 987-95.
12. Elmadfa I. European Nutrition and Health Report 2009. Karger Medical and Scientific Publishers, Basel, 2009.
13. Matsumura Y. Nutrition trends in Japan. Asia Pac J Clin Nutr 2001; 10: S?40-7.
14. An R. Fast-food and full-service restaurant consumption and daily energy and nutrient intakes in US adults. Eur J Clin Nutr 2016; 70: 97-103.
15. Tanaka T, Okamura T, Miura K et al. A simple method to

estimate populational 24-h urinary sodium and potassium excretion using a casual urine specimen. J Hum Hypertens 2002; 16: 97-103.
16. Powles J, Fahimi S, Micha R et al. Global, regional and national sodium intakes in 1990 and 2010: a systematic analysis of 24 h urinary sodium excretion and dietary surveys worldwide. BMJ Open 2013; 3: e003733.
17. Srinath Reddy K, Katan MB. Diet, nutrition and the prevention of hypertension and cardiovascular diseases. Public Health Nutr 2004; 7: 167-86.
18. Shizuo Tsuji. Japanese Cooking: A Simple Art. Kodansha USA, New York, 2007.
19. Ministry of Health, Labour and Welfare, Japan. The National Health and Nutrition Survey in Japan, 2005. http://www.mhlw.go.jp/bunya/kenkou/eiyou07/01.html
20. Ministry of Health, Labour and Welfare, Japan. The National Health and Nutrition Survey in Japan, 2015. http://www.mhlw.go.jp/bunya/kenkou/eiyou/dl/h27-houkoku.pdf
21. Cwiertka KJ. Modern Japanese Cuisine: Food, Power And National Identity. Reaktion Books, London, 2007.
22. Liem DG, Miremadi F, Keast RS. Reducing sodium in foods: the effect on flavor. Nutrients 2011; 3: 694-711.
23. Beilin LJ, Puddey IB. Alcohol and hypertension: an update. Hypertension. 2006; 47: 1035-8.
24. Ministry of Education, Culture, Sports, Science and Technology. Standard Tables of Food Composition in Japan - 2015 - (Seventh Revised Edition). http://www.mext.go.jp/en/policy/science_technology/policy/title01/detail01/sdetail01/sdetail01/1385122.htm
25. Ministry of Health, Labor and Welfare, Japan. The National Health and Nutrition Survey in Japan, 2013. http://www.mhlw.go.jp/bunya/kenkou/eiyou/dl/h25-houkoku.pdf
26. Hajjar I. and Kotchen T. Regional variations of blood pressure in the United States are associated with regional variations in dietary intakes: the NHANESIII data. J Nutr 2003; 133: 211-4.
27. Perrin AE, Dallongeville J, Ducimetière P et al. Interactions between traditional regional determinants and socioeconomic status on dietary patterns in a sample of French men. Br J Nutr 2005; 93: 109-14.
28. Mikkila V, Rasanen L, Raitakari T, Pietinen P, Viikari J. Consistent dietary patterns identified from childhood to adulthood: the Cardiovascular Risk in Young Finns Study. Br J Nutr 2005; 93: 923-31.
29. McGowan L, Cooke LJ, Gardner B, Beeken RJ, Croker H, Wardle J. Healthy feeding habits: efficacy results from a cluster-randomized, controlled exploratory trial of a novel, habit-based intervention with parents. Am J Clin Nutr 2013; 98: 769-77.
30. Khaw KT, Bingham S, Welch A et al. Blood pressure and urinary sodium in men and women: the Norfolk Cohort of the European Prospective Investigation into Cancer (EPIC-Norfolk). Am J Clin Nutr. 2004; 80: 1397-403.
31. Tabara Y, Takahashi Y, Setoh K et al. Prognostic Significance of Spot Urine Na/K for Longitudinal Changes in Blood Pressure and Renal Function: The Nagahama Study. Am J Hypertens 2017; 30: 899-906.

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Original article

A 4-week consumption of light or dark roast unfiltered (Turkish) coffee affects cardiovascular risk parameters of homocysteine and cholesterol concentrations in healthy subjects: a randomized crossover clinical trial

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Summary. *Background/aim:* The aim of this study is to investigate the impact of boiled, unfiltered (Turkish) coffee consumption on the plasma cardiovascular risk parameters of healthy subjects. The study also explores whether two unfiltered coffee beverages that differ in content due to varying degrees of roasting will affect cardiovascular biomarkers differently. *Methods:* In this crossover intervention study, healthy, nonsmoking, habitual Turkish coffee drinkers (n=28) were randomized to consume at least 3 cups of Light (LR) or Dark (DR) roast Turkish coffee brews per day for 4 weeks after a washout period (WO) of 2 weeks. Subsequent to each coffee abstinence period, both groups received the alternative intervention. After the first WO and the coffee intervention periods, anthropometric measures, blood pressure, heart rate and 10 biochemical parameters were collected and dietary records were completed. *Results:* The consumption of 3 ≥ cups Turkish coffee/day for 4 weeks, compared with the results after 2 weeks of coffee abstinence, led to a significant increase in homocysteine levels of habitual Turkish coffee drinkers in both coffee interventions ($p<0.01$). Anthropometric measurements, fasting blood glucose, blood pressure and heart rate did not change during the coffee consumption phase in either of the Turkish coffee groups. Both roasts increased concentrations of serum lipids compared to WO. However, only DR Turkish coffee intake significantly increased total cholesterol levels ($p<0.05$). *Conclusion:* Moderate amounts of LR or DR Turkish coffee consumption for 4 weeks, although differing in content, largely show similar biological effects as demonstrated by the tested cardiovascular biomarkers.

Key words: cardiovascular disease, cholesterol, homocysteine, coffee, diterpenes

Introduction

Coffee is widely consumed everywhere in the world and is prepared using several different methods (1). Turkish-style coffee, which is consumed in Greece, Cyprus, the former Yugoslavia, Turkey and the Middle East including Israel, is boiled coffee with a unique brewing procedure (2,3). Epidemiological studies suggest that consumption of boiled, unfiltered coffee is related to elevated risk of cardiovascular disease (CVD) (4). However, considerable controversy exists regarding the link between coffee consumption and CVD risk (5).

It has been shown that different coffee preparation and brewing methods influence the concentration of compounds present in the final coffee brew (6). Different from the coffee usually consumed in the western world, Turkish coffee is not drip filtered, but rather its preparation involves slowly boiling water that is mixed with finely ground Arabica coffee beans (7). This method causes a greater amount of biologically active

components (caffeine and diterpenes) to remain in the liquid (7,8).

Recent studies have shown that the diterpene content of a standard cup of coffee is highest in unfiltered preparation methods such as Scandinavian-style boiled coffee, French press (cafetiere) and Turkish coffee (with up to 88.7 mg/L in some Turkish brews) (1). The diterpenes (cafestol and kahweol) are compounds found in the lipid fraction of the coffee, and are associated with an increase in blood cholesterol (8). It was stated that the consumption of 10 mg cafestol per day- the amount present in three cups of unfiltered Turkish coffee- for 4 weeks increases serum cholesterol level by 5.0 mg/dL, whereas the consumption of 10 mg kahweol only rises it by 0.9 mg/dL (3,9). Nevertheless, favorable health effects have also been ascribed to diterpenes and data present in literature shows antioxidant activity, hepatoprotective, anticarcinogenic, anti-iflammatory and anti-angiogenic functions (10). Diterpene concentration in roasted coffee beans can be affected by various factors such as species, the cultivar, year harvested and degree of roasting (8). There are several other ingredients within coffee, which contribute to the biological activity (4). In addition to preparation method, roasting process greatly affects the chemical composition of the coffee (11,12). Coffee types which contain different major constituents have distinct cardiovascular health effects (13,14). Furthermore, coffee dose is defined as a potential modifier on CVD outcomes and in the meantime, current literature supports an association with 3-5 cups/day (5,15,16).

To the best of our knowledge, there are limited number of clinical trials investigating the relation between boiled coffee intake and cardiovascular health outcomes. Moreover, the effect of Turkish coffee has not yet been studied experimentally (3). This is the first study to investigate the impact of boiled, unfiltered (Turkish) coffee consumption on the plasma cardiovascular risk parameters of healthy subjects. The study also explores whether two boiled (Turkish) coffee beverages with different degrees of roasting produce distinctive cardiovascular biomarkers. Since Cyprus has a high number of regular boiled coffee drinkers, mean annual coffee consumption per capita is 6.1 kg which is higher than in the European community and United States (14). It would be interesting to see whether the effects on cardiovascular disease risk parameters would also be detectable in long-term habitual drinkers.

Materials and Methods

Study Subjects

Thirty healthy habitual Turkish coffee drinkers; Eastern Mediterranean University (EMU) staff, students or relatives who saw study information about the study published in the campus area or on the internet, were recruited to the study.

Eligibility criteria were: Regular Turkish coffee consumption of ≥ 1 cups/day, age 20-35 years, healthy, body mass index ((BMI) 18.5-24.9 kg/m2), non-smoker or former smoker (more than a year), willingness to abstain from coffee drinking and to consume ≥ 3 cups/day of Turkish coffee for 8 weeks.

Exclusion criteria were: Acute or chronic diseases, severe illness with in-patient treatment during the last 3 months, use of regular medication or any supplements, weight reduction >2 kg/week during the last month, pregnancy, breastfeeding, regular strong physical activity with ≥1h/day. We also excluded high intake of alcohol (defined as a weekly intake of >7 units for women and >14units for men), excess dietary consumption of total fat (>35% of daily calories), saturated fatty acid (>10% of daily calories) or cholesterol (>300mg/day).

Study Design

Ethical approval was obtained from the Faculty of Health Sciences of Eastern Mediterranean University (Famagusta, North Cyprus) and the study was registered as a clinical trial (NCT03495336). All subjects were informed about the aims of the study, agreed to participate and signed a consent form.

A survey was carried out to gather information regarding physical activity, medical condition and medication. Additionally to evaluate the inclusion to the study, a food frequency questionnaire (FFQ) and anthropometric measures (weight, height, body fat percent, fat free mass (FFM), waist circumference) were collected at the baseline interview. Our crossover clinical trial lasted 12 weeks (with two wash out and two coffee intervention periods (As shown in Figure)). After 2 weeks (wk)

washout period (coffee abstention phase), participants were assigned to one of the two coffee interventions (LR or DR) for 4-wk. At the end of this coffee phase subjects participated in an additional 2-wk wash out period and then for the next 4-wk period, they switched to the opposite/other coffee roast.

Participants were asked to maintain a stable exercise routine, sleep pattern and dietary habits throughout the study and were asked to avoid vitamin supplements, foods/beverages rich in caffeine (including coffee, cola beverages, cocoa, chocolate, energy drinks, and tea) and to keep 3-day food diaries (1 weekend day and 2 weekdays) prior to each measurement. Daily nutrient intake was calculated by using computer software (Ebispro, Stuttgart, Germany; Turkish version: BeBiS, Vers. 6.1). In order to assess physical activity levels, the validated Turkish version of the International Physical Activity Questionnaire (IPAQ)-short was administered (17).

Coffee Samples

Two commercially available Turkish Cypriot coffee blends differing in roasting were used in the study. Both coffee roasts were a blend of 100% Arabica (Coffea Arabica) and cultivated in the same geographic region (Brazil). They were vacuum-packed and provided by a different manufacturer. Roasting classification degree was measured according to "Roast Color Classification System" (Agtron/SCAA, Reno, NV,1995). The roasting processing time and temperatures were reported as 20 mins-200˚C and 40 mins-210 ˚C for LR and DR respectively. Coffee was distributed to participants in packages at the beginning of the each intervention. Subjects were instructed on how to prepare the beverage (5 g of Turkish coffee, 55 ml cold water) in a house hold Turkish coffee maker or cezve and were given standard cups and spoons. Participants were asked to drink at least 3 cups of the suggested Turkish coffee roast per day without a fixed schedule and to refrain from other types of coffee beverages throughout the study period. To control sugar intake only the customary (pre-study habitual) amount of Turkish coffee with sugar were allowed and the participants were asked to consume the rest of the coffee as plain (without sugar).

Coffee was brewed as instructed to the participants and analyzed for the caffeine and diterpenes content. Caffeine was determined by high-performance liquid chromatography (HPLC, Aligent Technologies, USA) with diode-array detector (DAD) and mass spectrometer. Cafestol and kahweol were analyzed in the unsaponified matter by HPLC-DAD.

Blood Sample Analysis

Subjects were advised to avoid the use of alcohol 72 hours before the blood sample collection. After an overnight fasting (min. 8 – max. 12 hours), blood samples were obtained at the end of the initial wash out period and after each 4-wk coffee intervention period, and sera were stored at –30˚ C, until analytical measurements were performed. The levels of serum glucose, triglycerides (TG), total cholesterol (TC), and high-density lipoprotein (HDL-C) cholesterol were determined using a Dimension Xpand Plus integrated clinical chemistry autoanalyzer (Siemens Healthcare Diagnostics, Deerfield, IL, USA). The serum levels of low-density lipoprotein (LDL-C) cholesterol were calculated using Friedewald's equation. EDTA-treated blood samples for total homocysteine analysis were immediately refrigerated (placed on ice) until the plasma was separated by centrifugation. All the samples were assayed for homocysteine by using High Performance Liquid Chromatography with fluorescent detection technique (HPLC-FLD). Serum Malondialdehyde (MDA) levels were determined with a colorimetric assay kit (Cayman Chemical, Ann Arbor, MI) according to the procedures provided by the manufacturer.

Assessment of other Measurements

Weight, height, abdominal circumference, body fat and blood pressure were measured after the washout period and after each intervention. Weight (in kilograms) was measured in light clothing, without shoes, to the nearest 0.1 kg; height was measured using a stadiometer to the nearest centimeter; and BMI was calculated (weight/height squared; in kilograms per square meter). The percentage of body fat and FFM was measured by Tanita Segmental Body Composition Analyzer BC-418 MA (Tanita Corp. Tokyo, Japan) and waist circumferences (midway between the rib cage and the iliac crest) were measured using a flexible tape. Blood pressure (BP) and heart rate was monitored using an automatic arm sphygmomanom-

eter (Pic Indolor Diagnostic, BS 150, Artsana, Italy) after a 5-min rest in a sitting position.

Statistical analysis

The results are expressed as means. Caffeine and diterpenes content of the two coffee beverages were reported as mean±standard deviation (SD) and Kruskal–Wallis test was used for statistical comparison of coffee roasts. Differences in human variables were analyzed by repeated-measures analysis of variance (ANOVA) for comparisons of LR coffee intake with DR and of each roast with the washout. All analyses were performed using SPSS 24.0 (IBM-SPSS Inc., Chicago, IL, USA). A two-tailed $P < 0.05$ was considered significant.

Results

Subject characteristics

Out of thirty healthy volunteers who were recruited for the study, one woman and a man dropped out during washout period because of a severe illness (this had been predefined as exclusion criteria). Thus, twenty-eight healthy nonsmoker habitual Turkish coffee drinkers (27. 50±5.30 y, range 20-35 y) were evaluated in the study. Half of the participants were female and all the subjects were sedentary (physical activity less than 1h/day, PAL: 1.52±0.12). According to the questionnaire results, the daily habitual Turkish coffee consumption of the subjects was 2.44±1.19 cups per day (min. – max. = 1- 5 cups/day). Participants' baseline characteristics are summarized in Table 1. During the first 4-wk intervention period half of the participants (n=14, 50% women) ingested LR coffee and other half (50% women) consumed DR coffee and in the next 4-wk period, they switched to the opposite coffee roast (Fig.1).

Coffee analysis

Coffee gave 1.71±0.07 mg of caffeine per mL in LR (94.05 mg per cup/55 mL) and 1.97±0.03 mg per mL in DR (108.35 mg per cup/55 mL). The mean concentrations of cafestol and kahweol were 1.79± 0.09 mg and 1.67±0.073 mg per cup/55 mL in LR and 6.83±0.27 and 6.17±0.12 mg per cup/55 mL in DR, respectively ($p=0.001$). DR provided more cafestol and kahweol than LR ($p<0.05$). Table 2 shows mean

Table 1. Baseline characteristics of the participants (N=28) [1]

Gender (male/female, n)	14/14, 28
Age (year)	27.50±5.30
Body mass index (kg/m²)	23.32±3.44
Fat mass (male/female, %)	20.74±5.36 (20,16 ± 4,53/21,32 ± 6,19)
Waist circumference (male/female, cm)	83,02±13,13 (92,00 ±10,8/73,35 ±7,20)
Habitual Turkish coffee consumption before the trial (cups/day)	2,44±1,19
Instant coffee(cups/day) (n=15)	1,67±0,82
Filter coffee(cups/day)(n=2)	1,00±0,00
Other coffee types	0
Carbohydrate (energy%)	47.11±6.82
Protein (energy%)	17.96±3.78
Total fat (energy%)	34.11±6.80
Cholesterol (mg/d)	372.99 ±244.61
Saturated fat (g/d)	33,93 ±18,44
Fiber (g/d)	20.81±8.20
Physical activity level (PAL)	1.52±0.12

[1] *Data expressed as mean ± standard deviation, numbers or %*

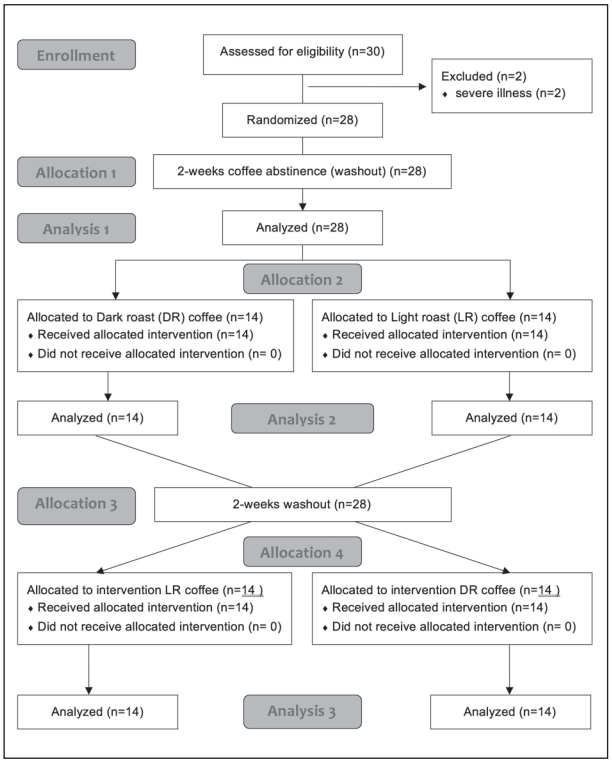

Figure 1. Study design.

Table 2. Mean ± SD concentrations of selected constituents in the commonly consumed Turkish Cypriot market blend Light roast (LR) and Dark roast (DR) Turkish Coffees.[2]

Compound	LR	DR
Caffeine (mg/mL)	1.71± 0.07	1.97±0.03
Cafestol (mg/55mL)	1.79±0.09[a]	6.83±0.27[b]
Kahweol (mg/55mL)	1.67±0.07[a]	6.17±0.12[b]

[2] Concentrations of components have been described as mean from duplicate measurements from five independent samples. Kruskal–Wallis test was used to analyze whether the repeated measurements within groups demonstrate any difference from the median. Different letters on the same line indicate significant difference (p<0.05).

± SD concentrations of selected constituents in the market blend Light roast (LR) and Dark roast (DR) coffee. Subjects consumed two different coffee blends with almost similar caffeine but substantially different diterpen (cafestol and kahweol) contents. The mean number of cups of coffee per day did not significantly differ between both groups.

Cardiovascular risk biomarkers

Cardiovascular effects of Turkish coffee consumption on food intake, anthropometric measures, physical activity, blood pressure and blood glucose

Self-reported diets (a 3-d food diary) showed that none of the participants consumed a significant amount of caffeine foods other than coffee during the study and the nutritional intake was similar before (during washout) and after each intervention period for all participants (P > 0.05). None of the dietary intake parameters showed statistical differences (Table 3) and volunteers did not report any changes in their physical activity throughout the study (data not shown).

Compared to no-coffee periods, subjects drank 3 ≥ cups/d (165 mL/d or more) of Turkish coffee in each 4-wk coffee periods. No adverse or side effects were seen in any of the coffee intervention groups.

There were no significant differences found between the coffee abstention period and the two coffee groups for primary anthropometric measures (body weight, body fat mass, FFM, BMI and waist circumference) (Table 4). However, notably, the body fat percent of subjects was nonsignificantly reduced following consumption of each coffee roast for 4-wk, by 1% (from 21.73±5.37 to20.63±5.38 in the LR and 20.66±5.47 in the DR p=0.057 each, Table 4). No significant change was observed for diastolic or systolic blood pressure, heart rate and fasting blood glucose (p>0.05).

Plasma Total Homocysteine (tHcy) Levels

The consumption of Turkish coffee (3 ≥ cups/d) for a 4-week period compared with the results after a 2-wk coffee abstinence, led to a significant increase in homocysteine (μmol/L) levels. The mean concentration of plasma homocysteine was 9.66±2.24 μmol/L at the end of the no-coffee period, 12.03±3.08 μmol/L at

Table 3. Average daily nutrient intake of volunteers on the basis of 3-day food records during the study.[3]

Energy and Nutrient Intake	WO	LR	DR
Energy (Kcal)	2103. 47±771.12	2102.36±763.50	2083.62±760.28
Proteins (g)	93.52 ±43.26	89.95 ±41.58	91.25 ±43.79
Lipids (g)	115.15±52.06	116.24±50.12	114.58±51.64
Saturated fatty acid (FA) (g)	32.98±17.25	33.83±18.45	33.60±17.46
Monounsaturated FA (g)	39.62± 18.72	36. 21± 18.51	36. 17± 18.70
Polyunsaturated FA (g)	22.52±11.79	22.80±11.64	23.12±11.69
Omega-3 (g)	2.26±1.40	2.35±2.42	2.41±1.85
Omega-6 (g)	20.26±10.87	20.45±12.08	20.69±9.68
Cholesterol (mg)	357.99±254.61	364.69±248.29	364.79±251.17
Carbohydrates (g)	173.26±59.39	174.10±54.91	171.85±57.13
Dietary fibre (g)	20.98±8.40	21.14±8.63	21.35±7.96

[3]Data are expressed as mean ± SD (N=28). Group comparisons were analyzed by repeated-measure ANOVA, followed by the Friedman test. No significant differences were detected between groups (p>0.05).

Table 4. Concentration of serum lipids, plasma total homocysteine and other cardiovascular risk parameters in coffee-free period and changes after 4 weeks of LR or DR coffee ingestion.[4]

Biomarkers	WO	LR	DR
Homocysteine (μmol/L)	9.66±2.24	12.03±3.08**	11.82±3.22**
Cysteine (μmol/L)	251±26.23	282.98±35.91**	289.69±41.33**
Cysteine/Homocysteine	27.05±5.42	24.99±6.8	25.67±5.18
Fasting blood glucose (mg/dL)	89.93±14,5	90.67±17.43	90.04±18.92
Total cholesterol (mg/dL)	182.93±33.87	190.63±29.02	192±31.94*
HDL cholesterol (mg/dL)	54.37±12.24	57.52±14.09	57.04±12.26
LDL cholesterol (mg/dL)	112.81±32.43	116.85±28.85	117.11±29.48
VLDL cholesterol (mg/dL)	3.94±1.8	4.31±1.35	4.55±1.61
Triglycerides (mg/dL)	78.74±35.99	86.15±27.04	91±32.15
Body weight (kg)	67.85±16.86	67.51±17.18	67.6±17.35
Body mass index (kg/m²)	23.25±3.57	23.16±3.61	23.18±3.65
Fat mass (%)	21.73±5.37	20.63±5.38	20.66±5.47
Systolic blood pressure (mmHg)	117.5±13.3	115.79±13.7	114.29±12.84
Diastolic blood pressure (mmHg)	76.54±9.19	75.61±7.8	76.04±8.83
Heart rate (beats/min)	78.75±11.15	80.96±13.73	81.5±11.89
Malondialdehyde (MDA) (μM)	28.16±35.9	27.92±25.34	18.02±12.74

[4] *ANOVA, analysis of variance;*
Data expressed as means ± SD (N=28).
***Significantly different from (coffee-free period) washout (ANOVA of repeated measures): P<0.01*
**Significantly different from washout (ANOVA of repeated measures): P<0.05*
§Significantly different from LR Turkish coffee (ANOVA of repeated measures): P<0.05

the end of the LR and 11.82±3.22 μmol/L at the end of the DR coffee periods. We thus observed an increase ($p<0.01$) in homocysteine concentrations after LR and DR intake with 24.5% (or 2.4 μmol/L) and 22.4% (or 2.2 μmol/L), respectively caused by unfiltered Turkish coffee. The significant effect of Turkish coffee on the homocysteine (μmol/L) and cysteine (μmol/L) concentration was seen during both coffee roast interventions. However, no differences between coffee effects on homocysteine and cysteine could be determined by group comparison. In addition, no noticeable changes were seen for the cysteine/homocysteine ratio.

Blood Lipid Concentrations

Although we observed a small rise in all serum lipid parameters after the consumption of either LR or DR coffee compared to abstention, there was no significant impact ($p>0.05$) on HDL-C, LDL-C, VLDL-C and TG plasma concentrations. Table 4 shows an increase in TC levels after each coffee roast intake compared to WO (from 182.93±33.87 mg/dL to 190.63±29.02 mg/dl in the LR-group and 192±31.94 mg/dl in the DR group). However, only DR intervention enhanced TC concentrations significantly (5.5%, $p<0.05$). Relative to baseline values, DR Turkish coffee raised mean TC concentrations by 10.0 mg/dL (0.56 mmol/L). No difference in TC levels between the coffee roasting intervention periods observed. In addition, no difference in other serum lipid parameters was monitored between coffee roasts.

Discussion

Previous randomized control trials reported that a high intake of unfiltered and filtered coffee (1 L/d) elevate plasma homocysteine concentrations (18,19). In our crossover study, although the coffee intake was lower (three-five cups) both coffee groups exhibited a greater increase of mean plasma total homocysteine (tHcy) levels compared to preceding control trials. In contrast, in a small clinical trial, Esposito et al. (20)

were unable to detect any significant rise in homocysteine levels, from drinking five cups of Italian style coffee per day for one week. Moreover, Mursu et al. (21) emphasized that consumption of coffee in a short-term did not increase tHcy levels. Possibly, in our study the intervention time of 4 weeks was long enough and/or the amount of coffee consumed was sufficient to promote changes within that parameter. Taken together, no difference between the effectiveness of both coffees on tHcy parameters was observed. Therefore, our results provide unequivocal evidence that Turkish coffee in moderate amounts would increase total plasma homocysteine concentrations, regardless of its roasting degree. In our study, the analysis of Turkish coffee blends showed different amounts of diterpenes (both cafestol and kahweol), but the concentrations of caffeine in both roasts were almost equal and the increase on tHcy levels were similar. This result might reflect the assumption that caffeine is one of the coffee constituents which increases plasma tHcy levels. In addition, Verhoef et al. (22) claimed that caffeine has been suggested to be partly responsible as the component in coffee, and coffee but not caffeine itself, as the factor that influences the rise in homocysteine levels in the blood after consumption. Thus in the present study, the changes can be related to the increased amount of coffee and caffeine intake during interventions which may have led to a significant increase in homocysteine levels. Moreover with our results, question still remains as to whether healthy subjects with increased homocysteine levels affects the cardiovascular system in the same way as it would in prediagnosed patients (23). It can be concluded that drinking large quantities of Turkish coffee could raise homocysteine in plasma. However, whether this raises the risk of cardiovascular disease is not yet certain (19). As previously reported by other authors, an important finding of this study was that there was a notable positive dose-response relation between Turkish coffee consumption and plasma tHcy, which was stronger than the relation between coffee and total serum cholesterol increase (23,24).

The results consistently show that unfiltered coffee has a negative effect on plasma serum lipid levels (25-27). In accordance with this, one of the key findings of the present study is that both Turkish coffee roasts increased serum lipid levels whereas only DR Turkish coffee intake significantly increased TC levels. This is believed to be due to the diterpenes (26,27) because the DR coffee contains higher amounts of cafestol and kahweol (ca. three times more of both) and is found more effective in significantly increasing serum TC than the LR coffee. It cannot be ruled out that the observed significant TC increase in our study was due to caffeine as seen in other studies (5,28) because both of the coffee roasts had similar amounts but exhibit different effects. In other words, we here demonstrated for the first time that roasting might affect the total diterpene profile of Turkish coffee (both cafestol and kahweol) profile and in turn, the TC levels of subjects. In several studies, specifically cafestol has been stated to be the most potent cholesterol raising compound of a coffee brew (5,26,27). The reason for the increase of plasma concentration due to the effect of cafestol is not entirely known (5). However, recently a mechanism that could explain this effect was proposed. It was stated that cafestol elevates serum cholesterol levels by activating farsenoid X and pregnane X receptors in the intestine causing the body to send a signal to the liver to stop the breakdown of cholesterol. As a result cholesterol accumulates in the serum and leads to an increase in concentrations (26).

An interesting finding that should be mentioned is that we did not observe a significant increase in serum LDL-C and TG levels, a cafestol consumption-related effect which was also seen in several studies (26,28, 29,30). Our potential explanation for this is the quantity of Turkish coffee intake and the relatively taken diterpen concentrations whereas the aforementioned increase in most of the literature data was observed with a marginal amount of unfiltered coffee (3,25,28,30). This would be in agreement with the results of a recent study where the daily volumes of (62.3±40.60 ml; 0.7±0.50 cup) Turkish coffee consumption caused no significant alteration in the serum lipid levels among the study population (13).

In addition to the relationship between the amounts of coffee consumed, the method of coffee preparation is also an important determinant of serum lipid levels (28,30). According to previous studies, shorter contact with hot water and retention of diterpenes by filter paper are the reasons for poor or no influence of filtered coffee on serum levels when com-

pared to boiled coffee (29,31,32). However, in a recent randomized clinical trial Corrêa et al. (5) found that drinking 3–4 cups of light- or medium-roasted paper-filtered coffee per day for 4 weeks, compared to our study, exhibited a stronger increase in TC and LDL-C levels in both groups even though the cafestol content is almost similar and has a lower-level of kahweol per cup. It was reported that the difference in results might be related to the higher caffeine intake (28,33) since it was almost twice as much compared to ours (28, 33). Therefore, in addition to the primary effect of diterpenes on TC levels, caffeine might have an impact in a dose dependent manner. Further studies should be conducted in order to investigate and observe the required doses.

Most of the interventions failed to demonstrate a significant decrease in lipid damage marker MDA with the exception of results found by Sirota et al. (34) who stated that consumption of 200 mL Turkish roasted coffee during a meal based on red-meat cutlets resulted in a significant inhibition of postprandial plasma MDA. In addition, Yukawa et al. (35) found a modest reduction of LDL oxidation susceptibility and a significant decrease in serum cholesterol, LDL cholesterol and MDA levels after consumption of 150 mL (8g Arabica) coffee 3 times per day for a week (36). No effect between treatments and control/placebo were found by other authors (21,37,38,39) which was consistent with our results. However, Leelarungrayub et al. (31) reports a significant higher level of MDA in men consuming 3 cups of caffeinated coffee (150 ml coffee, 8 g Arabica/cup) for a week, when compared to decaffeinated coffee or control, followed by a submaximal exercise test. This study is particularly relevant as similar results were found in previous tests where increased intramuscular fat oxidation was observed after caffeine-rich foods were consumed (31). However, it is not yet clear why there are certain discrepancies with a few of the previous reports. It may be due to the differences between study designs since especially, other study trials have observed a relatively large amounts of coffee consumption in contrast to this study.

None of the anthropometric measures were changed in either of the roasting groups after the consumption of coffee for 4-weeks. A similar finding has been reported in a recent study in overweight adults (40). An important finding of our study is that even though the body weight remained unchanged, we observed a slight loss in body fat percentage. In a crossover study consumption of coffee for 4 weeks resulted in a significant reduction in body fat levels of healthy subjects irrespective of roasting degree. In addition, it was demonstrated that coffee constituents other than those associated with roasting involved a significant, but weaker reduction in body fat content (41). Bakuradze et al. stated that the significant change in body fat and unvaried change in weight may be explained by the particularly counterbalancing effect of some gain in FFM (42). However, this result is not in accordance with our finding where there was no change observed in FFM after coffee intake.

The relation between regular coffee consumption and blood pressure (BP) was demonstrated with a J–shaped curve (43). Simply, it was stated that a moderate consumption of coffee may have a protective effect on BP, whereas high intake could increase the risk of hypertension (33,43). In normotensive habitual Turkish coffee drinkers in our study, we did not observe an increase in BP in 4 weeks. A Similar finding in a recent randomized control trial (33) explained that in habitual coffee drinkers a partial tolerance to caffeine might be developed as a result of the duration of consumption.

There are several strengths and limitations to be noted. Firstly, it can be stated that in our study, a placebo/control group was lacking. Secondly despite the subjects' coffee refinement between the interventions, the absence of the measurements for the second washout period is a potential limitation. Additionally our study has comprised a small sample size, which included a continued participation period of 12 weeks and a fixed dose of coffee intake. However, strength of this study lies in the amount of coffee used and the method of preparation which are similar to those used by the study population in real life. Therefore, to recommend consuming coffee in line with the amounts and preparation method used in this study is a suitable way to observe the desirable effects of Turkish coffee in real-life circumstances. Moreover, the crossover design of our study and the distribution of subjects eliminated gender differences as a cofounding factor. In addition, another limitation of this study is the possibility of a

discrepancy between the number of coffee pads given to participants and the mean number of cups of coffee they reported to have consumed during coffee intervention. For future studies in order to handle this problem at baseline and during coffee intervention we should recommend analyzing serum diterpene (cafestol and kahweol) and caffeine concentrations as objective measures for coffee consumption. Finally, another limitation of this study was that other metabolites as well as other components responsible for coffee's total effect on findings were not measured. This is the first clinical trial to show an increase in homocysteine and blood lipids of healthy habitual Turkish coffee drinkers after coffee consumption and to compare the effects of two different coffee roasts on CV disease risk parameters.

Moderate amount of Turkish coffee consumption has significant undesirable effects on total plasma homocysteine biomarkers, regardless of its roasting degree. This can be attributed to the changes related to the increased amount of coffee and caffeine intake during interventions. Although significant increase in cholesterol levels were only seen in the DR coffee intervention group, most likely because of higher cholesterol-raising diterpenes content, a rise in all lipid parameters was observed in both groups. To sum up, the results of this intervention study indicate that 3-5 cups/d of LR or DR Turkish coffee consumption for 4 wks., although differing in contents, largely exert similar biological effects as demonstrated by the cardiovascular biomarkers tested. More interventional studies and the evaluation of other coffee components are needed to clarify the effects of coffee on CV risk factors.

References

1. Zhang C, Linforth R, Fisk ID. Cafestol extraction yield from different coffee brew mechanisms. Food Res Int 2012;49:27-31.
2. Kıvançlı J, Elmacı Y. Characterization of Turkish-style boiled coffee aroma by gas chromatography and mass spectrometry and descriptive analysis techniques. Int J Food Prop 2016;19:1671-1686.
3. Urgert R, Katan MB. The cholesterol-raising factor from coffee beans. Annu Rev Nutr 1997;17:305-324.
4. 4.Ludwig IA, Clifford MN, Lean ME, Ashihara H ,Crozier A. Coffee: biochemistry and potential impact on health. Food Funct 2014;5:1695-1717.
5. Corrêa TA, Rogero MM, Mioto BM, Tarasoutchi D, Tuda VL, César LA, Torres EA. Filtered coffee increases cholesterol and inflammation biomarkers independent of roasting degree: A clinical trial. Nutr J 2013;29:977-981.
6. Caprioli G, Cortese M, Sagratini G, Vittori S. The influence of different types of preparation (espresso and brew) on coffee aroma and main bioactive constituents. Int J Food Sci Nutr 2015;66:505-513.
7. Church DD, Hoffman JR, LaMonica MB, Riffe JJ, Hoffman MW, Baker KM, Stout JR. The effect of an acute ingestion of Turkish coffee on reaction time and time trial performance. J Int Soc Sports Nutr 2015;12:37.
8. Rendón MY, dos Santos Scholz MB, Bragagnolo N. Is cafestol retained on the paper filter in the preparation of filter coffee? Food Res Int 2017;100:798-803.
9. van Tol A, Urgert R, de Jong-Caesar R, van Gent T, Scheek L M, de Roos B. The cholesterol-raising diterpenes from coffee beans increase serum lipid transfer protein activity levels in humans. Atherosclerosis 1997:132(2);251-254.
10. Moeenfard M, Silva JA, Borges N, Santos A, Alves A. Diterpenes in espresso coffee: impact of preparation parameters. Eur Food Res Technol 2015;240:763-773.
11. Kempf K, Kolb H, Gärtner B, Bytof G, Stiebitz H, Lantz I, Martin S. Cardiometabolic effects of two coffee blends differing in content for major constituents in overweight adults: a randomized controlled trial. Eur J Nutr 2015;54:845-854.
12. Dias RCE, de Faria-Machado AF, Mercadante AZ, Bragagnolo N, de Toledo Benassi M. Roasting process affects the profile of diterpenes in coffee. Eur Food Res Technol 2014;239:961-970.
13. Karabudak E, Türközü D, Köksal E. Association between coffee consumption and serum lipid profile. Exp Ther Oncol 2015;9:1841-1846.
14. Bae, J. H., Park, J. H., Im, S. S., & Song, D. K. Coffee and health. Integr Med Res 2014;3:189-191.
15. Tzoulaki I. Moderate coffee intake and cardiovascular health; no grounds for concern. Nutr Metab Cardiovasc Dis 2018;28:808-809.
16. Poole R, Kennedy OJ, Roderick P, Fallowfield JA, Hayes PC, Parkes J. Coffee consumption and health: umbrella review of meta-analyses of multiple health outcomes. Bmj 2017;359:j5024.
17. Saglam M, Arikan H, Savci S, Inal-Ince D. International physical activity questionnaire: reliability and validity of the Turkish version. Perceptual and motor skills. 2010: 111(1); 278-284.
18. Grubben MJ, Boers GH, Blom HJ, Broekhuizen R, de Jong R, van Rijt L, Katan MB. Unfiltered coffee increases plasma homocysteine concentrations in healthy volunteers: a randomized trial–. Am J Clin Nutr 2000;71:480-484.
19. Urgert R, van Vliet T, Zock PL, Katan MB. Heavy coffee consumption and plasma homocysteine: a randomized controlled trial in healthy volunteers–. Am J Clin Nutr 2000;72:1107-1110.

20. Esposito F, Morisco F, Verde V, Ritieni A, Alezio A, Caporaso N, Fogliano V. Moderate coffee consumption increases plasma glutathione but not homocysteine in healthy subjects. Aliment Pharmacol Ther 2013;17: 595-601.
21. Mursu J, Voutilainen S, Nurmi T, Alfthan G, Virtanen JK, Rissanen TH, Salonen JT. The effects of coffee consumption on lipid peroxidation and plasma total homocysteine concentrations: a clinical trial. Free Radic Biol Med 2005;38:527-534.
22. Verhoef P, Pasman WJ, van Vliet T, Urgert R, Katan MB. Contribution of caffeine to the homocysteine-raising effect of coffee: a randomized controlled trial in humans. Am J Clin Nutr 2002;76:1244-1248.
23. Rijal P. Coffee as a risk factor for cardiovascular diseases. A literature study (master's thesis). UiT Norges Arktiske Universitet; 2016
24. Nygård O, Vollset SE, Refsum H, Stensvold I, Tverdal A, Nordrehaug JE, Kvåle G. Total plasma homocysteine and cardiovascular risk profile: the Hordaland Homocysteine Study. Jama 1995;274:1526-1533.
25. Thelle DS, Strandhagen E. Coffee and disease: an overview with main emphasis on blood lipids and homocysteine. Scand J Food Nutr 2005;49:50-61.
26. Urgert R, Meyboom S, Kuilman, M, Rexwinkel H, Vissers MN, Klerk M, Katan MB. Comparison of effect of cafetiere and filtered coffee on serum concentrations of liver aminotransferases and lipids: six month randomised controlled trial. BMJ 1996;313:1362-1366.
27. Ricketts ML. Does coffee raise cholesterol? J Clin Lipidol 2007; 2:373-377
28. Cai L, Ma D, Zhang Y, Liu Z, Wang P. The effect of coffee consumption on serum lipids: a meta-analysis of randomized controlled trials. Eur J Clin Nutr 2012;66:872.
29. Naidoo N, Chen C, Rebello SA, Speer K, Tai ES, Lee J, van Dam RM. Cholesterol-raising diterpenes in types of coffee commonly consumed in Singapore, Indonesia and India and associations with blood lipids: A survey and cross sectional study. Nutr J 2011;10:48.
30. Jee SH, He J, Appel LJ, Whelton PK, Suh II, Klag MJ. Coffee consumption and serum lipids: a meta-analysis of randomized controlled clinical trials. Am J Epidemiol 2001;153:353-362.
31. Wierzejska R. Coffee Consumption and Cardiovascular Diseases–Has the Time Come to Change Dietary Advice? A Mini Review. Pol J Food Nutr Sci 2016;66:5-10.
32. Buscemi S, Marventano S, Antoci M, Cagnetti A, Castorina G, Galvano F, Mistretta A. Coffee and metabolic impairment: An updated review of epidemiological studies. NFS Journal 2016; 3:1-7.
33. Agudelo-Ochoa GM, Pulgarín-Zapata IC, Velásquez-Rodriguez CM, Duque-Ramírez M, Naranjo-Cano, M, Quintero-Ortiz MM, Muñoz-Durango K. Coffee Consumption Increases the Antioxidant Capacity of Plasma and Has No Effect on the Lipid Profile or Vascular Function in Healthy Adults in a Randomized Controlled Trial. J Nutr 2016;146:524-531.
34. Sirota R, Gorelik S, Harris R, Kohen R, Kanner J. Coffee polyphenols protect human plasma from postprandial carbonyl modifications. Mol Nutr Food Res 2013;57:916-919.
35. Yukawa GS, Mune M, Otani H, Tone Y, Liang XM, Iwahashi H, Sakamoto W. Effects of coffee consumption on oxidative susceptibility of low-density lipoproteins and serum lipid levels in humans. Biochem (Mosc) 2004;69:70-74.
36. Martini D, Del Bo C, Tassotti M, Riso P, Del Rio D, Brighenti F, Porrini M. Coffee consumption and oxidative stress: a review of human intervention studies. Molecules 2016;21:979.
37. Teekachunhatean S, Tosri N, Sangdee C, Wongpoomchai R, Ruangyuttikarn W, Puaninta C, Srichairatanakool S. Antioxidant effects after coffee enema or oral coffee consumption in healthy Thai male volunteers. Hum Exp Toxicol 2012;31:643-651.
38. Bloomer RJ, Trepanowski JF, Farney TM. Influence of acute coffee consumption on postprandial oxidative stress. Nutr Metab Insights 2013;6:NMI-S12215.
39. Ochiai R, Sugiura Y, Shioya Y, Otsuka K, Katsuragi Y, Hashiguchi T. Coffee polyphenols improve peripheral endothelial function after glucose loading in healthy male adults. Nutr Res 2014;34:155-159.
40. Kempf K, Kolb H, Gärtner B, Bytof G, Stiebitz H, Lantz I, Martin S. Cardiometabolic effects of two coffee blends differing in content for major constituents in overweight adults: a randomized controlled trial. Eur J Nutr 2015;54:845-854.
41. Riedel A, Dieminger N, Bakuradze T, Lang R, Parra GAM, Hochkogler CM, Richling E. A 4-week consumption of medium roast and dark roast coffees affects parameters of energy status in healthy subjects. Food Res Int 2014;63:409-419.
42. Bakuradze T, Parra GAM, Riedel A, Somoza V, Lang R, Dieminger N, Schipp D. Four-week coffee consumption affects energy intake, satiety regulation, body fat, and protects DNA integrity. Food Res Int 2014;63:420-427.
43. Zhang Z, Hu G, Caballero B, Appel L, Chen L. Habitual coffee consumption and risk of hypertension: a systematic review and meta-analysis of prospective observational studies–. Am J Clin Nutr 2011;93:1212-1219.

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Original article

Association of serum irisin levels with anthropometric, biochemical, and atherogenic indices in healthy adults

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Summary. *Objective:* Irisin, a myokine mostly expressed by muscle, is proposed to increase energy expenditure and reduce obesity and metabolic disorders. So, we evaluated the association between serum irisin levels and various anthropometric, biochemical, and atherogenic indices. *Methods:* This cross-sectional study was conducted on 90 apparently healthy males and females, aged 20-55 years, selected with simple random sampling. Anthropometric and atherogenic indices, dietary intake, physical activity levels, serum irisin levels, lipid profile, and fasting blood sugar (FBS) of the subjects were measured. *Results:* Median Irisin level was 1200 (500-8600) pg/ml which was higher in women than men (1250.00 (800-8600) VS. 1050 (500-7700)). In multivariate linear regression analysis, after controlling for potential confounders (age, total energy intake and physical activity), irisin levels were significantly associated with BMI (r=-0.214; P=0.003), waist circumference (WC) (r=-0.002; P=0.004), hip circumference (HC) (r=-0.245; P=0.004), waist-to-height ratio (WHtR) (r=-0.223; P=0.005), Body Roundness Index (BRI) (r=-0.214; P=0.008), Abdominal Volume Index (AVI) (r=-0.189; P=0.009), and Body Adiposity Index (BAI) (r=-0.207; P=0.046). In male subjects, it was significantly associated with BMI (r=-0.182, P=0.049), HC (r=-0.295; P=0.005), waist-to-hip ratio (WHR) (r=0.279; P=0.038) and BAI (r=-0.418; P=0.001), while in females with BMI (r=-0.268; P=0.005), WC (r=-0.236; P=0.030), WHtR (r=-0.223; P=0.047), and AVI (r=-0.226; P=0.047). No significant association was observed between irisin and biochemical and atherogenic indices. *Conclusions:* In the present study, irisin level was significantly associated with BMI, WC, HC, WHtR, BRI, and BAI. However, further studies are needed to clarify the role of irisin in obesity and its comorbidities.

Key words: abdominal obesity, atherogenesis, gender, Irisin, myokine

Introduction

The muscular tissue has recently emerged as an endocrine organ by releasing a variety of cytokines (termed as myokines) into the circulation. Myokines regulate physiological and metabolic pathways including energy metabolism and the underlying mechanisms of obesity, metabolic syndrome, and cardiovascular disease (CVD)(1, 2). A novel myokine, irisin, is the extracellular cleaved product of fibronectin type III domain containing 5 (FNDC5) gene prior to be released into the circulation and is regulated by the peroxisome proliferator-activated receptor-γ (PPAR-γ) coactivator-1-alpha (PGC-1-α). Irisin is mainly secreted during or after exercise and it is possibly responsible for the thermogenesis and many health benefits of physical-activity (3, 4). Moreover, irisin is able to stimulate brown-fat-like

development in white fat by increasing uncoupling protein 1 (UCP1) levels, and hence increase total energy expenditure (5, 6). Regarding to its modulatory effect in energy metabolism and thermogenesis, several studies have indicated a possible relationship between circulating irisin levels and diseases such as obesity, CVD, type 2 Diabetes (T2D), chronic kidney disease, nonalcoholic fatty liver disease, and cancer. Therefore, it has been suggested as a therapeutic agent against metabolic disorders (7-11). In rodents, irisin significantly increases total energy expenditure and reduces obesity and insulin resistance (12).

Human studies on the association between circulating irisin levels and anthropometric parameters are conflicting; some authors reported a negative correlation between circulating irisin levels and Body Mass Index (BMI) (13, 14), whereas others reported a positive correlation (15-17). Although limited human studies have examined the relationship between irisin and some abdominal obesity indices such as Waist Circumference (WC) and Waist-to-Hip Ratio (WHR) (18, 19), its association with other novel and more feasible indices such as Conicity Index (CI), A Body Shape Index (ABSI), and Abdominal Volume Index (AVI) has not yet been investigated. These indices evaluate body fat distribution effectively which is important in obesity-related co-morbidities. Body Roundness Index (BRI) is a new index to predict percentage of body fat and visceral adipose tissue (20, 21). Recent studies demonstrated a predictive ability of the BRI for predicting CVD and diabetes (22). Furthermore, Body Adiposity Index (BAI) has been proposed for estimating the percentage of body fat (23). Regarding the importance of abdominal obesity indices and body fat percentage indices in predicting obesity-related co-morbidities, exploring the association between different myokines and these indices seems valuable.

Studies on the association between circulating irisin levels and biochemical indices are conflicting, as well. Choi et al. (13) found that circulating irisin levels negatively correlated with oral glucose tolerance test (OGTT), glycated hemoglobin (HbA1C), and triglyceride (TG). However, Liu et al. (24) found that circulating irisin was positively correlated with total cholesterol (TC), TG, Low-density lipoprotein cholesterol (LDL-C) and fasting blood sugar (FBS). Although several studies have been carried out to determine the association between serum irisin levels and biochemical indices, no study have examined the relationship between irisin and atherogenic indices. Atherogenic indices such as atherogenic index of plasma (AIP), atherogenic coefficient (AC), castelli risk index I and II (CRI) and non-HDL Cholesterol (NHC) are derived from lipid profile and are better predictors of coronary heart diseases rather than the traditional lipid profile (25-27).

Controversial results were reported regarding the association of irisin and anthropometric and biochemical indices in previous studies. Moreover, the association of irisin with novel anthropometric and atherogenic indices has not yet been evaluated. Therefore, the aim of current study was to explore the association of serum irisin levels with abdominal and atherogenic indices along with body composition, lipid profile, total energy intake, and physical activity levels in healthy subjects.

Materials and Methods

Subjects

The present cross sectional study was conducted on 90 apparently healthy volunteers of both sexes (% 50 male), aging between 20 and 55 years. Participants were selected by simple random sampling from an outpatient clinic belonging to Tabriz University of Medical Sciences between October 2016 and February 2017. Subjects were excluded if they were pregnant, lactating, menopause or cigarette smokers.

The study protocol was approved by the Ethics committee of Tabriz University of Medical Sciences, Tabriz, Iran (ethical code: TBZMED.REC.1395.685). All subjects were made aware of the content of the study and a written informed consent document was obtained.

Anthropometric measurements

Weight was measured to the nearest 0.1 kg using Seca scale in light outdoor closing without shoes. Height was measured to the nearest 0.5 cm using a portable stadiometer in subjects standing in standard

position. BMI was calculated by dividing weight in kilograms by the square of the height in meters. WC was measured at the midpoint between the lowest rib and the iliac crest with a flexible anthropometric tape on the midaxillary line (28). HC was measured over thin clothing at the level of the maximum circumference of the buttocks posteriorly in a horizontal plane, without compressing the skin. Waist-to-Hip Ratio was calculated as WC (cm) divided by HC (cm), WHtR was calculated as WC (cm) divided by height (cm) and WHHR was calculated as WHR divided by height (cm) (21, 29, 30).

All anthropometric indices were calculated according to the following formula (30-37):

$$ABSI = \frac{WC(m)}{BMI^{\frac{2}{3}}(kg/m^2) * height^{\frac{1}{2}}(m)}$$

$$AVI = [2\,(WC)^2(cm) + 0.7\,(WC - HC)^2(cm)]/1000$$

$$CI = \frac{WC(m)}{0.109\sqrt{\frac{weight(kg)}{Height(m)}}}$$

$$BAI = \frac{(HC)\,(Cm)}{Height^{1.5}(m)} - 18$$

$$BRI = 364.2 - 365.5 \times \sqrt{1 - \left(\frac{(WC\,(m)/(2\pi))^2}{(0.5\,Height)^2\,(m)}\right)}$$

Dietary assessment and Physical activity

Participants' dietary intake was assessed using a validated interviewer-administered semi-quantitative 79 item food frequency questionnaire (FFQ) (38), which included all of the major food groups. To assess physical activity (PA), validated International Physical Activity Questionnaire – short form (IPAQ-SF) (39) was used, in which individuals reported the number of days and the duration of the vigorous, moderate, and walking activities during one week. According to IPAQ's scoring protocol, each individual was categorized to one of "inactive", "minimally active", and "Health Enhancing Physical Activity (HEPA)" categories.

Assessment of biomarkers

Blood samples were taken in the morning (8:00 – 9:00 AM) after 12 h of fasting. Serum lipid profiles, including TG, TC and high-density lipoprotein cholesterol (HDL-C) were also measured using enzymatic methods. Low-density lipoprotein cholesterol was calculated using the Friedewald's formula (40). Fasting blood sugar was measured by the hexokinase method. Circulating irisin levels were quantified in serum samples using commercial ELISA kits (ZellBio GmbH, Germany) (Cat. No: ZB-13253S-H9648) (13).

Lipid indices were calculated according to the following formulas (25-27):

AIP = log (TG / HDL)
CRI-I = TC / HDL
CRI-II = LDL / HDL
AC = (TC – HDL) / HDL
NHC = TC – HDL

Statistical analyses

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) software version 21 (SPSS Inc., Chicago, IL). Kolmogorov–Smirnov test was used to check the normality of distributions of continuous variables. Data were presented as mean ± standard deviation (SD) and median (maximum – minimum). The differences in mean between two groups were compared with independent samples t-tests or Mann–Whitney test. Chi-square test was used for between group comparisons, in case of categorical variables. Multiple linear regression analysis was used to identify variables independently associated with irisin levels. $P < 0.05$ was considered as statistically significant.

Results

The mean age of all participants was 35.59 ± 9.73 years and the mean BMI (Kg/m^2) was 27.83 ± 5.18 Kg/m^2 (Table 1). There was no significant difference between the BMI levels of male (27.52 ± 4.82 Kg/m^2) and female subjects (28.14 ± 5.56 Kg/m^2) in this study. Male subjects had significantly higher levels of weight (Kg) than female subjects ($P < 0.05$). The daily energy intake of men (2984.13 ± 623.22 Kcal) was significantly higher than women (2591.61 ± 729.20 Kcal) ($P < 0.05$) (Table 1). Based on three categories of IPAQ-SF, 68.2, 22.7 and 9.1 percent of men and 54.5, 40.9 and 4.5 percent of women were categorized as inactive, minimally active and health enhancing physical activity.

Laboratory characteristics of all participants are detailed in Table 2. There was no significant difference in biochemical characteristics including TC, LDL-C

Table 1. Baseline anthropometric characteristics, dietary intake and physical activity of total, male and female subjects

Variables	Total (n=90	Male (n=45)	Female (n=45)	P-value
Age (years) †	35.59± 9.73	37.47 ± 9.47	33.70 ± 9.73	0.069
Weight (kg) †	78.64 ± 15.92	83.20 ± 14.67	74.08 ± 15.97	0.006
BMI (Kg/m^2) †	27.83 ± 5.18	27.52 ± 4.82	28.14 ± 5.56	0.575
WC (cm) †	90.12 ± 13.94	93.88 ± 12.21	86.36 ± 14.67	0.011
HC (cm) †	105.09 ± 11.00	103.20 ± 9.09	106.97 ± 12.45	0.108
WHR † (cm/cm)	0.85 ± 0.08	0.90 ± 0.06	0.80 ± 0.07	<0.001
WHtR † (cm/cm)	0.53 ± 0.08	0.54 ± 0.07	0.53 ± 0.09	0.679
WHHR † (cm/cm/cm)	0.50 ± 0.04	0.52 ± 0.04	0.49 ± 0.05	0.010
CI † ($m^{2/3}/kg^{1/2}$)	1.21 ± 0.10	1.24 ± 0.08	1.17 ± 0.10	0.001
ABSI †($m^{11/6}\,kg^{-2/3}$)	0.07 ± 0.00	0.07 ± 0.00	0.07 ± 0.00	<0.001
AVI † (cm^2)	16.63 ± 4.99	17.92 ± 4.69	15.33 ± 5.00	0.014
BRI †	4.19 ± 1.64	4.24 ± 1.49	4.15 ± 1.79	0.796
BAI † ($cm/m^{1.5}$–18)	30.49 ± 6.45	27.08 ± 4.72	33.90 ± 6.18	<0.001
Total energy intake (kcal/d)†	2787.87 ± 702.67	2984.13 ± 623.22	2591.61 ± 729.20	0.008
Physical activity (Met-minutes/week) ‡	594 (0.00-23916)	480 (0.00-23916)	693 (0.00-3732)	0.341

bbreviations: BMI: Body mass index; WC: waist circumference; HC: Hip circumference; WHR: waist-to-hip ratio; WHtR: waist-to-height ratio; WHHR: waist-to-hip-to-height ratio; CI: Conicity Index; ABSI: A Body Shape Index; AVI: Abdominal Volume Index; BRI: Body Roundness Index; BAI: Body Adiposity Index. † The P value was obtained by independent samples t-test for normal distributed variables and represented as mean ± SD. ‡ The P value was obtained by Mann-Whitney test for non-normally distributed variables and represented as median (interquartile range). Bold values mean that the value is statistically significant, P < 0.05.

Table 2. Laboratory characteristics of total, male and female subjects

Variables	Total (n=90)	Male (n=45)	Female (n=45)	P value
FBS (mg/dl) †	89.98 ± 8.16	92.75 ± 8.50	87.21 ± 6.85	0.001
TG (mg/dl) ‡	133.50 (43-481)	174.50 (43-481)	114.50 (50-300)	<0.001
TC (mg/dl) †	181.62 ± 38.74	183.97 ± 35.07	179.26 ± 42.37	0.571
HDL-C (mg/dl) †	45.91 ± 8.34	43.20 ± 7.82	48.62 ± 8.05	0.002
LDL-C (mg/dl) †	93.13 ± 24.82	95.70 ± 28.16	90.56 ± 20.98	0.334
Irisin (pg/ml) ‡	1200 (500-8600)	1050 (500-7700)	1250.00 (800-8600)	0.086
AIP‡	0.46 (- 0.2-1.16)	0.62 (- 0.2-1.16)	0.35 (- 0.1-0.83)	<0.001
AC †	3.09 ± 1.15	3.36 ± 1.00	2.81 ± 1.22	0.023
CRI-I †	4.09 ± 1.15	4.36 ± 1.00	3.81 ± 1.22	0.023
CRI-II †	2.07 ± 0.62	2.25 ± 0.66	1.90 ± 0.52	0.008
NHC †	135.70 ± 40.11	140.77 ± 34.93	130.64 ± 44.52	0.238

Abbreviations: FBS: fasting blood sugar; TG: triglyceride; TC: total cholesterol; HDL-C: High-Density Lipoprotein Cholesterol; LDL-C: Low-Density Lipoprotein Cholesterol; AIP: Atherogenic Index of Plasma; AC: Atherogenic Coefficient; CRI-I: Castelli's Risk Index- I; CRI-II: Castelli's Risk Index- II; NHC: Non HDL Cholesterol. † The P value was obtained by independent samples t-test for normal distributed variables and represented as mean ± SD. ‡ The P value was obtained by Mann-Whitney test for non-normally distributed variables and represented as median (interquartile range).
Bold values mean that the value is statistically significant, P < 0.05

and NHC between male and female participants (P > 0.05). However, the FBS, TG, AIP, AC, CRI-I and CRI-II were significantly higher in men than women (P < 0.05). There were no significant differences in the levels of irisin between the men and women (P > 0.05). The range of minimum and maximum values of irisin were 500 – 8600 pg/ml in total, 500 - 7700 pg/ml in men and 800 -8600 pg/ml in women.

Table 3. The association of anthropometric indices with irisin

	BMI						WC						WHR						WHtR						WHHR			
	Total		Male		Female		Total		Male		Female		Total		Male		Female		Total		Male		Female		Total		Male		Female	
	β	P*	β	P*	β	P*	β	P*	β	p	β	P*	β	p	β	P*	β	p	β	P*	β	p	β	P*	β	P*	β	P*	β	P*
Irisin	-.214	.003	-.182	.049	-.268	.005	-.200	.004	-.078	.439	-.236	.030	-.061	.516	.279	.038	-.195	.169	-.223	.005	-.169	.126	-.223	.047	-.103	.296	.052	.728	-.157	.310
Age	.041	.549	.172	.066	.013	.878	.164	.014	.017	.864	.250	.013	.248	.007	-.058	.650	.318	.017	.213	.005	.174	.112	.289	.007	.324	.001	.223	.134	.331	.023
Total Energy Intake	.703	.000	.785	.000	.738	.000	.697	.000	.784	.000	.620	.000	.491	.000	.585	.000	.338	.013	.602	.000	.704	.000	.589	.000	.341	.001	.396	.010	.256	.079
Physical Activity	.020	.767	.028	.771	-.030	.705	.029	.655	.194	.063	-.037	.691	-.096	.276	.233	.086	-.211	.095	.040	.581	.127	.255	-.018	.857	-.089	.341	.087	.567	-.170	.216

Abbreviations: BMI: Body mass index; WC: Waist circumference; WHR: waist-to-hip ratio; WHtR: waist-to-height ratio; WHHR: waist-to-hip-to-height ratio. P values are from linear regressions adjusted for age, total energy intake and physical activity. Bold values mean that the value is statistically significant, P < 0.05

	CI						ABSI						AVI						BRI						BAI			
	Total		Male		Female		Total		Male		Female		Total		Male		Female		Total		Male		Female		Total		Male		Female	
	β	P	β	P	β	p	β	p	β	p	β	p	β	P*	β	p	β	P*	β	P*	β	p	β	P	β	P*	β	P*	β	p
Irisin	-.151	.114	-.001	.997	-.131	.347	-.078	.472	.086	.591	-.029	.852	-.189	.009	-.092	.363	-.226	.047	-.214	.008	-.174	.116	-.210	.072	-.207	.046	-.418	.001	-.178	.191
Age	.294	.002	-.030	.836	.474	.001	.304	.004	-.114	.470	.543	.000	.147	.034	.008	.936	.247	.019	.211	.006	.165	.130	.308	.005	.071	.486	.355	.002	.181	.149
Total Energy Intake	.407	.000	.425	.005	.337	.012	.166	.120	.085	.592	.088	.539	.692	.000	.778	.000	.609	.000	.593	.000	.705	.000	.575	.000	.273	.011	.487	.000	.560	.000
Physical Activity	.029	.745	.272	.074	-.036	.768	.018	.858	.275	.097	-.038	.778	.034	.607	.185	.078	-.035	.718	.049	.507	.125	.263	.000	.998	.127	.209	-.013	.912	.137	.252

Abbreviations: CI: Conicity Index; ABSI: A Body Shape Index; AVI: Abdominal Volume Index; BRI: Body Roundness Index; BAI: Body Adiposity Index. P values are from linear regressions adjusted for age, total energy intake and physical activity. Bold values mean that the value is statistically significant, P < 0.05

Table 4. The association of biochemical indices with irisin

	FBS						TC						HDL						LDL						TG			
	Total		Male		Female		Total		Male		Female		Total		Male		Female		Total		Male		Female		Total		Male		Female	
	β	p	β	P	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p
Irisin	.097	.368	.146	.341	-.061	.723	-.025	.831	-.272	.087	.142	.408	-.050	.646	.005	.974	-.165	.321	-.104	.371	.130	.421	-.328	.070	-.042	.690	-.050	.742	-.108	.527
Age	.236	.025	.294	.048	.061	.696	.104	.349	-.028	.855	.322	.045	-.241	.022	-.181	.257	-.250	.104	.007	.948	-.166	.299	.124	.448	.320	.002	.412	.008	.083	.598
Total Energy Intake	.245	.023	.181	.235	.109	.492	-.089	.438	-.219	.158	-.032	.843	-.276	.011	.002	.990	-.419	.009	.049	.670	.095	.552	-.113	.494	.261	.013	.123	.409	.337	.039
Physical Activity	.237	.021	.169	.281	.416	.009	.078	.476	-.217	.176	.324	.037	-.176	.085	-.152	.357	-.200	.175	.069	.527	.184	.266	.099	.527	-.016	.871	-.011	.942	-.099	.512

Abbreviations: FBS: fasting blood sugar; TC: total cholesterol; HDL-C: High-Density Lipoprotein Cholesterol; LDL-C: Low-Density Lipoprotein Cholesterol; TG: triglyceride

P values are from linear regressions adjusted for age, total energy intake and physical activity.

Bold values mean that the value is statistically significant, P < 0.05

Table 5 . The association of atherogenic indices with irisin

	AIP						AC						CRI-I						CRI-II						NHC					
	Total		Male		Female		Total		Male		Female		Total		Male		Female		Total		Male		Female		Total		Male		Female	
	β	P	β	P	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p
Irisin	-.017	.869	.019	.898	-.058	.718	.024	.831	-.134	.406	.135	.412	.024	.831	-.134	.406	.135	.412	-.060	.588	.131	.418	-.191	.251	-.014	.906	-.016	.925	.071	.653
Age	.336	.001	.378	.015	.190	.204	.276	.011	.196	.217	.379	.015	.087	.427	-.159	.315	.157	.304	.188	.086	.067	.674	.163	.289	-.028	.804	-.049	.762	-.056	.733
Total Energy Intake	.346	.001	.152	.311	.442	.005	.087	.427	-.159	.315	.157	.304	.087	.427	-.159	.315	.157	.304	.188	.086	.067	.674	.163	.289	-.028	.804	-.049	.762	-.056	.733
Physical Activity	.065	.494	.089	.565	.036	.800	.165	.117	-.069	.672	.373	.013	.165	.117	-.069	.672	.373	.013	.169	.108	.247	.137	.240	.104	.112	.303	.065	.687	.157	.323

Abbreviations: AIP: Atherogenic Index of Plasma; AC: Atherogenic Coefficient; CRI-I: Castelli's Risk Index- I; CRI-II: Castelli's Risk Index- II; NHC: Non HDL Cholesterol;
P values are from linear regressions adjusted for age, total energy intake and physical activity;
Bold values mean that the value is statistically significant, P < 0.05

To adjust the potential confounders involved in the association of irisin with anthropometric indices, we performed multiple linear regression analysis. As shown in Table 3, irisin was negatively and significantly associated with BMI (β= - 0.214, P= 0.003), WC (β= - 0.200, P= 0.004), HC (β= - 0.245, P= 0.004), WHtR (β= - 0.223, P= 0.005), AVI (β= - 0.189, P= 0.009), BRI (β= - 0.214, P= 0.008), and BAI (β= - 0.207, P= 0.046) after adjusting for age, total energy intake and physical activity. In addition, irisin had a significant association with BMI (β= - 0.182, P= 0.049), HC (β= - 0.295, P= 0.049), WHR (β= 0.279, P= 0.038), and BAI (β= - 0.418, P= 0.001) in men and BMI (β= - 0.268, P= 0.005), WC (β= - 0.236, P= 0.030), WHtR (β= - 0.223, P= 0.047) and, AVI (β= - 0.226, P= 0.047) in women. According to table 4, irisin was positively associated with FBS and negatively associated with TC, HDL, LDL and TG which was not statistically significant. As presented in table 5, irisin was positively associated with AC and CRI-I and negatively associated with AIP, CRI-II and NHC; however, these differences were not statistically significant.

Discussion

In this study, no significant difference was found between male and female subjects in circulating irisin levels , which was similar to Karan et al. study (41). On the other hand, some studies reported contradictory results. Al-Daghri and colleagues (42) observed higher irisin levels in girls compared with boys. While in another study, irisin levels were higher in men than women (43). This contradiction can be explained by the differences in characteristics of participants.

We also found a significant and negative association between serum irisin levels and BMI in total, male and female subjects. Existing evidence about association of irisin with BMI is controversial. Some studies reported a negative correlation between irisin and BMI (13, 14, 44, 45). Choi YK et al. (13) found that circulating irisin levels reduced in recently diagnosed T2D. In addition, in the study performed by Moreno-Navarrete et al. (14), serum irisin level was lower in obese patients compared with normal weight subjects. Reverse correlation between circulating irisin levels and BMI might

be due to a decreased expression of FNDC5 gene in adipose tissue, obesity-associated lower amounts of brown or beige adipocytes or impaired conversion of FNDC5 to irisin in obese patients. On the other hand, some studies reported a null or positive correlation. In a research performed in a relatively small sample size, Jameel et al. (18) reported that irisin was not associated with BMI. Whilst, Stengel et al. (16) reported that subjects with high degree of obesity (BMI > 35) had higher levels of serum irisin than those with normal weight. Also, Liu JJ et al. observed that in the healthy subjects, serum irisin levels had a positive correlation with BMI and metabolic factors. In another study performed by Park et al. (46), BMI was positively correlated with irisin. The controversial results in different studies along with different BMI levels, could be possibly due to methodological differences and variation in sensitivity and specificity of different kits used in different studies or other intervening variables such as age, gender, race and physical activity.

There is limited evidence about the association of irisin with abdominal obesity indices. In the present study, irisin was significantly associated with WC, HC, WHtR, and AVI in all study subjects. Also, it was associated with HC and WHR in male subjects and with WC, WHtR and AVI in female subjects, which indicates that irisin was significantly related to central obesity. Jameel et al. (18) reported that irisin was not associated with WHR. In a cross-sectional study conducted by Liu et al. (22) on more than 1000 Chinese adults with metabolic syndrome, irisin was associated with WC (an indicator of abdominal obesity). In addition, Moreno-Navarrete et al. (14) demonstrated that circulating irisin levels was negatively correlated with WHR. In contrast, Park et al. (47) showed that irisin levels were positively correlated with WC. In the current project, for the first time, we investigated the association of irisin with body fat percent indices including BRI and BAI. The results of our study showed a negative association in the total, male and female groups. In agreement with our results, Moreno-Navarrete et al. (14) reported that irisin was negatively correlated with percent fat mass. However, in the study performed by Jameel and co-workers (18), serum irisin levels inversely associated with percent body fat among men, but not women. In contrast, another study found that circulating irisin was positively correlated with body fat mass (47). Contradictory results may be due to the different experimental and physiological conditions, and the different health status of the participants.

In this study, no significant association was observed between circulating irisin levels and biochemical indices including FBS, TC, HDL, LDL and TG levels. Evidence indicating the association between irisin and biochemical indices are limited. Our results were similar to the Jameel et al. (18) findings who observed no significant relationship between serum irisin and glucose, TG, TC, HDL, and LDL levels. However, there are a number of studies which have reported significant relationships. Liu et al. (24) observed a positive correlation between serum irisin levels and TC, TG, LDL-C, and FBG. In contrary, Choi and colleagues (13) reported a negative correlation between irisin level and OGTT, HbA1c, and TG. The controversy over the associations between circulating irisin and glycaemic and lipid profile among different studies might be explained due to the variation in experimental design, differences in the biochemical analytical assays, different BMI levels, confounding variables such as age, race, sex, physical activity and the variation in health status of study participants.

In multivariate linear regression analysis, after controlling for potential confounders such as age, total energy intake, and physical activity, no significant associations between serum irisin levels and atherogenic indices were found.This result suggest that age, total energy intake, and physical activity are the main confounders.

In elderly subjects, the percentage of muscle tissue decreases, and subsequently the amount of irisin secretion decreases. Tanisawa et al. reported that serum irisin levels were significantly lower in elderly subjects than in young subjects (48). Scharf et al. reported that the endocrine release of myokines from skeletal muscle might be impaired in sarcopenia, and irisin is on the list of potential candidates in this regard (49).

This study had some strengths but also some limitations. The strengths included the homogeneity of the population in living conditions and health status, assessment of food intake and physical activity by validated questionnaires, consideration of the confounding factors such as smoking, age, total energy intake, and physical activity. This is also the first report that revealing the association between serum irisin levels

and new anthropometric and atherogenic indices. The limitations of this investigation were the low number of sample size and the cross-sectional design of the study that couldn't show the causality and provide information on a prospective manner.

In conclusion, our results indicated negative associations between serum irisin levels and various anthropometric measurements including BMI, WC, HC, WHtR, BRI, AVI and BAI in total healthy participants, without any associations with biochemical and atherogenic indices. The present research could raise credible hypotheses to be extended by future studies with prospective design, larger sample size and different BMI categories to clarify the role of irisin in obesity and its comorbidities.

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References

1. Pedersen BK, Febbraio MA. Muscles, exercise and obesity: skeletal muscle as a secretory organ. Nat Rev Endocrinol. 2012;8(8):457.
2. Pedersen BK. The diseasome of physical inactivity–and the role of myokines in muscle–fat cross talk. J Physiol. 2009;587(23):5559-68.
3. Bostrom P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, et al. A PGC1-alpha-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature. 2012;481(7382):463-8.
4. Boström PA, Fernández-Real JM, Mantzoros C. Irisin in humans: recent advances and questions for future research. Metabolism. 2014;63(2):178-80.
5. Castillo-Quan JI. From white to brown fat through the PGC-1α-dependent myokine irisin: implications for diabetes and obesity. Disease models & mechanisms. 2012;5(3):293-5.
6. Chen J-q, Huang Y-y, Gusdon AM, Qu S. Irisin: a new molecular marker and target in metabolic disorder. Lipids in health and disease. 2015;14(1):2.
7. Polyzos SA, Kountouras J, Anastasilakis AD, Geladari EV, Mantzoros CS. Irisin in patients with nonalcoholic fatty liver disease. Metabolism. 2014;63(2):207-17.
8. Wen M-S, Wang C-Y, Lin S-L, Hung K-C. Decrease in irisin in patients with chronic kidney disease. PloS one. 2013;8(5):e64025.
9. Sanchis-Gomar F, Lippi G, Mayero S, Perez-Quilis C, Garcia-Gimenez JL. Irisin: a new potential hormonal target for the treatment of obesity and type 2 diabetes. J Diabetes. 2012;4(3):196.
10. Aronis K, Moreno M, Polyzos S, Moreno-Navarrete J, Ricart W, Delgado E, et al. Circulating irisin levels and coronary heart disease: association with future acute coronary syndrome and major adverse cardiovascular events. Int J Obes (Lond). 2015;39(1):156.
11. Moon H-S, Mantzoros CS. Regulation of cell proliferation and malignant potential by irisin in endometrial, colon, thyroid and esophageal cancer cell lines. Metabolism. 2014;63(2):188-93.
12. Roberts MD, Bayless DS, Jenkins NT, Padilla J, Childs TE, Martin JS, et al. Elevated skeletal muscle irisin precursor FNDC5 mRNA in obese OLETF rats. Metabolism. 2013;62(8):1052-6.
13. Choi Y-K, Kim M-K, Bae KH, Seo H-A, Jeong J-Y, Lee W-K, et al. Serum irisin levels in new-onset type 2 diabetes. Diabetes Res Clin Pract. 2013;100(1):96-101.
14. Moreno-Navarrete JM, Ortega F, Serrano M, Guerra E, Pardo G, Tinahones F, et al. Irisin is expressed and produced by human muscle and adipose tissue in association with obesity and insulin resistance. J Clin Endocrinol Metab. 2013;98(4):E769-E78.
15. Huh JY, Panagiotou G, Mougios V, Brinkoetter M, Vamvini MT, Schneider BE, et al. FNDC5 and irisin in humans: I. Predictors of circulating concentrations in serum and plasma and II. mRNA expression and circulating concentrations in response to weight loss and exercise. Metabolism. 2012;61(12):1725-38.
16. Stengel A, Hofmann T, Goebel-Stengel M, Elbelt U, Kobelt P, Klapp BF. Circulating levels of irisin in patients with anorexia nervosa and different stages of obesity--correlation with body mass index. Peptides. 2013;39:125-30.
17. Pardo M, Crujeiras AB, Amil M, Aguera Z, Jimenez-Murcia S, Banos R, et al. Association of irisin with fat mass, resting energy expenditure, and daily activity in conditions of extreme body mass index. Int J Endocrinol. 2014;2014:857270.
18. Jameel F, Thota R, Wood L, Plunkett B, Garg M. Sex-dependent association between circulating irisin levels and insulin resistance in healthy adults. Journal of Nutrition & Intermediary Metabolism. 2015;2(3-4):86-92.
19. Yan B, Shi X, Zhang H, Pan L, Ma Z, Liu S, et al. Association of serum irisin with metabolic syndrome in obese Chinese adults. PloS one. 2014;9(4):e94235.
20. Maessen MF, Eijsvogels TM, Verheggen RJ, Hopman MT, Verbeek AL, de Vegt F. Entering a new era of body indices: the feasibility of a body shape index and body roundness index to identify cardiovascular health status. PloS one. 2014;9(9):e107212.
21. Tian S, Zhang X, Xu Y, Dong H. Feasibility of body roundness index for identifying a clustering of cardiometabolic abnormalities compared to BMI, waist circumference and other anthropometric indices: the China Health and Nutrition Survey, 2008 to 2009. Medicine (Baltimore). 2016;95(34):e4642.
22. Liu P, Ma F, Lou H, Zhu Y. Body roundness index and body adiposity index: two new anthropometric indices to identify metabolic syndrome among Chinese postmenopausal women. Climacteric. 2016;19(5):433-9.
23. Bergman RN. A better index of body adiposity. Obesity (Silver Spring). 2012;20(6):1135.

24. Liu JJ, Wong MD, Toy WC, Tan CS, Liu S, Ng XW, et al. Lower circulating irisin is associated with type 2 diabetes mellitus. J Diabetes Complications. 2013;27(4):365-9.
25. Tseng Y-H, Yeh Y-H, Chen W-J, Lin K-H. Emerging regulation and function of betatrophin. International journal of molecular sciences. 2014;15(12):23640-57.
26. Bhardwaj S, Bhattacharjee J, Bhatnagar M, Tyagi S. Atherogenic index of plasma, castelli risk index and atherogenic coefficient-new parameters in assessing cardiovascular risk. Int J Pharm Biol Sci. 2013;3(3):359-64.
27. Sujatha R, Kavitha S. Atherogenic indices in stroke patients: A retrospective study. Iran J Neurol. 2017;16(2):78-82.
28. Tabrizi JS, Sadeghi-Bazargani H, Farahbakhsh M, Nikniaz L, Nikniaz Z. Prevalence and Associated Factors of Prehypertension and Hypertension in Iranian Population: The Lifestyle Promotion Project (LPP). PLoS One. 2016;11(10):e0165264.
29. Fu S, Luo L, Ye P, Liu Y, Zhu B, Bai Y, et al. The abilities of new anthropometric indices in identifying cardiometabolic abnormalities, and influence of residence area and lifestyle on these anthropometric indices in a Chinese community-dwelling population. Clin Interv Aging. 2014;9:179-89.
30. Hardy DS, Stallings DT, Garvin JT, Xu H, Racette SB. Best anthropometric discriminators of incident type 2 diabetes among white and black adults: A longitudinal ARIC study. PloS one. 2017;12(1):e0168282.
31. Ehrampoush E, Arasteh P, Homayounfar R, Cheraghpour M, Alipour M, Naghizadeh MM, et al. New anthropometric indices or old ones: Which is the better predictor of body fat? Diabetes Metab Syndr. 2017;11(4):257-63.
32. Thomson CA, Garcia DO, Wertheim BC, Hingle MD, Bea JW, Zaslavsky O, et al. Body shape, adiposity index, and mortality in postmenopausal women: Findings from the Women's Health Initiative. Obesity (Silver Spring). 2016;24(5):1061-9.
33. Gowda V, Philip KM. Abdominal volume index and conicity index in predicting metabolic abnormalities in young women of different socioeconomic class. International Journal of Medical Science and Public Health. 2016;5(7):1452-6.
34. Cerqueira M, Amorim P, Magalhaes F, Castro E, Franco F, Franceschini S, et al. Validity of body adiposity index in predicting body fat in a sample of Brazilian women. Obesity (Silver Spring). 2013;21(12):E696-9.
35. Thomas DM, Bredlau C, Bosy-Westphal A, Mueller M, Shen W, Gallagher D, et al. Relationships between body roundness with body fat and visceral adipose tissue emerging from a new geometrical model. Obesity (Silver Spring). 2013;21(11):2264-71.
36. Biolo G, Di Girolamo FG, Breglia A, Chiuc M, Baglio V, Vinci P, et al. Inverse relationship between "a body shape index"(ABSI) and fat-free mass in women and men: insights into mechanisms of sarcopenic obesity. Clinical Nutrition. 2015;34(2):323-7.
37. Wang H, Liu A, Zhao T, Gong X, Pang T, Zhou Y, et al. Comparison of anthropometric indices for predicting the risk of metabolic syndrome and its components in Chinese adults: a prospective, longitudinal study. BMJ open. 2017;7(9):e016062.
38. Nikniaz L, Tabrizi J, Sadeghi-Bazargani H, Farahbakhsh M, Tahmasebi S, Noroozi S. Reliability and relative validity of short-food frequency questionnaire. British Food Journal. 2017;119(6):1337-48.
39. Vasheghani-Farahani A, Tahmasbi M, Asheri H, Ashraf H, Nedjat S, Kordi R. The Persian, last 7-day, long form of the International Physical Activity Questionnaire: translation and validation study. Asian J Sports Med. 2011;2(2):106-16.
40. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical chemistry. 1972;18(6):499-502.
41. Rana K. The metabolic regulation of cellular ageing: Aston University; 2016.
42. Al-Daghri NM, Alkharfy KM, Rahman S, Amer OE, Vinodson B, Sabico S, et al. Irisin as a predictor of glucose metabolism in children: sexually dimorphic effects. Eur J Clin Invest. 2014;44(2):119-24.
43. Crujeiras AB, Pardo M, Arturo RR, Navas-Carretero S, Zulet MA, Martinez JA, et al. Longitudinal variation of circulating irisin after an energy restriction-induced weight loss and following weight regain in obese men and women. Am J Hum Biol. 2014;26(2):198-207.
44. Hou N, Han F, Sun X. The relationship between circulating irisin levels and endothelial function in lean and obese subjects. Clin Endocrinol (Oxf). 2015;83(3):339-43.
45. Klangjareonchai T, Nimitphong H, Saetung S, Bhirommuang N, Samittarucksa R, Chanprasertyothin S, et al. Circulating sclerostin and irisin are related and interact with gender to influence adiposity in adults with prediabetes. Int J Endocrinol. 2014;2014:261545.
46. Park KH, Zaichenko L, Peter P, Davis CR, Crowell JA, Mantzoros CS. Diet quality is associated with circulating C-reactive protein but not irisin levels in humans. Metabolism. 2014;63(2):233-41.
47. Park KH, Zaichenko L, Brinkoetter M, Thakkar B, Sahin-Efe A, Joung KE, et al. Circulating irisin in relation to insulin resistance and the metabolic syndrome. J Clin Endocrinol Metab. 2013;98(12):4899-907.
48. Tanisawa K, Taniguchi H, Sun X, Ito T, Cao Z-B, Sakamoto S, et al. Common single nucleotide polymorphisms in the FNDC5 gene are associated with glucose metabolism but do not affect serum irisin levels in Japanese men with low fitness levels. Metabolism. 2014;63(4):574-83.
49. Scharf G, Heineke J. Finding good biomarkers for sarcopenia. Journal of cachexia, sarcopenia and muscle. 2012;3(3):145-8.

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Original article

Effects of nutritional intervention and dietary modification on the health status of pediatric acute lymphoblastic leukemia patients

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Summary. In order to ascertain the effectiveness of nutrient rich diet and dietary counseling on the health status of pediatric acute lymphoblastic leukemia patients, this experimental study was conducted at the Institute of Radiology and Nuclear Medicine, Peshawar. A sample of 30 leukemia patients were divided into experimental and control groups based on written consents. Data regarding demographic characteristics, anthropometric measurements, and retrospective food intakes were recorded on self-constructed questionnaire. Patients in the experimental group received dietary guidelines for nutrient rich diet. Anthropometry, dietary evaluation, and blood nutrients namely serum ferritin, albumin, globulin, total protein and creatinine at 30, 60 and 90 days intervals were assessed. The data showed low height for age and low weight or height at diagnosis indicating malnourishment and wasting among all the patients. After nutritional intervention mean weights of patients in the experimental group increased significantly over period of three months. Progressive weight loss was observed in patients of control group. Blood nutrients at diagnosis showed low Hb (9.19±3.11 and 9.31±1.07 g/dL) and serum ferritin (530±4.28 and 525±3.22 ng/mL) and increased total protein (7.28±3.07 and 7.67±2.76) in both groups. These indices improved in the experimental group over a period of 90 days. Nutrient intake in the experimental group patients improved significantly for all the nutrients.

Key words: acute lymphoblastic leukemia, nutrient rich diet, anthropometry, blood proteins, hemoglobin

Introduction

Leukemia though a rare disease accounts for 1 out of 3 cancers in children and teenagers. Genetic factors may increase the risk of childhood leukemia although most are not linked to any genetic cause. Inherited syndromes such as Down's syndrome, Li-Fraumeni syndrome, neurofibromatosis, Faconi anemia, and inherited immune system problems such as Ataxia telangiectasia, Luiskott-Aldrich syndrome, Bloom syndrome, and Schwachman-diamond syndrome have shown to increase the risk of leukemia. Life style and environmental related risk factors such as smoking, over-weight, alcohol and sun exposure, radiation exposure, chemo drugs an certain chemicals play a role but they unlikely cause most childhood cancers (1).

The exact incidence of childhood cancers is not well documented in Pakistan. The Karachi cancer registry estimated 600 children being diagnosed every year (2). The Shaukat Khanum memorial hospital cancer registry reported 11.44% malignant cases being diagnosed in children (3). Among these; acute leukemias were the most common being reported by many studies (4).

Children suffering from leukemia are at increased risk of nutrition related morbidity during and after

treatment. The overall survival in children and adolescents suffering from leukemia has increased from 10%-90% owing to the improved palliative care facilities (5). However, treatment related toxic effects still pose major problems. Children and adolescents suffering from leukemia experience a vast array of nutrition related disorders (6).

Some retrospective studies have shown that 35-68% children and adolescents develop hypertriglyceridemia, 68% had bone mass and about 30% survivors consumed bone-metabolizing nutrients (7-9). Studies have suggested that dietary modification help prevent the development of therapy related toxicity (10, 11). Leukemia has also been described as a "preobese state" as evident from a number of patients who developed obesity during and after treatment (12). Similarly, several studies have suggested remediation of both under or over weight during treatment to help lower the adverse association with survival (13, 14).

Despite gross prevalence of leukemia among children there is severe paucity of knowledge and data regarding the effect of nutritional interventions on the health status of these patients and remained a neglected area. The current study aimed at analyzing the impacts of nutrient dense diets on the health status of children undergoing leukemia and disease related treatments.

Materials and Methods

Experimental Design

This experimental study was carried out at Institute of Radiology and Nuclear Medicinal IRNUM from October 2014 to July 2015. Based on written consents 30 (17 males, 13 females) leukemia patients aging 5-15 years were divided into experimental and control groups. The experimental group followed dietary recommendations and counseling. The care givers were counseled for dietary modifications during and after therapy. While the control group received their usual diets, medical treatments, and supplements over a period of three months. The entire group underwent a preliminary interview and was clinically examined for protein energy malnutrition, micronutrient deficiencies, such as anemia and presence of infections. Demographic information for different parameters was recorded and patients' anthropometric measurements for height, weight, mid upper arm circumference and skin fold thickness were assessed. Hemoglobin, serum albumin, serum globulin, serum creatinine, serum ferritin and total protein of each patient were recorded thrice at 30, 60, and 90 days intervals.

Diet Therapy

Food intake record of the patients was taken on 65 items Food Variety Scale (FVS) and weekly meal plans along with recipes and portion serving were provided to the care givers. For facilitation they were provided with paper cups, plates and spoon for portion size estimation. The diets were nutritionally evaluated by Food Composition Tables of Pakistan (15). Based on individual patients' health condition, their food intake records, and therapies given following caloric distribution, proteins, Vitamin C, and Vitamin A were focused on.

1. Energy:* (Based of Resting Energy Expenditure)

Male BMR = 66.47 + (13.75 x weight in kg) + (5.003 x height in cm) - (6.755 x age in years)

Female BMR = 655.1 + (9.563 x weight in kg) + (1.850 x height in cm) - (4.676 x age in years)

(*Harris and Benedict equation for age and gender)

As per weight status of the patients a 1200-1800 Kcal/day diet was suggested with 20-30% from protein, 19% fats and 50% from carbohydrates.

2. Protein: 0.9 - 1.5 g/Kg body weight/day.

3. Vitamin C: 45 - 75 mg/day

4. Vitamin A: 4500 IU or 900 mcg (microgram) of retinol activity equivalent (RAE)

Foods rich in vitamin D, sulfur amino acids, carotene, selenium, and omega -3 fatty acids were focused upon during dietary modifications and recommendations. Diets were modified to liquid soft, low residue, post chemotherapy or post radiation diets. Care givers of the patients were counseled for the importance of hygiene, balanced diet, taking regular meals.

Statistical analysis

All the data was subjected to statistical tests such as mean, standard deviation, ANOVA, and co-efficient

of correlation. Two-way ANOVA was calculated to analyze the significance of dietary modifications from initial value till the end and from control group.

Results

Demographic characteristics of the sample

Demographic and family characteristics of the combined sample are given in Table 1. The sample comprised of 17 male and 13 female acute lymphoblastic leukemia patients. The disease was highly prevalent in the age range of 1-5 years. Educational background of both parents was quite low mostly being illiterate. The occupational background showed joblessness among fathers was common while 100% of mother were housewives. Greater percentage of fathers was engaged in private jobs or small- scale businesses with low monthly income and subsequent low spending on food. Mean family size showed five to six children in a nuclear setup being most common. The striking finding of the current study was that leukemia was most common among the last- born children.

Table 1. Demographic characteristics of the sample

Variables	No (% age)
i) Gender	
Males	17 (55%)
Females	13 (45%)
ii) Age at diagnosis	
0-5 years	18 (60)
6-10 years	6 (20)
11-15 years	6 (20)
iii) Father's education	
Illiterate	11 (35)
Elementary	10 (33)
Matric	4 (13)
Graduation	3 (10)
Postgraduates	2 (7)
iv) Father's occupation	
Unemployment	8 (26)
Government job	6 (20)
Private job/small-scale business	16 (53)
v) Mother's education	
Illiterate	18 (60)
Elementary	12 (40)
vi) Family size	
3-4	7 (23)
5-6	10 (33)
7-8	7 (23)
9-10	6 (20)
vii) Position in family	
First born	2 (61)
Middle	3 (10)
Second last	7 (23)
Last born	18 (60)

Anthropometric characteristics of the sample

Results of the anthropometric data of both control and experimental groups (Table 2) showed mean age of 8 years. Heights of both groups were low when compared with WHO standards for this age indicating stunting from early years. Weights of both groups were also low at the start of the trials and increased significantly in the children of experimental group. Improvement in MUAC and skin fold thickness was also observed in children of this group. Children in the control group lost weight significantly over period of three months.

Biochemical blood indices

Results of the blood nutrients over a period of 3 months are presented in Table 3. Among the patients of experimental group hemoglobin, serum ferritin and serum total proteins improved. The effects of nutritional intervention (as a result of nutritional counseling of the care givers) were modest on serum albumin, globulin and creatinine. Among the patients of control group a progressive increase in serum ferritin and total protein were observed. Hemoglobin also declined progressively indicating the catabolic effects of chemo drugs, radiation, and poor dietary intake due to therapy related toxic effects.

Nutrient Intake

Nutrient intake data of both the groups (Table 4) showed that energy, protein, iron, vitamin A and vitamin C intakes were quite below the recommended allowances for this age. Significant improvement occurred in the intake of all the nutrients in the patients of experimental group after 60 and 90 days. Intake of nutrients, particularly that of vitamin C and A remained subnormal in the patient of control group. The

Table 2. Anthropometric indices of the sample

Variables	At diagnosis		30 days		60 days		90 days	
	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control
Age (years)	8.2±13.55	8.3±4.30						
Height (cm)	124.7±21.58	124.3±26.1	126.0±21.6	124.9±25.9	125.7±11.9	124.9±25.8	126.1±21.6	125.0±11.2
Weight (cm)	24.7±11.67	23.4±13.32	[a]26.3±11.4	23.2±12.44	[ab]26.7±12.44	22.3±15.7	[abc]27.1±4.4	21.5±12.7
MUAC (cm)	17.5±3.9	16.3±2.77	17.8±3.63	16.9±3.22	[ab]18.8±6.01	16.0±3.81	[abc]19.0±3.81	15.6±4.58
Skinfold thickness (cm)	6.7±4.17	5.5±2.15	6.87±4.00	6.0±2.2	8.87±2.67	5.96±2.10	[abc]8.97±2.67	5.6±2.73

[a] *significantly different (P≤0.05) from control group*
[ab] *significantly different (P≤0.05) from control groupand at diagnosis*
[abc] *significantly different (P≤0.05) from control group, at diagnosis and 30days intervals*

Table 3. Biochemical blood indices for blood iron and protein

Variables	At diagnosis		30 days		60 days		90 days	
	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control
Hb (g/dL)	9.19±23.11	9.31±10.07	10.65±1.12	10.4±0.53	[ab]11.8±2.64	9.96±7.82	[abc]11.06±9.92	8.68±6.45
Serum albumin g/dL	3.03±7.26	3.07±11.87	3.3±9.40	3.85±11.87	3.52±9.85	3.69±9.79	3.39±7.96	3.69±9.79
Serum globulin (g/dL)	4.01±7.62	3.13±4.47	3.13±2.78	4.47±2.95	3.54±4.13	4.36±8.20	3.38±12.7	4.68± 12.34
Serum creatinine (mg/dL)	0.54±2.51	0.51±1.85	0.57±12.0	0.52±1.69	0.5±8.25	0.5±0.29	0.57±3.98	0.51±2.22
Serum ferritin (ng/ml)	530±4.28	525±3.22	574±3.96	619± 30.0	[a]427±4.28	[ab]674.5± 11.38	[*]438±13.98	[abc]761.3±13.9
Total protein (g/dL)	7.28±3.07	7.67±2.76	7.77±2.28	6.98±17.6	7.43±3.20	7.06±13.5	[abc]8.03±1.36	6.82±1.68

[a] *significantly different (P≤0.05) from control group*
[ab] *significantly different (P≤0.05) from control groupand at diagnosis*
[abc] *significantly different (P≤0.05) from control group, at diagnosis and 30days intervals*

Table 4. nutrient intake patterns of the sample

Variables	Pre-Test		30 days		60 days		90 days	
	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control
Hb (g/dL)	9.19±23.11	9.31±10.07	10.65±1.12	10.4±0.53	[ab]11.8±2.64	9.96±7.82	[abc]11.06±9.92	8.68±6.45
Energy (KaCal)	703.9±11.3	700.85±6.28	801.7±11.09	811.2±4.75	991.2±11.69	790±14.6	[abc]1105±4.76	851±21.5
Protein (g)	20.29±2.29	20.57±7.16	21.71±5.94	20.48±4.94	37.1±5.46	23.7±7.55	[abc] 39.3±10.45	23.0±16.6
Iron (mg)	5.8±3.69	5.76±4.20	7.6±11.4	5.92±2.81	[ab]8.2±1.25	5.5±8.15	[abc] 9.8±11.5	5.8±9.1
Vitamin A (I.U)	784.3±17.9	791.2±9.91	[a]1580.2±3.83	902.1±28.5	[ab]1889.6±22.6	622.6±8.89	[abc] 2967±9.35	1012±5.74
Vitamin C (mg)	10.4±40.7	11.70±6.10	[a]48.7±10.4	40.7±32.2	[ab]134.8±10.59	34.8±23.4	[abc] 139.9±5.29	20.08±8.20

[a] *significantly different (P≤0.05) from control group*
[ab] *significantly different (P≤0.05) from control group and at diagnosis*
[abc] *significantly different (P≤0.05) from control group, at diagnosis and 30days intervals*

major complaints given by this group were anorexia, nausea, oral hemorrhages, pain and oral lesions which restricted their intakes. The patients in the experimental group were motivated to eat and were recommended pureed fruits, and vegetable juices, which led to better intakes and improved health status.

Discussion

The global incidence of cancer has reached to 14.1 million new cases in 2012 and is estimate to rise to 21.4 million by the year 2030 (16). Results of the current study showed that acute lymphoblastic leukemia was most prevalent in male children. Majority of the patients belonged to low income and less educated families. These findings were in strong agreement with findings of other studies from Iran which reported high prevalence and fatality rate among males and impact of job related genetic and environmental factors contributing to the scenario (17). The low anthropometric indices among patients of both groups were indicative of childhood chronic malnutrition. Similarly, as reported weight loss occurs in majority of cancer patients as a result of infections and insufficient dietary intake during chemotherapy (18-21). Nutritional and dietary interventions brought about significant improvement in the health status of the patients in the experimental group. These findings were in strong agreement with other studies which suggested that nutritional interventions among all types of cancer patients prevent weight loss and improve the nutritional status of patients (18-22). The progressive improvement in the biochemical blood nutrient indices are suggestive of the facts that diet enriched with protein, iron and antioxidants help alleviating the toxic effects of drugs and therapy and might be of benefit in improving the survival rate and resistance to drugs among these children. Several studies have shown similar findings supporting the findings of the current study (23-25).

All children suffering from pediatric acute lymphoblastic leukemia in the current study showed chronic malnutrition and poor diets. Nutrient intakes reported by the participants were quite below the RDA's along that fluctuations were observed in the nutrient intake of control group. These results were in line with the findings of a cohort study which showed that malnourishment was present in children at diagnosis and that dietary intake of leukemia patients fell below the recommended values (26). However, the findings of the current study were in strong agreement with the reported results of other studies (24, 25). Although the weights of all children did not improve significantly in the current study which can be attributed to the facts that these children were already emaciated, however, the significant improvement in the blood nutrients was the remarkable achievement of the current study.

Conclusion

This study was successful in improving the health status of pediatric acute lymphoblastic leukemia patients at high risk of nutrition related morbidity. These results may help identify at risk patterns of nutrient and dietary intakes and can be used in future for designing intervention strategies in hospitals and clinics for children with cancers.

Acknowledgments

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References

1. American Cancer Society. Causes, Risk Factors, and Prevention. https://www.cancer.org/cancer/leukemia-in-children/causes-risks-prevention.html
2. Salvador C, Meister B, Crazzolara R, Kropshofer G. Management of hypertriglyceridemia in children with acute lymphoblastic leukemia under persistent therapy with glucocorticoids and L-asparaginase during induction chemotherapy. Pediatr Blood Cancer 2012; 59: 771
3. Kaste SC, Jones-Wallace D, Rose SR, Boyett JM, Lustig RH, Rivera GK, et al. Bone mineral decrements in survivors of childhood acute lymphoblastic leukemia: frequency of occurrence and risk factors for their development. Leukemia 2001;15: 728-734
4. Tylavsky FA, Smith K, Surprise H, Garland S, Yan X, McCammon E. Nutritional intake of long-term survivors of childhood acute lymphoblastic leukemia: evidence for bone health interventional opportunities. Pediatr Blood Cancer 2010; 55: 1362-69.

5. Kennedy DD, Tucker KL, Ladas EJ, Blumberg J, Rheingold SR, Kelly KM. Low antioxidant vitamin intakes are associated with increases in adverse effects of chemotherapy in children with acute lymphoblastic leukemia. Am J Clin Nutr 2004; 79: 1029-1036
6. Kennedy DD, Ladas EJ, Rheingold SR, Blumberg J, Kelly KM. Antioxidant status decreases in children with acute lymphoblastic leukemia during the first six months of chemotherapy treatment. Pediatr Blood Cancer 2004; 44: 378-385
7. Reilly JJ, Brougham M, Montgomer C, Richardson F, Kelly A, Gibson BE. Effect of glucocorticoid therapy on energy intake in children treated for acute lymphoblastic leukemia. J Clin Endrocrinol Metab 2001; 86: 3742-3745.
8. National Cancer institute. Common Terminology Criteria for Adverse Events (Ctcae) Seer Cancer Statistics Review 1975-2009. 2012 cited at https://seer.cancer.gov/csr/1975_2014
9. Picot J, Hartwell D, Harris P, Mendes D, Clegg AJ, Takeda A. The effectiveness of interventions to treat severe acute malnutrition in young children: a systematic review. Health Technol Assess 2012; 16(19): 1–316 doi: 10.3310/hta16190.
10. Sala A, Rossi E, Antillon F, Molina AL, de Maselli T, Bonilla M, Hernandez A, Ortiz R, Pacheco C, Nieves R, Naverret M, Barrentes M, Pencharz P,Valcecchi MG, Basrr R. Nutritional status at diagnosis is related to clinical outcomes in children and adolescents with cancer: A perspective from Central America Eur J Cancer 2012; 48: 243-52.
11. Antillon F, Rossi E, Molina AL, Sala A, Pencharz P, Valsecchi MG. Nutritional status of children during treatment for acute lymphoblastic leukemia in Guatemala. Pediatr Blood Cancer 2013; 60: 911-915
12. Brinksma A, Roodbol PF, Sulkers E, Kamps WA, de Bont ES, Boot AM. Changes in nutritional status in childhood cancer patients: A prospective cohort study. Clin Nutr 2015; 34: 66-73.
13. Ladas EJ, Sacks N, Brophy P, Rogers PC. Standards of nutritional care in pediatric oncology: Results from a nationwide survey on the standards of practice in pediatric oncology. A Children's Oncology Group study. Pediatr Blood Cancer 2006; 46: 339-44.
14. Hudson MM, Mertens AC, Yasui Y. Health status of adult long-term survivors of childhood cancer: a report from the Childhood Cancer Survivor Study. Jama 2003; 290: 1583-1592
15. Food Composition Tables for Pakistan. Department of Agricultural Chemistry and Human Nutrition, KPK, Agricultural University, 1990
16. Alwan, A. Global Status Report on Noncommunicable Diseases 2010. WHO Library Cataloguing-in-Publication Data, Geneva, Switzerland cited at www.who.int/nmh/publications/ncd_report2010/eng
17. Radmard, A.R. Five common cancers in Iran. Archives of Iranian Medicine 2010; 13: 143–146.
18. Jager-Wittenaar H, Dijkstra P U, Earthman C P, Krijnen W P, Langendijk J A, van der Laan B F A M, Roodenburg J LN. Validity of bioelectrical impedance analysis to assess fat- free mass in patients with head and neck cancer: An exploratory study. Journal of the Sciences and Specialties of the Head and Neck 2014; 36(4): 585-591.
19. Baldwin C, Spiro A., Ahern R, Emery PW. Oral nutrition intervention in malnourished patients with cancer: a systematic review and meta-analysis. J of National Cancer Institute 2012; 104: 371-388
20. Uster A, Ruefenacht U, Ruehlin M, Pless M, Siano M, Haefner M, et al. Influence of a nutritional intervention on dietary intake and quality of life in cancer patients: a randomized controlled trial. Nutrition 2013; 29(11-12):1342-1349
21. Brinksma A, Roodbol P F, Sulkers E, de Bont ESJM, Burgerhof JGM, Tamminga RYJ. Jager- Wittenaar, H. Tissing WJE. Finding the right balance: An evaluation of the adequacy of energy and protein intake in childhood cancer patients. Clinical Nutrition 2014; 3(2): 284-290
22. Tan SY, Poh BK., Nadrah MH, Jannah NA, Rahman J, Ismail MN. Nutritional status and dietary intake of children with acute leukemia during induction or consolidation chemotherapy. Journal of Human Nutrition & Dietetics 2013; 26 (1):23-33
23. Owen JL, Hanson S J, McArthur JA, Mikhailove TA. The Need for Evidence Based Nutritional Guidelines for Pediatric Acute Lymphoblastic Leukemia Patients: Acute and Long-Term Following Treatment. Nutrients 2013; 5(11): 4333-4346
24. Bauer, Jurgen H. Fruhwald MC. Important aspects of nutrition in children with cancer. Adv. Nutr 2011; 2(6): 67-77
25. Garmey EG, Liu Q, Sklar, CA, Meacham LR, Mertens AC, Stovall MA. Longitudinal changes in obesity and body mass index among adult survivors of childhood acute lymphoblastic leukemia; A report from the childhood cancer survivor study. J Clin Oncol 2008; 26: 4639-4645
26. Nelms MN, Sucher KP, Lacey K, Roth SL. Nutrition Therapy and Pathophysiology. 2nd Ed. 2011: 562-608. Cited at https://www.ebooks.com/.../Nutrition-Therapy-Pathophysiology-Titles-Diet.../plp

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Original article

Some elements and fatty acid profiles of three different wild edible mushrooms from Tokat province in Turkey

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Summary. Three wild edible mushrooms species were collected from the Tokat district to determine fatty acid profiles and some microelement contents. According to analyze results, the highest amount of Fe was found in *Coprinus atramentarius*, Cu was in *Suillus luteus*. The dominant fatty acid at all mushroom species was Oleic acid and the next three acids were Linoleic, Palmitic and Stearic acid ranging from 7.73-33.17%, 21.24-28.60% and 10.49-28.55% respectively. In addition to these acids, seven different kinds of fatty acids have been investigated too in this research. The results of the study showed that *Coprinus atramentarius, Laetiporus sulphureus* and *Suillus luteus* species have a rich measure of fatty acids and microelements.

Key words: fatty acids, mushrooms, microelements, Tokat Province

Introduction

Wild mushrooms distribute worldwide and they play important role in the ecosystem due to they are able to biodegrade the substrate and recycle valuable minerals and nutrient to nature back. They include a high quantity of protein, carbohydrates, and vitamin, besides minerals, fiber, and valuable fatty acids. Dried mushrooms contain 22% protein, 5% fat mostly in the form of the Linoleic acid, 63% carbohydrates, 10% minerals and provide several vitamins including thiamin, riboflavin, niacin, and biotin (1-2).

Wild edible mushrooms are become more important to human daily life due to their nutritional, pharmacological and economic potentials. Turkey has a rich diversity of edible mushroom species because of different geomorphology, weather conditions, and environmental features. Wild edible mushroom have been used as a natural food source since ancient times and are generally used in pharmacology and cosmetics, especially used as a source of income. Local people are collecting them to achieve economic income and so that Turkey is becoming a major exporter of edible mushrooms.

In addition to, wild edible mushrooms includes important fatty acids contents which are mostly not synthesized in the human body. Linoleic fatty acid is reported as omega 6 (W-6), Linolenic fatty acid as omega 3 (W-3). Omega 6 has important roles in blood circulation and omega 3 is considered the most valuable fatty acid and can be taken from only plants and animal nutrients. Fatty acids compositions have important effects on blood lipid profiles. Saturated fatty acids increase high-density lipoprotein (HDL) cholesterol and reduce low-density lipoprotein (LDL) cholesterol, triacylglycerol, and lipid oxidation (3).

Minerals from bioelements found in the living structure are composed of macro elements (Na, K, Ca, P and Mg) at the milligram level per kilogram (mg/kg), trace elements (Fe, Cu, Zn, Co, Mn and F) at the microgram per kilogram (μg/kg) level, (Ni, Al, Ag, As, Li, Pb and Au), and minerals also participate in the cofactor portion of enzymes that play a role in the regeneration of living organisms. Iron; is an essential mineral needed by all tissues and the lack of body structure is the cause of anemia. Copper; it catches free oxygen radicals and participates in the structure of many enzymes. Zinc; plays an important role in the function of

the male reproductive system in the production of pancreatic functions, insulin production, and cofactor for more than a hundred enzymes. Manganese; protein, polysaccharide and cholesterol, in fetal development and lactation, hydrolases, transferases and kinases (4).

Recommended Daily Allowance (RDA) provides information on the daily recommended mineral intake for a healthy lifestyle. RDA value for iron; 8 mg/day for men and 8-18 mg/day for women, for copper 900 micrograms/day, for manganese 2.3 mg/day for men and 1.8 mg/day for women, for zinc 11mg/day for men and 8mg/day for women.

This study aims to define some mineral and fatty acid positions in some edible fungus samples which were collected from Tokat (*Coprinus atramentarius, Laetiporus sulphures, Suillus luteus*) and it is desired to contribute to the knowledge of these mushroom which is not sufficient literature. According to the results; these mushroom specimens are rich in minerals and fatty acids and are therefore thought to can be used as human nutritional supplements.

Materials and Methods

The mushrooms were collected from different places in Tokat province and photographs were taken on, habitat characteristics were recorded and dried in the laboratory environment. Then the mushrooms were identified. After the dried mushrooms were milled with the homogenizer, the following treatments were carried out.

Microwave digestion: Cem brand and Mars 6 one touch (USA) model mikrowave digestion system was used. The process steps are as follows; 0.5 gr sample is weighed and transferred to teflon tubes of the device. Close the mouth tightly by adding 10 mL HNO_3. The maximum temperature is raised to 210°C in 15 minutes and it is kept at this temperature in 15 minutes, the total time is 30 minutes. At this time, the device works with 400-1800 W. Teflon tubing is pulled out under the oven and taken up with 10 mL ultra pure water in mouth-capped flasks, filtered if any particulates are present.

AAS analysis: Perkin Elmer brand and AAS 800 Model (USA) Atomic Absorption Spectrometry was used. In AAS analysis samples are read with each element specific lamp, wavelength and standard graphics. Each sample is read 3 times and the average is taken.

Wavelength and slit used in AAS are as follows;

Element	Wavelength (nm)	Slit (nm)
Fe	248.3	0.2
Cu	324.8	0.7
Zn	213.9	0.7
Mn	279.5	0.2

Fame (fatty acid methyl ester) Analysis: Fatty acids need to be derivatized in order to be analyzed in GC-MS. Derivatization with methyl esters is generally preferred. For this purpose Christie (1990) (5) method was preferred because it is practical and highly efficient. According to this method: the lipid extract was transferred to the lid-capped tubes to prepare methyl esters. 5 mL of 2% methanolic sulfuric acid was added and vortexed. This mixture was kept at 50°C for 15 hours of methylation. After 15 hours, the tubes were removed, cooled to room temperature, and vortexed with the addition of 5 mL of 5% NaCl. The fatty acid methyl esters (FAME) formed in the tubes was extracted with 5 mL of hexane. The hexane phase was removed from the top with a pasteur pipette and treated with 5 mL of 2% $NaHCO_3$ and waited for 1-2 hours to separate the phases. The solvent of the mixture containing the methyl esters was then evaporated under nitrogen. Fatty acids under the test tubes were dissolved in 1 mL of hexane and analyzed by GC-MS.

The chromatographic conditions of the GC-MS: Agilent brand GC-MS instrument (USA) 7890A / 5970C and SGE Analytical BPX90 100m x 0.25 mm x 0.25 um column (Australia) were used. The temperature program was gradually heated from 120°C to 250°C and the total time was set to 40 minutes. The temperature program: It is heated up to 120°C and 250°C at 5°C / min and is hold at this temperature for 14 minutes and the total time is 40 minutes.

Injection volume was 1 µL and split ratio was 25:1, solvent delay time was 12 minutes, carrier gas He was selected and the constant gas flow was set at 1 mL / min flow. H_2 flow 35 mL / min, dry air flow 350 mL / min, N_2 20.227 mL / min automatically set by

Table 1. Fatty acid profiles of mushroom samples

Mushroom species	C14:0	C15:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C22:0	C20:3	C20:4
Laetiporus sulphureus	0.71	0.00	28.60	0.47	28.55	33.94	7.73	0.00	0.00	0.00	0.00
Suillus luteus	0.49	0.34	25.97	0.65	17.09	24.60	29.05	0.00	0.33	0.00	1.47
Coprinus atramentarius	0.28	0.58	21.24	0.46	10.49	29.81	33.17	3.73	0.00	0.26	0.00

the program. GC-FID and MS results were recorded simultaneously. The results were evaluated by pairing with the NIST and WHILEY libraries registered on the device.

Result and Discussion

The present study gives an overview for the fatty acid profiles and the level of some microelements (Fe, Cu, Mn, and Zn) detected at *Coprinus atramentarius*, *Laetiporus sulphureus* and *Suillus luteus* species which were collected from Tokat's different localities in Turkey.

According to the result of the study, oleic, linoleic, palmitic and stearic acid levels were high and ranging from 24.60-33.94%, 7.73-33.17%, 21.24-28.60%, 10.49-28.55%, respectively.

While the highest measure of oleic, stearic, miristic and palmitic acids levels was detected at *Laetiporus sulphureus*, the highest linoleic and linolenic acids levels were noticed at *Coprinus atramentarius*. Behenic and palmitoleic acids were found in the very high range at *Suillus luteus*. The results of fatty acid profiles were given Figure 1-2.

Microelements levels are generally high in all mushroom samples and while the highest levels of Fe, Cu, Zn, and Mn was found at *Coprinus atramentarius*, 1183.60 mg/kg^{-1}, 57.12 mg/kg^{-1}, 288.40 mg/kg^{-1} and 64.20 mg/kg^{-1} respectively, the lowest levels of all elements were detected in *Laetiporus sulphureus*. The results of mineral levels were given table 2 and figure 3-4.

Comparison to literature data; Sesli and Tüzen (1999) studied different macrofungi which were collected from East Black Sea Region and they found 87.6 µg/g Fe, 10.3 µg/g Cu, 17.0 µg/g Mn, and 62.4 µg/g Zn at *Suillus granulatus* species which was familiar with *Suillus luteus* species (6). Again Tüzen et al. (2007) examined macrofungi species and detected 658

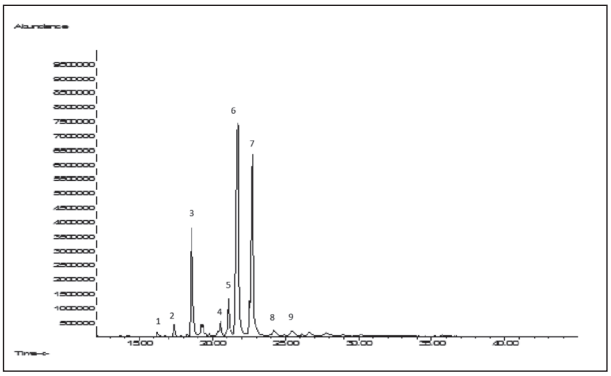

Figure 1. GC-MS chromatogram of *Suillus luteus*: number of 1.C14:0, 2.C15:0, 3.C16:0, 4.C16:1, 5.C18:0, 6.C18:1, 7.C18:2, 8.C22:0, 9.C20:4.

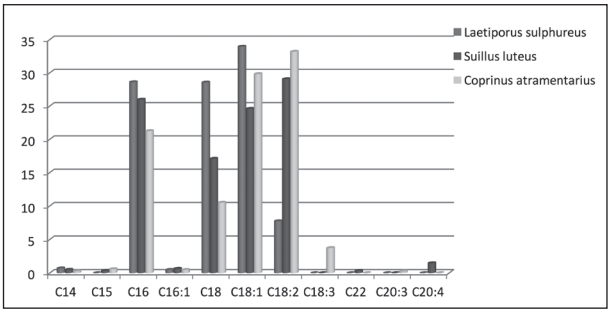

Figure 2. Fatty acid profiles of mushroom samples (C14:0 myristic, C15:0 pentadecanoic, C16:0 palmitic, C16:1 palmitoleic, C18:0 stearic, C18:1: linoleic, C18:3: linolenic, C22:0 behenic, C20:4 arasidonic acid)

Table 2. Mineral levels of mushroom samples (as mg/kg^{-1})

Mushroom species	Fe	Cu	Mn	Zn
Laetiporus sulphureus	162.920	5.000	19.360	28.360
Suillus luteus	283.240	13.360	22.840	118.840
Coprinus atramentarius	1183.600	57.120	64.200	288.400

μg/g Fe, 35.8 μg/g Cu, 77.5 μg/g Mn, and 59.8 μg/g Zn contents at *Suillus granulatus* species (7). Dursun et al (2006) studied mineral contents profiles of some wild growing mushroom species from Turkey and found 1425.6 mg/kg^{-1} Fe, 39.3 mg/kg^{-1} Zn, 9.6 mg/kg^{-1} Cu, 38.3 mg/kg^{-1} Mn at *Suillus luteus* and 63.8 mg/kg^{-1} Zn at *Laetiporus sulphureus* (8). Ayaz et al. (2011) were detected 28.6 mg/kg^{-1}Fe, 5.0 mg/kg^{-1} Mn, 38,6 mg/kg^{-1} Zn and 2.8 mg/kg^{-1} Cu in *Laetiporus bisporus* which was collected Black Sea region of Turkey and at another study levels of Fe 5.18 mg/kg^{-1}, Cu 0.08 mg/kg^{-1}, Mn 0.20 mg/kg^{-1}, and Zn 0.48 mg/kg^{-1} were found *Suillus luteus* that was collected from Western Black Sea region of Turkey (9). In addition to, *Laetiporus* species which was collected near the Balıkesir-Manisa highway from two different areas (from roadside and background area) was examined to determine levels of minerals. While levels of Cu 6.5 mg/kg^{-1}, Zn 38 mg/kg^{-1}, Mn 3.7 mg/kg^{-1} and Fe 162 mg/kg^{-1} were found at roadside samples, Cu 5.6 mg/kg^{-1}, Zn 33 mg/kg^{-1}, Mn 5.6 mg/kg^{-1} and Fe 90 mg/kg^{-1} were detected at background samples (Yılmaz et al. 2003) (10). Uzun et al (2011) detected mineral contents in *Suillus luteus* and *Laetiporus sulphureus* which were collected from Bingol and Selim district and according to the results while *Suillus luteus* species have 30 mg/kg^{-1} Fe, 146 mg/kg^{-1} Zn, 38 mg/kg^{-1} Cu and 10.8 mg/kg^{-1} Mn, *Laetiporus sulphureus* species have 1190 mg/kg^{-1} Fe, 314 mg/kg^{-1} Zn, 77 mg/kg^{-1} Cu and 28.5 mg/kg^{-1} Mn contents (11). Yamaç et al. (2007) investigated Central Anatolia mushroom samples and they found 57 mg/kg^{-1} Zn, 562 mg/kg^{-1} Fe, 32.60 mg/kg^{-1} Mn, and 20.40 mg/kg^{-1} Cu in *Suillus bovinus* and 45.20 mg/kg^{-1} Zn, 228 mg/kg^{-1} Fe, 6.20 mg/kg^{-1} Mn, and 26.60 mg/kg^{-1} Cu in *Suillus collinitus* species (12). Gençcelep et al. (2009) collected mushroom samples from the Erzurum province and examined samples to levels of mineral contents (13). They found 433 mg/kg^{-1} Fe, 111 mg/kg^{-1} Zn, 31.2 mg/kg^{-1} Cu and 43.4 mg/kg^{-1} Mn in *Suillus luteus* and 148 mg/kg^{-1} Fe, 94.3 mg/kg^{-1} Zn, 75.0 mg/kg^{-1} Cu and 13.9 mg/kg^{-1} Mn in *Coprinellus micaceus* which was close species to *Coprinus atramentarius.*

In addition to some studies from the foreign literature; S*uillus luteus* species was analyzed and except the Fe mineral, 130 μg/g Zn, 22 μg/g Cu, and 6.4 μg/g Mn were detected (14). Melczek et al. (2013) studied some Poland's mushroom species and they detected 38.94 mg/kg^{-1} Cu and 64.29 mg/kg^{-1} Zn in *Suillus luteus* (15). Konuk et al. (2006) also found iron as the highest mineral in a study on some edible mushrooms (16).

According to this study; while, Fe, Cu, Mn, and Zn elements levels of *Coprinus atramentarius, Laetiporus sulphures, Suillus luteus* are seen very close to literature data which were done examining mineral levels and literature data are very unsatisfactory about fatty acid levels of these mushroom species. Our study gives very qualified information about these species and provides sufficient literature on these mushroom species.

Conclusion

This research is give an information about some mineral elements and fatty acid profiles of some wild-grown edible mushroom species that are *Laetiporus sulphureus, Suillus luteus* and *Coprinus atramentarius.* According to results, mineral elements (Fe, Zn, Cu,

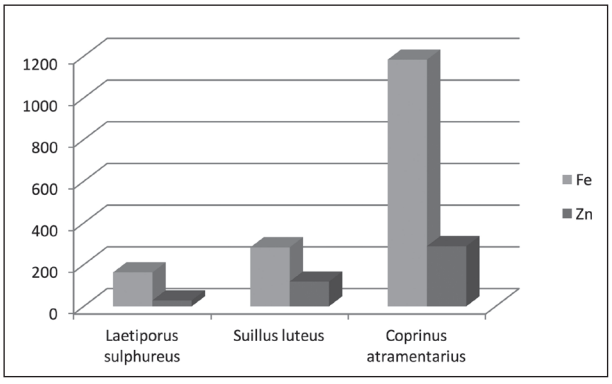

Figure 3. Mineral levels of mushroom samples (as mg/kg^{-1})

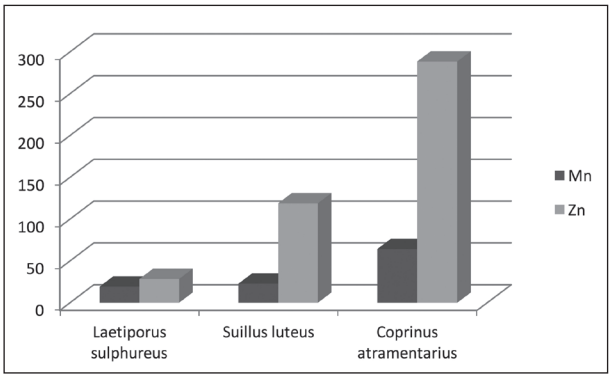

Figure 4. Mineral levels of mushroom samples (as mg/kg^{-1})

and Mn) levels were detected very high at *Coprinus atramentarius* species, fatty acid profile have some differences among these species. While, the highest levels of mineral elements were detected at *Coprinus atramentarius* species, *Laetiporus sulphureus* have the lowest. But if we look at the fatty acid profiles, some differences will be seen. *Laetiporus sulphureus* species have high quantity of myristic, palmitic, stearic, and oleic acids and *Suillus luteus* species have high levels of palmitoleic, behenic, and arachidonic acid, also again high level of pentadecanoic, linoleic which is called omega 6, and linolenic which is called omega 3 that is very important to human health and intake from the only vegetable and animal nutrition, in *Coprinus atramentarius*.

The results of this study provide important information in completing the literature data that is lacking in mineral contents and fatty acid levels of these mushroom species, and also it shows that these wild edible mushroom samples are very healthy to human because of the fatty acid profiles and rich mineral contents. It can be an alternative food supplement to those suffering from anemia, especially thanks to its rich iron content. Furthermore, RDA values for mineral will be met if a daily portion is eaten from any of types of mushrooms subject to our work.

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References

1. Breene W. Nutritional and medicinal valve of specialty mushrooms, *J Food Protect,* 1991, 53: 883-894.
2. Mattila P. Suonpaa K. and Piironen V. Functional properties of edible mushrooms, *Nutrition,* 2000, 16:694-696.
3. Kanu P.J. Zhu K. Kanu J.B. Zhou H. and Qian H. Biological active components and natraceuticats in sesame and related products; rewiev and prospect, *Trends Food Science Technology,* 2007, 18:599-608.
4. K. O. Soetan, C. O. Olaiya, and O. E. Oyewole, The importance of mineral elements for humans, domestic animals and plants: A review, African Journal of Food Science Vol. 4(5) pp. May 2010, 200-222.
5. Inger Wretensjö, Lennart Svensson, W.W. Christie, Gas chromatographic-mass spectrometric identification of the fatty acids in borage oil using the picolinyl ester derivatives, Journal of Chromatography A, Volume 21,1, 1990, 89-97.
6. Sesli E. and Tüzen M. Level of Trace Elements in The Fruiting Bodies of Macrofungi Growing in The East Black Sea Region of Turkey, *Food Chemistry*, 1999, 65: 453-460.
7. Tüzen M. Sesli E. and Soylak M. Trace elements levels of mushroom species from East Black Sea Region of Turkey, *Food Control,* 2007, 18: 806-810.
8. Dursun N. Özcan MM. Kaşık G. and Öztürk C. Mineral Contents of 34 Species of Edible Mushrooms Growing Wild in Turkey, *Journal of the Science of Food and Agriculture,* 2006, 86, 1087-1094.
9. Ayaz FA. Torun H. Özel A. Çöl M. Duran C. Sesli E. And Çolak A. Nutritional value of some wild edible mushrooms from Black Sea Region (Turkey), *Turk J Biochem,* 2011, 36(3); 213-221.
10. Yılmaz F. Işıloğlu M. and Merdivan M. Heavy metal levels in some macrofungi, *Turkish Journal of Botany,* 2003, 27: 45-56.
11. Uzun Y. Gençcelep H. Kaya A. and Akçay M.E. The Mineral Contents of some Edible Mushrooms, *Ekoloji,* 2011, 20, 80, 6-12.
12. Yamaç M. Yıldız D. Sarıkürkçü C. Çelikkollu M. and Solak MH. Heavy Metals in Some Edible Mushrooms From The Central Anatolia, Turkey, *Food Chemistry*, 2007, 103 263-267.
13. Gençcelep H. Uzun Y. Tunçtürk Y. Demirel K. Determination of mineral contents of wild-growing edible mushrooms, *Food Chemistry,* 2009, 113:1033-1036.
14. Falandysz J. Szymczyk K. Ichihashi H. Bielawski L. Gucia M. Frankowska A. and Yamasaki S. ICP/MS and ICO/AES elemental analysis (38 elements) of edible wild mushrooms growing in Poland, *Food Additives and Contaminants,* 2001, Vol. 18, No 6, 503-513.
15. Mleczek M. Siwulski M. Stuper-Szablewska K. Rissman, I. Sobieralski K. and Golinski P. Accumulation of elements by edible mushroom species: Part I. Problem of trace element toxicity in mushrooms, *Journal Enviromental Science and Health,* Part B, 2013, 48:69-81.
16. Konuk M, Afyon A, Yağız D. Chemical composition of some naturally growing and edible mushrooms, *Pak J Bot,* 2006, 38(3): 799-804.

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Original article

Synergistic effect of pretreatment, packaging and cobalt-60 gamma irradiation on nutrition and shelf life of pine nuts

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Summary. Pakistan is among the major producers of pine nuts but its export value is low which is attributed towards prevailing poor post-harvest conditions such as improper handling and storage and unhygienic conditions from field to the market. In this context, the present study was designed to evaluate the combinatorial effect of pretreatment, packaging and gamma irradiation on pine nut shelf life through evaluation of its microbial quality, sensory attributes and proximate analysis. Shelled pine nuts were pretreated (dried) under hygienic condition by two drying methods, solar drying (42°C) and cabinet drying (55°C). Two packaging strategies were undertaken in which samples were packed in inner aluminium and outer polythene packing whereas the other with only polythene packing. All the samples were irradiated with 7kGy radiation. Total bacterial load, Gram negative Enterobactereacae lactose and non-lactose fermenters and fungal count was observed in the range of log1 to none in all samples from initial till last day which showed the positive influence of drying treatment on samples. Insect infestation was not detected in any sample which showed that all combination of treatments were successful in eliminating the infestation. Oxidative stress when evaluated showed that oven dried control sample with aluminium and polythene packing showed high iodine value (78.8 $mg.g^{-1}$) and low Thiobarbituric acid value (TBA) (0.006 $mg.ml^{-1}$) whereas organoleptic properties showed good texture, colour and taste upto 20th day of storage. Conclusively, aluminium packing with polyethylene was effective with oven dried samples whereas if radiation is to be used then low dose with only polyethylene packing would be sufficient for long term preservation and to prevent post-harvest losses.

Key words: insect infestation, microbial analysis, organoleptic properties, oxidative stress, *Pinus gerardiana* (Pine nuts), sensory evaluation.

Introduction

The economy of any agriculture country depends largely upon its crop yield. The more the better, however certain circumstances affects the yield badly apart from the quality and quantity of it produced. Several stages underlay the process of moving the crop from field to the fork. Inadequate storage and transport conditions leads to massive post-harvest losses faced by the farmers and eventually severely disturbs the country's economy. Pakistan is an agricultural country and its economy is run by the export of its fresh produce and is among the major producers of pine nuts however its export value is very low and it is due to not meeting the international food export standards. The poor quality of pine nuts is attributed to poor prevailing post-harvest conditions. On one side these factors are the cause of decline in quantity

of the product that reaches the market while on the other hand affecting the quarantine attributes of the remaining ones.

Improper storage conditions develop rancidity in nuts and contamination from microbes along with their toxins (1). Number of studies has reported pine nuts contamination with *Salmonella* and *Escherichia coli* during processing (2, 3). Fungal mold can grow upon food when they are kept under parameter that allows sufficient moisture for their multiplication and produces various metabolites (4, 5). Food gets contaminated by fungal spores through wind, insects and rain (4). Fungal contamination and enzymatic degradation of structural components of the nuts causes reduction of grain quality, nutritional value, germination ability and increase in free fatty acid content (6, 7). The common toxic species belongs to the genera *Aspergillus*, *Penicillium* and *Fusarium* including molds such as *Scopulariopsis* and *Sporendonema* (8, 9). All these factors affect the quality of pine nuts thereby reducing its export value (10).In many developing countries, aflatoxins contamination of food by two fungi known as *Aspergillus flavus* and *Aspergillus parasiticus* are the main cause of both humans and animals illness (11). Now, the worldwide range for AFB_1 and aflatoxin total are 1-20ng.g^{-1} and 0-35ng.g^{-1}, respectively (12).

Dried fruits and nuts are also damaged by insect infestation another reason behind pre and post-harvest losses (3, 13). Pine nuts are known for their high quality of fats and unsaturated fatty acids. Storage stability is affected by fat content, degree of unsaturation and polysaturation, moisture content and temperature (14, 15). Lipid oxidation give rise to undesirable flavors, aromas and changes the nutritional quality of fats and oils which results in the production of unsafe compounds (1). At now, low moisture storage has proven beneficial to control respiration as a post-harvest treatment (16). Low moisture storage inhibits microbial growth and retard oxidative rancidity (17, 18).

Number of studies have shown the positive influence of dry heat treatment on shelf life of pine nuts where solar tunnel drier proved more fruitful mode for drying nuts as compared to the others (19). Open sun drying results in the reduction of crop yield in high amount every year but remains in use by many farmers as a method of drying which is due to the high cost for commercial dryers (20). The higher color, texture, taste and overall acceptability of nuts dried in tunnel drier revealed its superiority over other drying modes (19). Another drying method frequently used for drying of nuts is the cabinet drying at temperature 55°C. Packaging materials also plays an important role in enhancing the shelf life of pine nuts. The difference between packaging materials can be attributed to their thermal conductance properties which affect the internal decomposition reactions in the products during storage (21).

Food irradiation is used in the betterment of food hygiene, reduction in contamination and increase in shelf-life. Many studies have reported the effect of irradiation in preventing food quality from oxidation, insect and microbial contamination during storage and handling (22, 23). Recently, some countries have limited the irradiation dose above 10kGy for decontamination purposes (24). The aim of the present study was to observe the combine effect of dry heat treatment, packaging type and irradiation in enhancing the shelf life of pine nuts thereby reducing the post-harvest losses.

Materials and methods

Sample Preparation and Pretreatment

Pine nuts were shelled under hygienic conditions and were dried by two drying methods i.e. solar tunnel and cabinet drying (55°C for 160 hr). After drying, the samples were weighed and packed into two types of packaging, aluminium (inner packing) with additional packing of polythene (outside) and the other group with only polythene packing (14, 19).

Irradiation

Pine nut samples were irradiated at dose of 7kGy to find its effect regarding reduction in microbial flora and their toxins from the samples (25).

Microbial Analysis

Enumeration media used was nutrient agar for obtaining total bacterial count for each group of pine

nut samples. For lactose fermenters MaCconkey agar was used and for Gram negative enterobacteriaceae non-lactose fermenters *Salmonella Shigella* agar was used. Whereas for obtaining total fungal count Potato dextrose agar was used. Spread plate method was used to obtain colony forming unit per gram (26).

Microflora Characteristics

Cell morphology of the bacterial isolates were studied by Gram staining (26). Whereas for fungus cell morphology was studied by methylene blue staining (27).

Organoleptic properties

Taste, aroma, colour and texture of pine nuts kept under different conditions were analyzed by 9-point hedonic scale (28).

Proximate Analysis

Different tests were performed such as moisture content (%) and lipid profiling to find the quality of samples in each experiment.

Lipid profile

Lipid profile was analysed by measuring the samples peroxide value, acidity value, iodine value and thiobarbituric acid value.

Peroxide Value

Initial rancidity, peroxide value was studied (29).

$$\text{Peroxide value (mg.g}^{-1}\text{)} = \frac{\text{(S - B) X N sodium thiosulphate X 1000}}{\text{Weight of sample}}$$

S = titration of sample, B= titration of blank

Acid Value

Free fatty acids, acid value was estimated (30).

Acid value (ml.g^{-1}) = (56.1 x V x C)/ m

V = volume of Potassium hydroxide (KOH), C = concentration of KOH, m = mass in grams of the test portion, 56.1 = equivalent weight of KOH

Thiobarbituric Acid

For the estimation of conversion of free fatty acids into secondary oxidative products such as aldehydes and ketones, Thiobarbituric acid (TBA) value was estimated (31).

μM TBARS.g^{-1} fat = (A – b) / (a × m × 1000)

A= absorbance of the sample (oil), a= slope of the standard curve, b= intercept of the standard curve, m = amount of the sample (g)

Iodine Value

For the estimation of unsaturation present in sample, iodine value was estimated (29).

$$\text{Iodine value (mg.g}^{-1}\text{)} = \frac{\text{mL of blank – mL of sample × N (sodium thiosulphate) × 12.69}}{\text{Weight of sample in grams}}$$

Insect Infestation

The pine nut samples were checked from time to time for evaluation of different stages of insects with the help of magnifying glass.

Statistical Analysis

All the experiments were performed in triplicates. One way ANOVA, Duncan multiple range test was applied on the values to find the significance difference at $p \leq 0.05$.

Results and Discussion

Total bacterial count of control samples (solar dried and oven dried) for both packaged groups i.e. with or without aluminiumwaslog1 for up to 20th days of storage (Fig. 1a, b). The growth was acceptable for total bacterial count according to the international food export standards (32). Heat pretreatment given to the samples has reduced the moisture content considerably that prevented bacterial growth on pine nut samples. Low moisture content increases the shelf life of samples whereas excessive moisture content supports microbial activity (33). Both methods of packaging that is aluminium with polythene packaging and polythene alone were successful in eliminating the microbial growth. Gamma irradiated (7kGy) and control samples showed no sign of microbial contamination of any type for the entire storage period which is assumed to be due to shelling process done under hygienic conditions.

Extremely less fungal growth (log 1) was observed in control samples of both oven and solar

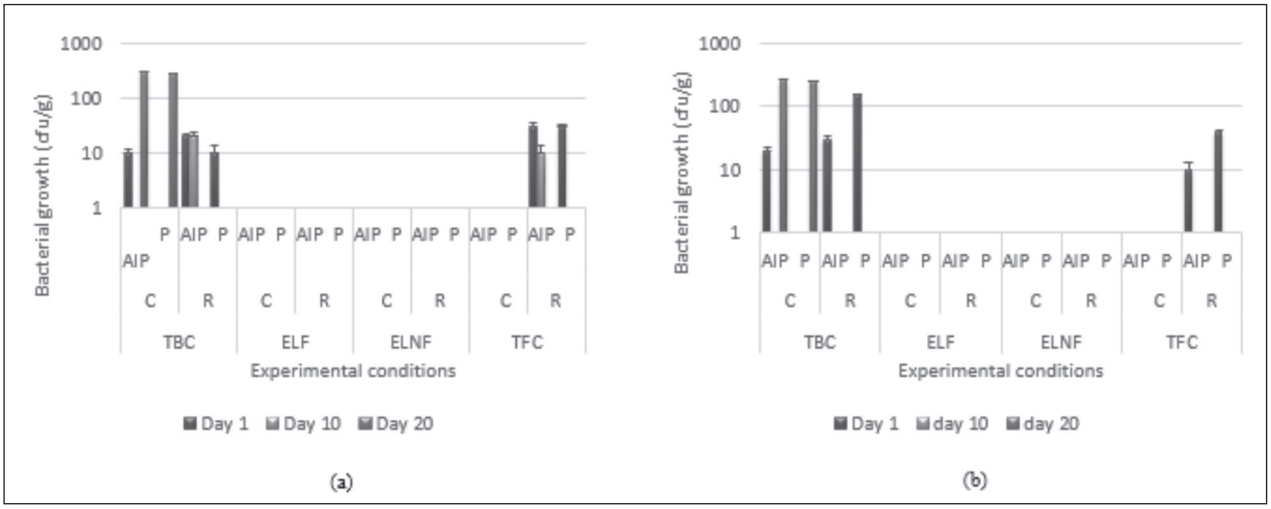

Fig. 1. Microbial evaluation of Pine nut samples at different experimental conditions (a) oven dried (b) solar dried.

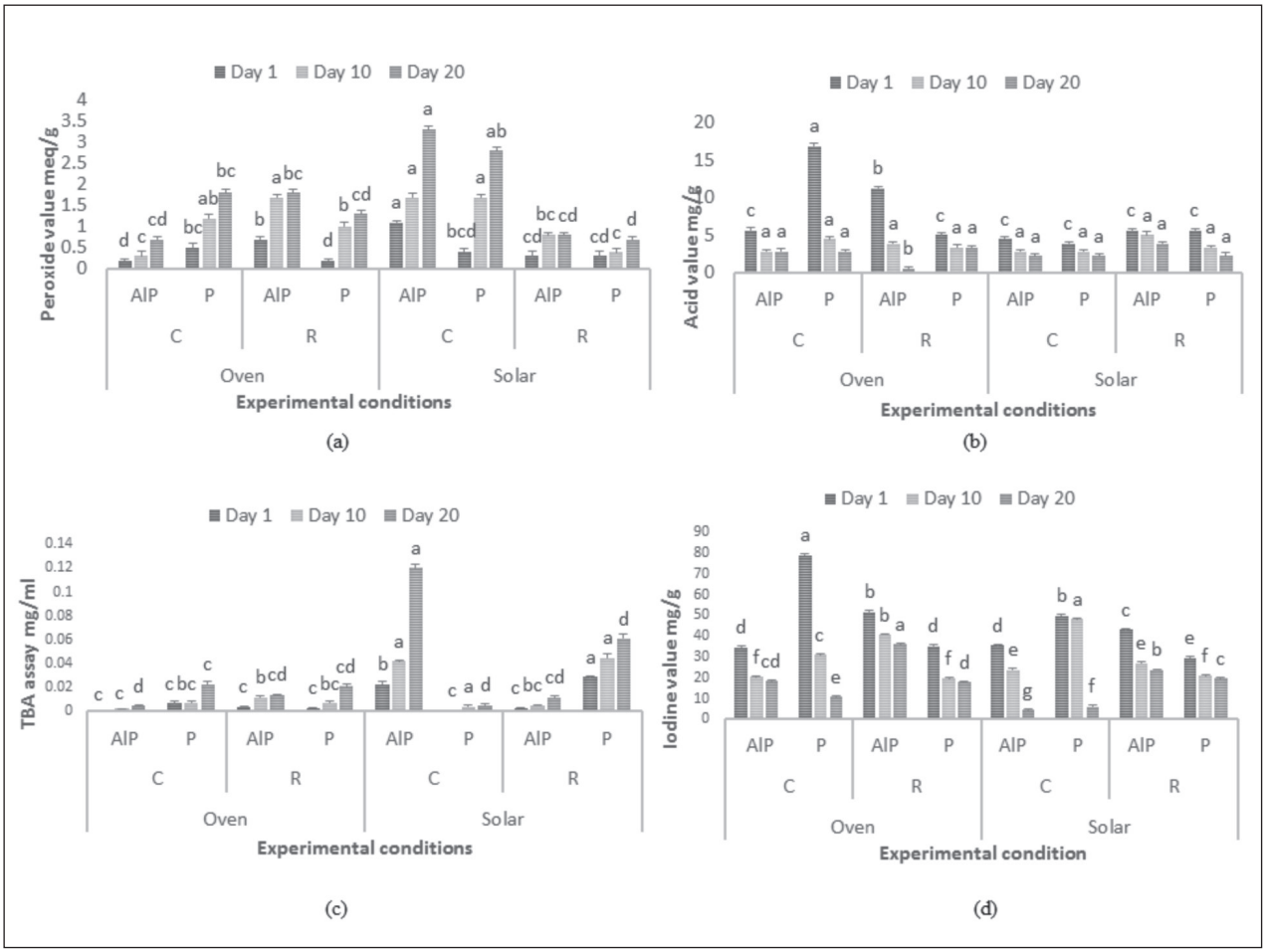

Fig. 2. Lipid profile of pine nut samples at different experimental conditions observed during 20th day of storage period (a) Peroxide value (b) Acid value (c) TBA value (d) Iodine value. Letters on bars show significant difference ($P \leq 0.05$) at different conditions.

dried experiments with both packaging types (Fig. 1a, b). The growth was under the accepted limits for fungal contamination according to the international food export standards (32). Dominant species were *Aspergillus niger* and *Penicillium* sp. Previous studies reported many fungal species such as *Aspergillus, Penicillium*, *Cladosporium*, *Tricoderma*, *Fusarium* from the dry nut samples (34, 35). Current findings indicated that the present strategies were successful in preventing food contamination to a greater extent during storage. Gamma treated (7kGy) samples showed no fungal growth during the entire study period. So, together pretreatment, packaging and irradiation were capable of reducing the fungal growth in pine nut samples during storage. The insect infestation was not detected in all samples. Many insects such as *Cydiacolorana*, *Eucosmabobana*, *Leptoglossus occidentalis* have been reported on pine nuts in other studies (36). So, all combination of treatments were fruitful in preventing insect infestation.

It is the lipid oxidation that changes the taste and aroma of pine nuts (37). The factors affecting the lipid oxidation are light, temperature, moisture and exposure to oxygen (38, 39). Different tests such as iodine value, peroxide value, thiobarbituric acid assay (TBRAS) and acid value were performed to check the rancidity in pine nut samples during storage. The peroxide value was less in oven dried samples as compared to solar samples. This increase in value might be due to the effect of solar heating and high oxygen concentration that caused unsaturated fatty acid formation. However radiated solar dried samples had less peroxide value as compared to non-radiated samples. Previously it has been reported that food irradiation increases the antioxidant property of a food generating radical scavengers that actually prevent radical generation (40). Same phenomenon occurred as radiated pine nut samples had less peroxide value and therefore at much better condition than the non-radiated solar dried samples. Peroxide value is the initial indicator of rancidity in nuts during storage (41). So, irradiation had positive impact on pine nuts during storage. Effect of packaging material when studied revealed that for oven dried samples aluminium with polyethylene packing was more suitable as it stopped the light from peroxidation of lipids whereas for radiated samples only polythene packing was effective presenting the positive long term effect of radiation on pine nuts storage (Fig. 2a). TBA value for oven dried non radiated samples with aluminum and polyethylene packing was less as compared to other experimental groups (Fig. 2c). This combination prevented the conversion of unsaturated fatty acids into aldehydes and ketones and rapid lipid oxidation (19). Degree of unsaturation was higher in oven dried samples as compared to solar dried samples. Whereas all radiated samples had high iodine value indicating less oxidation than the non-radiated samples (Fig. 2d). Iodine value of samples packed in aluminium and polythene packing was higher as compared to samples only packed in polyethylene packing. Thus if pine nuts were oven dried and packed in aluminium with polythene packing their shelf life can be increased by preventing lipid oxidation. Whereas if samples are gamma irradiated (oven dried) then only polythene packing can be used without effecting the shelf life of pine nuts. Decrease in iodine value indicates increase lipid oxidation due to degradation of unsaturated fatty acids after formation of hydroperoxides (42, 43).

The solar dried samples showed reduce acid value which showed less breakdown of unsaturated fatty acids (Fig. 2b). The higher acidity value means higher amount of free fatty acids which decreases the sample quality rapidly as seen in oven dried samples (44). According to AOCS, the acceptable acidity value for all oils should be $0.6 mg.ml^{-1}$whereas all the samples had above the limit. Decline in acidity value with time indicated conversion of free fatty acids into secondary oxidative products.

The oven dried samples of both packaging materials (aluminium plus polythene packing and polythene packing alone) showed smooth and shiny texture upto 11^{th} days of storage but gives moderate change in colour and taste due to lipid oxidation (Fig. 3a, c). The radiated oven and solar dried sample of both packaging material showed extremely unpleasant taste during the entire period of study (Fig. 3b, d, f, h). Conclusively, it can be said that solar drying method did not proved an effective strategy for long term preservation of pine nut samples. Aluminium packing with polyethylene was effective with oven

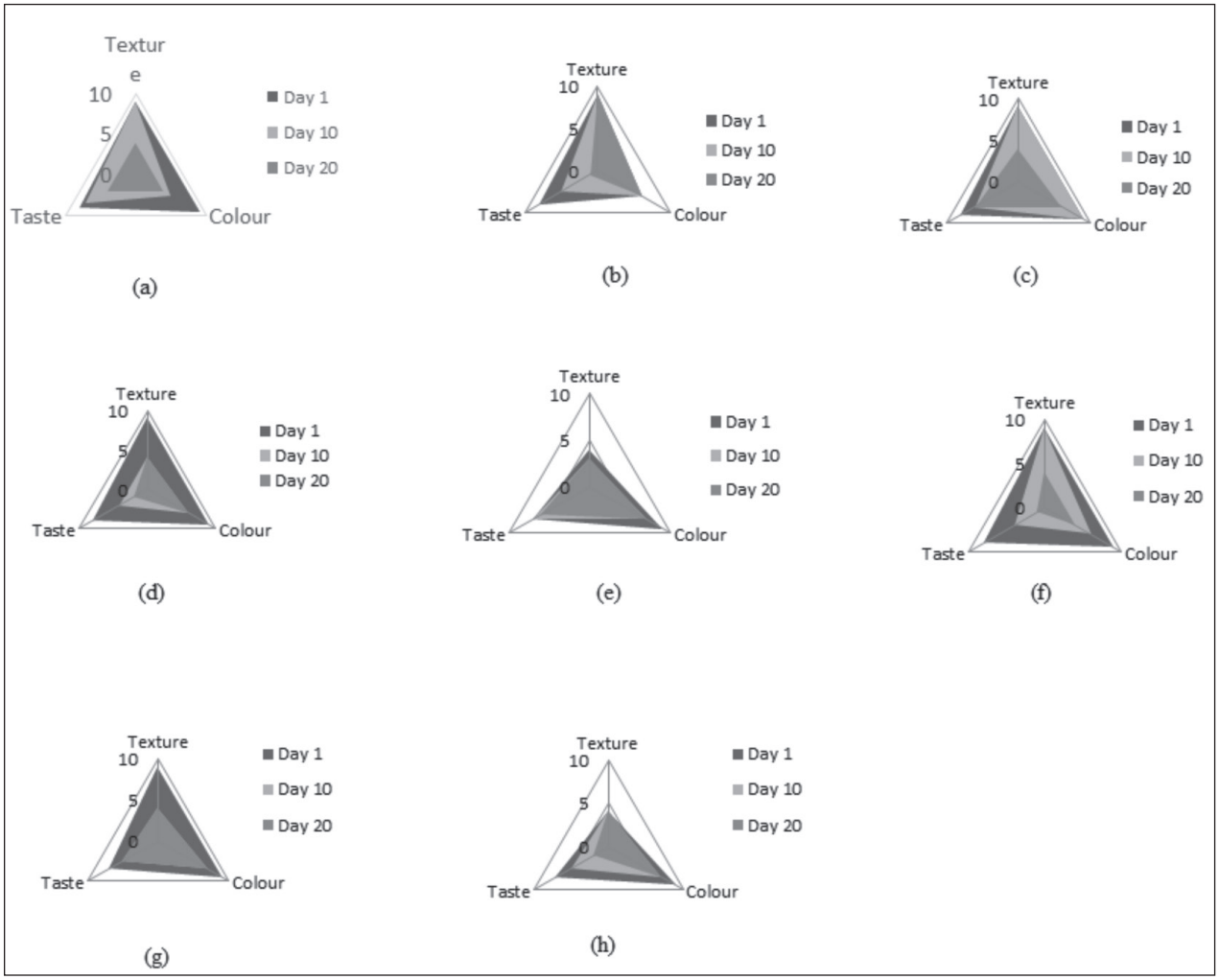

Fig. 3. Sensory evaluation of pine nuts at different experimental conditions for up to 20th day of storage period (a) oven dried pine nuts stored in polythene bag with aluminium packing (b) oven dried radiated pine nuts stored in polythene bag plus aluminium packing (c) oven dried pine nuts stored in polythene bag (d) oven dried radiated pine nuts stored in polythene bag (e) solar dried pine nuts stored in polythene bag with aluminium packing (f) solar dried radiated pine nuts stored in polythene bag plus aluminium packing (g) solar dried pine nuts stored in polythene bag (h) solar dried radiated pine nuts stored in polythene bag.

dried samples whereas if radiation is to be used then low dose with only polyethylene packing would be sufficient for long term preservation.

References

1. Frankel E. Recent advances in lipid oxidation. J Sci Food Agr 1991; 54: 495-511.
2. Isaacs S, Aramini J, Ciebin B, et al. An international outbreak of salmonellosis associated with raw almonds contaminated with a rare phage type of *Salmonella enteritidis*. J Food Prot 2005; 68: 191-198.
3. Miller W. Use of prescribed burning in seed production areas to control red pine cone beetle. Environ. Entomol 1978; 7: 698-702.
4. Brus W, Horn P, Joe W. Colonization of wounded Peanut seeds by soil fungi in Africa and south eastern Asia. Mycologia 2005; 97: 202-217.
5. Frank H. Moulds and mycotoxins in nuts and nut products. Schimmelpilze and mycotoxin in Nuessen und daraus hergestellt en producten. Mycotoxin in lebensmitteln, Federal Republic of Germany. 1981; 97-414.
6. Özdemir M, Devres O. Turkish hazelnuts: properties and effect of microbiological and chemical changes on quality.

Food Rev Int 1999; 15: 309-333.
7. White N, Jayas D. Microfloral infection and quality deterioration of sunflower seeds as affected by temperature and moisture content during storage and the suitability of the seeds for insect or mite infestation. Can J Plant Sci 1993; 73: 303-313.
8. Galvano F, Ritieni A, Piva G, Pietri A. Mycotoxins in the human food chain. The Mycotoxin Blue Book. 2005; 1:187-224.
9. Zinedine A, Brera C, Elakhdari S, et al. Natural occurrence of mycotoxins in cereals and spices commercialized in Morocco. Food Control 2006; 17: 868-874.
10. El-Magraby O, El-Maraghy S. Mycoflora and mycotoxins of peanut (*Arachis hypogaea*) seeds in Egypt. III. Cellulose-decomposing and mycotoxin-producing fungi. Mycopathologia 1988; 104: 19-24.
11. Wright G, Lamb D, Medway J. Application of aerial remote sensing technology for the identification of aflatoxin affected areas in peanut fields. 11th Australasian Remote Sensing and Photogrammetry Conference, Brisbane, Queensland, Australia. 2002; 3693-371.
12. FAO - Food and Agriculture Organization. 2004, Worldwide regulations for mycotoxins in food and feed in 2003.
13. Simmons P, Nelson H. Insects on Dried Fruits, USDA Agric. Handbook 464, USDA. ARS, Washington, DC. 1975.
14. Evranuz E. The effects of temperature and moisture content on lipid peroxidation during storage of unblanched salted roasted peanuts: shelf life studies for unblanched salted roasted peanuts. Int J Food Sci Tech 1993; 28: 193-199.
15. Pershern A, Breene W, Lulai E. Analysis of factors influencing lipid oxidation in hazelnuts. J Food Process Preserv. 1995; 19: 9-26.
16. Dillahunty A, Siebenmorgen T, Buescher R, Smith D, Mauromoustakos A. Effect of moisture content and temperature on respiration rate of rice. Cereal Chem 2000; 77: 541-543.
17. Genkawa T, Uchino T, Inoue A, Tanaka F, Hamanaka D. Development of a low-moisture-content storage system for brown rice: storability at decreased moisture contents. Biosys Eng 2008; 99: 515-522.
18. Rajarammanna R, Jayas D, WHITE N. Comparison of deterioration of rye under two different storage regimes. J Stored Prod Res 2010; 46: 87-92.
19. Thakur N, Sharma S, Gupta R, Gupta A. Studies on drying and storage of chilgoza (*Pinusgerardiana*) nuts. Arch Appl Sci Res 2014; 51: 2092-2098.
20. Murthy M. A review of new technologies, models and experimental investigations of solar driers. Sustainable Energy Rev 2009; 13: 835-844.
21. Kosoko S, Sanni L, Adebowale A, Daramola A, Oyelakin M. Effect of period of steaming and drying temperature on chemical properties of cashew nut. African J Food Sci 2009; 3: 156-164.
22. Braghini R, Pozzi C, Aquino S, Rocha L, Corrêa B. Effects of γ-radiation on the fungus Alternariaalternata in artificially inoculated cereal samples. Appl Radiat Isot 2009; 67: 1622-1628.
23. Thomas P, Diehl J. Radiation preservation of foods of plant origin. Part VI. Mushrooms, tomatoes, minor fruits and vegetables, dried fruits, and nuts. Crit Rev Food Sci Nutr 1988; 26: 313-358.
24. Aydas C, Engin B, Polat M, Aydin T. Electron spin resonance study of γ-irradiated Anatolian chickpea. Radiat Eff Defects Solids 2008; 1: 7-17.
25. Farkas J. Irradiation as a method for decontaminating food. Int J Food Microbiol 1998; 44: 189–204.
26. Benson H. Microbiological Applications. 5th Eds. Boston, USA. WC Brown Publications. 1990.
27. Leboffe M, Pierce B. Microbiology: Laboratory Theory and Application. Morton Publishing Company. 2012.
28. Al-Bachir M. Effect of gamma irradiation on fungal load, chemical and sensory characteristics of walnuts (*Juglans regia*). J Stored Prod 2004; 40: 355-362.
29. Zarinah Z, Maaruf AG, Nazaruddin R, et al. Extraction and determination of physico-chemical characteristics of Pili nut oil. Int Food Res J 2014; 21: 297 – 301.
30. Okpuzor J, Okochi V, Ogbunugafor H, Ogbonnia S, Fagbayi T,Obidiegwu C. Estimation of cholesterol level in different brands of vegetable oils. Pak J Nutr 2009; 8: 57-62.
31. Ke P,Woyewoda A. Microdetermination of thiobarbituric acid values in marine lipids by a direct spectrophotometric method with a monophasic reaction system. Anal Chim Acta 1979; 106: 279-284.
32. Joint FAO/WHO Codex Alimentarius Commission. *Codex alimentarius*. Rome: 15th Eds. Food and Agriculture Organization of the United Nations, 1992.
33. Kimbonguila A, Nzikou J, Matos L, et al. Proximate composition of selected Congo oil seeds and physicochemical properties of the oil extracts. Research J Appl Sci Engineering and Technology 2010; 2: 60-66.
34. Bayman P, Baker J, Mahoney N. Aspergillus on tree nuts: incidence and associations. Mycopathologia 2002; 155: 161-169.
35. Rezaeian F, Zamene Milani F, Kazemi A, Mohtadi Nia J, Ghaem Maghami S, Jabbari M. Contamination of tea and traditional vegetable distilled to mycotoxin producer fungi. 9th Iranian Nutrition Congress. 2006; 247.
36. Negron J. Cone and Seed Insects Associated with Pifton Pine. Desired Future Conditions for Pinon-Juniper Ecosystems. 1994; 97.
37. Tavakolipour H, Armin M, Kalbasi-Ashtari A. Storage stability of Kerman pistachio nuts (*Pistaciavera*). Int J Food Eng 2010; 6.
38. Koyuncu M, Aγkin M. Studies on the storage of some walnut types grown around Van Lake. Turkish J Agric For 1999; 23: 785-796.
39. Stark C, Mcneil D, Savage G. The effect of storage conditions on the stability of peroxide values of New Zealand grown walnuts. Proc New Zeal Soc An 2000; 43-54.
40. Bartosz G. Food oxidants and antioxidants chemical, biological and functional properties. CRC Press, Taylor and Franscis Florida, USA. 2013.
41. Ayora C, Lendl B. Rapid method for peroxide value deter-

mination in edible oils based on flow analysis with Fourier transform infrared spectroscopic detection. J Analyst 2001; 126: 242-246.
42. Magnus P. Food science and technology. Bristol Great Britain. 1992; 140-146.
43. Nkafamiya I, Maina H, Osemeahon S, Modibbo U. Percentage oil yield and physiochemical properties of different groundnut species (*Arachis hypogaea*). Afr J Food Sci 2010; 4: 418-421.
44. Atinafu D, Bedemo B. Estimation of total free fatty acid and cholesterol content in some commercial edible oils in Ethiopia, Bahir DAR. J Cereals Oilseeds 2011; 2: 71-76.

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Original article

Wild mint (*Mentha longifolia*) extracts in the production of non-alcoholic beverages

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Summary. Isolates of medicinal and aromatic herbs are used as additives in non-alcoholic drinks to improve the sensory characteristics and antioxidant potential. The method of drying the plant material, among other factors, has a profound influence on the chemical composition and pharmacological activities of plant extracts. This paper analyzes the effect of the drying technique (I - natural drying, II - in the laboratory oven, III - in low-temperature condensational drier) on the volatile fraction of the wild mint (*Mentha longifolia* (L.) Hudson) extract, in order to select an extract of the best quality for preparation of non-alcoholic drinks. The volatile profile of the extracts was determined by the GC-FID and GC-MS techniques, the antimicrobial activity by the microdilution technique, the antioxidant activity by the DPPH and FRAP assays, and the sensory acceptance according to the *Regulation on quality for refreshing non-alcoholic beverages*. The results showed that piperitone was the major component of the volatile fraction extract of the natural drying herb and low-temperature condensational drier herb (53.9% and 61.1%, respectively), while the extract of laboratory oven herb was rich in menthone (35.5%). At the concentrations in the range of 0.8-3.2 mg/mL the extracts better inhibited the Gram (+) bacteria. The beverage to which the extract of naturally dried wild mint was added, showed the antioxidant activity (9.09±0.17 μmol Fe^{2+}/mL by FRAP and 14.00±3.00 μL/mL by DPPH method) and good sensorial characteristics (concentration of the extract 0.8 g/L).

Key words: *Mentha longifolia*, drying, antioxidant activity, piperitone, beverage

Introduction

One of important product categories within the functional food segment is non-alcoholic beverages fortified with vitamins or other functional ingredients (1). For the preparation of non-alcoholic beverages with desired sensory and biological characteristics, medicinal and aromatic herbal raw materials (with different biological activities) are added. Species of the genus Mentha and the family Lamiaceae have enjoyed a rich tradition of use for flavouring, food preservation, and medicinal purposes, due to both their curative and their preventive properties (2).

Drying, as one of the oldest complex processes of food conservation, represents a very important phase in processing of medicinal and aromatic herbs. In conventional hot air-drying, high temperatures and long drying periods can cause thermal degradation or volatilization of important flavour compounds (3). Since there are negative concerns regarding the use of syn-

thetic ingredients for food preservation, natural alternatives such as the addition of plant extracts rich in phenolics are gaining popularity among consumers (4).

The influence of the effects of different drying methods on the yield and chemical composition of the essential oil obtained from the herb *Mentha longifolia* (L.) Hudson (wild mint, horsemint), was studied showing significant differences mainly concerning the chemical composition. Piperitone was the major compound in the all *three essential oil* from herb (low temperature drying 71.7%, natural drying 50.8% and laboratory drying oven 43.1%) (5). Besides volatile fraction (6), the non-volatile fraction of the ethanolic extract, in terms of chemical composition and antioxidant activities was assessed by Stanisavljević et al. (7) and the highest antioxidant activity and the greatest content of the total phenolics and flavonoids were found for the extract obtained from the raw material dried naturally.

In this paper the impact of different drying techniques on the composition and activity of ethanolic extracts of the herb *M. longifolia* was analysed aiming to select high quality isolates in order to be applied in the production of non-alcoholic beverages. Functional benefits of herbal extracts may provide value-added products and benefits to consumers.

Materials and Methods

Chemicals

All chemical substances were of analytical purity: Ethanol (Zorka-Pharma, Serbia), DMSO (dimethyl sulfoxide) and DPPH (2,2-diphenyl-1-picrylhydrazyl) (Sigma Chemical Company, USA), TPTZ reagent (2,4,6-tripyridyl-*s*-triazine) (TCI Europe, Belgium), Iron(II)sulfate-7-hydrate (VWR Prolabo, Belgium), Müller-Hinton and Sabouraud broth, Ampicillin, Amikacin and Nystatin (Torlak, Serbia), Sucrose and Citric acid (from the market).

Plant material

The *M. longifolia* herb in the phenological phase of blooming was gathered from the region of the municipality of Prokuplje (Rastovnica, 400 m.a.s.l., Serbia). The voucher specimen (N°16469) was deposited at the Herbarium of the Institute of Botany and Botanical Garden "Jevremovac" (Faculty of Biology, University of Belgrade).

Drying

The herb material was dried under three different types of drying techniques: I - natural drying (ND) in the shade on a draughty place for 15 days, II - in the laboratory oven (LOD) (Stockli, Switzerland) at 45°C for 2 days, III - in low-temperature condensational drier (LTCD) (LT-CD/60S, Freon Eko Kragujevac, Serbia) at 35°C for 2 days.

Extraction

Dried plant material was grinded in the electric coffee mill and extracted by the modified pharmacopoeia procedure (8) of the single percolation by ethanol 70% V/V as a solvent. Chopped herb (50 g) was soaked in Erlenmeyer with solvent (25 mL) and left two hours so that the herb could absorb the solvent and swell. Wet herbal material was moved to percolator, basted by a certain quantity of the same solvent and left at the room temperature for 24 hours. The quantity of solvent was determined by the preliminary extraction. The extract from the percolator was released in the Erlenmeyer flask at the regulated speed, 1-3 mL per minute. By the process of the one way percolation, the obtained quantity of the extract compared to the beginning quantity of the herb raw material was 2:1. Liquid extract was used for GC analysis. For all other analyzes dry extract was used. The obtained percolate was evaporated in the rotary vacuum evaporator (Ika-Werke, D-79219 Staufen, Germany), at 50°C, till dryness. Dried extract was milled into fine powder using the mortar with a pestle, dried into the vacuum-drier at 50°C until the constant mass and was kept in well closed glass vessels, on the dry, cold and dark place.

GC-FID and GC-MS analysis

Chromatographic analyses of the extract volatile fractions were performed by GC-FID and GC-MS techniques.

GC-FID analysis was carried out on a Hewlett-Packard system (HP-5890 Series II gas chromatograph), equipped with split-splitless injector and automatic liquid sampler (ALS), attached to HP-5 column

(25 m × 0.32 mm, 0.32 μm film thickness) and fitted to flame ionization detector (FID). Carrier gas flow rate (H_2) was 1 mL/min, injector temperature was 250°C, detector temperature 280°C, while column temperature was linearly programmed from 40-260°C (at rate of 4°C/min), and held isothermally at 260°C next 10 minutes. Undiluted extract were consecutively injected by ALS (1 μL, split mode, 1:30). Area percent reports, obtained as result of standard processing of chromatograms, were used as base for the quantification purposes. All measurements were performed in triplicate, and the results were presented as the mean values. Statistics has been covered by FID specification (results with a range of deviation for the level 1%).

The same analytical conditions as those mentioned for GC-FID were employed for GC-MS analysis, along with column HP-5MS (30 m × 0.25 mm, 0.25 μm film thickness), using HP G 1800C Series II GCD system (Hewlett-Packard, Palo Alto, CA, USA). Helium was used as carrier gas. Transfer line was heated at 260°C. Mass spectra were acquired in EI mode (70 eV), in m/z range 40-450. Sample solutions were injected by ALS (1 μL, split mode, 1:30).

The constituents were identified by comparison of their mass spectra to those from Wiley275 and NIST/NBS libraries, using different search engines. The experimental values for retention indices were determined by the use of calibrated Automated Mass Spectral Deconvolution and Identification System software (AMDIS), compared to those from available literature (9) and used as additional tool to approve MS findings.

Antimicrobial activity

The minimum inhibitory concentration (MIC) was determined by the broth microdilution method (10). Tests were performed in Müller Hinton broth for the bacterial strains, and in Sabouraud dextrose broth for *Candida albicans*. Further, the sensitivity of examined microorganisms to standard antibiotics ampicillin, amikacin and nystatin was evaluated.

The study was conducted against nine different bacterial strains: *Micrococcus luteus* ATCC 9341, *Micrococcus flavus* ATCC 10240, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212 and *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* NCIMB 9111, *Pseudomonas aeruginosa* ATCC 27853; and two strains of yeast *Candida albicans* (ATCC 10259 and ATCC 24433).

The dry extract was dissolved in DMSO and it was tested in the concentration range 0.81-30 mg/mL. Positive control of bacterial growth was set in the test as well as the sterility control of extract. Plates were incubated in aerobic atmosphere for 24 h at 37°C for bacteria and 48 h at 26°C for *Candida albicans*. Subsequently, the growth of bacteria and yeast was recorded semiquantitatively as turbidity of the medium and pellet at the bottom of the wells.

The preparation of non-alcoholic beverages

The base for beverages was made as the water solution of sucrose and citric acid (14% and 0.4%, respectively). The beverages were prepared with different percent of dry extract (0.6, 0.7, 0.8, 1.0, 1.2 g/L) obtained from naturally dried herbs ND (7), and kept in well closed glass dishes at dry, cold and dark place.

Antioxidant activity of non-alcoholic beverages

The antioxidant activity of the prepared non-alcoholic beverage as finished product was evaluated by FRAP and DPPH assays. For the Ferric Reducing Antioxidant Power (FRAP) assay, an aliquot of 0.1 mL of the sample was added to 3.0 mL of freshly prepared FRAP reagent. The measures were taken at 593 nm and the calibration curve was prepared using ferrous sulphate as the standard (11).

For determining antioxidant activity of samples the series of solutions in the range of concentrations 10.0; 50.0; 100.0; 500.0; 1000.0 **μ**Lof sample/mL were made for finshed product and DPPH radicals of 0.3 mM in 70% ethanol. 1.0 mL of DPPH radical solution and 2.5 mL of already prepared samples of finished product of different concentrations were mixed (12, 13):

$$\text{The capacity for neutralizing DPPH radicals (\%)} = 100 - [(A_s - A_b)\times 100/A_c] \quad (1)$$

where: A_s - absorbance in the presence of the product in the DPPH solution, A_c - absorbance of the control solution (containing only DPPH) and A_b - absorbance of the sample product solution without

DPPH. The EC_{50} value was calculated according to the experimental data by the use of the sigmoidal non-curve method and SigmaPlot 2000 Trial software.

The content of total phenolics in non-alcoholic beverages

The content of total phenolics was determined by Folin-Ciocalteu reagent. 0.2 mL of non-alcoholic beverage (concentration of 0.2 mL of beverage/mL of solution) was mixed with 1 mL of Folin-Ciocalteu reagent and 0.8 mL of 7.5% of water solution of Na_2CO_3. After thirty-minute incubation at room temperature and dark place, the absorbance of reactive compound was measured at 765 nm at spectrometer „VARIAN Cary-100". The content of total phenolic compounds was obtained by the equation of curve (R=0.9919) with gallic acid as a standard shown as mg of gallic acid/mL of beverage:

$$\text{Absorbance}=7.2328\ c_{\text{gallic acid}}(\mu g/mL) - 0.2286 \qquad (2)$$

Calibration was carried out with standard solutions of gallic acid. Regarding calibration curve and absorbance of the examined samples the content of total phenolics in samples was obtained.

The content of flavonoids in non-alcoholic beverages

The content of total flavonoids was determined by the spectrometric method. 0.1 mL of the solution of aluminum(III) - chloride (10%), 0.1 mL of the solution of potassium acetate (1M) and 2.8 mL of distillated water were added into 2.0 mL of non-alcoholic beverage (conc. of 0.2 mL of beverage/mL of solution). After thirty-minute incubation at room temperature the absorbance of reactive compound was measured at 415nm in relation to distilled water. Rutin was used as a standard, and the total content of flavonoids was shown as mg of rutin/mL of beverage and was determined by the equation of curve (R=0.9994):

$$\text{Absorbance}= 12.722\ c_{\text{rutin}}\ (\mu g/mL)+0.0034 \qquad (3)$$

Sensory evaluation of non-alcoholic beverages

The quality of readymade products was defined by sensory evaluation. The evaluation committee was composed of 15 members, the experts from the field of food technology, age between 30 and 65. The samples were offered to the evaluators at 20°C, in transparent glasses. Four attributes were evaluated: color intensity (max. 4 points), homogeneity (max. 4 points), fragrance intensity (max. 5 points) and taste (max. 7 points). Evaluation of the quality of the non-alcoholic beverages was carried out according to the *Regulation on quality for refreshing non-alcoholic beverages* (Serbian national regulative) (14).

Results and Conclusions

Chemical composition of volatile fraction of *M. longifolia* extracts are shown in Table 1. The greatest differences in the volatile profile of the extracts can be noticed in the content of piperitone, menthone and *iso*-menthone.

It is noticeable that monoterpenoids were found to be the most abundant class of compounds identified (ND 76.7%, LOD 77.7%, LTCD 81.4%), followed by sesquiterpenes. Chromatographic analysis showed that in ND and LTCD extracts the monoterpene piperitone was the major compound (53.9% and 61.1%, respectively). In the LOD extract was observed the lowest content of piperitone (18.5%), while the presence of menthone was recorded in the LOD extract as the dominant component (35.5%). Because of that, plant species from this locality can be inserted in the pipetitone chemotype. In the previous study of the essential oil of the same plant species, the authors found similar results (5). Piperitone dominated in all three oils (LTD 71.7%, ND 50.8%, LOD 43.1%), carvone in ND oil (20.0%), and menthone and *iso*-menthone in LOD oil (17.5% and 8.3%, respectively). It is obvious that there is an agreement in the content of menthone, *iso*-menthone and piperitone in extracts and essential oils, and way, temperature and drying time have an impact on the chemical composition of the studied isolates. Analyzing the essential oil *M. pulegium* originated from Iran (15), piperitone as the main component was obtained (38.0%).

According to the *Regulation on quality for refreshing non-alcoholic beverages* (16), the use of pulegone in food and beverages has limits of: 100 mg/kg for mint/peppermint containing alcoholic beverages; 20 mg/kg for mint/peppermint containing non-alcoholic bever-

Table 1. The chemical composition of volatile fraction of *M. longifolia* extracts

Constituents (%)	KIE	KIL	Drying method		
			ND	LOD	LTCD
α-thujene[m]	933	924	0.6	-	0.4
α-pinene[m]	n/a	932	0.9	0.9	0.8
sabinene[m]	973	969	-	-	0.4
β-pinene[m]	976	974	0.7	0.7	0.8
myrcene[m]	994	988	1.0	1.6	0.5
3-octanol[m]	1005	988	1.5	0.9	0.9
limonene[m]	1029	1024	1.9	0.2	2.0
1,8-cineole[m]	1032	1026	2.9	3.5	4.6
cis-β-ocimene[m]	1041	1032	1.1	0.6	0.7
benzene acetaldehyde	1051	1036	0.4	-	0.3
menthone[m]	1154	1148	-	35.5	-
iso-menthone[m]	1165	1158	0.7	9.5	-
α-terpineol[m]	1195	1186	0.4	-	0.4
cis-dihydrocarvone[m]	n/a	1191	0.7	0.6	1.3
trans-dihydrocarvone[m]	1200	1200	1.3	0.6	0.4
(3*Z*)-hexenyl 3-methyl butanoate	1239	1232	0.5	0.3	0.3
pulegone[m]	1243	1233	0.3	0.2	-
carvone[m]	1247	1239	3.0	1.7	4.1
piperitone[m]	1257	1249	53.9	18.5	61.1
cis-piperitone epoxide[m]	1258	1250	0.7	0.2	-
trans-piperitone epoxide[m]	1258	1252	0.5	-	0.2
carvacrol[m]	1303	1298	3.3	2.3	2.1
6-hydroxy-carvotanacetone[m]	1308	1309	1.0	-	0.5
para-vinyl guaiacol[m]	1322	1309	0.3	0.2	0.2
9-decenoic acid*[FAD]	1359	1359	0.8	0.2	-
β-bourbonene[s]	1386	1387	1.2	0.6	0.9
trans-caryophyllene[s]	1420	1417	4.7	2.4	5.6
α-humulene[s]	n/a	1452	0.7	0.6	-
cis-muurola-4(14),5-diene[s]	1459	1465	0.6	0.7	0.5
γ-muurolene[s]	1483	1478	3.4	2.0	3.6
bicyclogermacrene[s]	1498	1500	0.5	-	0.6
n-hexadecanoic acid[FAD]	1970	1951	0.4	0.6	-
ethyl hexadecanoate[FAD]	1999	1992	0.6	1.6	0.6
phytol[d]	2118	2114	1.7	1.4	2.0
cis-9,*cis*-12-octadecadienoic acid (linoleic acid)*[FAD]	2173	2132	0.9	2.3	0.8
Sum of contents (%)			92.9	90.5	96.4

*% (w/w) – mass percent defined by peak area percent determined by integration (GC-FID); m – monoterpenoids, s – sesquiterpenoids, d – diterpenoids, FAD - fatty acids and fatty acid derivatives; KIE - Kovats (retention) index experimentally determined (AMDIS), KIL - Kovats (retention) index, literature data, n/a – not available, * - tentative identification*

ages. As a pure ingredient, pulegone may not be added to foodstuff. The low content of pulegone in the studied isolates could be considered as a premium criterion due to extremely toxic properties, especially a high abortive potential and possible carcinogenic effect for humans (17). On the basis of the content of certain components it can be concluded that the quality of the extract depends on the drying process. This is specifically valid with the use of the laboratory oven at 45°C. At higher temperature the decrease in the content of piperitone was noticed, as well as of limonene, *cis*-β-ocimene, α-terpineol, carvone, β-bourbonene, *trans*-caryophyllene and γ-muurolene.

The results of antimicrobial activity of *M. longifolia* extracts and antibiotics against microorganisms are presented in Table 2.

Hand-made food products, which did not go under a thermal process, are at risk of contamination by *Staphylococcus* spp., which proves the significance of examining the antimicrobial activity of extracts. It is well known that the antimicrobial activity is the result of the presence of terpenes and their common influences (18). The examined extracts have shown moderate to low antimicrobial activity. The most sensitive were Gram (+) bacteria: *M. luteus, M. flavus, S. aureus* and *S. epidermidis*. Mahboubi and Haghi (15) stress the significance of piperitone as the oil component which has shown the germicide and antimicrobial effect especially against the Gram (+) bacteria *S. aureus*, while the least sensitive were Gram (–) bacteria, especially *Escherichia coli*. The present study confirmed the antifungal activity of Serbian *M. longifolia* extracts, as well.

Table 2. Antimicrobial activity of *M. longifolia* extracts

Microorganism	MIC					
	Amp. (μg/mL)	Amk. (μg/mL)	Nys. (μg/mL)	ND (mg/mL)	LOD (mg/mL)	LTCD (mg/mL)
Micrococcus luteus ATCC 9341	3.6	n.t.	n.t.	0.8	0.8	0.8
Micrococcus flavus ATCC 10240	n.t.	n.t.	n.t.	1.6	1.6	1.6
Staphylococcus aureus ATCC 25923	4.8	n.t.	n.t.	1.6	3.2	1.6
Staphylococcus epidermidis ATCC12228	2.6	n.t.	n.t.	1.6	1.6	1.6
Enterococcus faecalis ATCC 29212	4.0	n.t.	n.t.	15.0	7.5	15.0
Bacillus subtilis ATCC 6633	3.2	n.t.	n.t.	7.5	7.5	7.5
Escherichia coli ATCC 25922	6.4	n.t.	n.t.	7.5	7.5	7.5
Klebsiella pneumoniae NCIMB-9111	8.6	5.2	n.t.	7.5	7.5	7.5
Pseudomonas aeruginosa ATCC 27853	n.t.	12.5	n.t.	7.5	7.5	7.5
Candida albicans ATCC 10259	n.t.	n.t.	3.8	7.5	7.5	7.5
Candida albicans ATCC 24433	n.t.	n.t.	6.2	15.0	7.5	7.5

n.t. – not tested, Amp. – Ampicillin, Amk. – Amikacin, Nys. – Nystatin

The results obtained for the antimicrobial activity of the extracts of *M. longifolia* provide a foothold to the possibility of its use as a natural preservative in non-alcoholic drinks. It could help in addressing specific consumer needs as healthy diet is a part of the lifestyle that maintains or improves overall health.

Results of antioxidant activity, content of total phenolics and flavonoids of the non-alcoholic drink prepared with dry extract obtained from naturally dried herbs, are given in table 3.

Total antioxidant potential was 9.09±0.17 μmol Fe^{2+}/mL (FRAP method). The capacity of neutralising of DPPH radical shighly reaches 36.69% for the base of beverages, and 93.58% for non-alcoholic beverages, and the obtained value for EC_{50}, in the final product is (EC_{50}=14.00±3.00 μL/mL). The product obtained by adding the extract of wild mint dried naturally generally presents the best characteristics in comparison to the other two drying processes. The highest antioxidant activity was found for the ND extract (21.00±2.00 μg/mL) while LOD and LTCD showed significantly weaker activity (36.00±4.00 and 33.00±1.00 μg/mL, respectively) (7).

In the test of sensory evaluation, the sample of a beverage with the extract of wild mint in concentration of 0.8 g/L was significantly better than the other samples (Table 4). Well accepted by every evaluator it won 17.25 points, which is 86.25% out of maximum ideal value of 100%. The formulated product is light yellow and homogenous. It is of harmonic taste, typical, pleasant fragrance. The results of dry matter content 13.9% and ethanol content 0.13% (V/V) are in accordance to quality demands prescribed by the *Regulation on quality for refreshing non-alcoholic beverages* (14). Appreciating the results of FRAP and DPPH assays we can conclude that the addition of the extract to the finished product increases its antioxidant activity in relation to the prepared base.

In the category of the evaluated, the juice obtained from the addition of the extracts of wild mint dried naturally is the best of all others presented. However, it also has some failures, which should be removed. The highest individual mark ($\overline{X}$=3.61) it got for the sensory characteristic of quality (homogeneity) which is 90.25% of the maximum possible quality. Colour as the analyzed parameter of sensory quality was marked $\overline{X}$=3.49 out of maximum 4, which is 87.25% of the maximum possible quality. Sensory characteristics (fragrance and flavor), with the analysis of the product obtained $\overline{X}$=4.21 and $\overline{X}$=5.94 points of maximum 5 and 7, that is, 84.20% for fragrance and 84.85% for flavor of maximum quality. The experts showed disagreement when they evaluated fragrance when the coefficient of variation was 13.73, whereas they were in agreement when evaluated flavor of the juice.

Table 3. Antioxidant activity, content of total phenolics and flavonoids of the juice with the extract of *M. longifolia*

FRAP μmol Fe^{2+}/ mL of beverage	DPPH μL/mL	Total phenolics, mg of gallic acid/mL of beverage	Total flavonoids, mg of rutin/ mL of beverage
9.09±0.17	14.00±3.00	0.536±0.007	0.398±0.007

Table 4. Sensory evaluation of the juice with the extract of *M. longifolia*

Samples	Concentration of the extracts of Wild mint (g/L)	Colour max. 4	Homogeneity max. 4	Fragrance max. 5	Flavour max. 7	Total max. 20
1.	0.6	2.90	3.25	3.20	5.35	14.70
2.	0.7	3.20	3.70	3.70	5.45	16.05
3.	0.8	3.49	3.61	4.21	5.94	17.25
4.	1.0	3.35	3.80	3.85	5.50	16.50
5.	1.2	3.10	3.55	3.40	5.40	15.45

Preliminary results of the author, as well as the results presented in this work, indicate that drying technology of wild mint herb has a significant impact on the content of some compounds in the volatile fraction (essential oils or extracts). It is further reflected on the content of phenolics and flavonoids, the biological activity of the isolates and the product (antioxidant and antimicrobial activity), as well as on the sensory properties of the product. Non-alcoholic beverage based on wild mint extract may have a preventive effect on human health.

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References

1. Siró I, Kápolna E, Kápolna B, et al. 2008. Functional food. Product development, marketing and consumer acceptance – Areview. Appetite 51: 456.
2. Carović-Stanko K., Petek M., Grdiša M., et al. 2016. Medicinal Plants of the Family Lamiaceae as Functional Foods – a Review. Czech. J. Food Sci. 34: 377.
3. Karadağ A, Avcı N, Kasapoğlu KN, et al. 2016. Effect of microwave technology on some quality parameters and sensory attributes of black tea. Czech. J. Food Sci. 34: 397.
4. Eskicioglu V, Kamiloglu S, Nilufer-Erdil D. 2015. Antioxidant dietary fibres: Potential functional food ingredients from plant processing by-products – a Review. Czech. J. Food Sci. 33: 487.
5. Stanisavljević MD, Đorđević MS, Ristić SM, et al. 2010. Effects of different drying methods on the yield and the composition of essential oil from herb *Mentha longifolia* (L.) Hudson. Biol. Nyssana 1: 89.
6. Stanisavljević D, Đorđević S, Milenković M, et al. 2014. Antimicrobial and Antioxidant Activity of the Essential Oils Obtained from *Mentha longifolia* L. Hudson, Dryed by Three Different Techniques. Rec. Nat. Prod. 8: 61.
7. Stanisavljević MD, Stojičević SS, Đorđević MS, et al. 2012. Antioxidant activity, the content of total phenols and flavonoids in the ethanol extracts of *Mentha longifolia* (L.) Hudson dried by the use of different techniques. Chem. Ind. Chem. Eng. Q.18:411.
8. Pharmacopoea Jugoslavica (Ph. Jug. IV). 1984, 4th ed. Federal Institute of Public Health, Belgrade.
9. Adams R. 2007 "Identification of Essential Oil Components by Gas Chromatography/ Mass Spectrometry" 4th ed. Allured Publishing Corporation, Carol Stream, Illinois, USA.
10. Candan F, Unlu M, Tepe B, et al. 2003. Antioxidant and antimicrobial activity of the essential oil and methanol extracts of *Achillea millefolium* subsp. *millefolium* Afan. (Asteraceae). J. Ethnopharmacol. 87: 215.
11. Szöllösi R, Varga IS. 2002. Total antioxidant power in some species of Labiatae (Adaptation of FRAP method). Acta Biol. Szeged. 46: 125.
12. Choi CW, Kim SC, Hwang SS, et al. 2002. Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison. Plant Sci. 163: 1161.
13. Stanisavljević I, Stojičević S, Veličković D, et al. 2008. Screening the antioxidant and antimicrobial properties of the extracts from plantain (*Plantago major* L.) leaves. Sep. Sci. Technol. 43: 3652.
14. Sl. glasnik RS. 88/2017 "Regulation on quality for refreshing non-alcoholic beverages".
15. Mahboubi M. and Haghi G.J. 2008. Antimicrobial activity and chemical composition of *Mentha pulegium* L. essential oil. Ethnopharmacol. 119: 325.
16. European Commission. 2008 Regulation (EC) No 1334/2008. (Off. J. Eur. Union. 354) (http://eurlex.europa.eu/legal-content/EN/TXT/?uri=celex:32008R1334)
17. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. 2017 "Pulegone", (https://monographs.iarc.fr/ENG/Monographs/vol108/mono108-05.pdf. Accessed 23 August)
18. Bakkali F, Averbeck S, Averbeck D, et al. 2008. Biological effects of essential oils - A review. Food Chem. Toxicol. 46: 446.

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Original article

Lipide-soluble vitamin contents of some *Astragalus* species in Turkey

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Summary. *Backround:* It is believed that eight astragalus speices of this study have rich vitamin contents. *Purpose:* To determine vitamin levels of eight *Astragalus* species in Turkey for the first time. *Methods:* 1 g seed was homogenised in solvent isopropanol/hexane/ (2:3 v/v) and was treated at 10.000 g along five minutes. Afterwards, at 40°C, extracts were treated on a rotary evaporator. Then, samples were prepared. All of analysis were conducted by HPLC. Seeds were dissolved in mobile phase (methanol/acetonitrile; 25/75 v/v) and were injected 50 µL. The temperature of analytical column was performed at 40 °C. Detection of retinol acetate and retinol were done at 320 nm, and the detection of D2, D3, α-tocopherol acetate, α-tocopherol, δ-tocopherol were done 215 nm for, detection of K1 was done 235 nm. Authentic external standard mixtures were used to detect the vitamins. The findings obtined from analysis were represented as µg/g. *Results:* beta carotene, gamma tocopherol, vitamin D3 and retinol amounts of the Astragalus species were found to be quite high. High vitamin amounts of astragalus species provide the use of these plants in the treatment of various diseases. However, alpha tocopherol and vitamin K1 values of astragalus species in the study were found to be lower than the other species belonging to other fabaceae. *Conclusion:* Vitamin contents of eight Astragalus species; *Astragalus asterias* Steven, *Astragalus christianus* L., *Astragalus suberosus* Banks & Sol., *Astragalus barbatus* subsp. *Nanus* Ponert, *Astragalus lagopoides* Lam., *Astragalus camptoceras* Bunge ve *Astragalus cretaceus* Boiss. & Kotschy were determined.

Key words: *Astragalus*, lipide-soluble vitamins, HPLC

Introduction

The genus *Astragalus* L. (Fabaceae) is the richest genus of *Angiospermae* in the world, distributed around semiarid steppic regions (1). *Astragalus* L. has two phylogenetic branches; one of them is Old World (Africa, Asia, and Europe) and the other one is New World (America). It has about 2000 taxa with 136 sections in the Old World (2). It is represented by 478 taxa in 63 sections and 202 (42%) taxa endemic to the Turkish flora (3). 6 new endemic taxa were added to the Turkish flora in 2012 and 2018 (4-9) and the number reached 484.

Astragalus species is as forage for livestock and wild animals, although some of them have been recognized as of use in foods, cosmetics, as substitutes for tea or coffee, or as sources vegetable gums (10). The widespread use of legumes makes this food group an important source of lipid, fatty acids and protein in animal and human nutrition (11). Thus, species of Leguminosae have received considerable attention and their biochemical components (protein, fat, fatty acids, flavonoids) have been investigated (12). Although the fatty acid compositions of some Turkish *Astragalus* species were reported by different researchers (13-17) there was no studies about the lipide-soluble vitamin contents of eight *Astragalus* L. taxa. *Astragalus* species did not find enough studies about sources of vitamin content increased the importance of the research. Therefore, the aim of the present investigation is to determine the vitamin content of eight *Astragalus* L. taxa.

Materials and methods

Study area

Study area was located on the East of Anatolian diagonal, in the skirts of South-Eastern Taurus Mountains, in the Upper Euphrates Region of the Eastern Anatolia Region (18). Elazığ (**Fig. 1**) belongs to the Iran-Turan Plant Geography Region and falls within the B7 grid square according to the Grid classification system developed by Davis (19). Elazığ Province is bounded to the East by Bingöl, to the West by Malatya, to the South by Diyarbakır, and to the North by Tunceli. It is situated between longitudes 40–38° East and latitudes 38–39° North. The county is 1067 m above sea level (20).

Plant materials

Field study was carried out over a period of approximately one years. During this period, eight vascular *Astragalus* specimens were collected. The plants were pressed in the field and prepared for identification. Plants were identified using the standard text, "Flora of Turkey and the East Aegean Islands" (19) and were compared with the specimens in Firat University Herbarium. The names of plant families were listed in alphabetic order. Scientific names of plant species were identified according to the International The Plant Name Index: http://www.theplantlist.org.

Extraction of plant materials

1 g seed was homogenised in solvent isopropanol/ hexane/ (2:3 v/v) (21) and was treated at 10.000 g along five minutes. Afterwards, at 40°C, extracts were treated on a rotary evaporator. Then, samples were prepared based on the method of Sánchez-Machado (22).

HPLC analysis of vitamins

All of analysis were conducted by HPLC. Seeds were dissolved in mobile phase (methanol/acetonitrile; 25/75 v/v) and were injected 50 µL. The temperature of analytical column was performed at 40 °C. Detection of

Figure 1. Geographical location of the study area.

Table 1. Localities of studied *Astragalus* taxa

Plant No	Plant species	Voucher specimen	Locality and altitude
1	*Astragalus asterias* Steven	3020	Elazig Firat University campus 1060 m
2	*Astragalus christianus* L.	3002	Elazig Baskil, Belhan village 1520 m
3	*Astragalus suberosus* Banks & Sol.	3040	Elazig Firat University campus 1060 m
4	*Astragalus barbatus* subsp. *nanus* (DC.) Ponert	4129	Elazig Baskil, Bolucuk village 1600 m
5	*Astragalus lagopoides* Lam.	1917	Elazig Baskil, Kayabeyli village 1430 m
6	*Astargalus camptoceras* Bunge	3030	Elazig Firat University campus 1060 m
7	*Astragalus cretaceus* Boiss. & Kotschy	3234	Elazig Baskil, Hacimustafa village 1800 m
8	*Astargalus aduncus* Willd.	3010	Elazig Baskil, Kayabeyli village 1450 m

retinol acetate and retinol were done at 320 nm, and the detection of D2, D3, α-tocopherol acetate, α-tocopherol, δ-tocopherol were done 215 nm for, detection of K1 was done 235 nm. Authentic external standard mixtures were used to detect the vitamins (23). The findings obtined from analysis were represented as μg/g.

Results and discussion

In the study, vitamin contents of eight *Astragalus* species; *Astragalus asterias* Steven, *Astragalus christianus* L., *Astragalus suberosus* Banks & Sol., *Astragalus barbatus* subsp. *Nanus* (DC) Ponert, *Astragalus lagopoides* Lam., *Astragalus camptoceras* Bunge, *Astragalus cretaceus* Boiss. & Kotschy and *Astargalus aduncus* Willd. were determined. The lipide-soluble vitamin contents of studied *Astragalus* species were given table 2.

A large amount of beta carotene (β-carotene) was found in *Astragalus asterias* Steven species (1927,03 μg/g). Similarly, β-carotene *was found in Astragalus suberosus* Banks & Sol. (795,9 μg/g), *Astragalus christianus* L. (538,23 μg/g) and *Astragalus lagopoides* Lam. (360,57 μg/g). However in a study, 18 genotypes of the Fabaceae family, the highest amount of beta carotene was reported as 0,41 μg/g (24). The amount of beta carotene in some species in the present study is significantly higher than this value.

When the amount of gamma tocopherol (γ-tocopherol) was examined, the highest amount was determined in *Astragalus aduncus Willd*. (1486,45 μg/g). Different amounts of gamma tocopherol were measured in the species such as *Astragalus suberosus Banks & Sol.* (1230,95 μg/g), *Astragalus lagopoides Lam.* (574,0 μg/g) and *Astragalus camptoceras Bunge* (495,39 μg/g). These values are higher than the maximum amount of gamma tocopherol 95.3 μg/g in a previous study (25).

In the study, R-tocopherol and D2 were not found in eight *Astragalus* species, whereas vitamin D3 was found in all *Astragalus* species. The most biologically active form of vitamin D in humans is vitamin D3 (cholecalciferol), which is a fat-soluble steroid (26). In this study, the highest amount of vitamin D3 was found in *Astragalus christianus* L. (18,97 μg/g) while the lowest amount of *Astragalus asterias* Steven (1,61 μg/g) was measured. These values are higher than the maximum amount of vitamin D3 0,06 μg/100g in a previous study (27).

The level of α-tocopherol acetate was measured at the highest level of *Astragalus christianus* L. (3,43 μg/g) but not found in *Astragalus camptoceras* Bunge and *Astragalus cretaceus* Boiss. & Kotschy species. Alpha tocopherol (α-tocopherol) was detected in different amounts of all *Astragalus* species. The amount of alpha tocopherol was highest in *Astragalus aduncus* Willd.

Table 2. Lipide-soluble vitamin amounts of *Astragalus* species

Lipide-soluble vitamins (μg/g)										
Taxa Taxa	**β-carotene**	**γ-tocopherol**	**R-tocopherol**	**D2**	**D3**	**α-tocopherol**	**α-tocopherol acetate**	**K1**	**Retinol**	**Retinol acetate**
Astragalus asterias Steven	1927,03	-	-	-	1,61	3,01	1,4	0,07	-	-
Astragalus christianus L.	538,23	-	-	-	18,97	5,95	3,43	0,07	0,42	0,3
Astragalus suberosus Banks & Sol.	795,9	1230,95	-	-	15,82	12,46	1,47	0,91	0,49	0,77
Astragalus barbatus subsp. *nanus* (DC) Ponert	-	-	-	-	4,48	6,16	0,63	-	0,07	0,01
Astragalus lagopoides Lam.	360,57	574	-	-	10,92	12,95	1,96	-	0,35	0,42
Astragalus camptoceras Bunge	-	495,39	-	-	13,23	2,38	-	-	0,07	0,07
Astragalus cretaceus Boiss. & Kotschy	-	-	-	-	3,85	4,13	-	-	0,14	0,07
Astragalus aduncus Willd.	-	1486,45	-	-	4,06	20,85	0,42	-	0,07	0,14

type (20,85 µg/g). However, in a study, the amount of alpha tocopherol was found to be approximately 89.4 µg/g in many species of Fabaceae, and it was significantly higher than the species in present study (25).

Small amounts of vitamin K1 were found in *Astragalus asterias* Steven, *Astragalus christianus* L. and *Astragalus suberosus* Banks & Sol. species, while vitamin K1 was not measured in other *Astragalus* species. In a study, vitamin K1 value in species of fabaceae found to be 1,839 µg/g (28). This value is higher than the vitamin K1 values of all *Astragalus* species in the present study.

Retinol (0,07-0,49 µg/g) and retinol acetate (0,07-0,77 µg/g) were found in small amounts in *Astragalus* species. The highest amounts of these vitamins were found in the *Astragalus suberosus* Banks & Sol. species (retinol 0,49 µg/g, retinol acetate 0,77 µg/g), while could not be detected in *Astragalus asterias* Steven species. In a study of 18 genotypes belonging to Fabaceae family, the highest amount of retinol was determined as 0,0441 µg/g (24). The amount of retinol in most species in the present study is significantly higher than this value.

In the study, vitamin levels of eight *Astragalus* species were determined for the first time. The plants belonging to eight *Astragalus* species in this study were compared with other plants belonging to Fabaceae family. Beta carotene, gamma tocopherol, vitamin D3 and retinol amounts of the species in the present study were found to be quite high. High vitamin amounts of *Astragalus* species in the study provide the use of these plants in the treatment of various diseases. However, alpha tocopherol and vitamin K1 values of *Astragalus* species in the study were found to be lower than the other species belonging to other Fabaceae.

References

1. Akan H, Tatlidil S, Bicakci A. Pollen Morphology of Astragalus L. Section Alopecuroidei DC. (Fabaceae) in Turkey. International Journal of Botany 2005; 1(1), 50-58.
2. Podlech D, Zarre SH. A Taxonomic Revision of the Genus Astragalus L. (Leguminosae) in the Old World, Vienna, Austria: Naturhistorisches Museum Wien 2013; Vol. II, pp. 1039–1150.
3. Aytac Z, Ekici M, Akan H. Astragalus L. In: Guner A, Aslan S, Ekim T, Vural M, Babac MT, editors. Türkiye Bitkileri Listesi (Damarlı Bitkiler). Istanbul, Turkey: Nezahat Gokyigit Botanic Garden and Floristics Research Society 2012.
4. Taeb F, Uzunhisarcikli ME. Astragalus argentophyllus (Fabaceae), a new species from south Anatolia, Turkey. Ann Bot Fennici 2012; 49, 259–262.
5. Dinc M, Aytac Z, Dogu S. A new species of Astragalus (Fabaceae) from Turkey. Turk J Bot 2013; 37, 841–846.
6. Karaman Erkul S, Aytac Z. Astragalus yukselii (Leguminosae), a new species from Turkey. Turk J Bot 2013; 37: 836–840.
7. Cecen O, Aytac Z, Misirdali H. Astragalus unalii (Fabaceae), a new species from Turkey. Turkish Journal of Botany 2016; 40, 81-86.
8. Ilcim A, Behcet L. Astragalus topalanense (Fabaceae), a new species from Turkey. Turk J Bot 2016; 40: 74-80.
9. Donmez AA, Ugurlu Aydın Z. Astragalus ihsancalisii (Fabaceae), a new species from Erzurum province, E Turkey. Willdenowia 2018; 48(3), 399-404.
10. Rios JL, Waterman PG. A Review of the Pharmacology and Toxicology of Astragalus. Phytotherapy Research 1997; vol. 11, 411–418.
11. Yoshida H, Saiki M, Yoshida N, Tomiyama Y, Mizushina Y. Fatty acid distribution in triacylglycerols and phospholipids. Food Chem. 2009; 112, 924-928.
12. Akpınar N, Akpınar MA, Turkoglu S. Total lipid content and fatty acid composition of the seeds ofsome Vicia L. species. Food Chem. 2001; 74, 449-453.
13. Bagci E, Vural M. Fatty acid composition of Astragalus pycnocephalus and Astragalus condensatus, a chemotaxonomic approach. Gazi Univ. J. Sci. 2001; 14 (4), 1305-1311.
14. 14. Bagci E. Fatty acid composition of some Astragalus species from Turkey. Chem. Nat. Comp. 2006; 42(6), 645-648.
15. Adıguzel A, Agar G, Baris O, Gulluce M, Sahin F, Sengul M. RAPD and FAME analyses of Astragalus species growing in eastern Anatolia region of Turkey. Biochem. Syst. Ecol. 2006; 34, 424-432.
16. Keskin C, Kacar S. Fatty acid composition of root and shoot samples of some Astragalus L. (Fabaceae) taxa growing in the east and southeast of Turkey. Turk J Biol. 2013; 37: 122-128.
17. Emre I, Sahin A, Turkoglu I, Yilmaz O, Bahsi M, Kursat, M. Compositions of seed fatty acids in some Astragalus L. taxa from Turkey, Acta Botanica Gallica: Botany Letters 2011; 158:4, 487-491.
18. Sengun MT. The effect of Keban Dam Lake to Elazığclimate under the last valuations light. Fırat Univ J Res East Anatol Reg 2007; 5,116–121.
19. Davis PH (Ed.). Flora of Turkey and the East Aegean Islands 1965-1985; Vol.3, Edinburgh University Press, Edinburgh.
20. Polat R, Cakilcioglu U, Ulusan MD, Paksoy MY. Survey of wild food plants for human consumption in Elazığ(Turkey). Indian Journal of Traditional Knowledge 2015; 14(1), 69-75.

21. Hara A, Radin NS. Lipid extraction of tissues with a low-toxicity solvent. Anal.Biochem 1978; 90 (1) 420-426.
22. Sanchez-Machado DI, Lopez-Hernandez J, Paseiro-Losado P. High-performance liquid chromatographic determination of a-tocopherol in macroalgae. Journal of Chromatography A 2002; 976 (1) 277–284.
23. Yilmaz O, Keser S, Tuzcu M, Cetintas B. Resveratrol (trans-3,4',5-trihydoxystilbene) decreases lipid peroxidation level and protects antioxidant capacity in sera and erytrocytes of old female Wistar rats induced by the kidney carcinogen potassium bromate. Envir. Toxicol. Pharmacol 2007; 24, 79-85.
24. Arslan M. Diversity for vitamin and amino acid content in grass pea (Lathyrus sativus L.). Legume Research 2017; 40(5): 803-810.
25. Bagci E, Bruehl L, Ozcelik O, Aitzetmuller K, Vural M, Sahim A. A study of the fatty acid and tocochromanol patterns of some Fabaceae (Leguminosae) plants from Turkey. Grasas y Aceites 2004; vol. 55. Fasc. 4, 378-384.
26. Garcion E, Wion-Barbot N, Montero-Menei CN, Berger F, Wion D. New clues about vitamin D functions in the nervous system Trends Endocrinol. Metab 2002; 13 (3), pp. 100-105.
27. Hughes LJ, Black LJ, Sherriff JL, Dunlop E, Strobel N, Lucas RM, Bornman JF. Vitamin D Content of Australian Native Food Plants and Australian-Grown Edible Seaweed. Nutrients 2018, 10, 876; doi:10.3390/nu10070876.
28. Joshna A, Shiva M, Sree MSD, Prasanna KR, Himaja N, Radhika G. Vitamin K1 (Phylloquinone) – A review. JPR:BioMedRx: An International Journal 2013; 1(9),907-911.

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Original article

Preharvest and postharvest treatments for increasing the rate of ripening of date palm fruit (*Phoenix dactylifera* L.) cv. Medjool

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Summary. Date palm (*Phoenix dactylifera* L.) is an important subsistence crop for regions having water scarcity problems, however it requires significant amount of heat for ripening of the fruits; and fruits do not ripen on the tree in some climatic regions. This study aimed to hasten the ripening process of date fruits cv. Medjool by some preharvest and postharvest applications. Three doses (500, 750 and 1,000 ppm) of Ethephon, two controls and apple vinegar (4-5% acidity) were used in the preharvest studies with or without bunch bagging (perforated black polyethylene bags). Postharvest experiments were conducted with visually mature firm (Khalal) fruits of the cv. Medjool, and are subjected to i-) dipping into distilled water; ii-) dipping into Ethephon 500 ppm; iii-) dipping into Ethephon 750 ppm; iv-) dipping into apple vinegar (4-5 % acidity); v-) dipping into grape vinegar (4-5 % acidity); and vi-) freezing at -18°C for 3 days. Results showed that bunch bagging enhances fruit ripening on the tree, increase fruits' total soluble solids (TSS) and reduce titratable acidity (TA) at both Tamr and Khalal stages. Preharvest application of 1,000 ppm Ethephon (with bunch bagging) found to have highest ratio of Tamr (37%) and Rutab (46%) fruits where preharvest apple vinegar application had slight effect on the fruit ripening (12% Tamr) as compared with control (8% Tamr). On the other hand, postharvest application of apple vinegar, freezing at -18°C and grape vinegar found to enhance fruit ripening and the percentage of Tamr fruits were determined as 100%, 100% and 92% respectively.

Key words: ethephon, bunch bagging, apple vinegar, grape vinegar, freezing

Introduction

Nearly ¾ of the earth's surface consists of water, however only less than 1% of this amount is available for drinking and irrigation (1). On the other hand, human induced climate change reported to cause impacts on ecosystems, water resources and human nutrition (2). Water scarcity is an emerging problem for the earth (3) and for Cyprus (4). Therefore, to overcome these challenges, it is crucial for considering different types of agricultural adaptation, including crops with high irrigation use efficiency (5). Date palm (*Phoenix dactylifera* L.) is a drought tolerant crop which is known as an important subsistence crop for regions having water scarcity problems (6).

Date palm fruits include certain essential minerals and vitamins; and contain carbohydrates, lipids, proteins and dietary fiber (7). On the other hand, pharmacological studies noted that date palm fruits have high antioxidant capacity (8), anti-diabetic characteristics (9), antiviral activities (10) and anti-inflammatory properties (11). Medjool is a premium variety in the international market where it was reported to have large size fruits and excellent sensory qualities (12,13). The agronomic conditions in Cyprus are ideal for growing date palms including Medjool variety. However, although it is an early-maturing variety, total heat units are not enough in Cyprus for the ripening of Medjool varieties on the tree. Date can be harvested at three different stages: mature firm (Khalal), half ripe (Rutab) and fully ripe (Tamr). At the mature firm (Khalal) stage, the fruits have high amount of soluble tannins and they are astringent. Therefore, removal of tannins is required for making the fruits edible. Dates require cross pollination

and the pollination does not occur at the same time on the different trees, even at the same bunch. Thus, several harvests are necessary (14)

Early maturing date palm trees require at least 1,800 heat summation units (HSU) on the base of 18°C, where most of them need 2,600 HSUs for ripening of the fruits on the tree (15,16). Some agro climatic conditions i.e. Northern Cyprus do not supply the required amount of heat unit for the date palm fruits for ripening on the trees. Only about 10-20% of the total date fruits cv. Medjool is reported by farmers in Northern Cyprus to be ripen on the tree, in which the remaining un-ripe fruits cause important economic loss. Date palm fruits mainly ripen in August to September in Northern Cyprus, and decrease in temperatures occurring after September cause failure in ripening. Researchers reported that bunch bagging, by increasing the accumulation of heat units at the surrounding environment of the date palm fruits, and abscisic acid (1 mM) application hasten ripening in date palm fruits (17). It was also noted that ethephon application (500-1,500 ppm) induces ripening in date palm fruits cv. Zaghloul and cv. Samani (18) and cv. Helali (17). Al-Juburi et al. (19) reported that effectiveness of ethephon sprays differ in different climatic conditions. Therefore, this study aimed to hasten the ripening process of date fruits cv. Medjool by some preharvest and postharvest applications.

Materials and Methods

Preharvest applications

Preharvest studies were conducted at the Research and Application Farm of the European University of Lefke, near Güzelyurt city (35°11'10.23" N, 32°58'22.24" E, altitude 23 m a.s.l.) during the 2018 season. Thirty-six uniform 6-years old cv. Medjool date palm trees were selected for this study. Bunches of the all trees were hand pollinated with the same male flowers of a same tree during March. Preharvest treatments were arranged in a split-plot design with two main factors (no bagging and bunch bagging with perforated black polyethylene bags) and six treatments combined with each main factors. Treatments were as follows: control-1, control-2, Ethephon 500 ppm, Ethephon 750 ppm, Ethephon 1,000 ppm and apple vinegar (4-5% acidity). Each treatment replicated three times (one tree per replication). Bunch bagging (with 48 perforations) and spraying of ethephon was performed 4 weeks after fruits entered into Khalal stage (on 3rd of September 2018). Studies were continued for 35 days and the treatments, except control-2, were finalized on 8th of October (when daily heat summation units decreased to 2 C at the base of 18°C; and rains started). During this period, the Tamr fruit in each bunch of each treatment were periodically counted, collected and weighed. The control-2 treatment was continued until 28th of October, when the heat summation unit (HSU) decreased below zero (at the base of 18°C) and heavy rains started. At the end of the experiments, all fruits were harvested and categorized as mature firm (Khalal), half ripe (Rutab: softening more than 25%) and fully ripe (Tamr). Total 20 Tamr and 20 Khalal fruits per tree (replication) were randomly selected for quality measurements. Fruit weight (g), fruit diameter (mm) and fruit length (mm) were measured independently for each fruit. A homogenous juice sample was prepared from each replication of each treatment for the determination of total soluble solids (TSS) and acidity. Hand refractometer was used to measure the total soluble solids (TSS) as % Brix and titratable acidity (TA: g/100 g of malic acid) was evaluated according to AOAC (20) by titrating juice samples with 0.01 N NaOH until the end-point of pH 8.1.

Postharvest applications

Postharvest experiments were conducted with visually mature firm (Khalal: full yellow colored) fruits of the cv. Medjool, which were collected from a single date palm tree received regular cultural practices (neither bunch bagging nor Ethephon application) at the same orchard. The harvested Khalal fruits were immersed in water and ones which settled at the bottom were referred to as mature fruit; thus the experiments were continued with those fruits (approximately 95% of the fruits settled bottom). Fruits were subjected to six different treatments. The five of the six treatments are as follows: i-) dipping into distilled water under ambient conditions for 30 minutes; ii-) dipping into ethephon 500 ppm under ambient conditions for 3 minutes; iii-) dipping into ethephon 750 ppm under ambient conditions for 3 minutes; iv-) dipping into apple vinegar (4-5 % acidity) under ambient conditions for 30 minutes; and v-) dipping into grape vinegar (4-5 % acidity) under ambient conditions for 30 minutes. The final treatment of the postharvest experiments was keep-

ing the fruits under -18°C for 3 days. Each treatment was replicated three times (12 fruit/replicate). Each fruit was sorted and numbered; and the fruit weight was noted. All the treated fruits were kept under ambient conditions (25 ±1°C and 50-65% relative humidity) for 9 days for ripening. During this period, number of half ripe (Rutab: softening more than 25%) and fully ripe (Tamr) fruits were periodically noted. Fruit weight (g), color, TSS and TA were measured at the end of the experiments, as described above.

Effects of the applications on the fruit color was determined according to the formula of Konica Minolta (21). To measure the CIE L*a*b* colors of the samples, a mini light box (width:22 cm, lenght:30 cm, height:22 cm) was modified from Kim et al. (22) to isolate ambient light effects (Figure 1). One LED light was used to provide light to the mini light box with 5 V supply through a 220 R current limiting resistor. A smartphone (Samsung Galaxy S6) was placed on top of the light box and picture of the samples, both before and after ripening experiment, were captured 22 cm above the samples. The CIE L*a*b* colors of the fruit samples were then read on Photoshop CS6. The L* indicates lightness, a* indicates the red/green coordinate, and b* refers the yellow/blue coordinate. The differences (Deltas: Δ) between the color values (ΔL*, Δa* and Δb*) were calculated according to the following formulas, developed by Konica Minolta (21):

- ΔL* (L* after - L* before) = positive result (+) means "after" is lighter, negative result (-) means "after" is darker
- Δa* (a* after - a* before) = positive result (+) means "after" is redder, negative result (-) means "after" is greener
- Δb* (b* after - b* before) = positive result (+) means "after" is yellower, negative result (-) means "after" is bluer

Afterwards, above results were used in the following formula to calculate total difference (Delta E: ΔE*), which is always positive:

$$\Delta E^* = \sqrt{\{(\Delta L^*)2 + (\Delta a^*)2 + (\Delta b^*)2\}}$$

Data analysis

SPSS 20.0 software was used to perform analysis of variance (ANOVA) to analyze the data. Separation of the means of different treatments was then performed by Tukey's (HSD) multiple range test at $P \leq 0.05$.

Results

Preharvest studies

Heat summation units (HSUs) of the growing area during the studies were calculated on the base of 18°C and 10°C separately, and are given in Table 1. Flowering of the date palm trees began during mid-March. According to the climatic data, daily heat units were negative until the 23th of March, which was noted as the first day of flowering for this experiment. The preharvest applications of present study were performed 4 weeks after fruits entered into Khalal stage (on 3rd of September 2018). The total HSUs at the time of preharvest applications on the base of 18°C and 10°C, were calculated as 964 and 2,284, respectively. The experiments were performed with two controls, and one of the controls (control-2) was continued until the daily heat summation unit (HSU) decreased below zero (at the base of 18°C) and heavy rains started. At that time, HSU on the base of 18 and 10°C, were calculated as 1,279 and 3,039, respectively. Both HSUs are below the thresholds reported for cv. Medjool as 1,800 and 3,440 (16,23) and this result supports the findings of present study while ripening ratio of the control treatments were very low on the tree (Figure 2).

Results given in Figure 2. suggest that bunch bagging enhanced fruit ripening in both control and other treatments. It was also observed that bunch bagging increased the ratio of Rutab fruits. The lowest ratio of the Tamr fruits were obtained from control-1 without bunch bagging and followed by control-2 without bunch bagging. No statistical difference was de-

Table 1. Heat summation units of the growing area from flowering till the end of the experiments

Duration	HSU on the base of 18°C	HSU on the base of 10°C
From flowering till the preharvest applications	964	2,284
From flowering till the end of control-1 and experiments	1,209	2,809
From flowering till the end of control-2	1,279	3,039

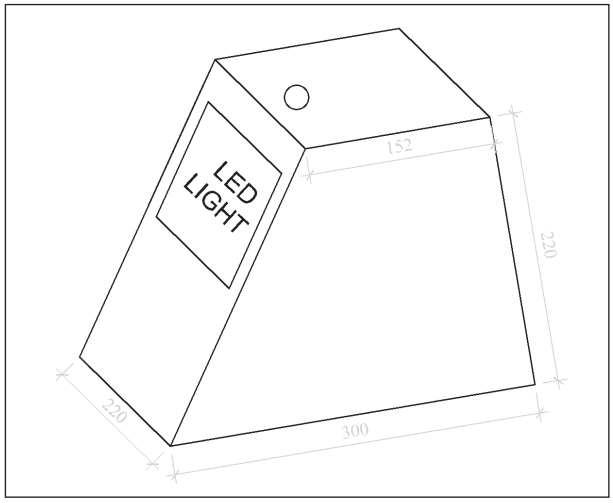

Figure 1: 3D printed drawing of mini light box.

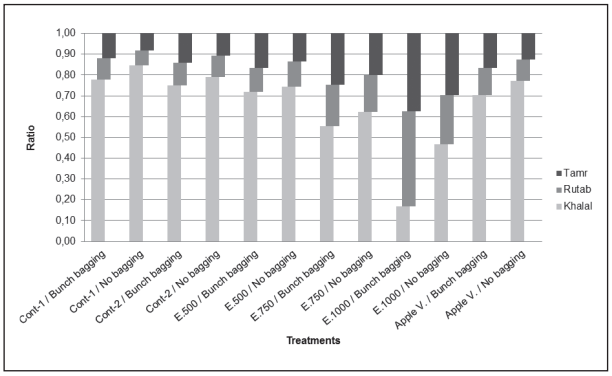

Figure 2: Effects of different preharvest treatments on the ripening of date palm fruits cv. Medjool

termined between these two treatments. The highest ratio of Tamr and Rutab fruits were obtained from the 1,000 ppm Ethephon application with bunch bagging. Other two lower doses of Ethephon (500 ppm and 750 ppm) and apple vinegar were also found to slightly increase the Rutab and Tamr ratio.

Pomological measurements showed that all applications, including bunch bagging, had significant influence on the fruit weight, fruit diameter and fruit length of Tamr and Khalal fruits (Table 2). The highest fruit of weight at Tamr fruits was obtained from 500 ppm Ethephon application without bunch bagging, while the lowest obtained from control-2 with bunch bagging. Significant differences were obtained between the bunch bagging and no bagging for all pomological measurements. Bunch bagging was found to reduce the fruit weight, fruit diameter and fruit length of Tamr fruits, while it was noted to increase these at Khalal fruits. Applications of Ethephon were also found to increase fruit weight, fruit diameter

Table 2. Effects of different preharvest treatments on the fruit weight, fruit diameter and fruit weight of date palm fruits cv. Medjool

Treatments		Fruit weight		Fruit diameter		Fruit length	
		Tamr	**Khalal**	**Tamr**	**Khalal**	**Tamr**	**Khalal**
No bagging	Control-1	11.2 ab	14.3 de	19.5 bc	23.7 d	34.3 a	39.7 bc
	Control-2	10.8 b	14.0 e	19.1 bc	23.1 d	33.5 a	39.3 c
	Ethephon 500 ppm	12.0 a	14.2 e	20.1 b	23.0 d	33.1 a	37.0 d
	Ethephon 750 ppm	11.8 ab	17.1 a	22.9 a	26.3 a	33.6 a	39.3 c
	Ethephon 1,000 ppm	10.8 b	15.5 bc	19.9 b	23.8 cd	34.0 a	43.2 a
	Apple Vinegar	8.6 c	11.0 g	18.9 bc	21.3 e	33.7 a	37.2 d
Bunch bagging	Control-1	7.5 cde	16.2 ab	15.7 e	24.8 b	31.2 bc	43.2 a
	Control-2	7.1 e	15.9 bc	15.3 e	24.4 bc	30.7 c	42.5 a
	Ethephon 500 ppm	7.3 de	16.9 a	17.3 d	24.6 b	32.9 ab	41.0 b
	Ethephon 750 ppm	8.3 cd	17.0 a	20.1 b	26.2 a	34.2 a	39.0 c
	Ethephon 1,000 ppm	7.5 cde	15.2 cd	18.4 cd	23.7 cd	32.6 ab	42.6 a
	Apple Vinegar	7.6 cde	12.8 f	20.2 cd	23.5 cd	33.4 a	36.2 d
No bagging / Average		*10.9* **	*14.4* **	*20.1* **	*23.5* **	*33.7* **	*39.3* **
Bunch bagging / Average		*7.6* **	*15.6* **	*17.8* **	*24.5* **	*32.5* **	*40.8* **

*Values followed by the same letter or letters within the same column are not significantly different at 5% level (Tukey's HSD multiple range test). Comparison of the no bagging and bunch bagging were done with independent samples t-test; and ** used to show significant differences at 99% and ns referred non-significant.*

and fruit length at both Tamr and Khalal stages. As expected, fruit weight, fruit diameter and fruit length were found to decrease from Khalal stage to Tamr stage, which represents the ripening of the fruits.

Treatments rather than bunch bagging found to have no significant effects on the total soluble solids (TSS) and titratable acidity (TA) of fruits (Table 3). Bunch bagging found to increase TSS of fruits at both Tamr and Khalal stages, however to reduce the TA contents of fruits at both Tamr and Khalal stages. Increase in TSS and decrease in TA are the signs of ripening and this result supports the other findings of present study, where bunch bagging found to enhance ripening. On the other hand, clear difference was found for the TSS and TA contents of the fruits between Khalal and Tamr stages. TSS increased from 29.5-31.9% to 65.0-68.7% during ripening and TA decreased from 1.14-1.32% to 0.14-0.23%.

Postharvest studies

Preharvest studies revealed that the date palm fruits cv. Medjool do not ripen on the tree in Northern Cyprus. Furthermore, postharvest studies were conducted to hasten ripening of the fruits. At the time of treatments, all fruits were in the Khalal stage. One day after application, all fruits which were subjected to freezing at -18°C showed ripening and entered into the Rutab stage (Figure 3). Around 15% of the fruits which were subjected to grape vinegar and 10% of the fruits which were subjected to apple vinegar also showed ripening and observed in Rutab stage. No changes observed in control and 500 ppm Ethephon applications, where only 3% of the fruits which were subjected to 750 ppm Ethephon moved into Rutab stage. At the 3rd day after application, all fruits of the freezing treatment found to be fully ripen (Tamr stage). Apart from the freezing treatment, 5% of the fruits of apple vinegar treatment showed full ripening. Ripening hastened 5 days after the applications of apple and grape vinegar and Ethephon applications. However, the effects of vinegars were much higher than the Ethephon applications. 22% of the control fruits also showed slight ripening and moved from Khalal stage to Rutab stage. However, no change observed on the control fruits after that. At the 9th day of applications, all fruits belonging to apple vinegar treatment and 92% of the fruits of grape vinegar treatment were fully ripened. Percentage of fully ripen (Tamr) fruits were found to be 33% and 50% for the applications of 500 ppm and 750 ppm Ethephon, respectively. The Khalal fruits in all treatments, including

Table 3. Effects of different preharvest treatments on the total soluble solids (TSS) content and titratable acidity (TA) of date palm fruits cv. Medjool

Treatments		TSS (% Brix)		TA (g/100g of Malic acid)	
		Tamr	Khalal	Tamr	Khalal
No bagging	Control-1	65.0 b	29.5 b	0.21 ab	1.28 b
	Control-2	65.2 b	29.7 b	0.21 ab	1.27 b
	Ethephon 500 ppm	65.2 b	29.2 b	0.21 ab	1.28 b
	Ethephon 750 ppm	65.3 b	29.5 b	0.22 ab	1.27 b
	Ethephon 1,000 ppm	65.2 b	29.7 b	0.22 ab	1.28 b
	Apple Vinegar	65.5 b	29.8 b	0.23 a	1.32 a
Bunch bagging	Control-1	68.7 a	31.8 a	0.14 cd	1.14 c
	Control-2	68.5 a	31.9 a	0.14 cd	1.15 c
	Ethephon 500 ppm	68.3 a	31.2 a	0.14 cd	1.15 c
	Ethephon 750 ppm	68.3 a	31.3 a	0.14 cd	1.18 c
	Ethephon 1,000 ppm	68.7 a	31.3 a	0.14 cd	1.15 c
	Apple Vinegar	68.5 a	31.5 a	0.16 c	1.18 c
No bagging / Average		*65.2* **	*29.6* **	*0.22* **	*1.28* **
Bunch bagging / Average		*68.5* **	*31.5* **	*0.14* **	*1.16* **

*Values followed by the same letter or letters within the same column are not significantly different at 5% level (Tukey's HSD multiple range test). Comparison of the no bagging and bunch bagging were done with independent samples t-test; and ** used to show significant differences at 99% and ns referred non-significant.*

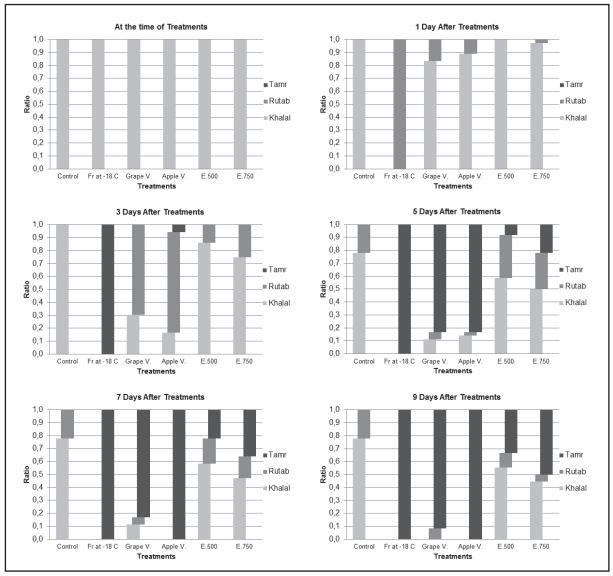

Figure 3: Effects of different postharvest treatments on the ripening of date palm fruits cv. Medjool

control, started to show drying and deterioration at the 9th day after applications.

At the time of postharvest applications to the fruits, all fruits were in Khalal stage and there were no significant differences among the weights of the fruits (Table 4). During the 9 days of storage/ripening, fruit weight in all treatments decreased. It was observed that the fruits treated with grape vinegar decreased more; and had significant difference from the other fruits. When comparing the percent (%) reductions in fruit weight, it was again observed that the highest reduction (26%) was occurred at the fruits treated with grape vinegar. It was followed by the applications of apple vinegar, control, freezing and Ethephon. These data are parallel to the results of ripening, except control treatment. Results suggest that decrease in fruit weight is not always a result of ripening, but might be a cause of deterioration.

Table 5 shows the TSS and TA of the fruits from Khalal and Tamr stages, 9 days after treated with differ-

Table 4. Effects of different postharvest treatments on fruit weight of date palm fruits cv. Medjool

Treatments	First weight	Final weight	Percent (%) Reduction in weight
Control	18.08 a	14.14 ab	21.4% bc
Freezing at -18°C	16.89 a	13.58 ab	19.4% cd
Apple Vinegar	17.58 a	13.61 ab	22.5% b
Grape Vinegar	17.94 a	13.28 b	26.0% a
Ethephon 500 ppm	16.78 a	13.56 ab	19.0% cd
Ethephon 750 ppm	17.81 a	14.44 a	18.3% d

Values followed by the same letter or letters within the same column are not significantly different at 5% level (Tukey's HSD multiple range test).

Table 5. Effects of different preharvest treatments on the total soluble solids (TSS) content and titratable acidity (TA) of date palm fruits cv. Medjool

Treatments	TSS (% Brix)		TA (g/100g of Malic acid)	
	Khalal	Tamr	Khalal	Tamr
Control	35.0 c **	53.5 d **	0.82 a **	0.39 a **
Freezing at -18°C	N/A	60.5 c **	N/A	0.28 b **
Apple Vinegar	N/A	67.3 a **	N/A **	0.25 bc **
Grape Vinegar	48.0 b **	65.5 ab **	0.45 b **	0.26 b **
Ethephon 500 ppm	53.3 a **	65.2 b **	0.38 c **	0.22 d **
Ethephon 750 ppm	53.7 a **	65.3 ab **	0.36 c **	0.23 cd **

*Values followed by the same letter or letters within the same column are not significantly different at 5% level (Tukey's HSD multiple range test). Comparison of the Khalal and Tamr fruits were done with independent samples t-test; and ** used to show significant differences at 99% and ns referred non-significant. N/A means not applicable.*

ent treatments. Results showed that there is a significant difference between the TSS of fruits at Tamr stage. Highest TSS measured from the fruits treated with grape vinegar. This treatment was also found to have the highest ripening ratio. Freezing at -18°C was also one of the best treatments for having highest percentage of fully ripened fruits, but fruits of this treatment had lower TSS than the other treatments. This might be due to the short period of ripening. Results suggest that, even the fruits are in same stage (Khalal or Tamr), the treatments highly influence TSS of the fruits. The TA of the fruits were found to decrease during ripening and significant difference was obtained among the treatments. Highest TA at the Tamr fruits were obtained from control fruits and the lowest from the Ethephon applications. The TA of the fruits treated with grape vinegar and Ethephon at Khalal stage was also found to decrease as compared to control treatments. This shows the biochemical changes in the fruit body which did not turned into pomological changes and did not resulted with ripening.

Postharvest treatments found to have different effects on the fruit color, as in fruit ripening (Figure 4). ΔL* of the fruits treated with different treatments found to have negative results which indicates the darkness. The fruits treated with grape vinegar, apple vinegar and 750 ppm Ethephon found to be darker than the others. These results are parallel to the ripening results. The fruits treated with freezing at -18°C found to be lighter than the fruits treated with apple vinegar. Results suggested that as the fruits ripen, darkness increases. Δa* values of the fruits were found to be positive and Δb* values of the fruits were noted as negative. These results showed that fruits become redder and bluer during ripening. All treatments found to have higher values than

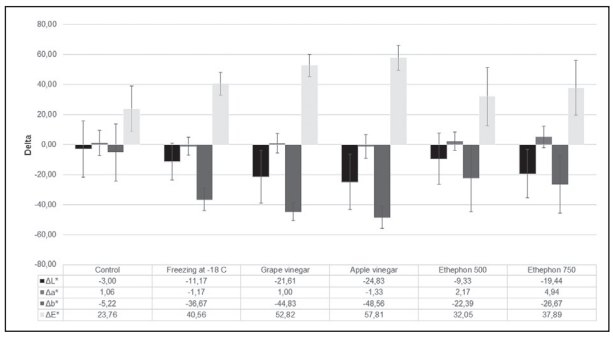

Figure 3: Effects of different postharvest treatments on the ripening of date palm fruits cv. Medjool

the control. Total color difference (ΔE*) was found to be highest at the fruits treated with apple vinegar (57.81) and followed by grape vinegar (52.82). The ΔE* of the control fruits was found to be lowest and measured as 23.76.

Discussions

Preharvest studies

It is well known phenomena that fruit trees require a certain amount of heat energy for growing, developing, flowering and/or ripening of the fruits. The mathematical expression of this energy is referred as heat summation unit (HSU) and is the sum of daily average temperature (°C) minus the base temperature of 18°C. It is calculated with the temperatures from flowering to harvesting. Swingle (15) reported that date palms require at least 1,833 HSUs (above 18°C) for maturation of the fruits. Numerous researchers noted that the HSUs are differing among the different varieties (24,25) and 1,800 HSUs are required to ripen early bearing cultivars and 2,600 HSUs are ideal for ripening many of them (16,26). There are a few studies conducted for the HSU requirements of cv. Medjool, but it is reported to be an early fruit bearing cultivar with around 1,800 HSUs (at the base of 18°C). On the other hand, Mertia and Kumawat (23) reported that cv. Medjool requires about 3,440 HSU for maturation, but the calculations were at the base of 10°C. In present study, two control treatments were tested (with two different time of harvesting) and ripening on the tree was found to be around 10% for both treatments. The HSUs for these treatments (on the base of 18°C) were calculated as 1,209 and 1,279, where both are far below the threshold reported as 1,800. The HSUs (on the base of 10°C) were measured as 2,809 and 3,039 which are also below the thresholds reported as 3,440 by Mertia and Kumawat (23) for cv. Medjool. All of these results supports the findings of present study where the ripening ratio on the tree (without any treatments) had been found to be below 10%.

Results of present study showed that bunch bagging increased the ratio of Tamr and Rutab fruits of cv. Medjool. The highest ratio of Tamr and Rutab fruits were obtained from the 1,000 ppm Ethephon appli-

cation with bunch bagging. Other two lower doses of Ethephon (500 ppm and 750 ppm) and apple vinegar were also found to slightly increase the Rutab and Tamr ratio. Results also showed that during ripening, from Khalal to Tamr, fruits lose weight and volume. The total soluble solids (TSS) and titratable acidity (TA) contents of the fruits also changed during ripening, while TSS increased and TA decreased as suggested by Ahmed et al. (27). Total soluble solid contents of Tamr fruits of present study were found to be similar with the finding of Salomon-Torres et al. (28) who reported 67.5% Brix when pollinated with itself. Muralidhara et al. (29) reported that fruits physiologically firm at Khalal stage and have its maximum weight and size; thus noted that bunch bagging improves ripening of date palms by increasing heat accumulation around the fruits. Fruits starts to soften and lose weight in Rutab stage mainly because of polygalacturonase and betagalactosidase (30). Glasner et al. (31) reported that as fruits soften and ripen, they start to lose tannins and gain sugars which increase TSS. The removal of astringency might be associated with the higher respiration due to heat accumulation; which increase CO_2 concentration around to fruits and lead acetaldehyde production (17). Higher doses of preharvest applied Ethephon was also found to improve ripening of date fruits, as agreed with Awad (17), and Al-Saif et al. (32) and disagree with the findings of Al-Juburi et al. (19). No previous studies noted for the effects of apple vinegar and/or grape vinegar on the ripening of the date palm fruits. However, previously Kader and Hussein (33) reported that acetic acid, which is the primary acid in apple and grape vinegar, is used postharvest to enhance ripening of date fruits.

Postharvest studies

Postharvest tests of present study found to be very effective in ripening of the date palm fruits cv. Medjool. Highest efficacy obtained from freezing at -18°C treatment and apple vinegar application, and followed by grape vinegar and Ethephon applications. Previously Kader and Hussein (33) reported that postharvest applied acetic acid and freezing enhance ripening, but no information was given about the required duration and their influence on the TSS and TA. Present study also showed that the application of grape vinegar significantly influences and increases the TSS. The treatments also found to influence the TSS and TA of the un-ripe Khalal fruits too. Previously Rouhani and Bassiri (34) reported that Ethephon application slightly affects fruit ripening. Awad (17) also noted that postharvest applied Ethephon and abscisic acid significantly enhance fruit ripening of cv. Helali. Matsuo and Ito (35) reported that Ethephon reacts with the tannins and form a non-astringent insoluble gel which promotes ripening. Postharvest treatments found to have different effects on the fruit color, as in fruit ripening. L* and b* values found to decrease and a* values found to increase during ripening. Similarly, Alhamdan et al. (36) reported that freezing enhances fruit ripening, decreases L* and b* values and increases a* values of cv. Barhi dates. Changes in color is closely related with the ripening stage of the fruits. Hazbavi et al. (37) also noted that storage time enhances ripening and reduce the lightness of date palm fruits.

Conclusions

Results of present study showed that heat summation unit (HSU) on the basis of 18°C was 1,279 in Northern Cyprus and is not enough for date palm fruits cv. Medjool to fully ripen on the tree. It was observed that bunch bagging enhances fruit ripening. Bunch bagging was also found to increase TSS of fruits at both Tamr and Khalal stages, however to reduce the TA contents of fruits. Preharvest application of 1,000 ppm Ethephon found to have highest ratio of Tamr and Rutab fruits where preharvest apple vinegar application had slight effect on the fruit ripening. On the other hand, postharvest application of apple vinegar, grape vinegar and freezing at -18°C were found to enhance date fruit ripening. Postharvest Ethephon application was also found to enhance ripening of the date palm fruits but the efficacy found to be less than 50%. It was also found that Tarm fruits are darker (negative ΔL^* values), redder (positive Δa^* values) and bluer (negative Δb^* values) than the Khalal fruits.

References

1. Evans RG, Sadler EJ. Methods and technologies to improve efficiency of water use. Water Resour Res 2008; 44: 1-15.

2. Magadza CHD. Climate change impacts and human settlements in Africa: prospects for adaptation. Environ Monit Assess 2000; 61: 193-205.
3. Kummu M, Guillaume JHA, de Moel H et al. The world's road to water scarcity: shortage and stress in the 20th century and pathways towards sustainability. Sci Rep 2016; 6: 38495.
4. EEA. Water resources across Europe—confronting water scarcity and drought. EEA Report 2; Printed at https://www.eea.europa.eu/publications/water-resources-across-europe. 2009; (Access date 11.03.2018).
5. Rickards L, Howden SM. Transformational adaptation: agriculture and climate change. Crop Pasture Sci 2012; 63: 240-250.
6. Botes A, Zaid A. The economic importance of date production and international trade. In: Zaid, A., Arias, E.J. (Eds.), Date Palm Cultivation, (FAO Plant Production And Protection Paper No. 156), 1999; 45-57.
7. Sumaira K, Nauman K, Rao SK, Haroon A, Asif A. A review on chemistry and pharmacology of Ajwa date fruit and pit. Trends Food Sci Technol 2017; 63: 60-69.
8. Bilgari F, Alkarkhi AFM, Easa AM. Antioxidant activity and phenolic content of various date palm (*Phoenix dactylifera*) fruits from Iran. Food Chem 2008; 107: 1636-1641.
9. Mard SA, Jalalvand K, Jafarinejad M, Balochi H, Naseri MKG. Evaluation of the antidiabetic and antilipaemic activities of the hydroalcoholic extract of Phoenix dactylifera palm leaves and its fractions in alloxan-induced diabetic rats. Malays J Med Sci 2010; 17: 4-13.
10. Jassim SAA, Naji MA. In vitro evaluation of the antiviral activity of an extract of date palm (*Phoenix dactylifera* L.) pits on a Pseudomonas phage. Evid Based Complement Altern Med 2010; 7: 57-62.
11. Zhang CR, Aldosari SA, Vidyasagar PS, Nair KM, Nair M. Antioxidant and anti-inflammatory assays confirm bioactive compounds in Ajwa date fruit. J Agric Food Chem 2013; 61: 5834-5840.
12. Sirisena S, Ng K, Ajlouni S. The Emerging Australian Date Palm Industry: Date Fruit Nutritional and Bioactive Compounds and Valuable Processing By-Products. Compr Rev Food Sci F 2015; 14: 813-823.
13. Khalloukia F, Ricarte I, Breuer A, Owen RW. Characterization of phenolic compounds in mature Moroccan Medjool date palm fruits (*Phoenix dactylifera*) by HPLC-DAD-ESI-MS. J Food Composit Anal 2018; 70: 63-71.
14. Lobo MG, Yahia EM, Kader AA. Biology and Postharvest Physiology of Date Palm. In: Dates: Postharvest Science, Processing Technology and Health Benefits, First Edition, (Eds. Siddiq M, Aleid SM, Kader AA), John Wiley & Sons Ltd. 2014.
15. Swingle WT. The date palm and its utilization in the South Western States. Bureau of Plant Industry, Bulletin No. 53, USDA, Washington, D.C. 1904.
16. Reilly D, Reilly A. Developing a date industry in Australia. Emir J Food Agric 2014; 26(11): 1000-1013.
17. Awad MA. Increasing the rate of ripening of date palm fruit (Phoenix dactylifera L.) cv. Helali by preharvest and postharvest treatments. Postharvest Biol Tech 2007; 43: 121-127.
18. Kamal HM. Effect of some growth regulators on the physical and chemical properties of date fruits. Bull Facul Agric Univ Cairo 1995; 46: 215-227.
19. Al-Juburi HJ, Al-Masry HH, Al-Banna M, Al-Muhanna SA. Effect of some growth regulators on some fruit characteristics and productivity of date palm trees (*Phoenix dactylifera* L.). II: Khaniezy cultivar. In: Proceedings of the Second International Conference on Date Palms, United Arab Emirates University, Al-Ain, March, 2001; 22-35.
20. AOAC. Official Method of analysis of the association of official analytical chemistry. AOAC, Arlington, Va, USA, 15th edition, 1990.
21. Konica Minolta. Identifying Color Differences Using L*a*b* or L*C*H* Coordinates. https://sensing.konicaminolta.us/blog/identifying-color-differences-using-l-a-b-or-l-c-h-coordinates/ 2018; (Accessed on 05.11.2018).
22. Kim SD, Koo Y, Yun Y. A Smartphone-Based Automatic Measurement Method for Colorimetric pH Detection Using a Color Adaptation Algorithm. Sensors 2017; 17: s17071604.
23. Mertia RS, Kumawat RN. Performance and field management of six prominent cultivars of date palm (*Phoenix dactylifera*) in extreme arid regions of Thar Desert, India. Acta Hortic 2010; 882: 65-68.
24. Barreveld WH. Date Palm Products. FAO Agricultural Services Bulletin No. 101. Rome, Italy http://www.fao.org/docrep/t0681e/t0681e00.htm#con, 1993; (Accessed on 05.11.2018).
25. Bhansali RR. Date Palm Cultivation in the Changing Scenario of Indian Arid Zones: Challenges and Prospects. In: Desert Plants (Ed. Ramawat KG), Springer-Verlag, Berlin Heidelberg, 2010; 423-443.
26. Al-Banna MF. The effect of climatic conditions and geographical distribution on the success of new date palm varieties in the Gaza Strip. Acta Hortic 2007; 736: 59-69.
27. Ahmed IA, Ahmed AWK, Robinson RK. Chemical composition of date varieties as influenced by the stage of ripening. Food Chem 1995; 54: 305-309.
28. Salomon-torres R, Ortiz-Uribe N, Villa-Angulo R, Villa-Angulo C, Norzagaray-Plasencia S, Garcia-Verdugo CD. Effects of pollinizers on production and fruit characteristics of date palm (*Phoenix dactylifera* L.) cultivar Medjool in Mexico. Turk J Agric For 2017; 41: 338-347.
29. Muralidhara BM, Singh RS, Bhargava R, Veena GL, Kumar MK. Morphological characterization of date fruits at different growth stages under hot arid conditions. Environment & Ecology 2016; 34(4): 1234-1237.
30. Serrano M, Pretel MT, Botella MA, Amoros A. Physicochemical changes during date ripening related to ethylene production. Food Sci Technol Int 2001; 7: 31-36.
31. Glasner B, Botes A, Zaid A, Emmens J. Date harvesting, packing house management, and marketing aspects. In: Date Palm Cultivation (Eds. Zaid A, Arias EJ), (FAO Plant

Production and Protection Paper No. 156), 1999; 177-198
32. Al-Saif AM, Alebidi AI, Al-Obeed RS, Soliman SA. Preharvest Ethephon spray on fruit quality and increasing the rate of date palm fruit (*Phoenix dactylifera* L.) cv. Helali. Prog Nutr 2017; 19(1): 97-1003.
33. Kader AA, Hussein AM. Harvesting and postharvest handling of dates. ICARDA, Aleppo, Syria. 2009; 15p.
34. Rouhani I, Bassiri A. Effect of ethephon on ripening and physiology of date fruits at different stages of maturity. J Hortic Sci 1977; 52: 289-297.
35. Matsuo T, Ito S. A model experiment for deastringency of persimmon fruit with the high carbon dioxide treatment: in vitro gelation of kaki-tannin by reacting with acetaldehyde. Agric Biol Chem 1982; 46: 683-689.
36. Alhamdan A, Hassan B, Alkahtani H, Abdelkarim D, Younis M. Freezing of fresh Barhi dates for quality preservation during frozen storage. Saudi Journal of Biological Sciences (in Press), 2016.
37. Hazbavi I, Khoshtaghaza MH, Mostaan A, Banakar A. Effects of postharvest hot-water and heat treatment on quality of date palm (cv. Stamaran). Journal of the Saudi Society of Agricultural Sciences 2015; 14: 153-159.

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Original article

In Vivo evaluation of therapeutic potential of heart of date palm extract on lipid profile and thyroid hormones in normal male wistar rats

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Summary. *Objective*: Reports exist on the use of *Phoenix dactylifera* as hypolipidemic, hypoglycemic or antioxidant but broad search in the literature revealed absence of studies on the effect of heart of date palm (HDP) on lipid profiles and thyroid hormone and to the best of our knowledge this is the first research carried out to investigate the effect of heart of date palm extract (HDP) on lipid profiles and thyroid hormones. *Method*: Extract of heart of date palm was prepared by mixing 50 gram of finely powdered heart of palm flour with 400 ml distilled water. Sixty healthy male albino rats were divided into ten groups, comprising of six rats in each group. One group was kept as control while other 9 group was divided into three batches of Sukkari, Naboat Saif, Solleg. Each batch has three groups served with 1, 2 and 3 ml of HDP. After the completion of the experimental period (60 days) blood samples were collected from rat's heart. Total Cholesterol, HDL Cholesterol, Triglycerides, Triodothyronine (T3), Thyroxin (T4) and TSH were determined for all groups. *Result:* HDP produced significant beneficial effect in the lipid profile of treated rats by significantly reducing total cholesterol and increasing HDL. LDLc, VLDLc and triglycerides also decreases with most of the treatment with some variations. Value of T3 and T4 significantly decreased, while the value of TSH significantly increased after treatment with HDP. Naboat Saif as compared to other two varieties (Sukkari and Solleg); exhibited better response towards lipid and thyroid hormones. *Conclusion*: This study shows that HDP supplementation may benefit management of lipid profile and thyroid hormones.

Key words: Hormones, cholesterol, triglycerides, dates, thyroxin, lipids

Introduction

Date palm (*Phoenix dactylifera* L.) is a monocotyledonous woody perennial belonging to the Arecaceae family. It is considered as the oldest plant cultivated in hot and dry climate as Arabian Peninsula, Africa, Middle East and Asia and comprises 200 genera and 3000 species (1). Kingdom of Saudi Arabia (KSA) has more than 23 million date palm trees and over 320 varieties with annual earning of over 500 million USD (2). It is one of the most important fruit crop in the KSA (3) and the importance of this kind of trees in that region refers to the nutritional value of the dates which is considered as a major human food (4,5). In today's world scientists and researchers are focusing more on natural plant products all over the world and various results have shown the massive potential of medicinal plants used traditionally (6). Various studies have shown beneficial effect of dates fruits, leaves and pits on health. They are considered to protect against many chronic diseases including cancer and heart diseases (7-9) and possess anti diabetic (10, 11), antimicrobial activity (12, 13), anti-inflammatory effect [14], antidiarrheal effects (15).

Heart of palm is a creamy white cylinder of variable length extracted from several palm genera and species (16, 17). It is a gourmet vegetable basically composed of the apical meristem of the palm and also the part of the young or immature leaves emerging from

the meristem and this edible meristem is normally consumed in soups and salads (16). A study has been done previously on chemical composition, minerals and antioxidants of different kind of hearts of date palm (HDP) from Saudi Cultivars (18) and they reported moisture as the predominant component (ranged from 80.44%-82.82%). Sucrose was predominant in sugar and ranged from 7.65%-82.82%, while the predominant mineral was potassium, sulphur and chloride. Furthermore this study illustrated that solleg had a higher total phenolic and flavonoid contents (56.05 mgGAE/100 gm and 6.82 MG QE/100 gm). Previous study on heart of palm shows that it contains unsaturated fatty acids, minerals (Zn, Fe, Mg, P, Mn, Ca, Cu, Na, K and Se) and crude fiber (19). Synthetic drugs are quiet expensive and shows adverse side effects. In contrast to this natural products are affordable and effective and good remedy in the treatment/management of diseases (20, 21). In addition to nutritional value and antioxidant effect (22, 23) many studies have shown the therapeutic effect of dates (24, 25,26), but to the best of our knowledge this is the first research carried out to investigate effect of HDP consumption (locally called as Al-Guomar) on metabolic response such as lipid profiles and thyroid hormones in male Wistar rats. Due to lack of any data and information on effect of heart of date palm on lipid and thyroid hormone, the authors compared their results with data available on dates fruits, leaves or pits.

Materials and methods

Collection of samples and Preparation

Samples of Sukkari, Naboat Saif, and Solleg (Saudi date palm varieties), consisting of leaves and stems were procured from Agricultural Experimental Station at Derab in Riyadh, King Saud University. HDP was peeled by an experienced staff affiliated to plant production department (Faculty of Food and Agriculture Sciences), considering minimal losses in heart of date palm tissue. After removing plant debris, HDP samples were washed with tap water and then freeze dried (Alpha 1-2 LDplus, Germany) and finely powdered in food grinder (National, MK-G30NR, Japan). Powdered HDP samples were passed to 60 mesh sieves and the flour was stored in air tight container at 4°C for further analysis.

Preparation of heart of palm (HDP) extract

Fifty gram of finely powdered heart of palm flour was mixed with 400 ml distilled water. The mixture was stirred for one hour and then filtered with cheese cloth to obtain a clear filtrate. The extract was prepared daily and used immediately in the rat bioassays (18).

Experimental design

Experimental Animals Center, College of Pharmacy, King Saud University provided sixty healthy male albino rats weighting between 180-200 grams. This study was conducted in accordance with research policies of the King Saud University Research Centre. Rats were housed individually under standard laboratory condition, light and dark cycles of 12h, in a polypropylene cages and allowed free access to commercial diet and water *ad libilum* for two months under strictly controlled pathogen free conditions with room temperature (25±2°C), humidity (50±5%). Commercial rodent chow diet was obtained from grain silos and flour mills, Riyadh Saudi Arabia. Stainless steel oral feeder was used to administer orally heart of palm extract to each rat at fixed time of the day, and with the specified volume of each extract. The initial weights of the rats and the weights every week were recorded. Sixty rats were randomly divided into ten groups, comprising of six rats in each group as discussed in Table 1.

Blood sample Collection

After the completion of the experimental period (60 days) blood samples were collected from heart of each rat after 12 hour fasting in lithium heparin tube. It was centrifuged at 3500 rpm for 10 minutes in megafuge and immediately plasma samples were prepared. After centrifugation supernatant was separated and stored at -80°C (U725-80 Freezers, New Brunswick, Massachusetts, United States) for further analysis.

Biochemical analyses

Lipid profile

High density lipoprotein kit (REF 041) was obtained from United Diagnostic Industry. This method was based on a non HDL precipitation followed by an enzymatic detection. Cholesterol (REF 024) and Triglycerides (REF 059L) kits were also obtained from United Diagnostic Industry. Enzymatic colorimetric

Table 1. Groups of experimental animals

Group No	Sample	No: of rats	Amount of extract consumed (ml)
1	Control	6	3 ml distilled water
2	Sukkari	6	1
3	Sukkari	6	2
4	Sukkari	6	3
5	Naboat Saif	6	1
6	Naboat Saif	6	2
7	Naboat Saif	6	3
8	Solleg	6	1
9	Solleg	6	2
10	Solleg	6	3

method was used for the determination of cholesterol and triglycerides (27, 28).The assays were performed according to the manufacturer's instruction. LDL cholesterol was calculated using following formula (29):
LDL Cholesterol (mg/dl) =Total cholesterol-HDL cholesterol- Triglycerides/5

Thyroid hormone

For the quantitative determination of triodothyronine (T3), thyroxin (T4) and TSH, Chemiluminescent micro particle immune assay was performed in the samples. Architect assays kit used for determination of triodothyronine (T3), thyroxin (T4) and thyroid stimulating hormone (TSH) kit was obtained from Abbot, Ireland (30, 31).

Statistical analysis

SPSS statistical software package was used to analyze the data. Data were expressed as mean ±standard deviation. The differences among the dietary treatment groups were analyzed by one way ANOVA at a significance level of $p \leq 0.05$; and if significant differences were found, a Post-hoc analysis using Duncan's multiple range tests was performed.

Results and discussions

Effect of heart of date palm extract (HDP) on lipid profiles in normal wistar rats

Results of the impact of the consumption of different kind of HDP extract on lipid profiles (total cholesterol HDLc, triglycerides and LDLc) are presented in Table 2. HDP produced significant beneficial effect in the lipid profile of treated rats by significantly reducing total cholesterol and increasing HDL. LDLc, and triglycerides also decreases with most of the treatment with some variations. Maximum effect of HDP treatment on LDLc and triglycerides has been observed in group 7 i.e rats treated with 3 ml extract of Naboat Saif but the least value of total cholesterol was observed in group 6 i.e rats treated with 2ml extract of Naboat Saif. Highest value of HDLc was observed in in group 3 i.e rats treated with 2ml extract of Sukkari.

Table 2. Effect of different kind of heart of palm extract (HDP) on lipid profiles

Group No:	Sample	Total cholesterol (mg/dl)	HDL Cholesterol (mg/dl)	Triglycerides (mg/dl)	LDL Cholesterol (mg/dl)
1	Control	73.11^{a} ±11.098	27.22^{ef} ±2.734	46.33^{e} ±5.368	36.72^{d} ±1.526
2	Sukkari (1ml)	61.61^{e} ±11.723	26.50^{fg} ± 2.526	44.50^{f} ±4.199	34.28^{f} ±1.674
3	Sukkari (2ml)	65.50^{cd}± 10.913	33.83^{a} ±3.148	58.50^{a} ±5.943	40.28^{b} ± 3.045
4	Sukkari (3ml)	65.00^{d} ±6.240	28.50^{d}±2.526	48.17^{d} ± 4.370	35.22^{e} ± 3.622
5	N Saif (1 ml)*	61.50^{e} ± 6.167	30.06^{c}±2.460	51.83^{c} ±4.033	38.89^{c} ±2.055
6	N Saif (2 ml)*	61.06^{e} ± 6.458	30.72^{c} ±2.469	52.33^{c}±4.409	39.17^{c} ± 5.159
7	N Saif (3 ml)*	57.83^{f} ±5.401	25.78^{g} ±2.157	42.50^{g} ±3.365	32.33^{g} ± 2.744
8	Solleg (1 ml)	70.44^{b} ± 7.905	31.89^{b} ±2.139	54.50^{b} ±4.007	41.22^{a}± 3.533
9	Solleg (2 ml)	67.00^{c} ± 10.466	27.39^{def} ±3.147	47.39^{de} ±5.147	34.33^{f} ±1.815
10	Solleg (3 ml)	69.94^{b} ± 9.142	26.78^{efg} ±1.477	44.56^{f} ±2.007	34.61^{f} ± 1.577

Data are expressed as the mean ± standard deviation; Model ANOVA, p values < 0.05 are significant. Superscript abc indicate significant differences among various groups as indicated by ANOVA followed by Duncan's multiple range test. N Saif- Naboat Saif

Effect of heart of date palm extract (HDP) on thyroid hormones in normal wistar rats

Table 3 depicts the thyroid hormone status of control and HDP groups. In control group TSH, T3 and T4 has been found to be 0.017 mg/100 ml, 0.486 mg/100 ml, and 3.90 mg/100 ml respectively. A significant effect of HDP has been found on thyroid hormone. Value of T3 and T4 significantly decreased, while the value of TSH significantly increased after treatment with HDP. Highest TSH (0.060 mg/100 ml) was observed in Sukkari (3ml) group, while lowest T3 and T4 were found in Naboat Saif (2 ml) (0.397 mg/100 ml) and Naboat Saif (3 ml) (3.21 mg/100 ml) respectively.

Discussions

Comprehensive exploration in the review of literature have shown dearth of studies investigating the effect of HDP on lipid profiles and thyroid hormone and to the best of our information this is the first study carried out to identify the effect of HDP extract on lipid profiles and thyroid hormones and such curtail make it difficult to discuss the result but it can be valuable to expand opportunities of thinking and ideas for further research in this field. During the past few decades, the eminence of herbal medicine has attained ground all over the world and witnessed a tremendous surge in acceptance which is mainly due to the faith that besides being cheap and locally available; herbal drugs are safe without any side effects (32, 33, 34). Date palm (Phoenix dactylifera L.) is very significant fruit crops in the palmacea family. Every part of this tree has its own uses. The influence of customary diet on lipid metabolism is a vital factor determining vulnerability to heart disease. Enhanced knowledge of the complexity of nutrient-disease relationships has shifted the framework for CVD prevention from a focus on macronutrient content of diets to foods and dietary patterns (35).

HDL and LDL are the two major groups of plasma lipoproteins involved in lipid metabolism and the exchange of triglycerides and cholesterol, cholesterol ester between tissues (36, 37). Addition of date to the diet of rats decreases plasma triglycerides and cholesterol and it is most likely facilitated by inhibition of absorption of dietary fats, cholesterol and bile acids (38). Erstwhile; studies have revealed that abnormalities of lipid and lipoprotein play substantial part in the pathogenesis and progression of CVD (39, 40). In controlled clinical trials almost 1.5% reductions in the incidence of coronary heart diseases has been observed after just 1% reduction in total and LDL cholesterol concentration (41).

Al Saif et al. (42) reported significant hypolipidemic effect of date diet in rats. Trabzuni et al. (18) in their previous study reported high content of total phenol and total flavonoid and 91%, 88% and 80% DPPH radical scavenging activity of Solleg, Naboat

Table 3: Effect of different kind of heart of palm extract (HDP) on thyroid hormones

Group No	Sample	TSH (mg/100 ml)	T3 (mg/100 ml)	T4(mg/100 ml)
1	Sukkari (1ml)	$0.049^{c} \pm 0.048$	$0.483^{a} \pm 0.061$	$3.42^{ef} \pm 0.357$
2	Sukkari (2ml)	$0.050^{c} \pm 0.004$	$0.446^{b} \pm 0.044$	$3.57^{de} \pm 0.250$
3	Sukkari (3ml)	$0.060^{b} \pm 0.007$	$0.437^{bc} \pm 0.050$	$3.72^{bcd} \pm 0.285$
4	Naboat Saif (1 ml)	$0.017^{a} \pm 0.005$	$0.412^{cd} \pm 0.052$	$3.75^{bcd} \pm 0.199$
5	Naboat Saif (2 ml)	$0.012^{a} \pm 0.005$	$0.397^{d} \pm 0.034$	$3.94^{ab} \pm 0.263$
6	Naboat Saif (3 ml)	$0.023^{d} \pm 0.045$	$0.401^{d} \pm 0.102$	$3.21^{f} \pm 0.236$
7	Solleg (1 ml)	$0.018^{a} \pm 0.010$	$0.446^{b} \pm 0.050$	$3.84^{bc} \pm 0.312$
8	Solleg (2 ml)	$0.027^{d} \pm 0.009$	$0.452^{b} \pm 0.045$	$3.94^{ab} \pm 0.275$
9	Solleg (3 ml)	$0.019^{a} \pm 0.013$	$0.424^{c} \pm 0.053$	$3.64^{cde} \pm 0.429$
10	Control	$0.017^{a} \pm 0.087$	$0.486^{a} \pm 0.051$	$3.90^{ab} \pm 0.201$

Data are expressed as the mean ± standard deviation; Model ANOVA, p values < 0.05 are significant. Superscript abc indicate significant differences among various groups as indicated by ANOVA followed by Duncan's multiple range test.

Saif and Sukkari respectively. Flavonoids in the HDP might also play a role in boosting the activity of lecithin acyl transferase which regulates blood lipids (43). Chaira et al. (44) reported that flesh and pits extracts of date palm fruit have free radical scavenging activities, and the significant effect of palmito extract on serum total lipid level could be attributed to the antioxidant potentials of palmito extract.

In the present study, concentration of HDLc significantly increased and this upsurge may be due to change in HDL composition which increases larger more cholesterol rich lipoprotein called HDLc and decreases the typical protein rich HDL (42). Coronary heart disease is strongly related to decrease in the concentration of high density lipoprotein cholesterol and increase in the LDLc. HDL can act as an acceptor of cellular cholesterol, scavenges extra cholesterol from peripheral tissues by lipid-poor apoA-I and HDL that is mediated by lipid transporter molecules and supply it to the liver for ultimate excretion into the feces as neutral sterols or bile acids and this role of HDL has been shown to be responsible for its athero protective properties (45,46). Long term use of anti hyperlipidemic drugs has been associated with few adverse effects, most importantly gastrointestinal upsets, general weakness, hepatic enzyme elevation and headache (47,48) and so herbal medicines can be better substitute.

Thyroid hormones (thyroxine (T4) and Triiodothyronine (T3)) are involved in the regulation of innumerable body functions such as reproduction, carbohydrate and lipid metabolism and oxygen consumption and any alteration in their normal levels leads to abnormalities (49). Follicular cells from free tyrosine and tyrosine residues of the protein called thyroglobulin synthesizes T4 (50) and almost 80% of the T4 gets converted to T3 by peripheral organs such as the liver, kidney and spleen (51). Trabezuni et al. (18) in their study mentioned that total flavonoids and phenols are the important pharmaceutical compounds of HDP and besides that, it also contains, carbohydrates, calcium salts, magnesium, zinc, potassium and even traces of iodine. The flavonoids can decrease thyroid hormones levels in various ways such as through inhibiting the activation of type 1 deiodinase that is specifically activated by TSH, through inhibiting thyroperoxidase and also by preventing the mineralization of iodine in the thyroid cells (52, 53). Presence of phenolic and flavonoid compounds may reduce both thyroid iodide uptake and thyroid peroxidase activity, which may be the reason for the observed depressing effect of HDP extract on thyroid hormones levels in normal rats. Similar result was reported previously by El Kaslan et al. (54) in normal rats after treatment with date palm pollen. Since HDP includes calcium and magnesium, it can contribute to fabricating and therefore increasing TSH as a mediator of second messenger via calcium-phosphatidylinositol mechanism (55, 56). Meanwhile; thyroid hormones play significant role in the pathogenesis of numerous diseases and any alteration in thyroid hormones levels can have hazardous effects on body physiology so it is necessary to conduct further studies on the effects of plants and their compounds on thyroid hormone secretion rates.

Conclusion

This study revealed that consumption of heart of date of palm as dietary component can be beneficial for lipid or thyroid control but more studies are required to assess its ameliorative role in hyperlipidemic and hyperthyroidism models and to expand vision into its possible mechanism of action and corroboration of these effects on animal and human models.

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References

1. Bokhari NA, Praveen K. Invitro inhibition potential of Phoenix dactylifera L. extracts on the growth of pathogenic fungi. J Med Plants Res 2012; 6:1083–88.
2. Al-Khalifa NS. First Arab Palm Conference on the Development of Date Palm and Dates Sector in the Arab World. Emir J Food Agric 2012; 24 Convener Note: 1-2.
3. Alkhateeb A. Comparison effects of sucrose and date palm syrup on somatic embryogenesis of date palm (Phoenix dac-

tyliferaL). Am J Biotechnol Biochem 2008; 4:19–23.
4. Shafiei M, Karimi K, Taherzadeh MJ. Palm Date Fibers: Analysis and Enzymatic Hydrolysis. Int J Mol Sci 2010; 11: 4285-4296.
5. Nasir MU, Hussain S, Jabbar S, Rahid F, Khalid N, Mehmood A. A review on the nutritional content, functional properties and medicinal potential of dates. Sci Lett 2015; 3:17-22.
6. Saeed MK, Deng Y, Dai R. Attenuation of biochemical parameters in streptozotocin-induced diabetic rats by oral administration of extracts and fractions of cephalotaxussinensis. J Clin Biochem Nutr 2008; 42: 21–8.
7. Habib HM, Ibrahim WH. Effect of date seeds on oxidative damage and antioxidant status in vivo. J Sci Food Agri 2011; 91:1674-1679.
8. Diab KAS, Aboul-Ela EI. In vivo Comparative studies on antigenotoxicity of date palm (phoenix dactylifera l.) pits extract against DNA damage induced by N-nitroso-N-methylurea in mice. Toxicol Int 2012; 19:279-86.
9. Essa MM, Akbar M, Khan MA. Beneficial effects of date palm fruits on neurodegenerative diseases. Neural Regen Res 2016;11:1071-1072
10. Miller CJ, Dunn EV, Hashim IB. The glycaemic index of dates and date/yoghurt mixed meals. Are dates 'the candy that grows on trees'? Eur J Clin Nut 2003; 57: 427-430.
11. Mard SA, Jalalvand K, Jafarinejad M, Balochi H and Naseri MKG. Evaluation of the antidiabetic and antilipaemic activities of the hydroalcoholic extract of Phoenix dactylifera palm leaves and its fractions in alloxan-Induced diabetic rats. Malays J Med Sci 2010; 17: 4-13
12. Ammar NM, Lamia T, Abou E, Nabil HS, Lalita MC and Tom JM. Flavonoid constituents and antimicrobial activity of date (Phoenix dactylifera L.) seeds growing in Egypt. In: Proceedings of 4th conference on research and development of pharmaceutical industries (Current Challenges). Med Arom Pl Sci Biotech 2009; 3: 1-5.
13. Jassim SAA, Naji MA. In vitro evaluation of the antiviral activity of an extract of date palm (Phoenix dactylifera L.) pits on a Pseudomonas phage 2007. Evid Based Complement Alternat Med 2010; 7: 57-62.
14. Eddine LS. Antioxidant, anti-inflammatory and diabetes related enzyme inhibition properties of leaves extract from selected varieties of Phoenyx dactylifera L. Innovare J Life Sci 2013; 1: 14-18.
15. Al-Taher AY. Possible antidiarrheal effect of date-palm (Phoenix dactylifera L.) spathe aqueous extracts in rats. Sci J King Faisal Univ (Basic and Applied Sciences) 2008; 9: 131-138.
16. Tabora PC, Balick Jr, MJ, Bovi, MLA, Guerra MP. Hearts of Palm (Bactris euterpe and Others). In: Williams J., Ed., Pulses and Vegetables, Chapman and Hall, London, 1993; p193-218.
17. Soto G, Luna-Orea P, Wagga MG, Smyth TJ, Alvarado A. Foliage residue decomposition and nutrient release in peach palm (Bactris gasipaes Kunth) plantations for heart-of-palm production in Costa Rica. Agron J 2005; 97:1396-1402.
18. Trabzuni DM, Ahmed SEB, Abu-Tarboush HM. Chemical composition, minerals and antioxidants of the heart of date palm from three Saudi cultivars. Food Nutr Sci 2014; 5:1379-1386.
19. Movahed A, Mohammadi MM, Akbarzadeh S, Nabipour I, Ramezanian N, Hajian N. The heart of date palm: its nutritional and functional constituents. Iran South Med J 2012; 2:100-105.
20. Rahmani AH, Aly SM, Babiker AY, Srikar S, Khan AA. Therapeutic effects of date fruits (Phoenix dactylifera) in the prevention of diseases via modulation of anti-inflammatory, anti-oxidant and anti-tumour activity. Int J Clin Exp Med 2014; 7:483-491.
21. Carmona F, Pereira AMS. Herbal medicines: old and new concepts, truths and misunderstandings. Brazilian J Pharmacog 2013; 23: 379-385.
22. Al-Farsi M, Alasalvar C, Morris A, Baron M, Shahidi F. Compositional and sensory characteristics of three native sun-dried date (Phoenix dactylifera L.) varieties grown in Oman. J Agric Food Chem. 2005; 53: 7586–7591.
23. Al-Farsi MA, Lee CY. Nutritional and functional properties of dates: a review. Crit Rev Food Sci Nutr. 2008; 48:877–87.
24. Ishurda O, John FK. The anti-cancer activity of polysaccharide prepared from Libyan dates (Phoenix dactylifera L.) Carbohyd Polym 2005; 59: 531-535.
25. Barh D, Mazumdar BC. Comparative nutritive values of palm saps before and after their partial fermentation and effective use of wild date (Phoenix sylvestris Roxb) sap in treatment of anemia. Res J Med Med Sci 2008; 3: 173-176.
26. Vayalil PK. Date fruits (Phoenix dactylifera Linn): an emerging medicinal food. Crit Rev Food Sci Nutr 2012; 52:249-71.
27. Saryono S, Eliyan J, Herdiati D, Khikmatullah AA, Silvana CP, di HPA. Anti-atherogenic properties of Deglet Noor date seeds (Phoenix dactylifera) methanol extract on diet-induced hypercholesterolemic rats. IOP Conf. Series: Mat Sci Eng 2017; 172:012046.
28. Nwaneri-Chidozie VO, Salemcity, James A, Sunday. Awe, Eke SC. Effect of Phoenix dactylifera fruit wine produced by Saccharomyces cerevisae on the haematological and some biochemical parameters in albino rats. Haya: Saudi J Life Sci 2017; 2:116-121.
29. Friedwald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Cin Chem 1972; 18:499-502
30. Fillee C, Cumps J, Ketelslegers JM. Comparison of three free T4 (FT4) and free T3 (FT3) immunoassays in healthy subjects and patients with thyroid diseases and severe non-thyroidal illnesses. Clin Lab 2012; 58:725-36.
31. Matyjaszek-Matuszek B, Pyzik A, Nowakowski A, Jarosz MJ. Diagnostic methods of TSH in thyroid screening tests. Ann Agric Environ Med 2013; 20:731–735.
32. Nitha A, Ansil P, Prabha S, Wills P, Latha M. 2011. Preventive and curative effect of Woodfordia fruticosa Kurz flowers on thioacetamide induced oxidative stress in rats. Asian

Pac J Biomed 1:395-400.
33. Kumar D, Kumar A, Prakash O. Potential antifertility agents from plants: A comprehensive review. J Ethnopharmacol 2012; 140:1-32.
34. Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol 2013:4:177.
35. Siri Tarino PW, Krauss RM. Diet, lipids, and cardiovascular disease. Curr Opin Lipidol 2016; 27:323-8.
36. Sviridiv D. Intracellular cholesterol trafficking. Histol Histopathol 1999; 14:305-319.
37. McNamara DJ. Dietary fatty acids, lipoproteins, and cardiovascular disease. Adv Food Nutr Res 1992; 36:253-351.
38. Chan PT, Fong WP, Cheung YL, Huang Y, Ho WK, Chen ZY. Jasmine green tea epicatechens are hypolipidemic in hamsters fed a high fat. J Nutr 1999; 129:1094-1101.
39. Glew RH, Kassam HA, Bhanji RA, Okorodudu A, VanderJagt DJ. Serum lipid profiles and risk of cardiovascular disease in three different male populations in northern Nigeria. J Health Popul Nutr 2002; 20:166-174.
40. Chrysohoou C, Panagiotakos DB, Pitsavos C, Kosma K, Barbetseas J, Karagiorga M, Ladi I, Stefanadis C. Distribution of serum lipids and lipoproteins in patients with beta thalassaemia major; an epidemiological study in young adults from Greece. Lipids Health Dis 2004; 3:1-8.
41. American Academy of Pediatrics. National Cholesterol Education Program (NCEP). Report of the expert panel on blood cholesterol levels in children and adolescents. Pediatrics 1992; 89:495-501.
42. Alsaif MA, Khan LA, Alhamdan AAH, AlOrf SM, Harfi SH, Al Othman AM, Arif Z. Effect of dates and gahwa (arabian coffee) supplementation on lipids in hypercholesterolemic hamsters. Int J Pharmacol 2007; 3:123-129.
43. Senecha C, Shama PK, D'Souza UP, Shastry CS. Anticholesteremic and antilipidemic activity of stem bark extracts of *Moringa oleifera* in diet induced hyperlipidemia models in rats. Int J Pharm Chem Biol Sci 2012; 1:916-923.
44. Chaira N, Ferchichi A, Mrabet A, Sghairoun M. Chemical composition of the flesh and the pit of date palm fruit and radical scavenging activity of their extracts. Pak J Biol Sci 2007; 10: 2202-2207.
45. Stein O, Stein Y. Atheroprotective mechanisms of HDL. Atherosclerosis 1999; 144:285-301.
46. Das DK. Cardioprotection with high density lipoproteins. Fact or friction? Circ Res 2003; 92:258-260.
47. Bellosta S, Corsini A. Statin drug interactions and related adverse reactions an update. Expert Opin Drug Saf 2018; 1:25-37.
48. Naci H, Brugts J, Ades T. Comparative tolerability and harms of individual statins: a study level network meta-analysis of 246 955 participants from 135 randomized, controlled trials. Circ Cardivasc Qual Outcomes. 2013; 6:390-399.
49. Kundu S Pramanik M, Roy S, De J, Biswas A, Ray A K. Maintenance of brain thyroid hormone level during peripheral hypothyroid condition in adult rat. Life Scie 2006; 79: 1450–1455.
50. Ekholm R, Bjorkman U. Glutathione peroxidase degrades intracellular hydrogen peroxide and thereby inhibits intracellular protein iodination in thyroid epithelium. J Endocrinol 1997; 138: 2871-2878.
51. Roshni PR, Rajan VK, Meenuvijayan, RemyaReghu. Evaluation of patient with thyroid disorders. Int J Res Pharm Chem 2013; 3: 244-249.
52. Aleebrahim-Dehkordy E, Ansaripour S, Rafieian-Kopaei M, Saberianpour S. Effects of substances on plants' active compounds on changes in the hormone levels of the pituitary–thyroid axis in hyperthyroidism and hypothyroidism. Phcog Rev 2018;12:1-6
53. Kar A, Panda S, Bharti S. Relative efficacy of three medicinal plant extracts in the alteration of thyroid hormone concentrations in male mice. J Ethnopharmacol 2002; 81:281-5.
54. El Kashlan AM, Nooh MM, Hassan WA, Rizk SM. Therapeutic Potential of date palm pollen for testicular dysfunction induced by thyroid disorders in male rats. PLOs one 2015; (10)10:e0139493.
55. Biswas SJ, Bhattacharjee N, Khuda-Bukhsh AR. Efficacy of a plant extracts (Chelidonium majus L.) in combating induced hepatocarcinogenesis in mice. Food Chem Toxicol 2008; 46:1474-87.
56. De Souza Dos Santos MC, Gonçalves CF, Vaisman M, Ferreira AC, de Carvalho DP. Impact of flavonoids on thyroid function. Food Chem Toxicol 2011;49:2495-502

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Original article

Physicochemical and antimicrobial effects of gelatin-based edible films incorporated with garlic peel extract on the rainbow trout fillets

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Summary. Influence of gelatin-based edible films incorporated with garlic peel extract (GPE) with concentration of 4% and 8% (by volume per mass of gelatin) on the microbiological, sensorial and physicochemical quality of rainbow trout fillets during refrigerated storage at 4±1 °C were evaluated. Gelatin films enriched with GPE retarted the total mesophilic and total psychrophilic bacteria, and Enterobacteriaceae counts during the storage period. Compared to control samples, lipid oxidation was delayed in the samples wrapped with gelatin films incorporated with GPE, especially in concentration of 8%. The sensory results are correlated with physicochemical and microbiological results. The shelf-life of rainbow trout fillets was found 5 days for control and the fillets wrapped with gelatin film without GPE, respectively, while the shelf life of fish was 10 days for the fillets wrapped with gelatin film incorporated with GPE. According to results of the study, the incorporation of GPE into gelatin film could enhance the both antimicrobial and antioxidative characteristics of the film. Therefore, gelatin film enriched with GPE efficient in maintaining the qualities of the rainbow trout fillets during refrigerated storage.

Key words: garlic peel extract, edible films, gelatin, rainbow trout, shelf life

Introduction

Fresh fish is one of the most important product due to its high nutritional value such as high level of omega-3 fatty acids, proteins and vitamins. However, it is one of the most perishable product which is susceptible to both microbiological and chemical deterioration during storage (1). Therefore, maintenance the freshness of fish with favorable preservation techniques is required. Temperature-based preservation techniques such as chilling and freezing had beed used in fish and fish products (2). With the increasing demand for high quality product with the extended shelf-life and minimal processing has promoted the development of several innovative techniques (3).

Edible films and coatings are a promosing trend in the last years to improve the shelf-life of perishable food products and to answer reliable product demands of the consumers (4,5). In addition, edible films and coatings can prevent lipid oxidation, color deterioration and enhance the product quality (6) by acting as moisture, oxygen, carbon dioxide or vapour barriers (7). Nowadays, there is an increasing attention to use of edible films incorporating with essential oils and plant extracts with antioxidant and antimicrobial properties.

Garlic (*Allium sativum* L.) is a popular food ingredient and also has been used as a medicine to treat various human diseases from time immemorial (8,9). Additionally, garlic has higher concentrations of phenolic compounds compared with other vegetables (10). In general, garlic is consumed as fresh, however, dried garlic slices and powder find approval in recent years as well (11). Because of the discarded peels after process-

ing and the consumption of garlic, massive garlic peel become one of the promising by-product. In recent years, evaluation of food by-products as a natural antioxidant and antimicrobial agent is gaining importance due to their inexpensiveness and simple extraction processes. Ifesan et al. (12) reported that ethanolic extract of garlic peel extract (GPE) demonstrated both antioxidant and antibacterial activities in cooked beef. There is very limited study on the evaluation of GPE. Additionally, there is no information on the incorporation of gelatine based edible film and GPE. Therefore, the objective of this investigation was to evaluate the effects of gelatin-based film incorporated with GPE on the chemical, sensorial and microbiological properties of rainbow trout (*Oncorhynchus mykiss*) fillets during storage at 4 °C.

Material and Methods

Extraction of garlic peels

Garlic peels (GPs) were collected from the local markets in Nigde, Turkey. After washed twice in tap water, the peels were dried at 45 °C for 48 h and ground into powder with a blender. For the extraction procedure, the method of Ifesan et al. (12) was used. GPs powder and etanol solvent (80 %) were stirred (1:10, g:mL) in a flask for 24 h. After extraction procedure, the garlic peel extracts (GPE) were filtered and concentrated by using rotary evaporator (IKA, HB-10 digital, Germany) at 45 °C under vacuum.

Preperation of gelatin films

Gelatin films were prepared according to method of Gomez-Estaca et al. (13) with slight modifications. Gelatin (food grade, Zag kimya, Turkey) dissolved in distilled water (8 g/100 mL) at room temperature. Then glycerol (0.1 mL per g of gelatin) and D-sorbitol (0.15 g per g of gelatin) were added to the solution and kept at 45 °C for 15 min. Garlic peel extract (GPE) was added to the film solution in concentration of 4 % and 8 % (by volume per mass of gelatin). 40 mL of the film solutions were poured into square polystyrene foam dishes in order to obtain films. All the film solutions were put into cabin for drying at room temperature for 48 h at 50 % relative humidity.

Preperation of samples

Rainbow trout (*Oncorhynchus mykiss*) fillets were provided from a fish farm in Niğde, Turkey and transported to the laboratory in ice boxes. They were washed after gutted, beheaded and filletted. The average weight and length of fish were 199.92±13.96 g and 17.44±1.29 cm, respectively. Two fillets were obtained from each fish. The fillets were divided into four groups. The fish fillets were wrapped according to Ahmad et al. (14) method with slight modifications. Dried gelatin films were peeled from the foam dishes and both sides of films were sterilized under UV for 10 min. First fish group was coated with gelatin film 0 % GPE (G0), second group was coated with gelatin film 4 % GPE (G4), third group was coated with gelatin film 8 % GPE (G8) and the last group left as control without wrapping. Each fillet was coated on both sides. Then, each sample wrapped with strech film and stored at 4±1 °C for 10 days.

Physicochemical analysis

For the determination of pH value, the probe of the pH-meter (Thermo Scientific Orion 2-star, Germany) was dipped into the fish homogenatas prepared with distilled water (1:1, w:v) (15).

Total volatile basic nitrogen (TVB-N) was determined according to Schormüller (16). 10 g homogenized fish sample was washed into the distillation flask, and 1 mg magnesium oxide was added. Samples were boiled and distilled into 10 mL of 0.1 mol equi/L HCl solution in a 500 mL conical flask with addition of tashiro-indicator. After distillation, the the flask were titrated with 0.1 mol equi/L NaOH. TVB-N results were expressed as mg nitrogen/100 g sample.

Peroxide value (PV) was determined according to method of AOAC (17). Approximately 2 g sample was stirred with 30 mL of solution including 3 chloroform:2 glacial acetic acid (v/v). After then 1 mL of saturated potassium iodide (KI) solution was added. The mixture was stored in a dark place for 5 min. Later on, 75 mL of distilled water was added and the mixture was titrated with sodium thiosulfate ($Na_2S_2O_3$) (0.1M) with the addition of starch solution as an indicator. The results were calculated as meq O_2/kg.

Thiobarbituric acid reactive substances (TBARS) analysis was conducted using the method of Tarladgis

et al. (18). 10 g of fish sample was steam distilled with 2.5 mL HCl: distilled water (1:2). 5 mL of the distillate was mixed with 5 mL thiobarbituric acid (TBA) solution (0.288 g/100 mL). Then the mixture put in the water bath at 110 ˚C for 35 min for the color reactions. The absorbance was measured with a UV–VIS spectrophotometer at 538 nm against a blank containing distilled water and TBA solution. The results were expressed as mg malonaldehyde/kg fish flesh.

Microbiological analyses

Fish sample (10 g) was mixed with 90 mL prechilled sterile ringer solution. Further decimal serial dilutions were used from this homogenate. For the determination of total psychrophilic bacteria and total viable counts Plate Count Agar (PCA) was used. Then the plates were incubated at 8 °C for 7 days and 37 °C for 24-48 h, respectively. For the Enterobacteriaceae determination, pour plating method was used in Violet Red Bile Agar (VRBA) and the plates incubated at 37 °C for 36-48 h.

Sensory analysis

For the sensory evaluation the method of Amerina et al. (19) was used with slight modification. Eight experienced panellists were evaluated the sensory characteristics of fish samples in terms of odour, texture, color and general acceptance by use of a nine-point hedonic scale. A score of 9-7 indicated "very good", a score of 6.9-4.0 "good", a score of 3.9-1.0 denoted as spoiled.

Statistical analysis

All measurements were performed in triplicate and analysis was carried out using the SAS software (Statistical Analysis System, Cary, NC, USA). Variance analysis (ANOVA) was used to evaluate the data and 5% significance level of Duncan's test was based to compare the differences between means of parameters.

Results and Discussion

pH value

pH value of the rainbow trout fillets wrapped with gelatine films incorporated with GPE and without GPE during storage at 4 °C for 10 days is shown in Table 1. The initial pH of the rainbow trout fillets was determined as 6.35. pH of the control samples was significantly ($P<0.05$) higher than those of the samples wrapped with gelatin film and increased to 7.18 at the end of the storage. Baygar et al. (20) reported that the pH value is between 6.0-6.5 in fresh fish. Additionally, according to Ludorf and Mayer (21) the acceptable limit of pH value for fresh fish is between 6.8 and 7.0. Generally, pH value of the all samples showed increase at the end of the storage and reached 6.76, 6.88 and 6.67 in the group G0, G4 and G8, respectively. It was reported that the accumulation of alkaline compounds such as ammonia and TMA, etc. results in the increase in pH value (22). Alparslan et al. (23) reported an increase in pH of rainbow trout fillets wrapped with gelatin film enriched with laurel essential oil and reached 6.61 at the end of the storage (26th day). They observed lower pH values in the fillets wrapped with gelatin film containing laurel esseantial oil. Fadiloglu and Coban (24) determined the initial pH of rainbow trout fillets treated with chitosan coating as 7.07 and finally increased to 7.77 after 12 days. In the present study, the lower increase in the pH value of rainbow trout fillets wrapped with gelatin film incorporated with 8% GPE was observed compared with unwrapped groups ($P<0.05$).

Total volatile basic nitrogen (TVB-N)

TVB-N is widely used as an indicator of the quality of fresh fish. TVB-N content of rainbow trout fillets coated with and without gelatin film (gelatin film or GPE film) during storage is shown in Table 1. At the beginning of the storage, TVB-N value of fresh fish was 18.2 mg N/100 g and showed increase in all groups during the storage period. It was observed that the control group demonstrated significantly ($P<0.05$) higher values than those of the samples coated with gelatin film incorporated with GPE. At the end of the storage, TVB-N values reached 42.70, 39.90, 34.30 and 30.10 mg N/100 g in control, G0, G4 and G8 groups, respectively. It was suggested that TVB-N content in freshly caught fish is typically between 5 and 20 mg N/100 g, and TVB content of 30-35 mg N/100 g is usually regarded as the limit of acceptability for fish (25). Control and G0 groups exceeded the limit value at the 5th and 7th day of the storage, where-

as fillets wrapped with gelatin film containing GPE reached unacceptable limit at the end of the storage. During the storage period, significantly (P<0.05) lower TVB-N values were found in G4 and G8 groups compared to the control and G0 samples. The increase of TVB-N is related to the the activity of spoilage bacteria and endogenous enzymes (26). This shows that the TVB-N results are related to microbiological findings. Similar studies reported that, fish fillets coated with edible films containing different concentrations of extracts and essential oils showed lower TVB-N values compared to uncoated samples during storage period (14, 23, 24).

Peroxide value (PV)

Lipid oxidation is the main cause of fish spoilage after microbial growth. Yuan et al. (27) reported that the application of edible films and coatings with incorpation of antioxidants represents a new approach to solve oxidation problem in food products. Peroxide value (PV) is a measurement of peroxides and hydroperoxides which are the primary oxidation products (28). The effect of gelatin film on the changes of PV of rainbow trout fillets is represented in Table 1. At the beginning of the storage PV in the fillets was determined as 2.0 meq O_2/kg and increased with the storage time in all samples. At the end of the storage, PV of the rainbow trout fillets reached 8.99, 8.74, 6.99 and 5.49 meq O_2/kg in control, G0, G4 and G8 groups, respectively. Control group exhibited higher value of PV than those of the samples coated with gelatin film. Significantly (P<0.05) lower PV was observed in the rainbow trout fillets wrapped with gelatin film incorporated with GPE. Varlık et al. (29) reported that a PV of less than 2 meq O_2/kg fish as "very good," up to 5 meq O_2/kg as "good," and 8-10 meq O_2/kg as at acceptability limit. According to literature, control group and the samples wrapped with gelatin film without the addition of GPE exceeded this limit value at day 5, while the samples wrapped with gelatin film containing 4% and 8% exceeded limit value at day 7 and 10, respectively. Similar results were observed in rainbow trout fillets coated with chitosan and gelatin film (23,24). In this study, incorporation of 8% GPE and gelatin film was much more effective in retarding the lipid oxidation of rainbow trout fillets during refrigerated storage.

Thiobarbituric acid reactive substance (TBARS)

Thiobarbituric acid reactive substances (TBARS) is an index of lipid oxidation which is widely used for the evaluation of secondary lipid degree (30). The initial TBARS value of rainbow trout fillet was 0.45 mgMDA/kg and increased in all samples during the storage priod (Table 1). TBARS values of the control and the samples coated with gelatin film without GPE were higher than those of the samples wrapped with gelatin film incorporated with GPE. At the end of the storage TBARS values of the rainbow trout fillets were found as 1.38, 1.42, 1.25 and 1.11 mgMDA/kg in control, G0, G4 and G8 samples, respectively. During the storage, fish fillets wrapped with gelatin film containing 8% GPE showed significantly (P<0.05) lower TBARS value. The results of TBARS revelaed that lipid oxidation in rainbow trout fillets could be retarded with the use of gelatin film enriched with GPE. Similar results were observed by Ahmad et al. (14) who described the delay of lipid oxidation in sea bass slices coated with gelatin films combined with lemograss essential oil (LEO) as a result of the antioxidant property of LEO. In the present study, it can be suggested that gelatin film incorporated with GPE could show low oxygen permeability due to antoxidant characteristics of GPE. Alparslan et al. (23) observed the the initial TBARS value of rainbow trout fillets as 0.03 mgMDA/kg and they reported lower TBARS value in the samples coated with gelatin films containing laurel essential oil. Martinez et al. (31) reported 0.62 mg MDA/kg of TBARS value for seabass at the beginning. Alsaggaf et al. (28) found lower TBARS value of Nile tilapia coated with chitosan film incorporated with pomegranate peel extract and TBARS value of the coated fish ranged between 0.21 and 0.32 mgMDA/kg during 30 days storage. It was reported that edible films and coatings serve as barriers against water vapor, gases, and flavor compounds and improving structural integrity and mechanical-handling properties of foods (32). Jeon et al. (30) observed that chitosan-coating reduced moisture loss and lipid oxidation of Atlantic cod and herring. Addition of GPE into the gelatin film enhanced the antioxidant property of gelatin film and showed lower TBARS value in the rainbow trout fillets than that of the uncoated samples.

Table 1. Changes in physicochemical properties of rainbow trout fillets wrapped with gelatin films containing GPE during storage at 4°C

	Storage period (days)	C	G0	G4	G8
pH	0	6.35±0.18^{Ad}	6.35±0.18^{Ac}	6.35±0.18^{Ad}	6.35±0.18^{Ab}
	2	6.75±0.00^{Bc}	6.88±0.00^{Aa}	6.54±0.01^{Cc}	6.49±0.08^{Cb}
	5	6.92±0.00^{Bb}	6.75±0.00^{Cb}	6.94±0.00^{Aa}	6.74±0.01^{Da}
	7	7.07±0.00^{Aa}	6.65±0.01^{Db}	6.74±0.01^{Bb}	6.69±0.00^{Ca}
	10	7.18±0.01^{Aa}	6.76±0.01^{Dab}	6.88±0.02^{Bab}	6.67±0.04^{Ca}
TVB-N	0	18.20±3.96^{Ae}	18.20±3.96^{Ae}	18.20±3.96^{Ad}	18.20±3.96^{Ad}
	2	26.60±1.98^{Ad}	25.90±0.99^{Ad}	21.70±0.99^{Bcd}	19.60±1.98^{Bd}
	5	35.70±0.99^{Ac}	30.10±0.99^{Bc}	25.20±1.98^{Cc}	23.10±0.99^{Dc}
	7	39.90±0.99^{Ab}	37.10±0.99^{ABb}	28.00±1.98^{Cb}	25.90±0.99^{Db}
	10	42.70±0.99^{Aa}	39.90±0.99^{Ba}	34.30±0.99^{Ca}	30.10±0.99^{Da}
Peroxide value	0	2.00±0.00^{Ac}	2.00±0.00^{Ac}	2.00±0.00^{Ac}	2.00±0.00^{Ac}
	2	3.00±0.00^{Ac}	2.97±0.00^{Bc}	2.00±0.00^{Cc}	1.98±0.00^{Cc}
	5	5.49±0.71^{ABb}	6.49±0.71^{Ab}	4.50±0.71^{ABb}	3.49±0.70^{Bb}
	7	9.49±0.69^{Aa}	9.00±1.41^{Aa}	5.50±0.71^{Bb}	3.00±0.00^{Cbc}
	10	8.99±0.02^{Aa}	8.74±0.70^{Aa}	6.99±0.01^{Ba}	5.49±0.71^{Ca}
TBARS	0	0.45±0.14^{Ac}	0.45±0.14^{Ad}	0.45±0.14^{Ad}	0.45±0.14^{Ac}
	2	0.63±0.02^{Ac}	0.60±0.01^{Ad}	0.62±0.03^{Acd}	0.47±0.01^{Bc}
	5	0.97±0.13^{Ab}	0.81±0.05^{ABc}	0.71±0.02^{Bc}	0.65±0.02^{Bc}
	7	1.09±0.02^{Ab}	1.05±0.07^{Ab}	0.99±0.03^{ABb}	0.90±0.03^{Bb}
	10	1.38±0.02^{Aa}	1.42±0.02^{Aa}	1.25±0.04^{ABa}	1.11±0.11^{Ba}

Means indicated by different capital letters in the same row differ significantly (P < 0.05). Means indicated by different lowercase letters in the same column differ significantly (P < 0.05). C: control samples, G0 film: samples wrapped with gelatin film, G4 film: samples wrapped with gelatin film incorporated with 4% GPE, G8 film: samples wrapped with gelatin film incorporated with 8% GPE.

Microbiological analyses

Total viable count (TVC) of rainbow trout fillets wrapped without and with films (gelatin film or GPE film) during storage at 4°C is shown in Fig. 1. At the beginning, the number of bacteria in trout samples was found as 2.27 log CFU/g which is lower than the initial number of rainbow trout fillets reported by the other researchers (7,33-35). In the control and gelatin film wrapped without GPE samples TVC increased significantly (P<0.05) and finally reached 7.47 and 7.30 log CFU/g, respectively. It was observed that TVC showed significant (P<0.05) increase in all groups entire the storage. However, this value did not exceed 7.0 log CFU/g which is upper limit value (36) in the samples coated with gelatin film contatining GPE. Significantly (P<0.05) lower TVC was found in gelatin coated (containing GPE) rainbow trout fillets during the storage period. Bakri and Dougles (37) reported that antibacterial effect of garlic resulted from interaction of sulphur compounds, allicin, with sulphur groups of microbial enzymes, leading to an inhibition of microbial growth. Ifesan et al. (12) observed that garlic peel extract showed bacteriostatic activity in the beef samples. Additionally, they concluded that the bioactive compounds present in the garlic bulb are likely to be available in the peel. In the present study, incorporation of garlic peel extract in gelatin film resulted in microbial spoilage delay.

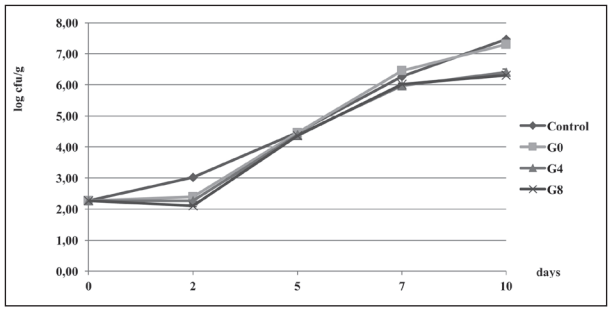

Figure 1. Changes in total viable counts of rainbow trout fillets wrapped with gelatin films containing GPE during storage at 4 °C. G0 film: samples wrapped with gelatin film, G4 film: samples wrapped with gelatin film incorporated with 4% GPE, G8 film: samples wrapped with gelatin film incorporated with 8% GPE.

It was reported that gram-negative psychrotrophic bacteria group are responsible from aerobic spoilage in chilled stored fresh fish (38). The changes in psychrophilic bacteria count (PBC) of rainbow trout fillets are presented in Fig. 2. A continuous increase of PBC was observed in all groups during the storage period. The PBC of control samples and gelatin wrapped without GPE samples were higher ($P<0.05$) than those of the samples coated with gelatin film containing GPE. Inititally, PBC was 2.59 log CFU/g in the rainbow trout fillets and reached 7.47, 7.38, 6.90 and 6.41 log CFU/g in control, G0, G4 and G8 groups, respectively. During the storage period, rainbow trout fillets coated with GPE gelatin film showed lowest PBC, in comparision with the control and gelatin film coated (without GPE) samples ($P<0.05$). Rezaei et al. (39) monitored the initial number of PBC of rainbow trout fillets as 2.3 log CFU/g and increased to 6.1 log CFU/g after 18 day of the ice storage. Ucak et al. (40) reported the initial PBC of rainbow trout fillets treated with onion peel extract (OPE) as 2.47 log CFU/g and PBC of the OPE treated fillets remanined lower than the control during the storage. Similarly, initial PBC was found as 3.1 log CFU/g in rainbow trout fillets wrapped with chitosan films incorporated with oregano or thyme essential oil (41).

Enterobacteriaceae is considered a hygiene indicator and one of the spoilage microorganisms of fresh rainbow trout (42). Papadopoulos et al. (44) reported that the contribution of Enterobacteriaceae to the microflora of fish should be considered during the assessment of fish spoilage. The initial number of Enterobacteriaceae was 1.53 log CFU/g which is lower than the initial number of rainbow trout fillets (2.27 log CFU/g) reported by Ozogul et al. (35), but this value reached 7.37 log CFU/g in the control group at the end of the storage (Fig. 3). The coating of fish fillets, either with gelatin-based film alone or with combinations of GPE, led to inhibition of microbial growth during the storage. However, the lowest Enterobacteriaceae was observed in gelatin coated samples incorporated with GPE ($P<0.05$). This indicated that gelatin film showed slight effect on the inhibition of Enterobacteriaceae without the addition of GPE. At the end of the storage, Enterobacteriaceae increased to 7.01, 6.93 and 6.48 log CFU/g in G0, G4 and G8 samples, respectively. Oz (43) concluded that the addition of garlic into rainbow trout diet reduced the number of Enterobacteriaceae in fish meat and kept it at a lower level during storage.

Sensory evaluation

The sensory results of the rainbow trout fillets coated without and with films are given in Table 2. The fillets coated with gelatin film incorporated with GPE received higher scores than those of the control and gelatin film wrapped without GPE samples ($P<0.05$). According to sensoy evaluation of color, odour, texture and overall acceptance of control samples was considered as unacceptable by the 5th day of the storage. However, the fillets wrapped with gelatin film containing GPE was reached unacceptable scores by the 10th

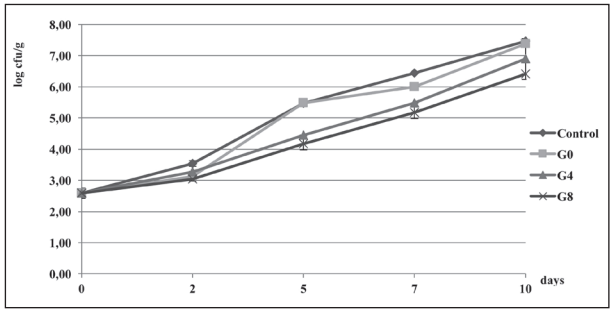

Figure 2. Changes in total psychrophilic bacteria count of rainbow trout fillets wrapped with gelatin films containing GPE during storage at 4 °C. G0 film: samples wrapped with gelatin film, G4 film: samples wrapped with gelatin film incorporated with 4% GPE, G8 film: samples wrapped with gelatin film incorporated with 8% GPE.

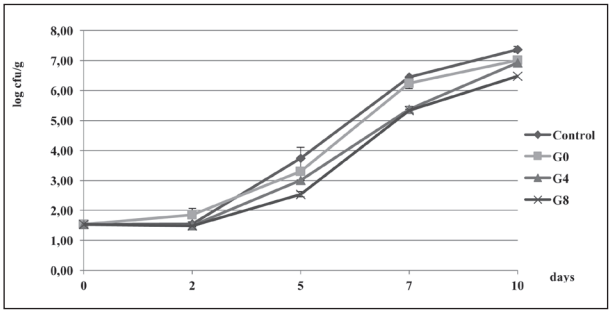

Figure 3. Changes in total Enterobacteriaceae count of rainbow trout fillets wrapped with gelatin films containing GPE during storage at 4 °C. G0 film: samples wrapped with gelatin film, G4 film: samples wrapped with gelatin film incorporated with 4% GPE, G8 film: samples wrapped with gelatin film incorporated with 8% GPE.

Table 2. Changes in sensory scores of rainbow trout fillets wrapped with gelatin films containing GPE during storage at 4 °C

	Storage period (days)	C	G0	G4	G8
Color	0	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}
	2	7.25±0.50^{Bb}	7.25±0.50^{Bb}	8.75±0.50^{Aa}	8.50±0.58^{Aa}
	5	3.75±0.96^{Cc}	6.00±0.00^{Bc}	7.50±0.58^{Ab}	7.50±0.58^{Ab}
	7	1.75±0.50^{Cd}	2.00±0.82^{Cd}	4.00±1.15^{Bc}	6.00±0.00^{Ac}
	10	1.25±0.50^{Ad}	1.50±0.58^{Ad}	2.00±0.00^{Ad}	2.00±0.82^{Ad}
Odour	0	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}
	2	6.75±0.50^{Bb}	6.50±0.58^{Bb}	9.00±0.00^{Aa}	8.75±0.50^{Aa}
	5	3.25±1.50^{Cc}	5.00±0.00^{Bc}	7.75±0.50^{Ab}	7.75±0.50^{Ab}
	7	1.00±0.00^{Cd}	2.25±0.96^{Bd}	4.00±1.15^{Ac}	4.50±0.58^{Ac}
	10	1.25±0.50^{Bd}	2.00±0.00^{Ad}	2.25±0.50^{Ad}	2.25±0.50^{Ad}
Texture	0	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}
	2	7.25±0.50^{Bb}	7.50±0.58^{Bb}	8.75±0.50^{Aa}	9.00±0.00^{Aa}
	5	3.75±2.06^{Bc}	6.00±0.00^{Ac}	7.75±0.50^{Aa}	7.75±0.50^{Ab}
	7	2.00±0.82^{Bd}	2.75±0.50^{Bd}	3.50±1.73^{ABb}	4.50±0.58^{Ac}
	10	1.25±0.50^{Bd}	1.50±0.58^{Bc}	3.00±0.00^{Ab}	3.00±0.00^{Ad}
Overall acceptance	0	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}
	2	6.50±0.58^{Bb}	7.00±1.15^{Bb}	8.75±0.50^{Aa}	8.50±0.58^{Aa}
	5	3.50±1.73^{Cc}	5.00±0.00^{Bc}	7.25±0.00^{Ab}	7.50±0.58^{Ab}
	7	1.75±0.96^{Bd}	2.25±0.96^{Bd}	4.50±0.58^{Ac}	5.50±0.58^{Ac}
	10	1.25±0.50^{Bd}	1.50±0.58^{Bd}	3.00±0.82^{Ad}	3.25±0.50^{Ad}

Means indicated by different capital letters in the same row differ significantly (P < 0.05). Means indicated by different lowercase letters in the same column differ significantly (P < 0.05). C: control samples, G0 film: samples wrapped with gelatin film, G4 film: samples wrapped with gelatin film incorporated with 4% GPE, G8 film: samples wrapped with gelatin film incorporated with 8% GPE.

day of the storage. This indicated that incorporation of gelatin film with GPE showed antioxidant and antimicrobial effect and delayed spoilage of rainbow trout fillets. Gelatin film without GPE retarded the spoilage in the fillets as well, but showed lesser extention in the shelf-life. The sensory a scores was correlated with microbiological and chemical results.

Similar results were observed by Jasour et al. (45) who reported 4 days shelf-life extension in the chitosan coated rainbow trout fillets compared with control samples. Jouki et al. (41) reported the shelf-life of rainbow trout fillets wrapped with edible film containing oregano or thyme essential oil as 18 days, while the shelf-life of control samples was 10 days. In many studies, it was found that the application of edible films enriched with essential oil or plant extract extended the shelf life of fish (7,23,24,28,46). The sensory results of this study showed that gelatin film containing GPE extended the shelf-life of rainbow trout fillets and the shelf-life was found as 5, 7 and 10 days for the control, G0 and GPE samples, respectively.

Conclusions

Based on the results of this study, GPE could inhibit bacterial growth and maintain sensory and chemical quality of rainbow trout fillets during refrigerated storage. Gelatin film without GPE has 2 days shelf-life extention effect on the rainbow trout fillets, while application of gelatin film enriched with GPE extended the shelf-life of fillets 5 days. Results showed that, addition of 4% concentration of GPE into gelatin film was much more effective, since the lowest microbiological and chemical scores were obtained from this group. Thus, GPE can be an effective antioxidant and antimicrobial agent in the gelatin based edible films and it can be used for the extention of shelf-life of fish and fish products.

References

1. Hassoun A, Coban OE (2017). Essential oils for antimicrobial and antioxidant applications in fish and other seafood

products. Trends Food Sci Technol, 68: 26-36.
2. Sampels S (2015). The effects of processing technologies and preparation on the final quality of fish products. Trends Food Sci Technol, 44: 131-146.
3. Maftoonazad N, Badii F (2009). Use of edible films and coatings to extend the shelf life of food products. Recent Pat Food Nutr Agric, 1: 162-170.
4. Chaparro-Hernandez S, Ruiz-Cruz S, Marquez-Rios E, Ocano- Higuera VM, Valenzuela-Lopez CC, Ornelas-Paz JJ, Del-Toro-Sanchez CL (2015). Effect of chitosan-carvacrol edible coatings on the quality and shelf life of tilapia (*Oreochromis niloticus*) fillets stored in ice. Food Sci Technol, 35(4): 734-741.
5. Vasconez MB, Flores SK, Campos CA, Alvarado J, Gerschenson LN (2009). Antimicrobial activity and physical properties of chitosan-tapioca starch based edible films and coatings. Food Res Int, 42: 762-769.
6. Gennadios A, Hanna MA, Kurth LB (1997). Application of edible coatings on meats, poultry and seafoods: a review. LWT-Food Sci Technol, 30(4): 337-350.
7. Ojagh SM, Rezaei M, Razavi SH, Hosseini SMH (2010). Effect of chitosan coatings enriched with cinnamon oil on the quality of refrigerated rainbow trout. Food Chem, 20: 193-198.
8. Ide N, Lau BH (2001). Garlic compounds minimize intracellular oxidative stress andinhibit nuclear factor-kappa b activation. J Nutr, 131: 1020-1026.
9. Iciek M, Kwiecien I, Wlodek L (2009). Biological properties of garlic andgarlic-derived organosulfur compounds. Environ Mol Mutagen, 50: 247-265.
10. Lanzotti V, Scala F, Bonanomi G (2014). Compounds from Allium species withcytotoxic and antimicrobial activity. Phytochem Rev, 13: 769-791.
11. Feng Y, Wu B, Yu X, Yagou AEA, Sarpong F, Zhou Z (2018). Effect of catalytic infrared dry-blanching on the processing and quality characteristics of garlic slices. Food Chem, 266: 309-316.
12. Ifesan BOT, Fadipe EA, Ifesan BT (2014). Investigation of Antioxidant and Antimicrobial properties of garlic peel extract (*Allium sativum*) and its use as natural food additive in cooked beef. J Sci Res Rep, 3(5): 711-721.
13. Gomez-Estaca J, Montero P, Fernandez-Martin F, Aleman A, Gomez-Guillen MC (2009). Physical and chemical properties of tuna-skin and bovine-hide gelatin films with added aqueous oregano and rosemary extracts. Food Hydrocoll, 23: 1334-1341.
14. Ahmad M, Benjakul S, Sumpavapol P, Nirmal NP (2012). Quality changes of sea bass slices wrapped with gelatin film incorporated with lemongrass essential oil. Int J Food Microbiol, 155: 171-178.
15. Manthey M, Karnop G, Rehbein H (1988). Quality changes of European catfish (*Silurus glanis*) fromwarm water aquaculture during storage in ice. Int J Food Sci Technol, 23:1-9.
16. Schormuller J (1968). Handbuch der Lebensmittelchemie. Band III/2. Heidelberg New York: Teil Springer-Verlag Berlin.
17. AOAC (Association of Official Analytical Chemists) (1990). Official methods of analyses of Association of Analytical Chemist (15th ed.). Washington: AOAC.
18. Tarladgis B, Watts BM, Yonathan M (1960). Distillation method for determination of malonaldehit in rancid food. J Am Oil Chem Soc, 37: 44-48.
19. Amerina MA, Pangborn RV, Roessler EB (1965). Principles of sensory evaluation of food. New York: Academic Press, p. 602.
20. Baygar T, Alparslan Y, Okumus M, Güler M (2012). Detecting the specific parameters that affect the maturation of farmed sea bass (*Dicentrarchus labrax*) fillets stored in sunflower oil, J Food Sci Technol, 51: 1197-1202.
21. Ludorf W, Meyer V (1973). Fish and Fish Products, Verlag Paul Parey, Berlin, Germany, p. 308.
22. Lopez-Caballero ME, Martinez-Alvarez O, Gomez-Guillen MC, Montero P (2007). Quality of thawed deepwater pink shrimp (*Parapenaeus longirostris*) treated with melanosis-inhibiting formulations during chilled storage. Int J Food Sci Tech, 42: 1029-1038.
23. Alparslan Y, Baygar T, Baygar T, Hasanhocaoglu H, Metin C (2014). Effects of Gelatin-Based Edible Films Enriched with Laurel Essential Oil on the Quality of Rainbow Trout (*Oncorhynchus mykiss*) Fillets During Refrigerated Storage. Food Technol Biotechnol, 52(3): 325-333.
24. Fadiloglu EE, Coban OE (2018). Effects of chitosan edible coatings enriched with sumac on the quality and the shelf life of rainbow trout (*Oncorhynchus mykiss*, Walbaum, 1792) fillets. J Food Saf, 38: 1-8.
25. Huss HH (1988). Fresh Fish-Quality and Quality Changes. FAO Fisheries, Roma. Series.
26. Kyrana VR, Lougovois VP (2002). Sensory, chemical and microbiological assessment of farm-raised European sea bass (*Dicentrarchus labrax*) stored in melting ice. Int J Food Sci Tech, 37: 31-328.
27. Yuan G, Chen X, Li D (2016). Chitosan films and coatings containing essential oils: The antioxidant and antimicrobial activity, and application in food systems. Food Resh Int, 89: 117-128.
28. Alsaggaf MS, Moussa SH, Tayel AA (2017). Application of fungal chitosan incorporated with pomegranate peelextract as edible coating for microbiological, chemical and sensorial quality enhancement of Nile tilapia fillets. Int J Biol Macromol, 99: 499-505.
29. Varlık C, Ugur M, Gokoglu N, Gun H (1993). Su Ürünlerinde Kalite Kontrol ğke ve Yöntemleri. Gıda Teknolojisi Dergisi Yayınları. Yay. No: 17, ğstanbul. 174.
30. Jeon Y, Kamil JYVA, Shahidi F (2002). Chitosan as an edible film for quality preservation of herring and Atlantic cod. J Agric Food Chem, 50(18): 5167-5178.
31. Martinez O, Salmeron J, Epelde L, Vicente MS, de Vega C (2017). Quality enhancement of smoked sea bass (*Dicentrarchus labrax*) fillets by adding resveratrol and coating with chitosan and alginate edible films. Food Control, 85: 168-176.
32. Galus S, Kadzinska J (2015). Food applications of emul-

sion-based edible films and coatings. Trends Food Sci Technol, 45(2): 273-283.
33. Frangos L, Pyrgotou N, Giatrakou V, Ntzimani A, Savvaidis IN (2010). Combined effects of salting, oregano oil and vacuum-packaging on the shelf-life of refrigerated trout fillets. Food Microbiol, 27: 115-121.
34. Arashisar S, Hisar O, Kaya M, Yanik T (2004). Effects of modified atmosphere and vacuum packaging on microbiological and chemical properties of rainbow trout (*Oncorynchus mykiss*) fillets. Int J Food Microbiol, 97: 209-214.
35. Ozogul Y, Yuvka Y, Ucar Y, Durmus M, Kosker AR, Oz M, Ozogul F (2017). Evaluation of effects of nanoemulsion based on herb essential oils (rosemary, laurel, thyme and sage) on sensory, chemical and microbiological quality of rainbow trout (*Oncorhynchus mykiss*) fillets during ice storage. LWT-Food Sci Technol, 75: 677-684.
36. ICMSF (1986). Microorganisms in foods. The international commission on microbiological specifications for foods of the international union of biological societies (pp. 181-196). Oxford: Blackwell Scientific Publications.
37. Bakri IM, Douglas CWI (2005). Inhibitory effect of garlic extract on oral bacteria. Arch Oral Biol, 50: 645-51.
38. Ibrahim Sallam K (2007). Antimicrobial and antioxidant effects of sodium acetate, sodium lactate, and sodium citrate in refrigerated sliced salmon. Food Control, 18(5): 566-575.
39. Rezaei M, Montazeri N, Langrudi HE, Mokhayer B, Mohammad Parviz M, Nazarinia A (2007). The biogenic amines and bacterial changes of farmed rainbow trout (*Oncorhynchus mykiss*) stored in ice. Food Chem, 103: 150-154.
40. Ucak I, Khalily R, Abuibaid AKM, Ogunkalu OA (2018). Maintaining the quality of rainbow trout (*Oncorhynchus mykiss*) fillets by treatment of red onion peel extract during refrigerated storage. Prog Nutr, 20(4): 672-678.
41. Jouki M, Yazdi FT, Mortazavi SA, Koocheki A, Khazaei N (2014). Effect of quince seed mucilage edible films incorporated with oregano or thyme essential oil on shelf life extension of refrigerated rainbow trout fillets. Int J Food Microbiol, 174: 88-97.
42. Mexis SF, Chouliara E, Kontominas MG (2009). Combined effect of an O_2 absorber and oregano essential oil on shelf- -life extension of Greek cod roe paste (tarama salad) stored at 4 °C. Innov Food Sci Emerg, 10: 572-579.
43. Oz M (2018). Effects of garlic (*Allium sativum*) supplemented fish diet on sensory, chemical and microbiological properties of rainbow trout during storage at -18° C. LWT-Food Sci Technol, 92: 155-160.
44. Papadopoulos V, Chouliara I, Badeka A, Savvaidis IN, Kontominas MG (2003). Effect of gutting on microbiological, chemical and sensory properties of aquacultured sea bass (*Dicentrarchus labrax*) stored in ice, Food Microbiol, 20: 411-420.
45. Jasour MS, Ehsani A, Mehryar L, Naghibi SS (2015). Chitosan coating incorporated with the lactoperoxidase system: an active edible coating for fish preservation. J Sci Food Agric, 95: 1373-1378.
46. Mohan CO, Ravishankar CN, Lalitha KV, Gopal TKS (2012). Effect of chitosan edible coating on the quality of double filleted Indian oil sardine (*Sardinella longiceps*) during chilled storage. Food Hydrocoll, 26: 167-174.

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Potential negative effects of caffeine in athletes

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Summary. Letter related to "Rahimi MR, Khabiri P, Faraji H. Effects of caffeine ingestion on resistance exercise-induced apoptosis in athletes: A randomized, double-blind, placebo-controlled, crossover study. PN 2Jul.2018 [cited 20Dec.2018];20(4):563-9."

Key word: caffeine, energy drinks, athletes

Dear Editor,

We have read with great interest the paper "Effects of caffeine ingestion on resistance exercise- induced apoptosis in athletes: A randomized, double-blind, placebo-controlled, crossover study" by Rahimi MR and coworkers (1) and we found his manuscript of importance with a view to clinical prevention. This study examined the effects of oral caffeine ingestion on biomarkers of apoptosis including Bax and Bcl-2 during strenuous Resistance Exercise in resistance trained men. Their results suggest that acute caffeine intake attenuated exercise induced apoptosis in resistance trained men, which was confirmed by attenuated percentage change of Bax/Bcl-2 ratio in the caffeine condition.

With reference to the findings reported in the paper, we would like to make the following contribution to the discussion.

Athletes are habitual consumers of highly caffeinated beverages to combat fatigue and improve performance, due to the well known effect of caffeine on muscle and on performance. Other than the positive effects reported in the paper by Rahimi and coworkers we have to consider the potential negative action of caffeine on arrhythmogenesis. Commercial energy drinks contain different quantity of other energetic substances such as guarana (containing guaranine, similar to caffeine), taurine, and gingko biloba (2, 3). Energy drinks have potential arrhythmogenic effect. This pro-arrhythmic effect could be dangerous in athletes due to the very high adrenergic activity developed during intense exercise (4). In previous studies we reported the development of atrial fibrillation in young subjects after acute ingestion of energy drinks mixed with alcohol during recreational activities (5, 6). Accordingly, several reports referred about episodes of arrhythmias induced by energy drinks consumption, sometimes associated with binge ingestion of alcohol (7). We suppose that the combined effect of highly dosage of caffeine and other stimulating substances included in energy drinks (i.e. taurine) can trigger the onset of arrhythmias. The reaction of individuals to caffeine consumption is variable. Caffeine, in fact, stimulates both the central and the peripheral nervous systems affecting the cardiocirculatory and breathing systems. Because of this stimulant effect, there is concern that caffeine might increase arrhythmic risk (2, 7). However, moderate doses of caffeine are well tolerated in patients with arrhythmia as well as in athletes. Caffeine may mediate AF by resulting in neurohormonal stimulation and sympathetic activation and the effects could be enhanced in nonhabitual caffeine consumers (7). It is plausible that only some subjects are

susceptible to developed arrhythmias during binge ingestion of EDs and alcohol and that alcohol support the development of side effects of EDs through unknown mechanisms (6, 7).

Further studies are needed in order to evaluate the effects of high caffeine consumption in athletes with a specific focus on arrhythmogenesis.

We agree with Dunican and coworkers that there is a need for a strategic approach to the use of caffeine in athletes.

References

1. Rahimi MR, Khabiri P, Faraji H. Effects of caffeine ingestion on resistance exercise- induced apoptosis in athletes: A randomized, double-blind, placebo-controlled, crossover study. Progr Nutr. 2Jul.2018 (cited 20Dec.2018);20(4):563-9
2. Mattioli AV. Effects of caffeine and coffee consumption on cardiovascular disease and risk factors. Future Cardiol. 2007 Mar;3(2):203-12. doi: 10.2217/14796678.3.2.203
3. Southward K, Rutherfurd-Markwick KJ, Ali A. The Effect of Acute Caffeine Ingestion on Endurance Performance: A Systematic Review and Meta-Analysis. Sports Med. 2018 Aug;48(8): 1913-1928. doi: 10.1007/s40279-018-0939-8.
4. Mattioli AV, Pennella S, Manenti, Ballerini Puviani M et al. Influence of energy drinks on obesity: A preliminary experimental study. Progress Nutr 2017; 19, Issue 4, 369-372
5. Mattioli AV, Pennella S, Farinetti A, Manenti A. Energy Drinks and atrial fibrillation in young adults. Clin Nutr. 2018 Jun;37(3):1073-1074. doi: 10.1016/j.clnu.2017.05.002. Epub 2017 May 6.
6. Mattioli AV, Pennella S, Manenti A, Farinetti A. Energy drink overconsumption can trigger atrial fibrillation. Journal of Cardiovascular Medicine 2016; 17: 902-03
7. Forward J, Akhurst J, Bruno R, Leong X, VanderNiet A, Bromfield H, Ern J, Bellamy T, Peacock A. Nature versus intensity of intoxication: Co-ingestion of alcohol and energy drinks and the effect on objective and subjective intoxication. Drug Alcohol Depend. 2017; 180, 292–303.

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Letter to Editor

Oral hyaluronic acid in patients with knee osteoarthritis

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Summary. The international guidelines agree that management of knee osteoarthritis (OA) requires both non-pharmacological, and pharmacological approaches and suggest initiating a background therapy with chronic symptomatic slow-acting drugs for osteoarthritis (SYSADOAs), such as hyaluronic acid (HA). Oral HA treatment is now widely used because of its safety, good results in daily clinical practice, and relative low cost for knee pain. On the other hand, oral HA has been the source of much research in the last years. Several trials have evidenced the good efficacy of oral HA in reducing pain and improving joint functionality in mild to moderate knee osteoarthritis, but critical issues concerning the parameters used in these studies to measure the end points still persist. In few cases objective parameters (i.e. ultrasound) have been considered and no study correlated them with specific scales like visual analogue score (VAS) and Knee injury and Osteoarthritis Outcome Score (KOOS) to improve patient assessment. This could be the goal of future researches.

Key words: knee osteoarthritis, hyaluronic acid, outcome assessment

Dear Editor,

We read with great interest the manuscript published by Guadagna S et al (1) titled "Oral hyaluronan for the treatment of knee osteoarthritis: a systematic review" in the Vol 20 No 4 (2018) of this journal. The authors have done a well conducted analysis to describe the current state of the art of the treatment of mild to moderate knee osteoarthritis (OA) with oral hyaluronic acid (HA) products. The authors have also mentioned the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO) recommendation to initiate a background therapy with chronic symptomatic slow-acting drugs for osteoarthritis (SYSADOAs), such as HA. The authors in their results evidenced the heterogenicity of HA preparations and that most trials used subjective measures (instead of objective parameters) to determine the efficacy of the HA treatment.

We argue that there are additional critical issues concerning the parameters used in the analysed clinical trials to measure the end points:

i) In the 7 trials performed in Japan, there were used only visual analogue scale (VAS) and/or scales for osteoarthritis symptoms tested on the Japanese population only, like Japanese Knee Osteoarthritis Measure (JKOM) and scoring of Japanese Orthopaedic Association (JOA) (2-4), and on no one a measurement using an objective tool, i.e. ultrasound (US) on isokinetic dynamometer; in the 10 trials conducted by the European and American investigators these instruments were used in only 4 of 10 studies.
ii) US (5-7) and isokinetic dynamometer (6-8) have given ambiguous results in several studies. US measurements evidenced a statistically significant difference with control group only in two trials (5,6) and it can be difficult to consider positive the results obtained in one of the three trials where the isokinetic test was used (statistically difference with control group only at isokinetic peak torque at 240° of the extensors) (2).
iii) The methods to apply and evaluate the US examination were not reported in two of the trials (5,7), while in the third study, the only US data collected was the synovial effusion in the suprapatellar recess measured in mm on the longitudinal axis (6), following the 2005 EULAR guidelines (9). Therefore, nei-

ther the recently proposed US score for large joints (10), nor the correlations between pain, radiological, and US findings (11), nor the several US features graded from 0 to 3 reported by Wu (12), were considered.

iv) The rationale to use the isokinetic test (Biodex Medical Systems, New York, USA) as primary efficacy assessment in 3 trials was that a decrease in knee OA pain could evidence an increased work, power and peak torque of leg muscles. Surely, this surrogate end point could be very useful to test HA on joint pain in trials with a different population, showing a clinical evidence i.e. in athletes with mean age 20 ±1.0 (13) or in soccer players with mean age 19.5 ±1.2 (14). Contrarily, the mean age of patients in the studies where isokinetic assessment was tested was 56.1±8.00 years (6), 42.38 ± 10.16 years (7), 59.6 ± 8.3 years (8); a difference, even statistically significant, in muscular strength obtained in a similar old population could not be considered important from a clinical point of view, because this parameter certainly did not modify patients' quality of life.

v) We agree with the authors when they say that the range of motion (ROM) of the knee joint measured with a goniometer is a simple tool and that it is commonly used in daily practices by orthopaedists. We also want to point out with the authors that ROM, unfortunately, has never been used as an end point in trials with oral HA, even if it could be easily correlated with specific scales like VAS and KOOS to improve patient assessment.

vi) No study has objectively evaluated the composite measurement of pain and basic activities like walking or performing daily activities. Tools able to record this data using accelerometery-based technology, are named actigraphs (15,16) and were already tested in clinical trials in patients affected by osteoarthritis of the knee. (17)

vii) When an Investigator is performing a ROM evaluation of the knee joint with a goniometer, overhead costs are near zero. The Biodex price is about 100 times the cost of a single actigraph; in addition, the Biodex system needs high qualified hospital centres and well-trained personnel. Finally, the ROM evaluation is part of the clinical practice of orthopaedics and the use of actigraphy is friendly with data collection by a standard personal computer (18). In this contest, Biodex system could represent the paradigm of the measurement tool not to be used in a pragmatic multicentre trial in a large population affected by pathologies like OA.

To address these topics we have planned a double blind randomized clinical trial (RCT) to compare a product based on oral HA with high molecular weight (Syalox® 300 Plus, River Pharma, Italy) versus placebo for a period of 4 months plus an optional period of 8 months; our goal will be to avoid the critical issues pointed out in the present letter, evidencing a correlation between improvements evaluated by subjective measurements (VAS, QoL) and by objective measurements (US and ROM). The project, extremely attractive from a scientific point of view, will be preceded by a pilot study (registered in clinicaltrials.gov as NCT 03421054) in order to assess the feasibility of the main study. This would save money, time and management for our team of investigators with limited resources.

We thank Guadagna S (Opera CRO) and the Progress in Nutrition journal for engaging in a challenging debate on the use of oral HA in knee osteoarthritis. We invite the authors of this systematic review to join and continue in this discussion. We also hope to collaborate with them to perform the future RCT, because our different and synergic competencies could contribute to define the correct role of oral HA as a new tool for the orthopaedists.

Disclosure

No potential conflict of interest relevant to this article was reported by the authors

References

1. Guadagna S, Barattini DF, Rosu S. Oral hyaluronan for the treatment of knee osteoarthritis: a systematic review. Progress in Nutrition 2018 20; 4
2. Tashiro T, Seino S, Sato T, Matsuoka R, Masuda Y, Fukui N. Oral administration of polymer hyaluronic acid alleviates symptoms of knee osteoarthritis: a double-blind, placebo-controlled study over a 12-month period. Sci. World J. 2012;doi:10.1100/2012/167928.
3. Iwaso H, Sato T. Examination of the efficacy and safety of oral administration of Hyabest® (J)-highly-pure hyalu-

ronic acid for knee joint pain. J Jpn Soc Clin Sports Med. 2009;58:566–72
4. Nagaoka I, Nabeshima K, Murakami S, Yamamoto T, Watanabe K, Tomonaga A, et al. Evaluation of the effects of a supplementary diet containing chicken comb extract on symptoms and cartilage metabolism in patients with knee osteoarthritis. Exp Ther Med. 2010;1:817–27
5. Möller I, Martinez-Puig D, Chetrit C. Oral administration of a natural extract rich in hyaluronic acid for the treatment of knee OA with synovitis: a retrospective cohort study. Clin Nutr Suppl. 2009;4:171–2
6. Sánchez J, Bonet ML, Keijer J, van Schothorst EM, Möllller I, Chetrit C, et al. Blood cells transcriptomics as source of potential biomarkers of articular health improvement: effects of oral intake of a rooster combs extract rich in hyaluronic acid. Genes Nutr. 2014;9:417.
7. Solà R, Valls RM, Martorell I et al. A low-fat yoghurt supplemented with a rooster comb extract on muscle joint function in adults with mild knee pain: a randomized, double blind, parallel, placebo-controlled, clinical trial of efficacy. Food Funct. 2015. DOI: 10.1039/c5fo00321k
8. Martinez-Puig D, Möller I, Fernández C, Chetrit C. Efficacy of oral administration of yoghurt supplemented with a preparation containing hyaluronic acid (Mobilee™) in adults with mild joint discomfort: a randomized, double-blind, placebo controlled intervention study. Mediterr J Nutr Metab. 2013;6:63–8
9. D'Agostino MA, Conaghan P, Le Bars M, Baron G, Grassi W, Martin-Mola E, Wakefield R, Brasseur JL, So A, Backhaus M, Malaise M, Burmester G, Schmidely N, Ravaud P, Dougados M, Emery P. EULAR report on the use of ultrasonography in painful knee osteoarthritis. Part 1: prevalence of inflammation in osteoarthritis. Ann Rheum Dis. 2005 Dec;64(12):1703-9
10. Hartung W, Kellner H, Strunk J, Sattler H, Schmidt WA, Ehrenstein B, Fleck M, Backhaus M. Development and evaluation of a novel ultrasound score for large joints in rheumatoid arthritis: one year of experience in daily clinical practice. Arthritis Care Res (Hoboken). 2012 May;64(5):675-82.
11. Serban O, Porojan M, Deac M, Cozma F, Solomon C, Lenghel M, Micu M, Fodor D. Pain in bilateral knee osteoarthritis - correlations between clinical examination, radiological, and ultrasonographical findings. Med Ultrason. 2016 Sep;18(3):318-25
12. Wu PT, Shao CJ, Wu KC, Wu TT, Chern TC, Kuo LC, Jou IM. Pain in patients with equal radiographic grades of osteoarthritis in both knees: the value of gray scale ultrasound. Osteoarthritis Cartilage. 2012 Dec;20(12):1507-13. Wu PT, Shao CJ, Wu KC, Wu TT, Chern TC, Kuo LC, Jou IM. Pain in patients with equal radiographic grades of osteoarthritis in both knees: the value of gray scale ultrasound. Osteoarthritis Cartilage. 2012 Dec;20(12):1507-13.
13. Yoshimura M, Aoba Y, Watari T, Momomura R, Watanabe K, Tomonaga A, et al. Evaluation of the effect of a chicken comb extract-containing supplement on cartilage and bone metabolism in athletes. Exp Ther Med. 2012;4:577–80
14. Yoshimura M, Aoba Y, Naito K, Watari T, Murakami S, Yoshimura K, Nakagawa T, Yamamoto T, Yamaguchi H, Nagaoka I. Effect of a chicken comb extract-containing supplement on subclinical joint pain in collegiate soccer players. Exp Ther Med. 2010; 3: 457-462
15. Yang CC, Hsu YL. A review of accelerometry-based wearable motion detectors for physical activity monitoring. Sensors (Basel). 2010;10(8):7772-88.
16. Steins D, Dawes H, Esser P, Collett J. Wearable accelerometry-based technology capable of assessing functional activities in neurological populations in community settings: a systematic review. J Neuroeng Rehabil. 2014 Mar 13;11:36.
17. Trudeau J, Van Inwegen R, Eaton T, Bhat G, Paillard F, Ng D, Tan K, Katz NP. Assessment of pain and activity using an electronic pain diary and actigraphy device in a randomized, placebo-controlled crossover trial of celecoxib in osteoarthritis of the knee. Pain Pract. 2015 Mar;15(3):247-55.
18. Baratto L, Capra R, Farinelli M, Gardella M, Jacono M, Morasso P.Wireless monitoring system of spontaneous daily motor activities. 6th National Congress SIAMOC Pisa October 26th-29th 200

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