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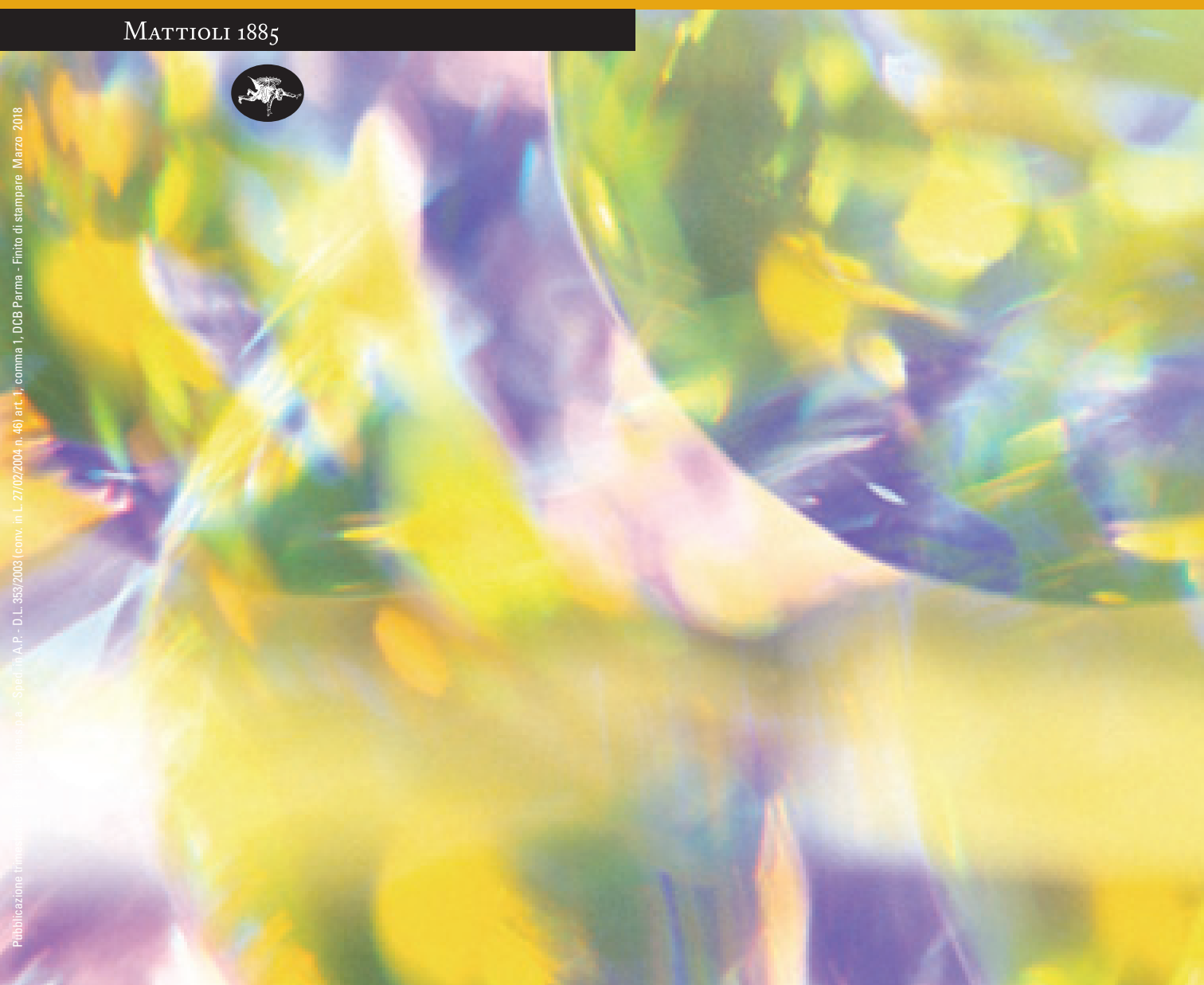

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Original article

Nutrition in dementia: a challenge for nurses

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Summary. *Aim*. The manuscript shows the presence of eating disorders in dementia in the elderly population and the risk of malnutrition. It is highlighted that the management of this patient is difficult and generate stress in the caregiver. Highlight the main questions that the medical staff provides the best form of nutrition for patients with dementia. *Methods*. The literature review and reported international guidelines analyze and propose measures to be carried into the environment of the meal, diet and encourages, as permitted, patient independence. *Results.* International guidelines suggest a multidisciplinary approach and the involvement of the family to carry out an individualized care plan. Strong are the recommendations to continue with assisted feeding by mouth. *Conclusions.*The literature shows that support proportionate to the real food needs of the person with dementia to ensure well-being and quality of life. It needs more nurse training and the definition of coded interventions involved, whether nurse acquire more awareness of their role.

Key words: dementia, nutrition, eating disorders, nurses, meal environment, diet, independence/patient involvement

Background

In the last years Italy has been a significant increase in the elderly population. The increase in years of life is related to better living conditions, but has produced an increase in chronic diseases, particularly dementia.

The ongoing and planned doubling of the number of cases every 20 years, estimated by WHO (World Health Organization) (1), indicate the urgent need to identify strategies to ensure support more complex. Cognitive disorders, mental and physical characteristics of dementia reduce the ability to play, effectively and independently, the activities of daily living, including the feed itself (2), making patient management tiring and complicated by having the nursing homes are the main setting in which it is treated (1). The ability to feed themselves, prior to being acquired and last to get lost (3), is compromised in about 50% of the elderly within 8 years from the beginning of dementia (4) and 45% of those institutionalized requires assistance with meals (2). The aim of the nurse is to solve practical problems such as hostility caused by the patient's difficulties with dementia to express verbally (5).

In advanced stages of the disease must be warded off the risk of malnutrition and dehydration (5) through a careful assessment of the patient's needs are real and genuine (2) and careful choice on the best mode of nutrition for the patient (6). The most important of Geriatrics Society have expressed their opinion on nutrition oral and enteral (7). In several studies confirms the opportunity to promote and ensure the physical and psychological well-being of the patient using just good will, attention to detail and time (8). Proposed actions concern the quality and presentation of food, the consideration of the tastes and eating habits of the patient, the prescription of a diet that takes into account the needs and difficulties the elderly, care for the environment used in the meal and preserve and ensure the greater degree of independence of the person in

this practice. The meal time is the activity of the most awaited day by the elders and represents an important opportunity for social interaction (8). This calls for a careful nurse training and the caregiver in charge supply of patients with dementia. Family involvement in the care process and, if possible, the patient and teamwork are important resources to define the individualized care plan and ensure the person's well-being (9).

Aim of the manuscript is to reflect the light of the literature data, the nurse's skills in identifying and promoting concretely, in daily practice, the patient's well-being with problems feeding.

Methods

A narrative review of literature. Scientific articles have been searched in the databases PubMed and CINAHL selecting the following key words in English: dementia, eating difficulties, feeding behavior, mealtimes difficulties, nursing, nutrition. They were selected approximately 50 papers published in the last ten years. Recommended interventions and responsibilities of the nurse in the field of nutrition to patients with dementia in nursing homes were investigated.

The bibliographic search began in December 2014 and ended in February 2015. All identified documents are in English. The selected studies include: the description of eating disorders; evidence supporting nutrition assisted orally; the type of care to be provided to the elderly and measures to be implemented in the environment of the meal, diet and directly on patients.

Results

Identify eating disorders

The problems feeding in the patient with dementia relate to the ability to recognize the food, to bring it to his mouth and swallowing (eating behavior) and / or the patient's behavior during feeding time, which manifests as food refusal, agitation , aggression, wandering and depression (feeding behavior) (10). Dysphagia is common, given the difficulty to remember how to chew, swallow and managing the food in the mouth (11) and is a cause of stress for patients and nursing staff (12). To these are added the typical disorders of old age problems such as alterations of the senses, arthritis and poor oral hygiene (13). Eating disorders are manifested with various severity levels at different stages of dementia (14), but are more detected in the advanced stage of the disease (2,15), affecting approximately 80% of patients (5,15). The behaviors adopted by the patient are not always easy to interpret (1,5). They can be, for the patient, the only effective mode of expression, indicating its inability to feed himself, or his desire to finish the meal. If the patient is unable to communicate could get away from the meal, leave open the mouth without spitting or swallowing food (14). The nurse, sometimes, might have difficulty understanding whether it is appropriate to continue to feed the patient against his will, or "let him die of hunger", chosen with considerable implications and essential by the ethical and ethical standards of the nursing profession. It may be useful contribution of the patient's family and other support professionals most appropriate interpretation of the behaviors adopted by the patient during the meal (16).

Malnutrition and nutrition assessment

The serious risk of dehydration and malnutrition, and thus the increase in morbidity and mortality and poor quality of life (17), associated with the presence of eating disorders, should be averted by their identification and early intervention (2,18). Dehydration is due to the patient's difficulty swallowing liquids (12,13). Malnutrition is clinically expressed by the weight loss (5) and is frequently found in elderly patients with dementia, particularly those living in care institutions (17). Weight loss is mainly determined by the rapid deterioration, but also by the stress associated with hospitalization; according to some studies is inevitable in the advanced stages of dementia (2,14,17,19), while for others it is uncertain whether it should be considered reversible or a predictor of terminal decline (15). Several studies show that the best nutritional assessment of the nurse is the observation of the patient during the meal (20), must be defined objective parameters such as the measurement (at least weekly) Weight (19) and the calculation of body mass index (weight in kilograms divided by height in meters square), which is less than the 18 value, it determines the need for intervention by a dietician (14). Other quality

parameters and combined methods to determine with greater suitability risk situations are: EdFED (Edinburgh Feeding Evaluation in Dementia Scale), defined in 2008 by the Hartford Institute for Geriatric Nursing New York University (HIGN) the best tool for the assessment of eating disorders and the corresponding level of support required for the elderly with dementia (10) and EdFEDQ (Edinburgh Feeding evaluation in dementia Questionnaire), a EdFED scale variant suitable for the detection of feeding behavior. Regular screening, performed with the scale MUST (Malnutrition Universal Screening Tool), represent the gold standard and are recommended by the National Institute and Care Excellence, especially in the Community (21).

The choice between hand or tube feeding

To use a device to feed the patient with dementia is easier to prevent malnutrition, aspiration and the occurrence of bedsores, the time savings and protection from legal problems for the support staff (14). About a third of residents in care services with cognitive deficits a PEG (Percutaneous endoscopic gastrostomy) is applied, especially in the end stage of the disease (11). There are numerous complications associated with its use, including, in particular, the accidental removal, due to the confusional state and / or patient agitation (18) that determines the increase in the use of physical and chemical restraints and the deterioration of the pressure ulcers (22). Scientific evidence suggests that the potential benefits of artificial nutrition does not exceed the adverse effects. There remains a high mortality, are not averted the suction operations, do not heal more easily pressure ulcers, or improve the nutritional status and in addition it has a higher risk of infection; increase oral secretions difficult to control, it also generates discomfort due to a possible malfunction of the device which sometimes requires the transfer to the hospital (6). The National Institute for Health and Clinical Excellence, the Royal College of Physicians (RCP), the British Society of Gastroenterology (BSG) and the Alzheimer's Society have determined that artificial feeding is not recommended in the progression of dementia and which can not only be used to prolong life in advanced stage of disease (7). According to the American Society for Parenteral and Enteral Nutrition (ASPEN), enteral feeding in advanced dementia is referred to as evidence of category E (18). Although most of the opinions of experts and observational data do not support the use, any randomized controlled trial has not yet been conducted to compare the benefits and adverse effects of this type of feeding against manual for ethical barriers. However articles published and which are based on the use of observational data, demonstrate a lack of effectiveness of nutrition through a device (6). Enteral nutrition does not determine improvements on the general state of health or increase the comfort and the patient's quality of life (11). The insertion of a PEG and the insertion time does not affect the survival of the patient (23).

However, it may be considered essential to use a device exclusively during the period of treatment of a concomitant acute problem which causes a difficulty in feeding (1,6,11) or in very advanced stages of dementia in which swallowing is strongly compromised (18) and the patient continues to refuse food (20). According to many studies, even in the end stage of dementia, nutrition assisted by mouth is considered more effective, is the most appropriate mode of human approach and guarantees better quality of life (18), the primary objective in the terminal stages of dementia.

The most important of Geriatrics Society suggest feeding the patient through the mouth with great care and patience, not force and insist (7), until it is tolerated and really possible (11). It is very important to follow strategies and precautions to avoid the risk of aspiration (7) and improve oral feeding (13).

At present the strategy is considered appropriate to define a personalized care plan that allows the identification of the most comfortable and suitable to the specific patient feeding mode ("comfort feeding only"), considering its will, the dangers and benefits of any possible intervention and providing for the involvement of the patient's family (1,6,14).

The nurse has a key role to play in explaining to the family the risks and benefits of various alternatives and acts as an intermediary between this and the multi-professional team (9) as a guarantee of the will of the patient (7).

The level of care provided and the appropriate assistance

Assistance feeding to ensure adequate intake of food, but reduces the autonomy of the person with dementia, promoting the development of a disability

(2,3). It has been reported that greater the degree of dependence, the lower the food intake (5). The failure to satisfy need assistance with meals is therefore a risk factor of malnutrition especially of the elderly living in institutions (17). It is crucially important to identify the actual and exact level of assistance needed for the patient based on his actual level of dependence and monitoring the situation that may progress rapidly (2).

Inadequate nursing feeding is mainly determined by the short time available (13), by the desire to finish soon the meal (5), the organization of the structure, from reduced staff (1) and its inappropriate training (3). Helping a person with dementia to eat takes a long time, even up to an hour for a person seriously affected (11).

For best results, care should be in the ratio of one to one (5). It would then be recommendable that always was the same person to feed the patient (2) so as to create an intimate relationship between nurse / caregiver and patient (4) able to reduce the appearance of aggressive behavior (2). Rather than on the person's deficits, attention should primarily be addressed to the still present capacity, quality and specifications of the patient resources (8). The nurse has the responsibility to integrate the skills lost and to encourage and maintain the present (3). Assistance should be the minimum necessary and possible; allow the patient to do as much as possible for himself (8,13) and guarantee respect for his dignity (19). To improve patient independence with dementia is important that the environment in which they live is familiar and safe while taking the meal (24). Encourage the presence of a relative while taking the meal improves patient care with dementia (19).

The role of training

Several studies have highlighted the need for and the value of a training program for caregivers and nurses (1,2,4,5,8,14,17). The National Institute for Health and Care Excellence (NICE, 2006) (21), has shown as important for the families and for the health workers, who often experience stress and suffering, and are not in possession of resources and tools useful (1).

Nurses trained show a better preparation to assist the patient and the environment, greater motivation, more patience and use new techniques. Without proper training program it is likely that some situations are incorrectly defined as difficulty in feeding and both made an inadequate interpretation of the patient's signs (5).

It is important a continuous and constant professional updating, to effectively address the diverse and articulate the patient's needs (10),

A recent literature review examined the effects of training programs, exercise and nutritional education: moderately increase the time of the meal, however, reducing the difficulties of the person to eat (4).

Suggested interventions to meet the needs feeding

Environment

Scientific evidence shows the benefits of interventions on the environment (14,11,19). Must be recreated, as much as possible, a family environment to the patient, the conditions in which he was fed in their own home to keep as much as possible its capacity to feed (20). The changes are not tolerated by the old and have a significant negative effect, regression (24).

In nursing homes, in the absence of very serious deficit, it has proved useful to put patients in the dining room (13) grouped according to their abilities and needs (11). The chairs should have armrests; wheelchairs, useful to better position patients, however, must be locked to prevent accidental falls. On the table must be present only the essential objects for the meal with contrasting color compared to the ground on which are placed to be more easily recognized by patients with agnosia (8). The use of circular tables encourages social interaction. Good lighting allows the elderly to see better and to recognize objects and people (20).

It is also recommended the placement of a large clock, with large numbers and hands, to enhance the sense of orientation of the patients (11).

A musical background enhances the experience of the meal (8). It is an inexpensive intervention, practical and safe, which decreases the agitation (25) and increases food intake (11). Reduce noise and other distractions helps the person with dementia to recognize the time of the meal, to concentrate on the actions that performs preserving its ability to feed (1,20).

Food and diet

To guarantee essential nutrients the choice of food and their preparation should be accurate. The

poor quality of food causes an appreciation reduced by the elderly and reduces appetite (13). The nurse should be always take into account the cultural and ethnic aspects (5,8), eating habits and tastes of individual patients trying as much as possible to respect them (6). The ways in which food is served has a significant impact on the type of experience lived by the patient with dementia (12).

The nurse's collaboration with the physician, the speech therapist and dietitian allows the development of a varied and balanced diet that cures in particular the consistency of the food, in order to reduce the high and menacing risk of aspiration (7). Remember and show the patient how to swallow and urge him to cough to each bite and let him introduce small amounts at a time. According to the degree of dysphagia, food must be of an increasingly homogeneous and unique texture. We should avoid chewy food, brittle or hard and be careful to the presence of possible shells, skins or seeds. The thickener makes dense liquids thus reducing the risk of aspiration and facilitating swallowing. It also allows to obtain a different consistency, according to need, from a pudding syrup, reduces the staff concerns of assistance and increases the safety and the patient compliance in taking liquids preventing dehydration (12). If properly administered oral supplements increase the caloric and protein intake and body weight by reducing the incidence of comorbidities associated with malnutrition (26). However their use is not supported by all (11), as they may decrease appetite, could not be tolerated and create gastrointestinal disorders and is also the considerable cost (21). In some cases contribute to increase the weight (18); it is uncertain whether they constitute an initial or exclusive remedy. A valid and able to actually increase the weight, it is represented by snacks between meals and the other. The sweet foods are preferred by older people and can be an important food for their high calorie content (12,14). In nursing structures is often present the healthy habit of setting up an afternoon snack with drinks and snacks, as opportunities for increased caloric intake and fluid and socialization (11). Some authors reveal that, dietary recommendations for fat, carbohydrate and dietary fibre are the same for older people as for the rest of the population and similar healthy eating guidelines apply. (27). Alzheimer's Disease International (ADI) suggested that simultaneous supplementation with multiple micronutrients (fatty acids, phospholipids, vitamins E, C, B6 and B12, and folic acid) might be required synergistically to increase brain levels of molecules that are essential building blocks of brain synapses (28).

Patient

Involve the patient in the meal preparation produces awareness of the proximity of this event, gives a sense of independence and enhances the well-being and self-esteem (8,13). The elderly should be assigned tasks according to its real possibilities and capacities (19). There is no consensus in defining which meal represents the time of day in which the patient with dementia is more alert and therefore better able to feed itself (14); for some it's lunch (19); for others, however, breakfast (11).

If the patient shows aggressive reactions, it is better to stop and try again when the meal be more composed (11). Activities based on the Montessori method are found to be effective in this type of patients develop since the residual capacity, increase the practice of activities of daily living, stimulate the senses especially the sense of touch (with an exercise in object recognition) and hearing (background music during meals), increase the capacity for coordination of movements and reduce agitation. This method is an alternative to preserve the independence of the elderly and generates long-term effects. Applying it correctly people are even able to acquire new skills thus demonstrating that dementia does not exclude the possibility of rehabilitation treatments (3). In order to guarantee patient autonomy literature shows the "finger food" because it preserves the ability to feed itself longer than willful use of cutlery even if the caregiver support (8,12,14, 20).

Conclusions

Eating disorders are a major challenge for the nurse, all the medical staff and the patient's family (17,19).

The feeding in dementia is influenced by several factors (social, cultural, organizational and environ-

mental) that make its management complex (29). The range of specific interventions is still poorly examined and marked. Further studies are therefore needed to validate those published so far, in particular there are no randomized studies and the work carried out have no common criteria, thereby making it impossible to a meta-analysis. The reduced sample is then examined a limit of many articles analyzed (30).

Very important is the personnel training to recognize and correctly interpret difficulties and the patient's needs, especially if not clearly expressed (1,16). More and comprehensive knowledge allows nurses to be more confident in acting and have interventions that will have a better impact. There are no studies on the work of the nurse in relation to the manifestation of aggressive behavior during the meal (14, 16).

Ethical dilemmas are not indifferent involving the figure of the nurse, especially on the end of life and artificial nutrition (30). It therefore seems necessary to drafting of guidelines in clinical practice, which define better the role of caregiver (1) and national programs that allow to carry nutrition for made conscientiously mouth (11). authoritative guidelines could provide crucial scientific bases considerations in mind to allow the identification of the best form of nutrition for the patient by bridging the limited knowledge on this topic. At the local level would enable each district to identify the resources (human and otherwise) to which you can refer and professionals to identify the state of dementia, to avoid interventions, therapies and inadequate hospital admissions (7). It seems necessary that the nurse is more involved in this field and able to effectively educate the support staff and family members (31). The nurse, especially the geriatric nurses, must play a leading role and participate in the drafting of programs and protocols on nutrition (11).

The new tools (assessment scale), the different possibilities of intervention, the available resources (literature, guidelines, the multi-professional team, the patient and members of his family) and the progress of scientific and technological research does not justify more poor nutrition of the elderly with dementia. These must then acquire and maintain a nutritional state that contributes to the best possible quality of life (12, 32).

According to current knowledge and literature consulted for this study shows that, although the specific role of the nurse in the field of nutrition is still not clearly defined, this professional can not deny his role as "advocate and guarantor" of the patient , the expert guidance, support, educator, or delegate their responsibilities to others. The nurse must act correctly to ensure the welfare of the person taking care, taking particular attention to their needs, without preventing his even minimal involvement.

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Review

The effects of catechins on related risk factors with Type 2 diabetes: a review

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Summary. Insulin resistance in patients with diabetes mellitus causes complications such as cardiovascular and renal diseases. Studies have shown that catechins can be effective in controlling hyperglycemia and preventing the complications of diabetes by improving insulin sensitivity and reducing the risk factors for Type 2 Diabetes Mellitus such as oxidative stress, dyslipidemia and obesity. The aim of the present study is a review of the studies conducted in the field of the effect of catechins on the improvement of the risk factors associated with Type 2 Diabetes Mellitus. This review study was conducted by searching in the databases of *Science Direct*, *Scopus*, *PubMed* and using the keywords, such as catechins, green tea, insulin resistance, diabetes mellitus, hyperglycemia, obesity, dyslipidemia and oxidative stress. In this study, articles published between the years 2000-2016, were used. The results of the review of the studies showed that the catechins and food containing them can improve hyperglycemia, oxidative stress, dyslipidemia and obesity in patients with Type 2 Diabetes Mellitus.

Key words: catechin, Diabetes Mellitus Type 2, dyslipidemia, obesity, oxidative stress

Background

Diabetes mellitus is one of the most common metabolic diseases. The prevalence of diabetes has increased dramatically in recent decades (1). Global diabetes prevalence increased from 4.3% (in 1980) to 9.0% (in 2014) in men, and from 5.0% to 7.9% in women. The number of adults with diabetes in the world increased from 108 million in 1980 to 422 million in 2014 (2). Lifestyle and changing dietary habits, which with the industrialization of society causes a wide range of diseases, are a major cause of the increasing prevalence of diabetes type 2 (3-5). Uncontrolled type 2 diabetes lead to complications, such as coronary artery diseases, peripheral vascular diseases, retinopathy, cerebral vascular diseases, neuropathy and nephropathy (6). Researchers attempt consistently in order to achieve a safe and efficient therapeutic approach for the treatment of type 2 diabetes. Many chemical agents are available for the control and treatment of diabetes, but to date, no full recovery of the disease has been reported. Furthermore, more oral drugs are costly and have side effects. On the other hand, numerous medicinal plants have been introduced that have shown the potential for reducing the blood glucose and preventing the complications of type 2 diabetes (7-10). Many of the properties of the medicinal herbs are originated from their active ingredients that mainly are classified

in the category of antioxidants. Catechin is one of the antioxidants contained in the medicinal plants that have potential for the treatment of type 2 diabetes.

A large number of interventions and epidemiologic studies reported that Catechin reduces the risk of chronic diseases such as cardiovascular disease (11). Some human and animal Interventional studies suggest that green tea extract or Epigallocatechin gallate (EGCG) can have beneficial effects on blood sugar control (12, 13). EGCG can cause removal of ROS and decrease oxidative stress (14). It is likely that catechins and their food sources prevent the complications of diabetes by reducing the expression of proinflammatory cytokines. While the beneficial effects of catechins in the health and treatment of diseases have been reported, but high doses of catechins may have side effects (15). Some animal studies have reported that taking high doses of catechin can induce oxidative stress in the liver and pancreas tissue (16).

Despite conflicting studies on the effects of catechin in the treatment of type 2 diabetes, limited review studies have been published in this area. So this review article has been designed in order to study the effects of catechin on the risk factors for type 2 diabetes.

Catechins and their sources

Catechins are considered as family polyphenolic antioxidants. Due to their carbon structure, Polyphenols are mainly divided into categories of phenolic acids, flavonoids and lignans. Flavonoids are the most abundant polyphenols in the diet. Flavonoids are divided into flavones, flavonols, isoflavones, anthocyanins, flavanols, the proanthocyanidins and flavanones. Some of these flavonoids are found only in a small number of foods. Soy in isoflavones, citrus fruits in flavanols citrus, fruits and vegetables in flavonols, are considered as their major food sources (17).

Catechins are considered as the most main flavones. Catechin types include: catechin, gallocatechin, catechin-3-gallate, Gallocatechin 3- gallate, epicatechin, epigallo catechin, epicatechin 3- gallate, epigallo catechin-3-gallate. Green tea is considered as the richest source of catechins. Among the catechins, EGCG is the most abundant and strongest catechins in the green tea and includes 65% of the total content of catechin in green tea (18). In addition, catechins are also abundantly found in chocolate. The content of polyphenols is different in types of chocolate, and dark, milk and white chocolates include the maximum value, respectively (19).

Materials and Methods

This review study has been conducted by searching scientific databases such as *Scopus*, *Science Direct*, *Pubmed*. Articles were searched using keywords: Catechins, green tea, insulin resistance, diabetes mellitus, hyperglycemia, obesity, dyslipidemia and oxidative stress in the articles published between from 2000 to 2016. In this study, a variety of studies, including interventional, cohort, case-control, cross-sectional and meta-analysis were reviewed. Based on the objectives of the study the articles were included. These objectives were: The effect of catechins on blood glucose, the effect of catechins on obesity, the effect of catechins on oxidative stress and the effect of catechins on dyslipidemia. At first, on the basis of titles related to the objectives of this study, the articles were reviewed. After studying abstract for eligibility, if it was qualified, the main body of the article was studied and analyzed. In the beginning, 534 articles, which were potentially associated with the objectives of the study were identified. After studying the full text of these articles, finally, 74 articles were included in this study.

Results

Catechins effect on hyperglycemia

In vivo studies have shown that green tea can improve insulin sensitivity (20). Animal studies have reported the hypoglycemic effect of green tea extract as a rich source of catechins (21). Epidemiological studies have reported that green tea consumption may reduce the risk of type 2 diabetes (22). A clinical trial study showed that supplementation with green tea extract reduces the glycated hemoglobin in people with abnormal blood sugar (23). It is reported that EGCG causes an increase in insulin sensitivity and facilitates entry of glucose into cells (24). Liu et al.'s study showed the EGCG supplementation for 12 weeks in

Table1. The effects of catechins on related risk factors with Type 2 diabetes

Variable	Catechins	Models	Effects	Reference
	Green tea-extract powder containing 544 mg polyphenols (456 mg catechins) for 2 months	Subjects with glucose abnormalities	Decrease in hemoglobin A1c level. No significant changes in FBS and Insulin resistance	(23)
	EGCG supplementation for 12 weeks	SAMP8 mice	Improved glucose homeostasis	(25)
Hyperglycemia	Green tea (containing 582.8 mg of catechins), 12 weeks	patients with type 2 diabetes	Decrease in hemoglobin A1c	(26)
	Green tea extract containing 856 mg of EGCG	obese individuals with type 2 diabetes	Decrease in HbA1C, HOMA-IR index and insulin level	(27)
	400 mg EGCG, twice daily, 8 weeks	Overweight or obese male subjects	No effect on insulin sensitivity, insulin secretion and glucose tolerance	(65)
	30 ml of EGCG-supplemented olive oil	Patients with early atherosclerosis	Decrease in inflammatory parameters: sICAM, white blood cells, monocytes, lymphocytes and platelets.	(66)
	high-fat diet containing 0.1%, 0.2%, or 0.5% EGCG (w/w), 25 weeks	non-obese type 2 diabetic GK rats	Decrease in OHdG and MDA by supplementation with EGCG at 0.1%. Significant reductions in the mRNA levels of genes related to inflammatory responses (IL-1β, IL-6, IL-18, TNF-α, IFN-γ, MCP-1), 8-OHdG, and total MDA by EGCG supplementation at 0.1%.	(42)
Oxidative stress	Green tea extract for 8 weeks	Obese subjects with metabolic syndrome	Decrease in MDA and HNE	(67)
	high-dose green tea extract for day 28	Obese diabetic mouse	Decrease in sICAM-1	(68)
	Catechin-rich green tea (catechins 615 mg) beverage per day, 4 weeks	Postmenopausal women	Improved serum postprandial derivatives of reactive oxygen metabolites concentrations. A significant increase in serum postprandial thioredoxin concentrations	(69)
	0.1% EGCG for 34 weeks	Obese and diabetic C57BL/KsJ-db/db Mice.	Decrease in TNF-α. also, decreased the expression of TNF-α, interleukin (IL)-6, IL-1β, and IL-18 mRNAs in the livers	(70)
	high-dose green tea extract (EGCG) at a daily dosage of 856.8 mg for 12 weeks	women with central obesity	Improve in weight loss, as well as decreases in BMI and waist	(72)
	Decaffeinated green tea extract (catechins 400 mg), twice daily for 6 weeks	overweight and obese men	Decrease in body-weight	(71)
Obesity	beverage containing 625 mg of catechins with 39 mg caffeine for 12 wk	overweight and obese adults	Decrease in total abdominal fat, subcutaneous abdominal fat area	(50)

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Table1. The effects of catechins on related risk factors with Type 2 diabetes

Variable	Catechins	Models	Effects	Reference
	1 bottle oolong tea/d containing 690 mg catechins, for 12 wk	Healthy men	Decrease in Body weight, BMI, waist circumference, body fat mass, and subcutaneous fat area	(49)
	High-catechin drink (886 mg catechins, 198 mg caffeine/day) for 90 days	overweight subjects	Decrease in intra-abdominal fat area, waist circumference, body weight, total body fat and body fat %	(53)
	Tea (4.5 g green tea/330 ml, 400 mg catechins)	patients with coronary artery disease	Improved levels of postprandial Pancreatic lipase, total cholesterol, HDL-C and LDL-C	(63)
	Green tea extract containing 1315 mg catechins (843 mg EGCG) for 12 month	postmenopausal women	Decrease in TC, LDL cholesterol and non-HDL cholesterol. No change in HDL-cholesterol concentration	(62)
Dyslipidemia	Catechins and EGCG 550 mg, for 56 days	Rats (Sprague Dawley)	cholesterol and LDL were substantially reduced	(73)
	EGCG 25mg/kg/day for 8 weeks	diabetic rats	Imoroved levels of serum triglyceride, HDL- and LDL cholesterol	(22)
	500 mg green tea extract, three times a day for 16-week	patients with type 2 diabetes and lipid abnormalities	Decrease in triglyceride, increase in HDL-cholesterol, Adiponectin, apolipoprotein A1 and apolipoprotein B100	(74)

SAMP8: senescence-accelerated mice prone 8; OHdG: 8-hydroxydeoxyguanosine; MDA: malondialdehyde; HNE: hydroxynonenals

mice can reduce the levels of FBS and fasting insulin by a change in the expression of GLUT4 gene (25).

A clinical trial conducted by Nagao et al. showed that the 12-week intervention of a catechin-rich drink, including green tea containing 528.8 mg of catechins than a green tea drink containing catechins can significantly 96 mg of the reduce insulin levels in type 2 diabetic patients, although no significant difference was observed in fasting glucose levels and glycosylated hemoglobin (26). The results of the clinical trials conducted by Hsu et al. showed that 1500 mg of green tea extract supplementation (856 mg EGCG) for 16 weeks on obese people with type 2 diabetes can cause a significant reduction in fasting insulin, insulin resistance and glycated hemoglobin (27). On the other hand, some studies have reported failing to influence the catechin in improving the glycemic status. In an intervention cross-over study, Baer et al. showed that a five-day intervention using tea along with catechins, on the healthy people cannot change a significant change in the levels of FBS, fasting insulin, area under the oral glucose tolerance curve and area under the insulin level curve (28). In addition, Toolsee et al. reported that intervention of 200 ml of green tea (containing 234 mg of EGCG) for 14 days in patients with pre-diabetes cannot have significant changes in fasting blood glucose and glycosylated hemoglobin (29). On the other hand, some studies have suggested that green tea can increase the level of FBS. A study by Josic et al. showed that an intervention of 300 mg of green tea compared to placebo (drinking water) can increase glucose levels after meals (Postprandial), although the increase in the levels of fasting insulin and area under the curve the blood glucose was observed (30). A meta-analysis of randomized controlled trials reported that the administration of green tea catechins with or without caffeine resulted in a significant reduction in fasting blood glucose (31).

Catechin effect on the oxidative stress

Hyperglycemia in type 2 diabetes causes several complications such as nephropathy, decreased insulin secretion from the pancreas, insulin resistance, retinopathy and cardiovascular disease. The major cause is side effects caused by oxidative stress hyperglycemia and leukocyte activity that causes inflammation (32). Recent studies have shown that hyperglycemia causes inflammation directly through increased pro-inflammatory cytokines such as IL-1β, IL-6, IL8 and TNF-α (33, 34). Through apoptosis in the pancreatic Langerhans islet beta cells, cytokines cause an increase in capacity and a reduction in the risk of developing type 2 diabetes as well as a reduction in insulin secretion capacity (35). Several studies have reported that the expression of pro-inflammatory cytokines are induced by reactive oxygen species (ROS) and are followed by oxidative stress (36). By increasing the activity of mitochondrial respiratory chain, hyperglycemia induces production of ROS (37). On the other hand, antioxidants can reduce oxidative stress and inflammation. Studies show that among antioxidants, EGCG is one of the antioxidants that reduce the risk of cardiovascular disease (38). EGCG can clear ROS in in- vitro (39). In addition, EGCG can reduce the 8-hydroxy guanosine doxycycline (8-OHdG) as one of the DNA oxidation products (40). Through an effect on NF-κB signaling pathway, EGCG reduces the production of inflammatory cytokines (41). Recently, an experimental study examined effects of EGCG supplementation in non-obese diabetic rats. The results of the study showed that EGCG supplementation can cause a significant reduction in oxidative stress markers such as 8-OHdG and Malondealdeide (MDA). In addition, EGCG supplementation could cause a significant reduction in the level of inflammation-related genes mRNA (TNF-α, IFN-γ, IL-1β, IL-6, IL-18, MCP-1), 8-OHdG and MDA in the peripheral leukocytes (42).

Catechins effect on obesity

Through a variety of mechanisms, obesity causes the development of type 2 diabetes. In obesity, growing up in fat cells reduces the antilipolytic activity of insulin on the enlarged fat cells. The reduced antilipolytic activity of insulin increases the free fatty acid level in Free fatty acid (FFAs) level in the blood circulation. High FFAs circulating in the blood, impair glucose metabolism and increase insulin resistance (43). Adipose tissue is a source of production of inflammatory cytokines. With the increase in the amount of fat tissue, production of inflammatory cytokine also increased. Inflammatory factors released from adipose tissue by reducing lipoprotein lipase activity and increasing the intracellular lipolysis, cause an increase in the level of FFAs in the blood circulation. In addition, adipokines can increase insulin resistance by different mechanisms (44).

Several epidemiological studies have shown that catechins may have anti-obesity effects (45, 46). There have been reports on inverse association between intake of catechins with body mass index (47). A cross-sectional study showed that tea consumption is inversely associated with BMI and waist size (48). A clinical trial showed a 12-week intervention of Oolong tea containing 690 mg catechins than the control group (Oolong tea containing 22 mg catechins) can cause a significant reduction in body weight, BMI and body fat tissue (49). Another study found that intervention of 625 mg of catechins along with 39 mg of caffeine for 12 weeks in adults obese can cause weight loss, total abdominal fat area and subcutaneous abdominal fat area compared to the placebo group (50). Another study found that green tea consumption along with resistance exercise increase lean body mass and waist circumference in addition to reducing body fat (51). A clinical trial study showed that administration of EGCG supplementation (300 mg daily) can decrease respiratory quotient compared to the placebo group (52). In a double blind clinical trial study, anti-obesity effects of catechin-rich drink on overweight subjects were studied. Results showed that consumption of catechin-rich drinks for three months can cause a significant reduction in body fat percentage, body weight, total body fat, body fat, intra-abdominal fat and waist circumference (53).

Catechins effect on dyslipidemia

Disturbances in the metabolism of fatty acids lead to increased levels of FFAs in the bloodstream and will be followed by high triglyceride accumulation in the tissues. Changes in the metabolism of fatty acids by pancreatic beta cell dysfunction and insulin resis-

tance induced in body tissues (such as liver, muscle) will develop type 2 diabetes (54,55). Dyslipidemia is abundantly found in patients with type 2 diabetes (56). Dyslipidemia in diabetic patients can underlie atherosclerosis and cardiovascular disease (57). Therefore, treatment of dyslipidemia in diabetic patients can prevent cardiovascular complications of the disease (58).

Recent studies indicate can used as an effective and safe treatment to improve the lipid profile (59). It has been reported that the green tea, through reducing the amount of oxidized LDL, improves vascular performance (60). With the intervention of EGCG, decrease in MDA a major lipid peroxidation byproduct has been observed (61). A double blind clinical trial study has investigated the effect of supplementation of catechin-rich green tea extract on lipid profiles in postmenopausal women. The results of the study showed that the intervention of green tea extract containing 1315 mg for one year can cause a significant reduction in the level of total cholesterol, LDL cholesterol and non-HDL cholesterol (62). Another study examined the effect of tea containing 400 mg of catechin on lipid profile changes after breakfast in coronary artery disease patients. The results of the study showed that the catechin-rich green tea after breakfast can cause control level of Serum triglycerides, total cholesterol, HDL-C, LDL-C and pancreatic lipase (63). Recently, the results of a systematic review study showed that consumption of 107- 856 mg/day EGCG for 4 to 14 weeks can reduce LDL-C (64).

Conclusions

The studies reviewed suggest that catechins can be used as an auxiliary treatment for controlling blood sugar and risk factors associated with type 2 diabetes (Obesity, Dyslipidemia, Oxidative stress).

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Original article

Evaluation of students' dietary behaviours depending on gender

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Summary. It is believed that university students commit many nutritional errors due to changes in lifestyle such as moving away from the family home, irregular meals, long hours spent studying, and frequently taking part-time jobs. Thus, we aimed to a) describe the baseline dietary intake of university students, b) identify differences in healthy eating between genders, and c) explore the relationship between lipoproteins and anthropometric data. In total, 500 students, 339 females and 161 males from Lithuanian universities and colleges participated in the study. The Food Frequency Questionnaire was used to evaluate dietary habits. The body composition values and blood analysis were estimated. Most participants (74.3%) reported regularly eating breakfast on weekdays, but of those, less than half ate breakfast 1-2 days a week. Females were more likely to consume cooked vegetables, salad/raw vegetables, fresh fruits, and curd-/cream cheese/yoghurt (p<0.001). By comparison, consumption of red meat, poultry, sausages, fish, and hard/soft cheeses (p<0.001) was common among males. In addition, males ate fast food (p<0.001) more often than females. Females consumed chocolate more often than males did (p<0.001). In males, total cholesterol, triglycerides, and low density lipoprotein (LDL) cholesterol were correlated with body weight and body mass index (BMI) (p <0.01). In females, high density lipoprotein (HDL) cholesterol was negatively correlated with body weight and BMI (p<0.01). The main barriers to healthy eating were identified as skipping breakfast and deficiencies in the consumption of specific food groups such as fruits and vegetables as recommended by the World Health Organisation (WHO). With respect to gender, differences in healthy eating were found in the consumption of meat and regular meals. The degree of obesity, triglycerides, and LDL cholesterol were higher in males, suggesting possible association with chronic disease incidence such as hyperlipidaemia and hypertension.

Key words: health behavior, students, dietary habits, lipoproteins, body composition

Introduction

According to the World Health Organisation (WHO) (2016), a healthy diet should include a high consumption of fruits, vegetables, and whole grains, in addition to low consumption of saturated fats, salt, and refined carbohydrates. Research suggests young adults engage in poor eating behaviours, such as low fruit and vegetable consumption and high consumption of energy-dense snack foods (1). In addition, they frequently fail to consume meals regularly (2). The regular omission of meals, particularly the breakfast meal, has been associated with poorer diet quality (3); lower intakes of total energy, vitamins, and minerals (4); increased risk of central adiposity (5); markers of insulin resistance (6); and cardio metabolic risk factors (7). It is believed that university students commit many nutritional errors due to changes in lifestyle. This may include moving away from the family home, irregular meals, long hours spent studying, and frequently taking part-time jobs. Therefore, students are more likely to pay less attention to the amounts and quality of food they consume (8).

Recent studies provide evidence linking the habit of not healthy eating with a lower risk of weight gain,

obesity, and metabolic syndrome (9). Increased body fat mass in turn increases total body mass and lipid concentration in the blood. High total cholesterol (Tchol), low density lipoprotein cholesterol (LDL-chol), and low high density lipoprotein cholesterol (HDL-chol) are associated with vascular death. In addition, high levels of triglyceride (TG) is an important and independent predictor of cardiovascular disease (10). Therefore, healthy levels of blood lipids and blood glucose are crucial to preventing cardiovascular disease. That is one of the reason to pay attention to the quality of food students consume. Surveys from Western countries show gender differences in food consumption, nutrient intake, and attitudes towards food. Females are more concerned about healthy diet and more often classify foods according to the assumed nutrient content than males (11). Research (12) has shown that males of all ages consume more saturated fat and dietary cholesterol than females do. Cholesterol intake of males was substantially higher that recommended levels, while dietary cholesterol of most females of all ages fell within the recommended range for classes of age (12). The combination of food categories characterizing the diet according to gender therefore plays a central role in determining the amount of energy consumed. Males have a higher energy intake, and a higher percentage of the energy in males' diets is derived from animal products, whereas the share of products of vegetable origin is higher in females' diets (13). Thus, it can be inferred that differences in food consumption, nutrient intake, and attitudes towards food are less healthy in males' diets than in females' diets.

In addition, little is known about the preventative measures that can be taken for university students to follow a healthy diet. Thus, we aimed to a) describe the baseline dietary intake of university students, b) identify differences to healthy eating between the gender, and c) explore the relationship between lipoproteins and anthropometric data.

Subjects and Methods

The analyses are based on the online survey data from the Food Frequency Questionnaire (FFQ) conducted across Lithuania among university students from February 2015 to July 2015. University students were recruited via fliers, mailing lists, social networks, and advertising the study during classes and lectures. Participants provided informed consent by selecting the "agree" button during the online survey, which then directed them to the first question of the survey. We put the exclusion criteria in the informed consent. These were: pregnancy; breastfeeding; genetic factors such cystic fibrosis and sickle cell anaemia; non-communicable chronic conditions, such as diabetes, hyperlipidaemia, inflammatory bowel disease, and multiple sclerosis; medical conditions; and regular consumption of recreational drugs and excessive alcohol. If a respondent identified with any of these exclusion criteria, they were not allowed to continue with the online survey. After completing the questionnaire, the participants were asked to evaluate their blood by using biochemical blood analysis and to assess their body composition values.

In total, 500 students from Lithuanian universities and colleges participated in the study. There were 339 females and 161 males and their mean age was 23 years old. The subjects were moderately physically active (<2 hours per week) but did not participate in any formal physical exercise or sport programme. Each subject volunteered to participate after being informed of the purpose and experimental procedures. A large proportion of respondents lived in the country (36%), in towns with 20,000 -100,000 inhabitants (30%), and with their families (68%). Generally, they defined their financial situation as average (69%).

Questionnaire

The Food Frequency Questionnaire (FFQ) was used to evaluate dietary habits in terms of dietary rate. The FFQ consisted of 22 questions regarding food items, and the rate of consumption was assessed by the following response categories: never, less than once a week, one to three times a week, four to seven times a week, or several times a day. Based on a food pyramid developed by the German state-funded Agency for Consumer Information (14), the 22 food items were grouped into six food groups: 1. vegetables, salad; 2. fruits; 3. bread, grains, side dishes; 4. dairy products; 5. meat, sausages, fish, eggs; 6. sweets and snacks.

Estimation of Biochemical Blood Analysis

Upon arrival, subjects were requested to rest in a seated position for approximately 20 minutes before blood collection. The hand used for the blood collection was placed into warm water (37–38 °C) for 5 minutes to encourage blood flow to the capillary sites. The hand was dried and an alcohol swab was used. The capillary blood from the fingertip was collected using a disposable 2 mm contact-activated sterile lancet (BD Biosciences, Australia) to the lateral portion of the distal phalanx of the third or fourth metacarpal into a 300 μL heparinised capillary tube (Kabe Labortechnik, Germany). The first blood drop for the fingertip was discarded to minimize excess tissue fluid. The blood sample from finger was taken to establish total cholesterol (Tchol), high (HDL-ch) and low (LDL-ch) density lipoprotein cholesterol and triglyceride (TG) concentration by an enzymatic method using a standard Cardio Check biochemical analyser (USA). The device operated on the principle of dry chemical reagent using test strips. LDL-ch was calculated by formula: LDL-ch = Tchol – HDL-ch - TG/5.0 (15).

Estimation of Body Composition Values

Weight (kg), body mass index (BMI), body fat mass (%) and lean body mass (%) were estimated by a body composition analyser, "Tanita body composition analyzer TBF – 300a" (USA), where we set subject's height, age, and physical capacity (Table 1). We used BMI categorization as underweight (BMI < 20), normal weight (BMI - < 24.9), overweight (BMI - < 30), and obesity (BMI ≥ 30) (16).

Statistical Analysis

Statistical analysis was performed by using *SPSS 20.0* software for *Windows*. Descriptive statistics were applied. The nutrition habits were conducted by Chi^2 tests to explore if eating behaviour differs between male and female university students. The correlation among variables was evaluated by Chi^2 tests. Links among nutrition habits and food additives were determined using Chi^2 tests applying to discrete variables and a Kruskal-Wallis H test. For all tests $p<0.05$ was considered significant.

Results

Table 1 presents the anthropometric data. The males had higher weight, height, and skeletal muscle ($p<0.001$) values than the females. Body fat values were higher in females ($p<0.001$) than males. There were no differences in BMI between the two groups ($p>0.05$).

The subjects (74.3%) reported regularly eating breakfast on weekdays (3-5 times), while 9.33% stated they rarely or never ate breakfast on weekdays. But 31.86% females and 36.02% males ate breakfast only 1-2 days a week. During the week, 63% of students ate 3-4 times a day (Table 2).

Subjects reported eating cooked vegetables (3.2%) as well as raw vegetables and salad (3.6%) several times a day (Table 3). Fresh fruits were consumed by 26.9% of students several times a day. Brown bread was eaten by 10.3% less than once a week. While 18% of the students reported they never ate red meat, 12.6% stated that they consumed it 4-7 times a week. More than half of the students (55.4%) ate poultry 1-3 times a week, and 43.1% consumed fish 1-3 times a week. More than half (52.5%) of the students reported consuming fast food less than once a week, and 1.9% reported eating fast food frequently (4-7 times a week) (Table 3). Females were more likely to consume cooked vegetables ($p<0.01$), salad/raw vegetables, fresh fruits, and curd-/cream cheese/yoghurt (all: $p<0.001$) compared to males. Consumption of red meat, poultry, sausages, fish (all: $p<0.001$), and hard/soft cheeses ($p<0.001$) was more common among male students. Males ate fast food ($p<0.001$) and side dishes like pasta/rice ($p<0.001$) and fried potatoes/chips ($p<0.001$) more frequently than females did. Females consumed chocolate more often than males ($p<0.001$). Chocolate was eaten by 4.5% several times a day.

Table 1. Subjects' characteristics

	Female	**Male**
Height, m	1.61 ± 1.1	1.72 ± 1.7*
Weight, kg	67.71 ± 5.1	81.37 ± 5.8*
Body mass index, kg/m^2	24.74 ± 0.03	27.21 ± 0.01
Lean body mass %	29.68 ± 2.9	41.82 ± 4.1*
Body fat, %	28.67 ± 3.1	16.61 ± 3.9**

Statistically significant difference; significance level p<0.05

Table 2. Baseline dietary for breakfast consumption and eating times per day

	Female % (n)	Male % (n)	Age 19 - 25 % (n)
Breakfast consumption a week			
6 – 7 days	23.01 (78) *	14.28 (23)	22.2 (111)
3 – 5 days	35.10 (119) *	74.30 (65)	74.3 (173)
1 – 2 days	31.86 (108)	36.02 (58)	33.4 (167)
Rarely or never	10.03 (34)	9.33 (15)	9.33 (49)
χ^2 P*	0.442		0.001
Eating times a day			
1 – 2 times	24.19 (82)	25.47 (41)	24.8 (124)
3 – 4 times	63.12 (214)	63.35(102)	63 (315)
5 and more	12.68 (43)	11.18 (18)	12.2 (61)
χ2 P *	0.079		0.001*

Statistically significant difference; significance level p<0.05; χ^2 – Chi square

Table 3. Baseline dietary intake

	Never (%)	< 1 times/week (%)	1-3 times/week (%)	4-7 times/week (%)	Several times a day (%)
Cooked vegetable	0	20	40	60	5
Salad and raw vegetables	5	10	40	40	5
Fresh fruits	0	10	20	40	25
Canned fruits and compote	30	30	20	5	0
Pasta and rice	2	5	60	60	5
Boiled potatoes	0	20	70	30	0
Brown bread	0	15	20	60	0
White bread	0	15	20	60	0
Breakfast cereals	5	10	25	35	5
Curd, cream, yogurt	5	10	25	35	10
Eggs	0	5	20	60	0
Poultry	0	20	10	30	20
Red meat	0	19	21	45	15
Fish	0	20	35	40	5
Sausage and ham	0	23	15	40	45
Chocolate	10	20	40	30	10
Cake and pastries	0	15	55	55	10
Ice cream	0	20	65	40	3
Fast food	0	15	55	30	5
Chips and fried potatoes	0	12	50	30	5

TG and LDL-chol (p<0.05) values were higher, and HDL-chol was lower (p<0.001), in males than females. There was no difference in triglyceride and Tchol level between the two genders (p>0.05) (Table 4). In males, Tchol, LDL-chol and TG were correlated with body weight and BMI (p<0.01). In females, HDL-chol was correlated with body weight and BMI (p<0.01) (Table 5).

Discussion

The main barriers to healthy eating were identified as skipping breakfast and the deficiencies in the consumption of specific food groups such as fruits and vegetables compared to WHO recommendations. With respect to gender, differences in healthy eating were found in the consumption of meat and regular meals. The degree of being overweight ~~obesity~~, triglycerides, and LDL cholesterol were higher in males, suggesting possible associations with chronic disease incidence such as hyperlipidaemia and hypertension. Such knowledge is necessary to inform health promotion strategies in the university setting.

Table 4. Blood lipid profile levels of subjects by gender

	Female	Male
Tchol (mmol/l)	4.65 + 0.88	4.67 + 1.27
HDL-ch (mmol/l)	1.83 + 0.43	1.41 + 0.34*
LDL-ch (mmol/l)	2.62 + 0.74	3.99 + 1.05*
TG (mmol/l)	1.03 + 0.39	2.07 + 0.69**

Statistically significant difference; significance level p<0.05; Tchol – total cholesterol, HDL-ch – high density lipoprotein cholesterol, LDL-ch – low density lipoprotein cholesterol, TG – triglyceride.

Table 5. Correlation coefficient between anthropometric and blood clinical indices for gender

	Female		Male	
	Age	**BMI**	**Age**	**BMI**
Tchol (mmol/l)	0.139	0.350	0.145*	0.097*
HDL-ch (mmol/l)	0.011*	0.123*	0.058	0.337
LDL-ch (mmol/l)	0.147	0.452	0.177*	0.111*
TG (mmol/l)	0.112	0.282	0.060*	0.134*
Glucose (mmol/l)	0.239	0.460	0.163	0.134*

Statistically significant difference; significance level p<0.05; Tchol – total cholesterol, HDL-ch – high density lipoprotein cholesterol, LDL-ch – low density lipoprotein cholesterol, TG – triglyceride, BMI – body mass index.

The students' life at university has a variety of components affecting their diet: irregular lifestyle; a change in residence; a lack of student cafeterias; stress; a hectic lifestyle; jobs taken during non-class time; inappropriate habits and eating behaviors adopted from their family homes; meeting energy requirements by consuming energy-packed snacks, or the use of stimulants (8). Kowalska (8) observed that a frequent problem among students relates to irregular consumption of meals, particularly breakfast. In our study, we found that 74.3% of participants reported regularly eating breakfast on weekdays (3-5 days), but 31.86% of females and 36.02% of males ate breakfast only 1-2 days per week.

Our literature review identified that young adults skipped breakfast more frequently than other main meals. A sample of American elderly participants reported the prevalence of breakfast skipping was highest (10.7%) when compared to lunch skipping (8.6%) and dinner skipping (5.8%) (17), with similar results seen in children and adolescent populations (18). Nine of the ten studies reported time as the biggest perceived influence on meal skipping when ranked against other important correlates of young adult meal skipping (19). Smith et al. (5) reported that skipping breakfast over an extended period time may be associated with cardio-metabolic health.

Van der Heijden et al. (20) found the habit of eating breakfast contributed to the prevention of weight gain. A possible explanation is a higher insulin stimulus of hydroxyl methyl glutaryl Co-A (HMG-CoA) reductase. Compared with participants who ate breakfast, those who skipped breakfast had higher fasting insulin concentrations and, therefore, might have higher HMG-CoA reductase (5). Through these possible mechanisms, skipping breakfast might induce higher LDL cholesterol and, therefore, atherosclerosis. Furthermore, recent studies found that skipping breakfast clusters were associated with risk factors of hypertension such as smoking and lower levels of physical activity (21).

According to WHO (2016), a healthy diet includes the consumption of at least five portions of fruits and vegetables a day. In relation to this recommendation, the intake of fruits and vegetables in our sample was quite low with less than 30% of all students reporting to eat fruit and vegetables several times a day. This finding aligns with other studies focusing on university students from various countries: the United States (22), Spain (23), Italy (24), and Germany (25). In accordance with other studies (24), males consumed fast food, red meat, poultry, sausages, hard/soft cheeses, side dishes like pasta/rice, fried potatoes/chips and meat products more often than females did. Males also consumed fruits and vegetables less often than females did. Reasons for such gender differences could be a generally higher health awareness (24), better nutrition knowledge (26), and better knowledge about what constitutes a "healthy diet" (22) among females.

A previous study conducted among 479 Swedish university students found that female students had healthier habits than male students, despite being stressed, whereas male students showed a high level of overweight and obesity and were less interested in nutrition advice and health-enhancing activities (26). Also, the authors reported that female students were more interested in changing their dietary habits than male students were (26). It has also been shown that men give lower priority to health compared to other considerations, such as taste and convenience, in making their food choices (27) and that they feel more ambivalent about healthy dietary choices (28).

The International Health and Behaviour survey (IHBS) examined a range of health behaviours in a total of 19298 university students from 23 different countries using a study approach based on a self-report questionnaire (29). In almost all 23 countries, a higher percentage of women reported avoiding high fat-foods, eating fibre-rich foods, and eating fruit daily. In comparison, males have a higher energy intake, and a higher percentage of the energy in males' diets is derived from animal products (13). However, men reported liking fruit slightly more than women liked fruit. However, there was no significant gender difference in attitudes towards fruit and vegetables (29).

The females within our sample reported eating chocolate more frequently than the males did. Previous studies also reported that female students consumed sweet foods more frequently than their male counterparts (22). They found an association between such eating habits and higher levels of perceived stress in female students (22). Also, women are more often affected by the problem of craving (i.e., the strong desire for certain foods) than men, with the women more likely crave sweet foods. Extensive research showed that women often experience the so called "carbohydrate craving" and there is an association between the wish for sugar- and fat-rich foods (like chocolate and other sweets) and menstrual cycle (30).

Problems with eating behaviour have a strong female prevalence emerging in childhood and adolescence (31). Girls often eat less and pay attention to calories, sugar, and fat intake under the pressure of "feeling obliged" to be slim (31). Consequently, in part due to a specific social pressure, girls are more likely than boys to develop eating disorders (i.e., anorexia, bulimia, binge eating disorder) (31). Among school children, girls were found to consume much less energy than boys and have reduced micronutrient intakes (31).

In our study, males' BMI showed higher incidence of being overweight, higher triglycerides and higher LDL cholesterol than women did, and the blood lipids showed positive correlation with males' BMI. Chang et al. (10) reported similar results; part of the reasons for higher triglycerides and LDL-cholesterol levels in male students was the higher rate of obesity among males. It has been reported that higher obesity rate was related to increased blood triglycerides and total cholesterol and decreased HDL-cholesterol (17) in male college students (10).

Most researchers now agree that being overweight increases the risk of developing chronic diseases and premature death (32). In comparison with normal weight individuals, overweight individuals have 4.7 times higher probability of suffering from metabolic syndromes, which can lead to abdominal obesity, high serum triglycerides, low HDL cholesterol, and elevated plasma glucose (33). Both lipid profile and BMI have been shown to be important predictors for hypertension, diabetes, and cardiovascular diseases (33), and clinical data indicate that elevated levels of total cholesterol, triglycerides and low-density lipoprotein are risk factors for cardiovascular events (34).

Consequently, male students could possibly have higher risks for chronic disease incidence such as hyperlipidaemia and hypertension. It is well established that obesity or being overweight is often associated with increased plasma TG and decreased HDL-cholesterol concentrations. Women typically have lower plasma TG and higher HDL-cholesterol concentrations than men (35), as our results confirm. Several authors have demonstrated that oestrogen may have an agonistic effect on peroxisome proliferator-activated receptor (PPAR) activity and thus may underlie gender differences in lipid homeostasis (36). It has been reported that men may have higher insulin resistance than women, possibly due to more visceral and hepatic adipose tissue in the setting of lower oestrogen levels, which may be protective, as well as lower adiponectin levels (37).

In our study, we have found the correlation between Tchol, LDL-chol, TG and BMI in males. We also found HDL-chol was correlated with BMI in females. The researchers analysed the relationship between BMI categories and 16 commonly tested blood biochemical indicators in low-income female population (38). It is interesting to note that, along with increasing BMI, the levels of 11 bio-indicators show a trend of linear increase, including TG, LDL-cholesterol, Tchol, and glucose, but negatively with the levels of 5 bio-indicators including HDL cholesterol (38). These results help focus our data where BMI was overweight in males and correlated with Tchol, LDL-chol and TG values in comparison to women, where HDL-chol was correlated with BMI.

Importantly, the effect of obesity on the lipoprotein profile is evident in the absence of clinically significant imbalances in plasma glucose and lipid homeostasis, and is qualitatively the same in men and women; the females' and maless chronic heart disease risk advantage is largely related to the traditional lipid risk factors (e.g., plasma TG and HDL-cholesterol concentrations) because the differences between men and women in lipoprotein particle concentration is comparably minor (35).

In conclusion, this study demonstrated that the main barriers to healthy eating were identified as skipping breakfast and deficiencies in the consumption of specific food groups, such fruits and vegetables as recommended by WHO. With respect to gender, differences in healthy eating were found in the consumption of meat and regular meals. This study demonstrated, that the degree of being overweight ~~obesity~~, triglyceride levels, and LDL cholesterol levels were higher in males than in females, suggesting possible association with chronic disease incidence such as hyperlipidaemia and hypertension among males.

The findings of this study can inform health promotion strategies in the university setting. Students, faculty, staff, and administrators can work together to make meaningful changes within the university environment. Nutrition educators can partner with dining service personnel to provide guidance for students when making food choices. They can also write articles for campus newspapers addressing barriers and enablers to healthful dietary practices, including quick recipes. Educators with expertise in nutrition or physical activity can partner to develop service learning projects for students to share with their peers ways to overcome barriers and facilitate enablers to healthful dietary practices.

Researchers may want to explore the differences in barriers and enablers for maintaining a healthful diet based on whether students subscribe to meal plans and live on or off campus. Also, they can create opportunities as well as motivate students regularly to make test of lipids in the blood, not only in medical institutions, but also on campus, or even in areas where the studies may take place.

Limitations

Our study aimed to describe the baseline dietary intake in Lithuanian university and college students, identify gender-specific differences to healthy eating, and explore the relationship between lipoproteins and anthropometric data. We found that the main barrier for healthy eating was skipping breakfast. Gender-specific eating behaviors were found in the consumption of meat and the regularity of meals. Finally we concluded that the degree of being overweight and concentrations of triglycerides, and LDL cholesterol were higher in males than in females. The knowledge of this work is important to promote optimal health strategies in high education institutions.

Nevertheless, our study has some limitations. First, due to the cross-sectional design of our study,

no causal relationships can be drawn from our data. In addition, the study recruited university students from across Lithuania. Therefore, we cannot rule out the possibility of a participation bias, which may limit the generalizability of our results. Furthermore, due to the self-reported variables, reporting bias and recall bias may have occurred.

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Effects of nutrition education on adipocytokines levels in cord blood at birth

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Summary. *Background/aims:* Adipocytokines play a role in intrauterine growth but the effects of the nutrition education on adipocytokines and physical measurements at birth remains unclear. The aim of this study was to evaluate effects of nutrition education on adipocytokines levels in cord blood and physical measurements at birth. *Methods:* The present study involved 52 pregnant women who were in pregnancy followed up in Gazi University Medical School Hospital at Ankara, Turkey. They were randomly divided into two groups and experimental group involved in nutrition education. *Results:* There was a positive association between the cord blood leptin values of the both groups. Nutrition education had no significant effect on cord blood adiponectin, visfatin and IL-6 values of the newborns. Also there was not a significant difference between the average values of weight and the head circumference of newborns in both groups. In experimental group, the average birth length was observed to be higher than that of control group. *Conclusion:* Nutrition education might improve leptin levels of the newborns in the experimental group. In the lights of these results, it may be suggested that higher cord blood leptin levels could play an important role in higher birth length values of the newborns.

Key words: nutrition education, pregnancy, adipocytokines, newborns, anthropometry, birth

Introduction

Pregnancy is an occasion when women become more aware of the importance of healthy nutrition. A healthy and balanced "diet" during pregnancy is one of the most significant components affecting both the health of the mother and the health of the fetus. Since it is essential for a better pregnancy outcome pregnant women should receive nutrition education from their healthcare professionals during pregnancy. Nutrition education programmes are important as enhancing subject dietary intakes by promoting behavioral changes such as food choice and cooking ability. The maternal diet must provide sufficient energy and nutrients for the mother's usual requirements and the needs of the growing fetus (1-4). The prenatal life is a time of rapid cellular growth and replication and of functional maturation of organs in the intrauterine milieu. In pregnancy physiological adaptations are aimed to facilitate delivery of nutrients to the fetus. Since the discovery of adipocyte-derived hormones, commonly called adipocytokines, the adipose tissue is no longer considered an inactive fat store tissue, but an endocrine organ, secreting a variety of biological molecules, which regulate energy homeostasis as well as metabolism and inflammation in pregnant and non-pregnant women. In addition, adipocytokines have been recently implicated in fetal growth (5,6). Fetal growth is governed by multiple factors.

In pregnancy, adiponectin, leptin, visfatin and interleukin-6 (IL-6) like adipose tissue markers affect

intrauterine metabolism (7,8). Adiponectin is an adipocyte-secreted hormone that modulates a number of metabolic processes, including glucose regulation and fatty-acid oxidation, and has an insulin-sensitizing effect. The umbilical cord blood adiponectin levels found to be positively associated with birth weight and fat mass. In addition to adiponectin, another adipocytokine, leptin is also found to be related to other physiological conditions beyond appetite control and obesity. Leptin has recently been proposed as a biomarker of fetal adiposity and provide a biological link for the fetal programming of later adult metabolic health. Adiponectin and leptin are present in cord blood and the positive correlation of their concentrations with neonatal birth weight as well as the high production of these adipokines may play important roles in fetal development (9-11). Visfatin is a protein that is produced in adipose tissue. Both tissue and plasma levels of visfatin increase in obesity. Moreover, circulating visfatin concentrations were shown to increase in hyperglycemia. It has insulin-mimetic effects and lowers plasma glucose levels (12,13). Cord blood IL-6 levels describe the intensity of the fetal inflammatory response. It has recently been shown that cord blood levels of IL-6 is of fetal origin and do not reflect maternal or placental cytokine production (14,15).

The effects of nutrition education on adipocytokines and physical measurements at birth remains unclear. The aim of this study was to evaluate effects of nutrition education on adipocytokines levels in cord blood and physical measurements at birth.

Methods

This was a randomized controlled trial conducted at Gazi University Medical School Hospital at Ankara, Turkey. 96 pregnant women who were in pregnancy followed up in Department of Gynecology and Obstetrics of Gazi University Medical School Hospital were assessed for eligibility. The mothers whose pregnancies are not older than 8 weeks, who have no diabetes, hypertension, acute or chronic diseases, who does not smoke, use alcohol or drugs were included in the research. Of the 32 women who met the inclusion criteria but refused to participate in this study. On the other hand 12 women had chosen to give birth in other hospitals or obstetrics clinics. In total, 52 pregnant women were randomized to the study. They were randomly divided into two groups: 26 and 26 pregnant women were included in the experimental and control groups, respectively. All the pregnant women were Turkish and they didn't have a migration background. In addition, they had an educational level of high school or higher. All women received oral and written information and signed an informed consent before entering the study. The control group did not receive dietary advice. Experimental group received dietary advice. The first study visit took place during the first trimester (pregnancy weeks 8–12). Follow-ups were done in the second trimester (pregnancy weeks 24–26) and the third trimester (pregnancy weeks 35–37).

Dietary advice was provided by trained dietitians throughout face to face interviews and dietitians followed up of the pregnant women during their pregnancy. Newborn infants born in Gazi University Medical School Hospital between October 2010 and March 2011 by normal, vaginal delivery and spontaneous were enrolled in this study. Newborns born with infection, thyroid, bone, renal, diabetes and gastrointestinal disorders were excluded. This study includes all births from 37 to 42 weeks of gestation. After birth, totally cord blood of 52 infants of mothers from both groups were taken and information related to height, weight and head circumferences of the infants were recorded.

All woman participants gave informed consent for themselves and their infants. The study was approved by the Gazi University Medical Faculty Ethic Commitee was received and informed consent was obtained before the blood samples were taken. The study was carried out in compliance with the Helsinki Declaration. This work was supported by Gazi University, Scientific Research Projects Committee '[08/2010-09]' (Figure 1).

Dietary Intervention

Participants received dietary counseling on the day of their visit to Gazi University Hospital. Dietary advice focused on enhancing the quality of the diet, by educating women on which foods and what quantities they need to consume in order to achieve the dietary intake. The recommended energy intake for pregnant women is: no additional energy requirement in the first trimester, 340 kilocalories (kcal) extra energy per day for the

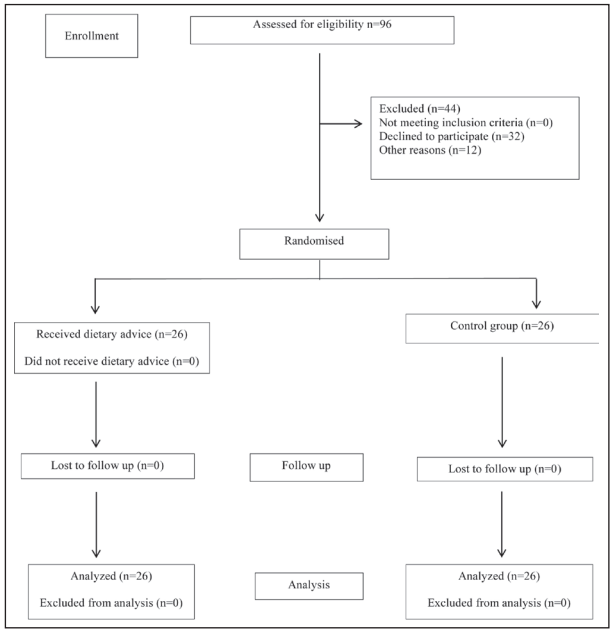

Figure 1. Participant flow in CONSORT-recommended form.

second trimester and 450 kcal extra energy per day for the third trimester. The recommended daily protein intake in the first trimester is 45 g per day (0.75 g/kg/day). In the second and third trimesters the required protein intake for pregnant women is 60 g per day (1.00 g/kg/day). They were advised to consume two meals of fish per week. The recommended carbohydrate intake is 170 g and dietary fibre intake is 20 g per day. Dietary intake of linoleic acid is 10 g per day, 1 g per day a-linolenic acid, and 110 mg per day total eicosapentaenoic acid, docosahexaenoic acid. The recommended daily intake of milk or yogurt is 500 g. In addition to these, all participants received advice on dietary sources of iron, calcium, zinc, copper, selenium, magnesium and iodine.

The participants were encouraged to consume of variety of fruits and vegetables each day by reducing their intake of sugar sweetened soft drinks and fruit juices. Diet quality was individually adjusted as needed, and counseling was also given on fat quality, food frequency, fibre intake, and nutrient density according to the Dietary Guidelines of Turkish Ministry of Health. After their first visit, women were repeatedly (twice a week) called by telephone to remind them of the recommendations and were followed-up the second and third trimesters.

Anthropometric Measurements

All the measurements were done while the newborn has been lying supine on the examining table and on the right side of its body. Each baby was measured 30 minutes after being feed. Birth weight (gm), length (cm), head and circumference measurements (cm) were recorded. Newborn anthropometric measurements were obtained within 24 hours of delivery.

Biochemical Analysis

Cord blood which was placed into biochemical tubes for the analysis were centrifuged for 5 minutes at 3000 RPM and plasma and serum were separated and placed in Eppendorf tubes and were kept at -80 ° C until the day of analysis in the Department of Biochemistry. SIGMA (6K15) laboratory type centrifuge and BIOTEK (microplate reader/Snergy HT) ELISA (Enzyme-Linked Immuno Sorbent Assay) Analyzer were used for analysis of the cord blood samples. Determination of leptin and IL-6 were performed using Immunoenzymetric assay kits (DIAsource - Louvain-la-Neuve, Belgium), while adiponectin levels were measured with commercial ELISA kit (enzyme immunoassay kit - Biovendor (Brno, Czech Republic). Visfatin levels were measured by Visfatin C-Terminal (Human) Enzyme Immunoassay Kit (Phoenix Pharmaceuticals, CA, USA), according to the manufacturer's instructions.

Statistical Analysis

Statistical analysis was performed with SPSS version 19.0 (SPSS Inc., Chicago, IL, USA). Clinical and anthropometrical data of the study populations are given as means ± SD. To check the normality of the data distribution Kolmogorov-Smirnov test and Shapiro-Wilk test was performed. Differences in the means of variables were tested using both parametric and nonparametric tests depending on the distribution of the variables.

In order to detect the differences between the groups Paired samples t-test and Mann-Whitney U test were used. For all statistical analysis p-values below 0.05 and 0.01 were considered significant.

Results

The baseline characteristics and measurements are presented in Table 1. The mean maternal age of women were 29 ± 2.9 (years) in the control and 29 ± 3.1 (years) in the experimental group. Maternal Body Mass Index (BMI) was calculated during the first trimester (pregnancy weeks 8–12). Follow-ups were done in the second trimester (pregnancy weeks 24–26) and the third trimester (pregnancy weeks 35–37).

Mean birthweight, length and head circumference values of the newborns in the control group were 3590 ± 322 gm, 52 ± 2.1 cm and 34.8 ± 1.2 cm respectively. While in the experimental group, mean birthweight, length and head circumference values of the newborns were 3870 ± 319 gm, 53 ± 3.3 cm and 33.6 ± 1.8 cm respectively (Table 1).

Table 2 shows that, cord blood adiponectin average values of the experimental group (31,446 ± 1,6446 μg/ml) were higher than average values of the control group (30,023 ± 1,6007 μg/ml). However, there was no significant difference between the cord blood adiponectin values of the experimental and control groups (p>0.05). Average leptin values determined in the experimental group (4,3227 ± 0,9262 ng/mL) were higher than the control group values (2,6881 ± 0,56056 ng/mL). There was a positive association between the cord blood leptin values of the experimental and control groups (p<0,05). Average visfatin values of the experimental group (4,031±0,2939 ng/ml) were slightly lower than the values of the control group (4,123 ± 0,3321 ng/ml). But, there was no significant association between the cord blood visfatin values of the experimental and control groups (p>0,05). IL-6 average values (9,127 ± 1,3408 pg/ml) of the experimental group were lower than the average values of the control group (23,538 ± 16,397 pg/ml). There was no significant association between the cord blood IL-6 values of the control and experimental groups (p>0,05). Except for the leptin values, nutrition education during pregnancy had no significant effect on cord blood adiponectin, visfatin and IL-6 values of the newborns.

Table 1. Maternal and infant characteristics

Characteristics	Control (n=26)	Experimental (n=26)
Maternal age (years)	29 ± 2.9	29 ± 3.1
Early-pregnancy BMI (kg/m2)	23.1 ± 3.8	23.0 ± 4.2
Mid-pregnancy BMI (kg/m2)	25.6 ± 3.9	25.8 ± 3.8
Late-pregnancy BMI (kg/m2)	27.9 ± 4.4	28.1 ± 4.2
Parity		
0	12 ± 3	11 ± 2
1	10 ± 3	11 ± 3
≥2	4 ± 4	4 ± 2
Infants		
Female/male	11/15	14/12
Birthweight (gm)	3590 ± 322	3870 ± 319
Length (cm)	52 ± 2.1	53 ± 3.3
Head circumference (cm)	34.8 ± 1.2	33.6 ± 1.8
Gestational age at delivery (weeks)	38.1 ± 2.4	39.0 ± 2.1

Data are medians (SDs).

In Table 3, it is seen that the average birth weights of newborns in the experimental group (3295,63 ± 62,492 gm) was slightly higher than the average birth weights of newborns in the control group (3230,02 ± 65,232 gm). The results indicate that there was not a significant difference (p>0,05) between the average weights of newborns in control and experimental groups. In the experimental group, the average birth length (49,771 ± 0,2879 cm) was observed to be higher than that of control group (48,604 ± 0,2675 cm). In other words, on average, the newborns in the experimental group were taller than the newborns in the control group. The statistical analyses proved that there was a significant difference (p<0,01) between the birth lengths of the newborns in the experimental group and those in the control group.

The head circumference average of newborns in the experimental group (34,156 ± 0,1349 cm) was seen to be higher than the average head circumference measured in the control group (33,781 ± 0,2068 cm). However, statistical analyses showed that the head circumference rates did not differ significantly (p>0,05) between control and experimental groups.

Table 2. Comparison of cord blood adipokines values between the groups

	Groups	N	X	Std. Error	min	max	p
Adiponectin, (μg/ml)	Experimental	26	31,446	1,6446	17,4	45,5	,538
	Control	26	30,023	1,6007	15,5	45,5	
	Total	52	30,735	1,1406	15,5	45,5	
Leptin, (ng/mL)	Experimental	26	4,3227	,69262	,79	13,00	,035*
	Control	26	2,6881	,56056	,60	14,30	
	Total	52	3,5054	,45573	,60	14,30	
Visfatin, (ng/ml)	Experimental	26	4,031	,2939	2,6	8,1	,811
	Control	26	4,123	,3321	2,6	9,6	
	Total	52	4,077	,2197	2,6	9,6	
IL-6, (pg/ml)	Experimental	26	9,127	1,3408	1,9	27,8	,735
	Control	26	23,538	16,397	2,4	433,0	
	Total	52	16,333	8,2072	1,9	433,0	

*Mann-Whitney U test was conducted. *p<0,05*

Table 3. Comparison of anthropometric measurements between the groups

	Groups	N	X	Std. Error	min	max	p
Birth weight (gm)	Experimental	26	3295,63	62,492	2410	4180	,470
	Control	26	3230,02	65,232	2210	4030	
	Total	52	3262,82	45,055	2210	4180	
Birth Length (cm)	Experimental	26	49,771	,2879	46,0	53,0	,004**
	Control	26	48,604	,2675	44,0	52,0	
	Total	52	49,188	,2044	44,0	53,0	
Head circumference (cm)	Experimental	26	34,156	,1349	32,5	36,5	,132
	Control	26	33,781	,2068	30,5	37,0	
	Total	52	33,969	,1243	30,5	37,0	

*Mann-Whitney U test was conducted. **p<0,001; *p<0,05*

Discussion

Pregnancy is characterized by endocrine and metabolic maternal adaptations including increase in weight, body fat mass, and insulin resistance. These changes are physiological adaptations necessary to meet the energy demand of the fetus and prepare the maternal organism for delivery and lactation. A group of cytokines, collectively known as adipocytokines, have endocrine and paracrine effects. The most studied adipokines are leptin, adiponectin, resistin, IL-6 and visfatin. Markedly high levels of adipokines have been detected in umbilical plasma, hence, suggesting a possible role on fetal development and metabolism (16).

Good nutrition during pregnancy is one of the most significant components affecting both the health of the mother and the health and development of their unborn babies. Poor maternal nutrition has been linked with poor infant outcomes. These include inadequate development, low birth weight and an increased risk preterm birth or even miscarriage. Also poor maternal nutrition associated with maternal excess weight gain, pre-eclampsia, increased risk of developing chronic diseases (17).

Systematic review and meta-analysis of 34 studies providing nutrition education with and without nutrition support in the form of food baskets, food supplements or micronutrient supplements found that nutrition education improved gestational weight gain, reduced the risk of anemia in late pregnancy, increased birth weight and lowered the risk of preterm delivery [18]. According to Ota et al., nutrition advice improves nutrient intakes during pregnancy, reduces the risk of preterm birth by 54% and increases head circumference at birth [19]. It has been demonstrated that cord blood concentrations of leptin and adiponectin are associated with body mass index and adiposity in neonates (20).

In 2004, Tsai et al. examined the relationships between cord plasma concentrations of adiponectin and leptin in healthy term neonates. They found that, adiponectin and leptin levels were positively correlated with birthweight and adiposity. They suggested that these adipocytokines positively associated with intrauterine growth [21]. In 2009, Cekmez et al. examined the relationship between adiponectin, visfatin, insulin and birthweight at birth in healthy term infants. They found that, cord plasma adiponectin and visfatin levels were positively correlated with birthweight. They also suggested that, adiponectin and visfatin might be involved in regulating fetal growth (22). Conversely we did not find a significant association between adiponectin and visfatin values of the both groups.

IL-6 during pregnancy mainly due to placental production and has been related to pregnancy-associated insulin resistance. Cord blood IL-6 could be related with hypoxia, nutrient deficiency and early-onset neonatal sepsis (23-25). Moreover, high cord blood IL-6 concentrations in premature infants with fetal inflammatory response syndrome were found to be predictive for respiratory distress syndrome and death (26). In our study, cord blood IL-6 levels were found to be lower in experimental group with respect to control group. Nutrition education during pregnancy might be responsible for low IL-6 levels in the cord blood. In 2011, Nakano et al. conducted a study on 52 Japanese newborns. They found a positive relationship between cord plasma leptin levels and anthropometry. Higher levels of leptin appeared to correlate with an increased amount of fats accumulated in the fetus, resulting in higher BMI. However they did not find any association between cord adiponectin levels and anthropometry. They concluded that, leptin was one of the key hormones for controlling fetal lipid metabolism in addition to fetal growth (27). In another study, Kahveci et al. found a positive correlation between newborn leptin levels and anthropometrics. They suggested that leptin could play a role in newborn growth and development (28).

In our study, we found a positive association between leptin and birth length levels of the newborns whose mother received nutrition education during their pregnancy. Accordingly, it can be said that the nutrition education during pregnancy is effective on leptin values and leptin plays an important role in the process of fetal growth. A major limitation of this current study is the relatively small sample size. The relatively small sample size decreased the ability to detect significant differences across groups.

Conclusion

In conclusion, we showed that cord plasma leptin and birth length values of the newborns in the experimental group were significantly higher than the control group. Taken together these findings suggest that nutrition education might improve leptin levels of the newborns in the experimental group. In the lights of these results, it may be suggested that higher cord blood leptin levels could play an important role in higher birth length values of the newborns. Further studies are needed to better understand whether these adipocytokines are differentially regulated in the course of pregnancy.

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Nutritional and lifestyle habits of European pharmacy undergraduate students

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Summary. *Aim:* Balanced nutrition and healthy lifestyle habits are very important, especially in young population. By this way it is possible to prevent many non-communicable diseases. As a health care professionals, pharmacists have very important role in this mission. The aim of this study was to evaluate basic nutritional knowledge and lifestyle habits of pharmacy undergraduate students in the context of their future health profession. *Methods:* The study group consisted of 591 European undergraduate pharmacy students. The data related to anthropometry, eating and lifestyle habits were obtained based on self-administered cross-sectional survey. Analysis of gender differences was performed using the chi-square test. Statistically significance was set at p value < 0.05. *Results:* Anthropometric characteristics of students showed that 10.5 % female students were underweight, while 62.6% of the males were overweight and 0.7% were obese. Regular breakfast had 80.9% of the students. Breakfast skipping was statistically higher in male participants ($p<0.05$). Only 35% of students reported daily intake of vegetables. Also, low fruit consumption was observed in all students with statistically lower fruit intake in males ($p<0.05$). Consumption of alcohol, fried food and tobacco was not common among students. Basic principle of balanced nutrition was recognized by 58.4% of study population. *Conclusion:* Results of our study indicate that European pharmacy students have some unsatisfactory eating habits and nutritional knowledge which is already related to their inadequate nutritional status. Our finding suggests that increased level of nutritional education should be incorporated into European pharmacy curriculum.

Key words: Nutritional status, lifestyle habits, diet, European pharmacy students, nutritional education

Introduction

In European modern society, nutrition evolves from under-nutrition to over-nutrition (1). Data suggest that obesity has become a global epidemic disease, and it is the fastest growing form of malnutrition in developed and developing countries (2). Improper nutritional habits and physical inactivity contribute to overweight which can later cause obesity, which has proved to be an independent risk factor for many diseases such as certain cancers, type II diabetes mellitus and coronary heart disease (3, 4).

The student population is the important target for the promotion of healthy lifestyles, including healthy nutrition. Several studies indicated that University students make various mistakes in their nutrition. The most common reported nutritional faults observed among students include: consumption of snacks between meals, avoiding main meal, fast food consumption, night snacking, unhealthy weight loss diets (5). Beside unhealthy dietary behavior, there was evidence of low physical activity in student population (6). There have been various studies on the nutritional status, and eating habits among the non-European university students (7-9), and European students (10-14), however data on dietary patterns focused on pharmacy students are limited.

Nutritional knowledge of pharmacy students as future health professionals is particularly important. Pharmacists, especially those working in community

pharmacy, could provide various public health services. Pharmacist in some cases is the first and only health-care professional which can give advice regarding self-care to the patient. Activities of pharmacists evaluated by Pharmaceutical Group of European Union (PGEU) showed that European (EU) community pharmacists play important role in self-care support of patients, including counseling about selecting appropriate over-the-counter medication; healthy lifestyle; nutritional counseling regarding the type and quality of food eaten and selection of dietary supplements (15). Also, pharmacists can benefit by participating in educational programs aimed at public health promotion (16).

Nutritional knowledge of pharmacy students as future health professionals is important. Unfortunately, nutrition-related courses are incorporated in undergraduate programs of pharmacy studies among European countries in various degree so it could be expected that understanding of basic dietary recommendations in health and disease also varies. Some good examples of nutritional courses incorporated in pharmacy curriculum can be observed in Eastern Europe (17, 18), South European countries (19) as well as in Western Europe (20).

Since dietary habits of pharmacy students in Europe have rarely been determined, the primary purpose of this study was to evaluate eating and selected lifestyle habits among European undergraduate pharmacy students, including the usage of dietary supplements. One of the goals of this study was also to test the hypothesis if pharmacy students adopt healthy nutritional and lifestyle habits since it is expected that this population is better informed about their importance.

Methods

Design and Participants

The cross-sectional study was carried out between April and November 2012. during the Annual Congress of European Pharmaceutical Student Association (EPSA) in Istanbul, Turkey, International Pharmaceutical Federation (FIP) World Centennial Congress of Pharmacy and Pharmaceutical Sciences in Amsterdam, The Netherlands, and EPSA Autumn Assembly in Sofia, Bulgaria. Students from non-European countries were excluded and a total number of 591 undergraduate pharmacy students took part in this investigation. All participants were asked to sign informed consent according to the Declaration of Helsinki. This study received approval from the Ethic Committee for Human Research of Faculty of Pharmacy, University of Belgrade.

Data Collection

A self-reported height and weight data were used to calculate body mass index (BMI) as the ratio of body mass in kg and the square of height in meter (kg/m^2). Students were classified as overweight if their BMI was 25-29.99 kg/m^2 and obese with BMI ≥ 30 kg/m^2, as well as underweight with BMI <18.5 kg/m^2 (21). Eating habits of students were evaluated with a self-administered questionnaire which was adopted from previous studies (22, 23). The questionnaire was firstly used and standardized on University students from Japan and South Korean population by Sakamaki et al. (22). This questionnaire was also used in the study conducted by Yahia et al. (23) without standardization for Lebanon students since it contains general questions which are not country specific. The questionnaire consisted of 11 closed questions, divided into three sections. Four questions examined meal frequency; two questions examined fruit and vegetable intake. Next four questions explored lifestyle habits including fried food and alcohol consumption, smoking history, and social interaction during meals. One question was used to estimate the basic knowledge of students regarding balanced nutrition. To the original questionnaire, additional question was added to dietary supplements usage among pharmacy students. Anyone who reported currently taking, at least one dietary supplement, as well as using it one month previously, was defined as a dietary supplement user.

Statistical Analysis

The statistical analysis was performed using SPSS 18.0 for Windows (Chicago, IL, USA) software. Data are presented as the means and standard deviations for quantitative data and as frequencies for category data. Non-parametric variables were analyzed using the chi-square test. All reported p values were made on the

basis of two-tailed tests. Differences were considered statistically significant at p value < 0.05.

Results

Demographic and anthropometric characteristics of the study participants are presented in Table 1. This study included 591 European pharmacy students, 305 women and 286 men, the mean age 24.09 ± 2.26. Average weight and height of female study population were 58.68 ± 4.67 kg and 168.8 ± 4.8 cm, while the male population had average 84.91 ± 7.95 kg and 182.5 ± 6.3 cm. The average BMI of female students was 20.63 ± 1.84 kg/m^2 while for male students it was 25.52 ± 2.28 kg/m^2. Based on the classification of nutritional status by BMI, most the students were normal weight (63.1%). Among female students 10.5 % were underweight, 88.2 % were normal weight, while overweight and obese counted only 1.3%. Results for the nutritional status among male students were different, none of them were underweight, about two-thirds (62.6%) of the male students were overweight, and only 1% was obese. In general, obesity was observed in only 0.7% of the study population, an overweight problem was noticed in 30.8%, and undernutrition in 5.4% of the total study population.

The results of lifestyle and eating habits investigation among the study population are shown in Table 2. Obtained results were analyzed and compared by gender. Only half of students (55%) reported having regular meals during the day. Obtained results also revealed that majority of pharmacy students had three meals during the day (60.7%) and two meals (28.3%), without statistically significant difference between the genders. The regular breakfast habit was reported by 80.9% of the students, where statistically significant difference among genders was noticed (p=0.04). The habit of skipping the breakfast was more pronounced in male pharmacy students. Beside main meals, the snack consumption was also reported by the students. The frequency of daily snacking among students was 19.3%, while 40.9% consumed snacks between meals once or twice per week, with no gender differences.

The results of this study suggested an inadequate daily intake of colored vegetables among European pharmacy students. This is supported by the result that only 35% of the students ate colored vegetables daily while 23.4% students ate colored vegetable only once or twice per week. Low daily fruit consumption was also observed in study population because only 34% of students consumed fruit daily. While there was no gender difference in reported vegetable consumption, daily intake of fruits was statistically higher in female students (p< 0.05). Concerning fried food consumption, the results showed that more than 80% of pharmacy students rarely or 1-2 times per week use this type of food but the result that 6% of students reported eating fried food every day is of concern.

Two lifestyle habits, alcohol and tobacco consumption, were recorded. According to the obtained

Table 1. General Demographic and Anthropometric Characteristics of the Study Group (n=591)

	All	Female	Male	P
Number, n (%)	591	305 (51.6)	286 (48.4)	-
Age, years	24.09 ± 2.26	23.74± 2.17	24.46 ± 2.31	-
Weight, kg	71.37 ± 14.62	58.68 ± 4.67	84.91 ± 7.95	-
Height, cm	175.4 ± 8.8	168.8 ± 4.8	182.5 ± 6.3	-
BMI, kg/m^2	22.99 ± 3.19	20.63 ± 1.84	25.52 ± 2.28	-
Underweight, n (%)	32 (5.4)	32 (10.5)	-	P < 0.001*
Normal, n (%)	373 (63.1)	269 (88.2)	104 (36.4)	P < 0.001*
Overweight, n (%)	182 (30.8)	3 (0.98)	179 (62.6)	P < 0.001*
Obese, n (%)	4 (0.7)	1 (0.33)	3 (1.0)	P < 0.001*

*Significant differences between gender were determined by Chi-square analyses (*P< 0.05)*

Table 2. Lifestyle and Eating Habits Among the Study Participants (n=591)

Questions	Levels	Total		Female		Male		P
		n	%	n	%	n	%	
Do you take your meals regularly?	always regular	325	(55)	171	(56.0)	154	(53.8)	0.62
	irregular	266	(45)	134	(43,9)	132	(46.1)	
Do you always take breakfast?	daily	478	(80.9)	260	(85.2)	218	(76.2)	0.04*
	three or four times per week	75	(12.7)	29	(9.5)	46	(16.1)	
	once or twice per week	21	(3.6)	9	(3)	12	(4.2)	
	rarely	17	(2.9)	7	(2.3)	10	(3.5)	
How many times do you eat meals except snacks during the day?	one time	29	(4.9)	14	(4.6)	15	(5.2)	0.92
	two times	167	(28.3)	87	(28.5)	80	(28)	
	three times	359	(60.7)	187	(61.3)	172	(60.1)	
	four times	36	(6.1)	17	(5.6)	19	(6.6)	
How often do you take snacks apart from regular meals?	daily	114	(19.3)	56	(18.4)	58	(20.3)	0.94
	three or four times per week	49	(8.3)	26	(8.5)	23	(8.0)	
	once or twice per week	242	(40.9)	126	(41.3)	116	(40.6)	
	rarely	186	(31.5)	97	(31.8)	89	(31.1)	
How often do you eat green, red or yellow colored vegetables?	daily	207	(35)	110	(36.1)	97	(33.9)	0.55
	three or four times per week	173	(29.3)	90	(29.5)	83	(29.0)	
	once or twice per week	138	(23.4)	73	(23.9)	65	(22.7)	
	rarely	73	(12.3)	32	(10.5)	41	(14.3)	
How often do you eat fruits?	daily	203	(34.3)	115	(37.7)	88	(30.8)	0.02*
	three or four times per week	195	(32.9)	90	(29.5)	105	(36.7)	
	once or twice per week	142	(24.0)	67	(22)	75	(26.2)	
	rarely	51	(6.9)	33	(10.8)	18	(6.3)	
How often do you eat fried food?	daily	38	(6.4)	19	(6.6)	19	(6.3)	0.70
	three or four times per week	70	(11.8)	31	(10.5)	39	(13.3)	
	once or twice per week	172	(29.1)	90	(30.5)	82	(27.6)	
	rarely	311	(52.6)	165	(52.5)	146	(52.8)	
How often do you take alcohol?	never	159	(26.9)	90	(29.5)	69	(25.1)	0.01*
	two or three times per week	135	(22.8)	55	(18.0)	80	(27.9)	
	rarely	297	(50.3)	160	(52.5)	137	(47.9)	
How often do you eat with friends and family?	daily	334	(56.5)	177	(56.1)	157	(57)	0.70
	three or four times per week	199	(33.7)	101	(32.8)	98	(34.6)	
	once or twice per week	47	(8.0)	20	(9.2)	27	(6.6)	
	always alone	11	(1.9)	7	(2.0)	4	(1.7)	
Please state your smoking history	current smoker	41	(6.9)	25	(8.2)	16	(5.6)	0.25
	non-smoker	550	(93.1)	280	(91.8)	270	(94.4)	
What type of food do you think you should eat to have a balanced nutrition?	mainly meat	96	(16.2)	40	(13.1)	56	(19.6)	0.06
	mainly vegetable	110	(18.6)	63	(20.7)	47	(16.4)	
	meat, vegetable and other variety of food	345	(58.4)	185	(60.7)	160	(55.9)	
	others	40	(6.8)	17	(5.6)	23	(8.0)	

*Significant differences between gender were determined by Chi-square analyses (*P< 0.05).*

results, daily consumption of alcohol was not common among students. A half of studied population (50.2%) reported rare alcohol drinking, while 26.9% of students never drink. As expected, the alcohol consumption was statistically significantly higher in the male compared to the female students ($p< 0.05$). Also, consumption of tobacco was not common among European pharmacy students. The most of the students reported being non-smokers (93.1%). More than half of participating students (56.5%) eat daily with friends and family without a difference in gender. Interestingly, the simple question regarding balanced nutrition was answered correctly only by 58.4% students.

In Table 3. Results regarding dietary supplements were presented. It was found that 19.3% of the students reported their use, with vitamins and minerals supplements as the most commonly used.

Discussion

Investigation of dietary habits and nutritional knowledge of student population has recently attracted the attention of various research groups. Health profession university students are of special interest because of their future involvement in health promotion practices. The aim of this study was to assess nutritional status among European pharmacy students, and also to investigate the eating and lifestyle habits among them. According to our knowledge, this kind of studies was rarely conducted among European pharmacy students (12, 13). In our study, participating students were from 26 European countries covering an entire geographical area of Europe.

We have found that depending on gender there are two different problems concerning nutritional status among European pharmacy student population. Within female population, overweight and obesity were of no importance, but each tenth female student was undernourished. Among male population undernutrition and obesity were not problems, but almost two-thirds of participating students were overweight. Other investigations have also found that overweight status was a predominant problem among male university students in European (24, 25) as well as in non-European countries (23, 26, 27). Better nutritional status among female students may be explained with their generally healthier dietary and lifestyle habits compared to the male students. Von Bothmer and Fridlundt (28) have found that female Swedish university students had healthier habits related to alcohol consumption and nutrition. Also, the possible contributing factor to the normal, but also to the undernourished status can be mass media influence on the picture of the ideal female body shape (29). The similar results were obtained for female participants in the previ-

Table 3. The Usage of Dietary Supplements Among Student Populations (n=591)

Questions	Levels	Total		Female		Male		P
		n	%	n	%	n	%	
Do you use dietary supplements?	No	477	(80.7)	239	(78.4)	238	(83.2)	0.135
	Yes	114	(19.3)	66	(21.6)	48	(16.8)	
	one supplement	87	(76.3)	48	(72.7)	39	(81.3)	0.386
	≥ 2 supplements	27	(23.7)	18	(27.3)	9	(18.8)	0.124
What kind of dietary supplements do you use?	Multivitamins	52	(45.6)	25	(37.9)	27	(56.3)	0.664
	Mineral	23	(20.2)	18	(27.3)	5	(10.4)	0.010*
	Vitamin	22	(19.3)	15	(22.7)	7	(14.6)	0.124
	Fish oil	14	(12.3)	10	(15.2)	4	(8.3)	0.178
	Herbal	10	(8.8)	7	(10.6)	3	(6.3)	0.342
	Probiotics	9	(7.9)	6	(9.1)	3	(6.3)	0.507
	Proteins	8	(7.0)	-	-	8	(16.7)	0.003*
	Multivitamin-minerals	7	(6.1)	5	(7.6)	2	(4.2)	0.073
	Royal jelly	7	(6.1)	5	(7.6)	2	(4.2)	0.768
	Yeast	5	(4.4)	4	(6.1)	1	(2.1)	0.374

*Significant differences between gender were determined by Chi-square analyses (*P< 0.05)*

ous study conducted among students from Faculty of Pharmacy, University of Belgrade (30).

Regarding the meal regularity, we found that even 45% of students had irregular meal pattern, especially in terms of daily breakfasting in male students. This result is in line with previous studies among medical students in northern Greece (10) and France (31). Breakfast is the most important meal of the day because irregular breakfasting is a precondition for low nutritional status (32). In fact, there is evidence that regular daily breakfast decrease dietary fat intake and frequent snacking (33). The additional calories from fat and snacks can contribute to the positive energy balance and to risk in developing overweight and obesity (34). In contrast with earlier studies conducted in Chinese, Lebanese, and Saudi Arabian students, we have found less snack consumption among European pharmacy students (9, 23, 26). Additionally, we did not find statistically significant difference in snack consumption among genders.

Fruits and vegetables are high water and fiber sources, and their consumption in the balanced diet can reduce energy intake and prevent overweight and obesity development (35). In the present study, it was noticed the unhealthy eating habits concerning fruit and vegetable consumption. Only 1/3 of participating students reported consuming fruits and colored vegetables daily. This was in line with low intake of fruits and vegetables among students reported in several previous studies (10, 11, 26, 36). Additionally, our results confirmed that female students had healthier habits in term of the fruit consumption than male study participants (11, 31, 37). Also, in line with the previous study of Sakamaki et al. (9) conducted on Asian students, we have found that consumption of fried food among European pharmacy students was low. Similar results were obtained for drinking and smoking habits. However, opposite to the previous investigations, we noticed more inappropriate knowledge about balanced diet (9, 23). Merely 58% pharmacy students answered correctly to a basic question what balanced nutrition should consist of, while Sakamaki et al. (9) and Yahia et al. (23) found 70.7% and 74%.

One of the aims of this study was to evaluate students' habits of dietary supplements use. Although some studies reported that students frequently consume dietary supplements (38,39), in our study only 19,3% of the students reported using its. Within the supplement users' group, 27% used two and more supplements. Multivitamin was the category of dietary supplements most frequently consumed (8.8% of students), both by female and male students. This result is in line with the most popular supplements reported in a recent survey among pharmacy and nursing students (40). A statistically significant difference between genders was observed in the use of other supplements such as minerals and proteins. The data showed that women consumed more mineral and men consumed more protein supplements.

Investigation of nutritional knowledge and dietary and lifestyle habits of pharmacy students is interesting in the context of their future profession. Beside physicians, pharmacists have an important role in the prevention of some chronic non-communicable diseases. Pharmacists can give advice regarding the proper nutrition to the patients with diabetes mellitus and cardiovascular disease (41), obese patients (42), and those with eating behavior diseases (43). The examples from some countries show how it looks like in practice when the pharmacist is involved in nutritional counseling. In Denmark community pharmacists organized two programs for weight control during the 90-es with individual weight reduction, counseling and weight reduction programs became an important part of pharmacy practice in Denmark (44). In some South European countries, such as Spain, pharmacists also participate in health prevention activities by giving advice regarding the proper nutrition (45). In UK community pharmacists have the important role in weight management control programs through a promotion of healthy eating and lifestyle habits (46).

Conclusions

The maintaining an optimal weight and adoption of healthy eating habits are very important at university age for overall health and can help prevent and control malnutrition. The prevention of chronic non-communicable diseases can be essential for improving quality of life as well as reducing the need and costs of potential therapy in the future. The obtained results of this study indicate that there are a few topics related

nutrition in pharmacy students should mainly focus on. The first one is activities for the promotion of diet and healthy behaviors, with special attention to male students. Thus, observed high level overweight in male students requires interventions that should improve eating habits, such as regularly breakfasting. Also, despite satisfactory results related to smoking, alcohol and fried food consumption, it is necessary to stimulate higher daily intake of fruit and vegetables among student populations. Nutritional education of pharmacy students deserves special attention too, especially in respect to their future community role in the health promotion. Examples from some European countries are encouraging because they indicate how the knowledge of pharmacists in this field actually utilized in the pharmaceutical practice. The fact that about one-third students answered false on the question about balanced diet, gave an indication of the risk of unsatisfactory nutrition knowledge level among pharmacy students. Although in some European countries nutritional education in pharmacy curriculum is present for a long time, in other countries it found place recently. Nutritional education related to weight management and maintain of adequate nutrient supply is recommended so nutrition related courses should be obligatory included to the European pharmacy curriculum.

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Original Article

The effects of glucose and fructose on body weight and some biochemical parameters in rats

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Summary. *Objective:* Dietary fructose from added sugar as high fructose corn syrup may causes major risks in obesity, hyperlipidemia, cardiovascular diseases, hyperuricemia and fatty liver. The aim of this study was to investigate and compare the effects of high fructose and high glucose intake on body weight and some biochemical parameters in rats. *Subject and methods:* The study was conducted on adult, 32 Wistar albino male rats (300-350 g weeks) which fed with standard laboratory chow. In each group, 8 rats was selected randomly and which was be composed four groups. The rats in each group, in addition to standard meal, different amount of glucose and fructose containing solutions (10% and 30% glucose-fed group, 10% and 30% fructose-fed group) was given by oral gavage for 6 weeks. At baseline and after 6 weeks total cholesterol, VLDL-cholesterol, triglycerides, uric acid, AST and ALT as biochemical parameters and liver histopathological examination of rats were determined. Body weight of the rats was evaluated every week. *Results:* The 30% fructose group caused higher AST levels according to 10% glucose group, 30% glucose group and 10% fructose group. At the end of 6 weeks, the mean body weight in the fructose-fed groups was higher than the glucose-fed groups ($p>0.05$). No statistically significant difference between rat groups' portal inflammation rates were found and the moderate and severe ballooning were observed in 30% fructose rats ($p<0.05$). *Conclusions:* As a result, dietary fructose from added sugar as high fructose corn syrup may causes major metabolic disorders.

Key words: fructose, glucose, body weight, biochemical parameters, portal inflammation

Introduction

Fructose, commonly known as fruit sugar, is also a major component of sweeteners such as table sugar, honey and high fructose corn syrup (HFCS) (1). Although fructose is a simple sugar that exists naturally in fruits and vegetables, the majority of dietary fructose comes from two sweeteners, sucrose and high-fructose corn syrup, which are commonly used in manufactured foods and beverages (2). Since the beginning of 20th century, fructose consumption has increased 4-fold by the introduction of HFCS (1). Especially, fructose consumption has increased as usage of HFCS in the Western diet. Based upon disappearance data, the annual per capita intake of HFCS from 1967 to 2006 increased from 0.03 to 58.2 lbs, whereas sucrose decreased from 98.5 to 62.3 lbs. Sucrose is a disaccharide and consists of 50% fructose and 50% glucose. The HFCS form used in soft drinks compose of 55% fructose, 42% glucose, and 3% oligosaccharides. Because of the higher fructose dose, soft drinks sweetened with HFCS would provide more fructose into the systemic circulation than soft drinks sweetened with sucrose. Furthermore, HFCS provides an immediate source of free fructose and glucose, whereas sucrose must first be broken down by sucrase (2,3). An increasing amount of fructose in the diet is suggested to play a causal role in the pathogenesis of the metabolic syndrome, insulin resistance, impaired glucose tolerance, type 2 diabetes, obesity, hyperlipidemia, cardiovascular diseases, hype-

ruricemia and fatty liver (4). Fructose does not increase the satiety signals of blood glucose and insulin to the same extent as does sucrose or glucose. Short-term food intake is inversely related to the glycemic and insulin responses to sugars, and it has been proposed that fructose does not suppress gastric appetite hormone and reduced insulin and leptin signaling in the brain. High fructose causes an increase in the synthesis of non-esterified fatty acids production. Fructose is lipogenic and stimulates triglyceride synthesis. Acute oral or intravenous administration of fructose results in a rapid increase in serum levels of uric acid through accentuated degradation of purine nucleotides and increased purine synthesis. The aim of this study was determined the effect of different amounts of fructose and glucose in rats to body weight and some biochemical parameters.

Material and Methods

Experimental design

This research conducted in Baskent University Production and Research Centre for Experimental Animal, Ankara, Turkey. This study was approved by Baskent University Ethical Committee for Experimental Resarch on Animals (Project no: DA14/14) and supported by Baskent University Research Fund.

Male rats were divided into four groups with each group comprising of eight animals. Male Wistar albino rats (32 weeks) weighing 300-350 g were randomly assigned to one of the four groups; 10% glucose-fed group, 30% glucose-fed group, 10% fructose-fed group and 30% fructose-fed group.

Group 1 n(8): Standart pellet+10% HFCS
Group 2 n(7): Standart pellet+30% HFCS
Group 3 n(8): Standart pellet+10% glucose solution
Group 4 n(7): Standart pellet+30% glucose solution

Sample size calculated on the basis of probability distribution of the measured values with a given significance level (e.g., 5%), medium effect size (e.g., 0.35) and the power of test (e.g., 85%). This analysis was performed using G*Power 3.1.3 software program.Thus, the total sample size was obtained in 32 rats. All animals were housed in cages and subjected to a 12 h light-dark cycle at 24 ± 2 oC and animals were fed on a standard pellet diet and water ad libitum. The solutions have been prepared by feeding to rats, at four concentrations, 10 and 30 g/ 100 milliliter glucose; 10 and 30 g/ 100 milliliter fructose. Solutions to be administered by gavage were stored at 4°C and warmed to room temperature. The follow-up terminated at the end of 6 weeks.

Evaluation of Measurements

At baseline and at the end of the 6 weeks, total cholesterol (TC), VLDL-cholesterol (VLDL-C), triglyceride (TG), uric acid (UA), alanine aminotransferase (ALT), aspartate transaminase (AST) measurements were sampled. For the experiment; the animals were starved overnight for 12 h before the blood collection process and approximetly 1mL blood sample was collected from each rat by snipping the tail using heparin anti-coagulant under diethyl ether anaesthesia. Then, plasma was obtained from the blood using a centrifuge at 4 °C for 15 min. Serum total cholesterol, triglyceride and uric acid levels were assayed by enzymatic tests, using an AbbottÒ Architect C8000 Analyzer according to the manufacturers specifications. (Abbott Park, IL, USA). VLDL cholesterol was calculated from measurements obtained for triglyceride using the following formula: VLDL = Triglyceride/5 (mg/dL). Serum ALT and AST levels were assayed by an UV test according to standardized method, using an Abbott© Architect C8000 Analyzer according to the manufacturers specifications. (Abbott Park, IL, USA). Body weight was measured weekly during the follow-up.

Liver histopathology

Histopathologic examination was carried out at the end of 6 weeks. Steatohepatitis was evaluated using the grading and staging system of Brunt et al. (5). The grades were classified as grades 0-4, which were based on the percent of hepatocytes involved in the biopsy (0: none, 1: 10%, 2: 10–33%, 3: 33–66%, 4: 66%).

Statistical analysis

The results were expressed as mean±SD or mean (95% CI). Paired t-tests were used to estimate the presence of changes in study parameters for each experiment group (e.g., Group 1: 10% HFCS; Group 2: 30% HFCS; Group 3: 10% glucose solution; Group

Table 1. Effect of fructose and glucose feeding on biochemical parameters and systolic blood pressure for 6 weeks

	10% Glucose		p[1]	30% Glucose		p[2]	10%Fructose		p[3]	30% Fructose		p[4]
	Baseline	After 6 Weeks		Baseline	After 6 Weeks		Baseline	After 6 Weeks		Baseline	After 6 Weeks	
Cholesterol	75.6±14.95	110.8±64.19	0.184	92.6±36.88	93.8±57.32	0.879	69.5±10.83	72.7±11.10	0.554	69.8±8.11	74.1±16.11	0.249
VLDL-C	14.0±3.52	37.2±37.43	0.177	17.9±9.25	20.3±15.49	0.418	16.0±5.14	17.1±4.67	0.596	14.0±2.94	16.9±5.55	0,242
Triglycerid	70.0±17.60	186.0±187.34	0.179	89.5±46.25	101.5±77.47	0.418	80.0±25.71	85.3±23.36	0.596	70.0±14.71	84.7±27.77	0.242
Uric asid	1.46±0.51	2.0±0.36	0.061	1.33±0.19	1.53±0.60	0.359	1.3±0.26	1.3±0.34	0.547	1.4±0.29	2.0±0.36	0.006*
ALT	74.8±64.19	65.4±20.05	0.620	54.2±17.81	53.5±10.23	0.907	65.6±16.68	74.6±31.05	0.544	69.6±24.64	89.0±19.44	0.015*
AST	124.8±71.36	177.7±50.6	0.010*	116.3±7.53	119.1±24.13	0.754	97.3±11.85	134.8±48.34	0.067	106.0±27.39	206.3±47.23	0.002*

p^{1-4}:The significance test of differences between baseline and after six weeks values for each group.
VLDL-C:VLDL-Cholesterol

4: 30% glucose solution), In addition, the absolute changes (the difference between baseline values and after six weeks values) were tested between groups using one-way ANOVA. The distribution of changes was evaluated for normality assumptions using One Sample Kolmogorov–Smirnov test. The Fisher exact test was used for proportions. SPSS version 21.0 was used to analyze the recorded data. Significant values of p<0.05 were considered to be statistically significant.

Results

The mean of plasma UA, TG, TC, VLDL-C, ALT and AST at baseline and after the six weeks were shown in Table 1. It was found that the significant differences in mean values of AST in 10% glucose-fed group (p=0.010); uric acid, ALT and AST in 30% fructose fed group (p=0.011, p=0.015, p=0.002, respectively). The 30% fructose group caused the difference in AST levels according to 10% glucose group, 30% glucose group and 10% fructose group.

The difference of the initial and final body weight were shown in Figure 1. After a 6 week trial, the mean body weight in the fructose-fed groups was higher than the glucose-fed groups, but there were no significant differences in body weight gain among groups (p>0.05) (Figure 1).

The effect of fructose and glucose feeding on portal inflamation and hepatocyte ballooning in rats' livers for 6 weeks were shown in Table 2. No statistically significant difference between rat groups' portal inflammation rates were found. Both 10% fructose and 30% fructose groups, 2 of the 8 rats were observed mild inflammation. There were statistically significant differences between the rat groups in terms of hepatocyte ballooning (p=0.025). Mostly, the moderate and severe ballooning were observed in 30% fructose rats (Table 2).

Discussion

When we analyzed the difference of body weight during a 6 week treatment, the mean body weight in

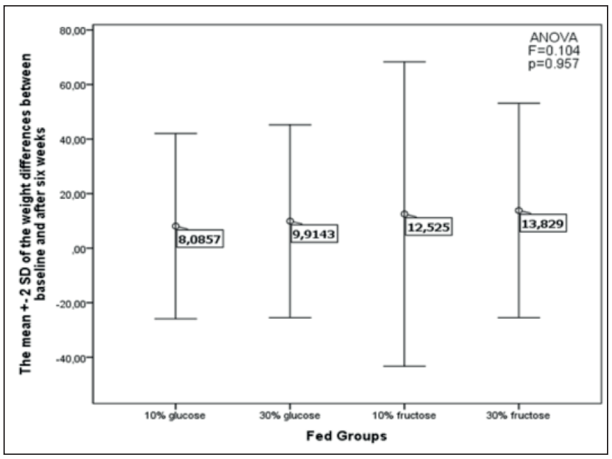

Figure 1. Effect of fructose and glucose feeding on body weight in rats for 6 weeks

Table 2. Effect of fructose and glucose feeding on portal inflamation and hepatocyte ballooning in rats' livers for 6 weeks

	10% Glucose		30% Glucose		10% Fructose		30% Fructose		
	n	%	n	%	n	%	n	%	
Portal Inflamation									
No	7	26.9	7	26.9	6	23.1	6	23.1	p=0.886
Mild	1	25.0	-	-	2	50.0	1	25.0	
Hepatocyte Ballooning									
No	3	25.0	5	41.7	-	-	4	33.3	p=0.021*
Yes	5	27.8	2	11.1	8	44.4	3	16.7	

the fructose-fed groups has shown higher than the glucose-fed groups (p>0.05) (Figure 1). This study was suggested that the weight gain by fructose feeding as previous studies.Over the past several years, the reasons for the increase in obesity prevalence have shown that increased the sugar added to food and it has taken the place of the sucrose to HFCS by researchers (6-8). HFCS caused an increase in body weight greater than sucrose in both male and female rats. This increase in body weight was accompanied by an increase in fat cumulation and circulating levels of TG (9).

In recent studies having drawn attention to fructose has emphasized the absence of satiety such as other sugars. Plasma glucose and insülin levels effected the state of satiety after food consumption. Although fructose does not contribute to the feeling of fullness, has the same energy load with the blood sugar glucose. Therefore, as long as the amount of glucose decreases and the amount of fructose increases, the feeling of fullness occurs later and it is consist of more eating behavior (10,11). The excessive consumption of HFCS may contribute to the incidence of obesity by reducing insulin and leptin levels (12). The intake of HFCS would not lead to insulin or leptin-induced satiety. Because fructose leads to increased plasma free fatty acids, leptin, adiponectin, abdominal adipose tissue and impaired insulin sensitivity (13,14). The recent studies demonstrate that compared to pure glucose, chronic fructose feeding does not suppress the appetite hormone ghrelin and does not provide enough insulin and leptin secretion (15,16). In a study which was analyzed the long-term effects of HFCS on body weight, the rats with access to HFCS gained significantly more body weight than sucrose groups (9). Fructose (or sucrose) administration to humans and rats also induces attributes of liver diseases and may have a role in the pathogenesis of fatty liver diseases (17,18). The fatty liver disease includes a broad spectrum of manifestations of fatty liver, ranging from steatosis alone, steatosis with inflammation, steatosis with hepatocyte injury, or steatosis with sinusoidal fibrosis in relation to the progress of the pathological state (19,20). In this study we investigated whether fructose could play a role metabolic disorders in liver.

Administration of high doses fructose can also cause elevation of portal inflamation rates hepatocyte ballooning. The moderate and severe ballooning were observed in most 30% fructose rats. But there were no statistically significant difference between rat groups' portal inflammation rates were found. If only both 10% fructose and 30% fructose groups, 2 of the 8 rats were observed mild inflammation. Ackerman et al., demonstrated that implementation of fructose to rats results in hepatic steatosis with a 198% increase in hepatic triglycerides and an 89% increase in hepatic cholesterol concentration and Davail et al., evidenced high fructose diets also develop fatty liver (21,22)

Conclusion

As a conclusion, dietary fructose from added sugar as high fructose corn syrup may causes major risks in obesity, fatty liver disease, insulin resistance, hyperlipidemia, impaired glucose tolerance, Type 2 diabetes, cardiovascular diseases, hyperuricemia, gout and meta-

bolic syndrome. So, the healthy preference of fructose source in diets is fruit and the amount of safe dietary intake of fructose may accept as 10% of total energy.

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An open label, non-comparative pilot study to assess the efficacy and safety of a food supplement containing manna in pediatric functional constipation

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Summary. *Objective*. The primary objective of this study was to evaluate the efficacy and safety of the food supplement Physiomanna Baby® in pediatric patients with a history of functional constipation defined by Rome III criteria; secondary objective was to evaluate the adherence to the tested product in the enrolled children. *Methods*. The trial was designed as an open label, non-comparative pilot trial. In 3 Romanian sites (one community hospital and two private medical practice offices) 49 children (20 males, 29 females) aged 0 - 8 years were enrolled. The study was conducted between February 2016 and April 2016. The investigational product was administered as 1 g/kg in single daily oral administration from the first day and continued for a maximum of 3 days in the first week. If the constipation symptoms persisted, the children were treated in an additional cycle of treatment for a maximum of 3 days. *Results*. The number of Spontaneous Bowel Movements (SBM) per week has increased to normal after Physiomanna Baby® administration (from 1.80 ± 0.41 at baseline to 6.04±1.54 at day 8) evidencing a statistically significant difference (P-value <0.0001). The efficacy was also demonstrated in the subpopulation of children <4 years where the mean values per week increased from 1.69±0.47 at baseline to 6.15±1.59 SBM at day 8 (P-value <0.0001). According to Investigator Global Assessment of Efficacy (IGAE), Physiomanna Baby® shows immediate and excellent efficacy after one or two doses for 79.60% of the children, a very good efficacy after three doses for 12.24% and good efficacy after the second cycle of administration for 6.12% of children. Both Investigator Global Assessment for Safety (IGAS) and Patient Global Assessment for Safety (PGAS) were rated as 100% excellent for all patients. *Conclusions*. The food supplement Physiomanna Baby® provided immediate efficacy, offering to pediatricians a safe solution in the care of mild to moderate functional constipation, even if the study design characteristics were limited (pilot trial with a small sample size and without control group).

Key words: children, functional constipation, manna, food supplement, fiber

Introduction

Functional constipation is one of the most frequent pediatric gastrointestinal complaint with a prevalence ranging from 0.7% to 29.6% (1) and describes all children in whom constipation does not have an organic etiology (2). It is characterized by infrequent and painful defecation, hard or large stools, fecal incontinence and abdominal pain, causing a significant distress to both the child and family and is associated with a significant impact on health care (cost per year is 3 times than that in children without constipation) (3). One third of children with untreated chronic constipation continue to have problems beyond puberty, that will impact their social life (4).

Functional constipation in children has been defined by Rome III criteria (5,6) as presented in Table 1.

A medical history and complete physical examination was undertaken to confirm the diagnosis. The literature enphasises that laboratory or radiological investigations should only be performed in severe, refractive cases of constipation (7), to exclude an underlying disease or organic cause (present in less than 5% of cases) (8).

International guidelines underline that the first step in treatment should consist of education, dietary, and behavioural modifications. Actually, osmotic and stimulant laxatives represent the most frequent way to manage children constipation (9), even if scientific evidence does not yet support this practice and the risk of adverse events is possible (10-12). On the other hand, the role of fiber in the treatment is becoming well-known, because pediatricians would like to solve this affection in a less invasive approach as possible (13). Food supplements could represent useful tools for these problems (15). Natural plant exudates containing fibers like glucomanna have been used as the fiber supplement in such cases (16). One of the most promising candidates is Manna, an exudate containing oligosaccharides and polyols like mannitol with a mild laxative action (17).

Physiomanna Baby® is a food supplement containing Manna in addition to natural extract from Fennel (*Foeniculum vulgare*), Chamomile (*Chamomilla recutita*) and lemon balm (*Melissa Officinalis*) (18,19) that could play a role to treat functional constipation in children.

Despite the wide use of Manna and the consideration that many pediatrics already prescribe it to children, this product is almost absent in clinical trials. Therefore, we have planned the present pilot trial to have a preliminary assessment of Physiomanna Baby® efficacy and safety and to obtain data to calculate the sample size for future randomized studies.

Material and Methods

Design

The study was designed as a pilot, open label, non-controlled, multicenter trial with a prospective design on one cohort of pediatric patients.

Study population

The study included all infants aged less than 8 years addressed for functional constipation in one community hospital and two private medical practice offices located in Timisoara, Romania (Emergency Children Hospital "Louis Țurcanu"; Private Practice SCM Gados; Private Practice CMI Dr. Herteg) between February and April 2016. The diagnosis was made using the Rome III criteria (6) (Rome IV was not available at the time the study was performed).

To be included in the study, each subject had two or fewer SBM during the previous week and was otherwise in good health as judged by a physical examination at the baseline visit.

The exclusion criteria consisted of any organic cause for constipation, history of known obstruction

Table 1. Rome III criteria for functional constipation

Age <4 years[a]	**Age ≥4 years**[b]
1. <3 defecations per week	1. <3 defecations in the toilet per week
2. ≥1 episode of fecal incontinence per week after acquisition of toileting skills	2. ≥1 episode of fecal incontinence per week
3. History of excessive stool retention	3. History of retentive posturing or excessive volitional stool retention
4. History of painful or hard bowel movements	4. History of painful or hard bowel movements
5. Presence of a large fecal mass in the rectum	5. Presence of a large fecal mass in the rectum
6. History of large diameter stools, which may obstruct the toilet	6. History of large diameter stools, which may obstruct the toilet

[a] *Must fulfill ≥2 criteria for ≥1 month prior to diagnosis;* [b] *Must fulfill ≥2 criteria at least once per week for ≥2 months prior to diagnosis, with insufficient criteria for diagnosis of irritable bowel syndrome*

or perforation, congenital malformations able to produce constipation (i.e. Hirschsprung's disease, imperforate anus, children with cerebral palsy), the use in the 4 previous weeks of concomitant medication, herbs or dietary supplements that can cause constipation; TSH clinically significant elevation or abnormal plasma electrolytes; positive Fecal Occult Blood Test (FOBT); known allergy to manna, mannitol or other Physiomanna Baby® ingredients. No enrolled child had participated in a clinical trial in the past 30 days and a written informed consent was signed by parents before enrollment.

Food supplement in study

At baseline, the parents received all the necessary tested product for one subject (1 jar containing 100 g manna food supplement in powder). A second additional jar was available as a back-up.

Physiomanna Baby® (Iuppa Industriale S.r.l. - Alice Bel Colle (AL), Italy) was prescribed as a weight-dependent dose of 1 g/kg body (half of the little spoon attached to the jar). The food supplement was orally administered by the parents once a day mixed in water. It was recommended that the child drink plenty water during the day. The administration started from the first day of study and continued for a maximum of 3 days in the first week (1st cycle); if the constipation symptoms persisted the food supplement administration should continue for additional 3 days during the second week (2nd cycle).

Framework of the study

Two mandatory onsite visits (at baseline and day 8), and three phone contacts (on day 2, 3, and 4) were performed if the efficacy endpoint was reached within the first week of administration (Figure 1).

When the target was not reached after the first week and therefore Physiomanna Baby® administration was continued in the second week, three more phone contacts (on day 9, 10, and 11) and a final visit on day 14 were performed. In addition, Investigators could ask the children and their parents for any number of unscheduled visits.

The parents were taught how to administer and when to stop the investigational food supplement, and how to record the number of stools per day during the whole study period. Parents were also instructed to contact the Investigator if any adverse events occurred.

Outcome variables for Efficacy

Assessment of efficacy consisted of the number of Spontaneous Bowel Movements (SBM) recorded per day and per week; SBM was defined as BM not preceded by a 24 h administration with a laxative or enema.

During the trial, the administration of Physiomanna Baby® continued if 2 SBM or less occurred in the last 7 days. When the number of SBM increased to 3 times a week or more the parents stopped the administration and the Investigator planned the final visit in the same week.

At the final visit, the Investigator Global Assessment for Efficacy (IGAE) was registered for each child using the following 4-point scale: 0 = worse: no

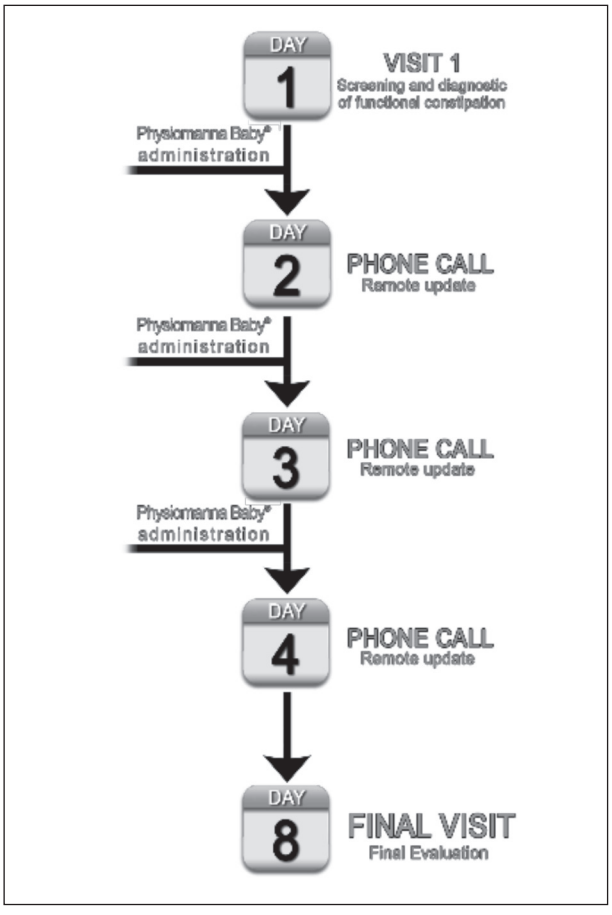

Figure 1. Study flow chart

effect of treatment; 1 = good: the treatment effect appeared after the second cycle of administration, only; 2 = very good: the treatment effect occurred after three days of administration; 3 = excellent: the treatment effect occurred after one or two days of administration.

Outcome variables for Safety

As far as tolerability of the tested product was concerned, the Investigators monitored the occurrence of any adverse event during the study. Investigator Global Assessment for Safety (IGAS) was registered for each child using a 4-point scale: 0 = worse; 1 = mild; 2 = good; 3 = excellent.

In addition, at the final visit was also recorded the Patient Global Assessment for safety (PGAS) that, in children less than 4 years old, was performed directly by parents. The used 4 point scale was the following: 0 = worse tolerability; 1 = mild tolerability; 2 = good tolerability; 3 = excellent tolerability.

Adherence

The patients' parents were reminded of the importance of strictly complying with the instructions received from the Investigator. The evaluation of product's administration adherence was performed as secondary outcome checking diary cards and checking the unused content of the returned bottles.

Statistical analysis and sample size

The null hypothesis of the study was that there will be no statistically significant difference between the number of SBM recorded in the week immediately before enrollment and the number reported after 1 or 2 weeks of Physiomanna Baby® administration.

Since this trial was planned as a pilot study, a prior estimation of sample size was not performed. To have 45 evaluable patients (a sample size often used for the new tested product in trials on pediatric functional constipation (20-22)) it was estimated that the subjects included in the study could be about 50.

SBM per week were analyzed comparing pre- and post- study administration status by using a Wilcoxon paired test for matched samples. Statistical significance level was set at 5%.

Quantitative variables (i.e. demographic) were described through counts, mean, standard deviation (SD), standard error, 25, and 75 percentiles, minimum and maximum. Statistical analyses were performed using SAS 9.2 (SAS Institute Inc., Cary, NC, USA) with an intention to treat approach.

Ethical aspects

The study has been approved on February 10th, 2016 by the Comisia de Etică a Colegiului Medicilor Timiș (Timișoara, România). The study was conducted under the ethical principles for medical research involving human subjects of the Declaration of Helsinki. The informed consent was obtained in writing from parents before performing any study specific procedures. The study was registered on www.ClinicalTrials.gov with identification number NCT02732743.

Results

Of 50 children included in the trial, one resulted as a screening failure and forty-nine (20 males and 29 females) were analyzed. Only one patient was withdrawn during the study period due to prohibited medication assumption: therefore, 48 children (mean age 3.51±2.31 years) completed the study and were considered as evaluable.

Table 2 underlines the consistent number of children less than 12 months (12 on 49) enrolled.

During study monitoring, no protocol deviations were recorded.

In the population analysed, mean SBM weekly frequency was 1.80±0.41 at baseline visit and after Physiomanna Baby® administration it increased to

Table 2. Patients distribution at baseline by gender and demographic

Age range	Female	Male	Total
1-6 months	4	3	7
6-12 months	3	2	5
1-5 years	16	9	25
5-8 years	6	6	12
TOTAL	29	20	49

Table 3. Spontaneous Bowel Movements evolution

	Children <4 years		Children ≥4 years		All	
	Baseline Visit	**Day 8**	**Baseline visit**	**Day 8**	**Baseline visit**	**Day 8**
N	26	26	23	23	49	49
Mean± Std Dev	1.69±0.47	6.15±1.59	1.91 ±0.29	5.91±1.50	1.80 ±0.41	6.04±1.54
Std Err	0.09	0.32	0.06	0.31	0.06	0.22
Min-Max	1-2	2-8	1-2	2-8	1-2	2-8
Percentiles 25	1	6	2	6	2	6
Percentiles 75	2	7	2	7	2	7

6.04±1.54 on day 8 (P-value <0.0001, by using a Wilcoxon signed rank test) (Table 3).

This positive result was also confirmed considering separately the two sub-population of children less or more than 4 years (Figure 2).

In the younger group after Physiomanna Baby® administration, the mean values per week increased from 1.69±0.47 to 6.15±1.59 SBM (P-value <0.0001), and in the children >4 years from 1.91±0.29 to 5.91±1.50 (P-value <0.0001). A Wilcoxon Signed-rank test at a 5% significance level was used to assess the differences between age groups.

In both the analyzed subpopulations the results belonging to males were slightly better than those of females, even if no statistically difference between gender was evidenced.

Table 4 shows the mean increase of SBM during the study period (from Baseline to Day 8).

Approximately 85% of the subjects have gained 3 or more SBMs during the first week of study period (3 SMB/week is limit indicated for functional constipation by Rome III criteria).

Only for 3 on 48 patients was necessary to repeat the treatment during the second week and no relapse of functional constipation was evidenced from day 14 to day 21.

IGAE evidenced a significant performance of Physiomanna Baby® in the management of functional constipation: 79.6% of children were rated as excellent, 12.24% as very good and 6.12% as good.

No adverse event was evidenced during the trial period and also IGAS was rated as 100% excellent.

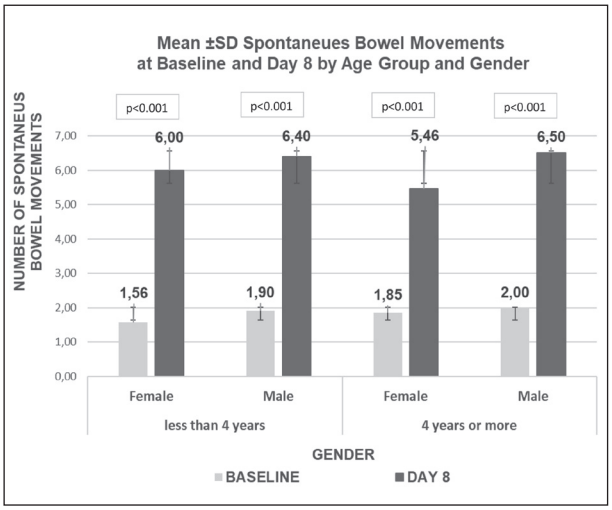

Figure 2. Spontaneous Bowel Movements mean ±SD values at Baseline visit and Day 8 for age 2 groups (Less than and More than 4 years) and by Gender. P-values were obtained by performing Wilcoxon Signed-rank tests at a 0.05 significance level.

Table 4. Spontaneous Bowel Movements change from Baseline visit to day 8

	Children <4 years	**Children ≥4 years**	**All**
N	26	23	49
Mean± Std Dev	4.46±1.65	4.00±1.41	4.24±1.55
Std Err	0.31	0.29	0.22
Min-Max	0-6	0-6	0-6
Percentiles 25	4	4	4
Percentiles 75	6	5	5

These positive evaluations were confirmed by patient diaries data where tolerability was considered as excellent in 100% of the cases by means PGAS.

The adherence to product's administration was 100%.

Discussion

The emotional and psychological attitude of parents (expressed by anxiety and questionable therapeutic behavior) plays an important role in daily management of pediatric functional constipation. For such a frequent gastrointestinal disease (for example, it is 7 times higher than asthma in children 7–8 years of age (23)), usually there is no need for drug treatment. But, all too often, parents and healthcare providers, seem scared in front of a slight prolonged functional constipation.

Therefore, providing a correct information to parents can be very important because, in absence of a doctor's advice, they risk to rely to ineffective remedies based on popular tradition or bought directly based on media advertising. When choosing between the different therapeutic options, the doctor will obviously evaluate the child's needs (possibly combining them with the parental ones) and likely prescribe an effective approach. At the same time, however, the doctor must strike a balance between the expected benefits and the safety of the treatment in children. In this context, functional constipation treatment should: reduce the children' symptoms, improve the frequency and consistency of stools, reduce discomfort, abdominal pain, and restlessness and, at the same time, ensure the clinician about prescribing a product that is safe and non-toxic. Considering the above factors, in children with functional constipation the use of osmotic and stimulant laxatives should be reserved for the most severe cases (24) and used under medical supervision, due to the risk of adverse events (which can be serious, though rare), whereas folk remedies do not offer sufficient guarantees of the expected activity. Moreover, utmost safety is essential for infants, and a safe food supplement like Physiomanna Baby® able to resolve the constipation symptoms in 45 out of 49 children treated by one administration cycle only (single dose or two), and assure the absence of constipation relapse after an additional week, should be considered as an important therapeutic tool for the pediatrician. This indication is also strengthened by the findings of its tolerability in the treatment of constipation, a disease present in 17% to 40% of children in the first year of life (4). In this regard, Physiomanna Baby® administration was found to be totally safe, without any adverse events also in a population with mean age of 3.51±2.31 years.

These preliminary results, even with the limits given by the design of a pilot study (small sample population and without control group), show that Physiomanna Baby® is beneficial in the care of children with mild to moderate constipation. Follow-up studies should be designed to assess if it can prevent chronic constipation and recurrent impactions.

Conclusions

For children with acute or mild chronic functional constipation, with no chronic digestive diseases and no organic diseases, Physiomanna Baby® could offer to the pediatricians a safe and clinically tested solution in the care of pediatric functional constipation. It could be prescribed in weight-dependent doses as maintenance therapy to achieve regular evacuation, to avoid recurrent constipation, to maintain soft stools and to reduce the laxative intake.

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Original article

Serum zinc level in Iron Deficiency and Iron Deficiency Anemia of children aged 6 months to 5 years

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Summary. *Background:* Iron Deficiency Anemia (IDA) is the most common anemia in all age groups. The coexistence of iron deficiency anemia and zinc (Zn) deficiency is quite common, for most of the etiologic factors are common. The purpose of this study is to determine the prevalence of zinc deficiency in children with iron deficiency and/or iron deficiency anemia. *Method:* One hundred and sixty child patients within 6month-5 year age range diagnosed with iron deficiency and/or iron deficiency anemia and 91 children with no iron deficiency and/or iron deficiency anemia diagnosis were included in the study. The relationships between serum zinc levels and other biochemical and hematological parameters were examined. *Results:* It was observed that the study and control groups had similar demographic characteristics, and there was no statistically significant difference between two groups in terms of average age (months), distribution of age groups, and gender groups. Statistically significant differences were observed between hemoglobin (Hb), haematocrit (Hct), mean corpuscular volume (MCV), red cell distributionwidth (RDW), iron (Fe), total iron binding capacity (TIBC), transferrin saturation (TS) and Ferritin values of the study group and control group, and the cases in the study group were determined to be highly anemic. Statistically significant difference was determined between groups in terms of zinc levels, and zinc levels in the study group were determined to be lower than those in the control group. Zinc deficiency (10%) in children diagnosed with iron deficiency and/or iron deficiency anemia was found more common compared to the control group (2.2%). A positive correlation between serum zinc levels and Hb, Hct, MCV, Fe, TS and Ferritin, and a negative correlation between serum zinc levels and RDW and total TIBC were detected in the study group. *Conclusion:* According to these results, it was concluded that children with iron deficiency could simultaneously have zinc deficiency, the iron-zinc combination might be more effective for the treatment of iron deficiency than treatment with only iron, and early age supplementation programs might be useful for the highrisk groups such as childhood until zinc deficiency tests gave routine results.

Key words: iron deficiency anemia, zinc deficiency, children

Introduction

Zinc (Zn) and iron (Fe) are essential micronutrients for human body and these are involved many complex enzyme systems (1). Children with Iron Deficiency Anemia (IDA) have low serum Zn levels. Inadequate intake of Zn is considered to be responsible for 20 % of global child mortality (2). Zinc and Fe affects the absorption of each other level in intestinal mucosa. Phytate and excessive intake of mineral supplements may be impair Zn and Fe absorption (3). Deficiency of trace element nutrients is seen common in children. Because of the increased requirements, inadequate intake, and recurrent infections, in developing countries especially infant and young children are vulnerable to Zn and Fe deficiency (4). Zinc is an important, trace elements that affects catalytic, structural and regulatory functions in human body. Iron is important for synthesis of hemo-

globin and myoglobin (5). Iron deficiency (ID) can be seen a total of 25% of the world's population. Infants, young children, females, adolescents and pregnant and lactating mothers are most affected by ID in developing countries (6-8). Only 35–55% of cases of IDA in children are solely due to iron deficiency and others are associated with changes in status of multiple trace elements. ID and IDA are more common in Turkey than developed countries. Previous studies had reported that ID rate between 1.5% to 62.5% in pediatric age group in Turkey (9-11). Nutritional zinc deficiency is also quite common in the world and in Turkey at rates as high as 15.7% (12,13). Although Fe and Zn deficiency had been reported different region of world, frequency rate can be various by country (14-16). Deficiency of zinc, is frequently associated with IDA in developing countries and in Turkey (2).

Symptoms in Zn and Fe deficiency cases are abundant and they can show similarities. Most importantly co existence of deficiency of these two trace elements aggravate the symptomps.

Most of the researchers have shown lower serum zinc levels in patients with iron deficiency anemia in comparison to nonanaemic patients (2, 17, 18), but others have not found significant differences in serum zinc between iron deficiency anemia patients and healthy controls (19-21). Studies are limited related with iron deficiency anemia and micronutrient deficiency in children.

In this present study, we aimed to measure serum Zn level in children between the ages of 6 months and 5 years with Fe deficiency or Fe deficiency anemia in order to determine if Zn deficiency accompanies it.

Materials and Method

The study was conducted by Bagcilar Training and Research Hospital Pediatrics Outpatient Clinic, Istanbul, Turkey November 2014 and July 2015. One hundred sixty patients whose ages were between 6 months and 5 years and who had ID and/or IDA were included in the study. Within the control group, zinc level in the serum samples obtained from 91 healthy children who had no iron deficiency and iron deficiency anemia presented in the same period was studied.

Patients with findings supporting the absorption and bleeding disorder in the gastrointestinal system and patients with findings supporting a chronic disease and thalassemia or other hemoglobinopathies were excluded from the study. The study approved by Bagcilar Training and Research Hospital Board Ethics Committee. The complaints of the patients were recorded; their physical examination was performed after measuring their weight and height.

After obtaining the informed consent, 2cc of venous blood were taken into the standard purple cap CBC tube containing 0.072 ml 7.5% K3-ethylenediaminetetraacetic acid for a complete blood count; 4cc of blood were taken into the yellow cap biochemistry tube not containing anticoagulant in order for Fe, TIBC, ferritin and zinc levels were to be studied.The peripheral blood smear stained with Wright's stain was examined by a light microscope. For full blood count, automatic hemocytometer Roche XT 1802 daily calibration of which was performed daily was used. Fe and total iron binding capacity (TIBC) were studied with Cobos C602 device.

The serum samples were used by dissolving, which were separated from the full-blood at the moment they were received and frozen at -80°C for serum ferritin and zinc values to be studied. The serum ferritin levels were measured by the immune chemiluminescence method using Cobos 6000 device. Full blood cell count, Fe, TIBC, ferritin measurements were performed at the central laboratory of Bagcilar Training and Research Hospital. Zinc levels were studied by the atomic absorption spectrophotometer method (Atomic Absorption Spectrophotometer AA-6701F SHIMADZU, Japan) at a private laboratory.

The transferrin saturation index (TSI) was obtained by proportioning the serum iron to TIBC. Patients with hemoglobin (Hb), hematocrit (Hct) and mean corpuscular volume (MCV) levels under the lower limits according to the age group and with TSI level below 16% and ferritin level below12 ng/dl were considered as IDA. Patients with normal hemoglobin, hematocrit and MCV level according to the age group and having TSI level below 16% and ferritin value below12 ng/dl were considered to be in the iron deficiency group. However, when the ferritin levels of some patients were found high (due to the acute phase

reactant of ferritin) although no findings were detected in favor of infection, they were considered to be in the iron deficiency group if TSI was low. Hemoglobin lower limits for age groups were determined based on Dallman anemia criteria. Hemoglobin lower limit was set as 10.5 gr/dl for 0.5-2 age range and as 11.5 gr/dl for 2-6 age range (22). According to this, the cases with higher hemoglobin values than lowerlimits set for age groups, with TSI higher than 16% and with ferritin higher than 12 ng/ml were considered as the control group. Zinc levels were evaluated according to the age groups (Normal values for the age groups; 70-120 μg/dl for 1-5 age range group). Zinc deficiency was defined if <65 μg/dl (23).

Statistical Evaluation

The statistical analysis in this study was performed using NCSS (Number Cruncher Statistical System) 2007 Statistical Software (Utah, USA) package program. Together with the descriptive statistical methods (mean, standard deviation) in the evaluation of the data, an in dependent t-test was used to compare the pairs, the chi-square test was used to compare the qualitative data, and the Pearson correlation test was used in order to determine the relationships of the variables with each other. The linear regression analysis was performed between zinc and other variables. The results were evaluated at $p < 0.05$ significance level and in 95% confidence range.

Results

The study group consisted of 101 male (63.12%), 59 female (36.88%), and the control group consisted of 48 male (52.75%) and 43 female (47.25%) cases. The average age of the study group was 34.86 ± 19.6 months, and the average age of the control group was 39.37 ± 18.96 months. All patients with iron deficiency anemia had a complaint of loss of appetite. In addition to the complaint of loss of appetite, 50 patients (31.25%) were suffering from failure to thrive, 20 patients (12.5%) were suffering from anxiety, 30 patients (18.75%) were suffering from asthenia and getting tired quickly, 10 patients (6.25%) were suffering from pica, 20 patients (12.5%) were suffering from getting ill often, 20 patients (12.5%) were suffering from dizziness and tachycardia, 10 patients (6.25%) were suffering from a headache. All of 16 patients with zinc deficiency were suffering from the loss of appetite and failure to thrive. In addition to these findings, 4 patients had recurrent lower respiratory tract infection, 2 patients had atrichiosis, 4 patients had a decline in school performance, 2 patients had anxiety and 4 patients had recurrent diarrhea history.

The most common physical examination finding was paleness of the skin and mucosa; the other findings were tachycardia, murmur, and a slowdown in growth (Table 1).

Within the study group, there were 81 cases (50.63%) within the 0-2 age range and 79 cases (49.38%) within the 2-5 age range. Within the control group, there were 39 cases (42.96%) within the 0-2 age range and 52 cases (57.14%) within the 2-5 age range. No statistically important difference was observed between the median ages (months) of the study group and control group ($p=0.077$). No statistically important difference was observed between the age distribution of the study and control groups ($p=0.236$). No significant difference was observed between the groups in terms of gender ($p=0.0108$).

The assessment of the study group and control group in terms of zinc, hemoglobin, hematocrit, MCV, RDW, Fe, TIBC, TSI, and ferritin can be seen in Table 2. (Figure 1, 2). Based on these results, the average zinc of the study group was found lower than that of the control group, which was statistically significant ($p=0.0001$).

Table 1. Examination findings of the patients with anemia, anemia + zinc deficiency

	Paleness of the skin and mucosa	Tachycardia	Murmur	Hair and skin changes	Height 3 percentile	Height 3-10 percentile
Anemia (n)	100	20	10			
Anemia+zinc deficiency (n)	12	2	1	4	5	5

Table2. The Assessment of the Study group and Control Group in terms of zinc, hemoglobin, hematocrit, MCV, RDW, Fe, TIBC, TSI and Ferritin

		Control Group n:91	Study Group n:160	p
Zinc		1.27±0.3	0.94±0.19	**0.0001**
Zinc	**<65** µg/dL	2 2.20%	16 10.00%	**0.021**
	>65 µg/dL	89 97.80%	144 90.00%	
Hemoglobin		12.69±0.68	10.49±1,07	**0.0001**
Hematocrit		38.35±2.26	33.2±3.11	**0.0001**
MCV		79.63±3.44	70.38±6.4	**0.0001**
RDW		12.19±0.75	14.65±1.98	**0.0001**
Fe		88.52±19.88	34.96±14.22	**0.0001**
TIBC		360.53±77.67	404.3±62.98	**0.0001**
TSI		0.25±0.07	0.08±0.04	**0.0001**
Ferritin		39.33±25.55	22.64±21.55	**0.0001**

Zinc deficiency in the study group was found higher than that of the control group, which was statistically significant (p=0.021). While zinc deficiency of 10% was seen in the study group, this ratio was 2.2% in the control group. The zinc deficiency risk in the study group was found to be 4.94 (1.11-22.02) times higher than that in the control group on average.

A statistically significant correlation in a positive way was observed between zinc value and age, hemoglobin, hematocrit, MCV, Fe, ferritin, TSI. Besides, a statistically significant correlation was observed between zinc and RDW, TIBC in a negative way (Table 3). The linear regression analysis was performedwith hemoglobin, hematocrit, MCV, RDW, Fe, TIBC, TSI and ferritin values among which a significant correlation was observed during the univariate tests, and R= 0.599, R2: 0.359, Corrected R2: 0.336 were found (p= 0.0001).

During the multivariate analysis, Fe (p=0.001) showed a statistically significant relationship (Table 3).

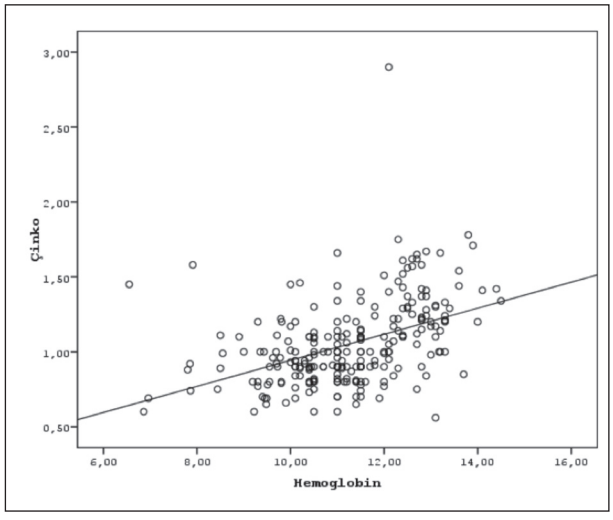

Figure 1. The relationship between zinc and hemoglobin in the study group

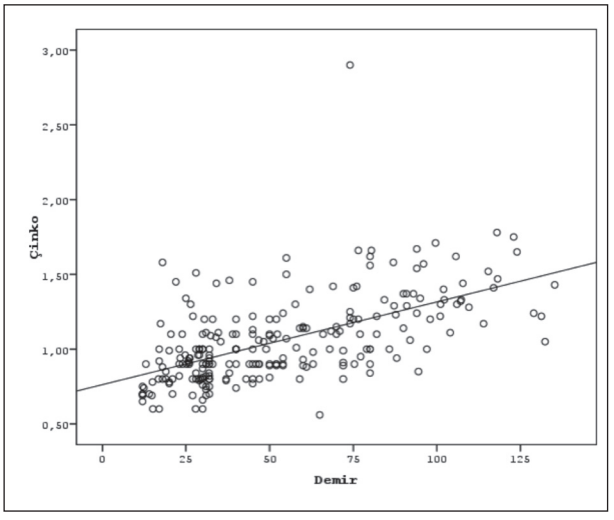

Figure 2. The relationship between zinc and hemoglobin in the study group

Table 3. The correlation between zinc and age, hemoglobin, hematocrit, MCV, RDW, Fe, TIBC, TSI and ferritin values.

		Zinc
Age	r	0.152
	p	0.016
Hemoglobin	r	0.436
	p	0.0001
Hematocrit	r	0.424
	p	0.0001
MCV	r	0.344
	p	0.0001
RDW	r	-0.298
	p	0.0001
Fe	r	0.583
	p	0.0001
TIBC	r	-0.158
	p	0.015
TSI	r	0.580
	p	0.0001
Ferritin	r	0.263
	p	0.001

Discussion

According to the 2001 World health Organization (WHO) report, 30% of the children aged between 0 and 4 years and 48% of the children aged between 5 and 14 years are anemic in developing countries (24). Iron deficiency anemia often shows association with low serum zinc levels, in infancy and young children (2,17).These changes in zinc status are frequently explained by coexisting deficiencies of iron and zinc due to the common dietary sources of both micronutrients and decreasing their intestinal absorption by the same dietary factors (25). Zinc deficiency was reported to occur along with the iron deficiency in the studies on the ID and IDA as well as trace element deficiency (2,5,14,15, 25,26-28). The International Zinc Nutrition Consultative Group concluded that breast milk is a sufficient source of zinc for normal birth weight term infants until approximately 6 months of age. Children start to lose endogenous zinc from non-intestinal sites, such as the urinary tract and skin, after 6 months of age, because infants need more Zn after 6 months of age (29). In children, the highest prevalence of Fe deficiency is found between 4 months and 3 years of age because of the rapid growth and inadequate dietary intake of Fe (26). In the present study, the levels of serum zinc in patients with IDA were lower in comparison to reference levels and controls. The patients in our study were in the 6 month-5 years age range, which is the period of rapid growth. The need of the body for these elements rapidly increases along with the rapid growth. The period following the first6 months is at the same time the transition period to the complementary feeding, and the external intake decreases and, based on this, iron and zinc deficiencies increase within this period.

Duque et al (28) reported in the study they conducted iron and zinc deficiencies constitute the prin-

Table 4. The linear regression results for the multivariate tests.

	Unstandardized Coefficients		Standardized Coefficients		
	B	**Std. Error**	**Beta**	**t**	**p**
Hemoglobin	-0.006	0.032	-0.03	-0.196	0.845
Hematocrit	0.011	0.009	0.141	1.249	0.213
MCV	-0.004	0.004	-0.09	-0.02	0.368
RDW	0.006	0.01	0.045	0.626	0.532
Fe	0.006	0.001	0.576	10.71	**0.001**
TIBC	0.001	0.000	0.029	0.347	0.729
TSI	0.853	0.608	0.273	1.402	0.162
Ferritin	0.001	0.001	0.104	1.762	0.079

cipal micronutrient deficiencies in Mexican children younger than 2 years old and the prevalence of simultaneous iron and zinc deficiencies was 9.2% and 2.7% in urban and rural areas. Ece et al. (2) reported in the study they conducted that zinc deficiency was observed along with IDA in children in the1-14 age group. Additionally, in this study, a positive correlation was identified between serum zinc level and MCV, and no significant correlations were identified among other hematologic parameters and zinc level. In this present study, a statistically significant correlation in a positive way was observed among zinc, hemoglobin, hematocrit, serum iron level, ferritin, and MCV. That a positive relationship of zinc deficiency was identified with these parameters supports the assumption that zinc deficiency occurs along with IDA. In a study carried out in Mexico, iron deficiency prevalence was reported to be 26% and zinc deficiency prevalence was reported to be 13% among children aged 1-4 years (30). In a study carried out in India on children aged 6 months to 5 years, iron deficiency anemia was reported to be 55.8% and zinc deficiency prevalence was reported to be 17.9% (28). It was reported in the study conducted by Özden et al (5) that IDA and zinc deficiency were observed at the same time in the infants in the 6-12 month age range, and these deficiencies were associated with the iron and zinc deficiencies in mothers. The lack of micronutrients in infant period begins with the lack of maternal intake.

Angelova et al (15) reported that trace elements deficiencies including zinc deficiency in the iron deficiency anemia in children under 3 years of age were due to the lack of nutritional intake. A significant correlation in a positive way between age and zinc level was observed in our study. As reported in the literature, zinc deficiency is most commonly seen around 6 months (29). This period is the period of rapid growth in which the need increases and the intake is insufficient. Increased request of zinc due to rapid growth and decreased intake of zinc due to inadequate feeding practices predispose infants and pre-school children, especially living in society of low socioeconomic level, to an elevated risk of zinc deficiency (31). The socioeconomic levels of the patients living in our region are below the average, and it is believed that it is effective in the insufficient intake.

Additionally in this present study, statistical significance in a positive way was also identified between serum zinc level and TSI. Iron and TSI values of the patients, serum zinc levels of whom were low, were lower than those of the patients with higher zinc levels. This finding confirmed the statement that zinc deficiency could be seen in ID without the emergence of anemia. In the study, a negative correlation was observed between RDW and TIBC. RDW and TIBC increases are the expected parameters in iron deficiency anemia, and these are the parameters supporting the statement that zinc deficiency and iron deficiency anemia occur together. In a study carried out on children with IDA aged 6 months to 24 months, a linear correlation was observed between serum zinc level and iron and iron binding capacity (32). Iron deficiency anemia was observed to increase zinc deficiency 5 times. When explaining this correlation, they supported their finding with the opinion that zinc administration to children with IDA decreased the symptoms depending on ID. Furthermore, it was reported that in the co-existence of zinc deficiency and iron deficiency anemia, zinc protoporphyrin increased; when iron decreased in the body, zinc could be used in hemoglobin synthesis instead of iron (33). Park et al. explained the relationship between iron and zinc in their study with this opinion as well. Another mechanism explaining the co-existence of zinc and iron deficiency during infancy is the deficiency in meat intake which is the major source of these two elements. Infants in this age group are fed with grain-based food. Fibre and phytates in grain-based food inhibit the intestinal absorption of zinc and iron (34,35). Our findings are in parallel with the results of the study conducted by Park et al. Zinc deficiency in children leads to morbidity from many diseases such as diarrhea, pneumonia. Diarrhea and pneumonia are still important causes of mortality among children under 5 years of age in developing countries. In this present study, the patients with the co-existence of anemia and zinc deficiency were observed to have recurrent lower respiratory tract infection and diarrhea history. Iron and zinc are the key elements for both physical and mental development of children in the period of rapid growth. Zinc plays a very important role in normal growth and development in human and animal metabolisms through a variety of metalloenzymes and zinc-dependent enzymes. The

patients diagnosed with zinc deficiency were observed to have a slowdown in height and weight development. The infants brought to the hospital with the slowdown in growth, loss of appetite and malnutrition complaints are often found to have complementary feeding with low zinc level (such as fruits, vegetables, dairy products and water) in addition to breast milk. These infants also have iron deficiency anemia. Plasma zinc concentration is generally normal in mild zinc deficiency, therefore, normally, it is not recommended to be determinated. In such cases, in addition to the iron deficiency treatment, the implementation of empirical zinc supplementation (1 mg/kg/day) for 1-2 months is recommended. It is difficult to measure the effectiveness of this approach, but the most common clinical result is the improvement in the growth and food intake (36).

However, it was observed in a study conducted that zinc administration in IDA treatment had no positive or negative effect. Therefore, zinc administration was recommended in iron deficiency anemia at different times in addition to iron administration if there was zinc deficiency as well (37). Unfortunately, zinc level is not checked routinely in many centers.

Conclusion

Iron and zinc are the 2 micronutrients that are deficient in the diet of the people of Third World countries. It was found out in this study that 10% of children with iron deficiency anemia had zinc deficiency as well. It is thought that iron zinc combination can be more effective in iron deficiency treatment compared to iron alone treatment, and early period supplementary programs can be useful for high-risk groups such as infant period and early childhood period until zinc deficiency tests give routine values, or to utilize more widely the routine control of serum zinc level is considered to be helpful.

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Original article

The relationship between plasma total antioxidant capacity and dietary antioxidant status in adults with type 2 diabetes

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Summary. *Background and aim:* There is limited information available on the association of plasma total antioxidant capacity (TAC) with dietary TAC in DM. The aim of this study was to evaluate the plasma TAC and its association with dietary antioxidant status in adults with Type 2 diabetes. *Methods:* Thirty outpatients diagnosed with type 2 DM (diabetics, n = 29) and 15 healthy subjects (control, n = 15) aged 40-70 with BMI≤ 30 kg/m^2 were recruited to the study. Energy and nutrients intake, anthropometric measurements, dietary and plasma TAC, and some biochemical parameters were evaluated. The calculation of dietary TAC was based on previously published databases in which modified version of the FRAP. *Results:* Serum triglyceride, uric acid, and HbA1c levels in diabetics were higher than controls. A negative and statistically significant correlation was found between plasma TAC and HbA1c for diabetics. A negative and statistically significant correlation was observed between dietary TAC, HbA1c and fasting plasma glucose in diabetics. A positive and statistically significant correlation was found between plasma TAC and dietary intake of niacin in diabetics. No remarkable differences were found between dietary and plasma TAC in either group.*Conclusion:* This study provides evidence that dietary TAC is not an important modulator of antioxidant status in diabetic subjects. But, it showed that the increase in niacin and antioxidant taken with foods can be effective in controlling HbA1c and fasting plasma glucose.

Key words: antioxidant, HbA1c, metabolic control, TAC, type 2 diabetes mellitus

Introduction

According to the World Health Organization (WHO), approximately 150 million people have diabetes mellitus (DM) worldwide, and this number may double by the year 2025 due to population growth, age, unhealthy diet, obesity and sedentary lifestyle (1). There is concern about an emerging diabetes epidemic in Turkey. A cross-sectional survey, "The Turkish Epidemiology Survey of Diabetes, Hypertension, Obesity and Endocrine Disease" (TURDEP-II) conducted in 2010, included 26.499 randomly sampled adults aged ≥ 20 years showed that the prevalence of diabetes was 16.5% translating to 6.5 million adults with diabetes in Turkey (2).

The role of oxidative stress and inflammation in several chronic diseases is receiving increasing attention due to identified links with chronic diseases such as atherosclerosis, obesity, or type 2 DM (3). One of the pathogenic mechanisms that can explain this increased risk in DM is the imbalance between pro-oxidants and antioxidants, which results in oxida-

tive stress (OS) (4). Hyperglycemia results in glucose auto-oxidation, no enzymatic glycation and monocyte dysfunction, which lead to increased production of free radicals (5). This is further aggravated by the decreased levels of antioxidants (6) and leads to oxidative damage, illustrated by the high levels of lipid and DNA peroxidation products found in diabetic patients (7).

Food intake has been related to oxidative stress modulation (8, 9), being described as energy restriction that might decrease the levels of oxidative stress mediators (10). Antioxidant intake has been suggested to protect against oxidative damage (11).

Dietary antioxidants have been hypothesized to have a protective effect against the development of DM by inhibiting peroxidation chain reactions (12). Fruit and vegetable consumption has long been reported to be associated with lower incidence and mortality rates of several chronic diseases (13, 14). One hypothesis of the protective effects is that all types of antioxidant compounds, including vitamin C, vitamin E, carotenoids, and phytochemicals such as flavonoids and proanthocyanidins could protect cells from free radical–induced oxidative damage (15).

An increasing number of studies have reported that an increase in plasma TAC is associated with intake of fruits and vegetables which are rich in antioxidants (16, 17). Given that the concentration of single antioxidants may not reflect the total antioxidant power of food, the concept of TAC was introduced (18). Dietary TAC has been shown to be a good indicator of diet antioxidant status (19, 20).

Nevertheless, there is limited information available on the association of plasma TAC with dietary TAC in DM. It has been hypothesized that dietary TAC is a good indicator of diet quality with respect to reflecting the antioxidant capacity of a diet, as well as a predictor of plasma antioxidant status. Thus, the aim of this study was to evaluate the plasma TAC and association with dietary TAC in adults with type 2 DM.

Methods

Study population and design

This descriptive study was performed at Internal Medicine Clinic of Kayseri Acıbadem Hospital, a tertiary referral Centre in Turkey, the between 2013 and 2014.

Thirty outpatients diagnosed with type 2 DM (diabetic group, n = 30) and 15 healthy subjects (control group, n = 15) aged 40-70 with BMI ≤ 30 kg/m^2, and who did not report any physical activities were recruited to this study (5). All controls were in good health as determined by a medical history questionnaire, physical examination and normal results of clinical laboratory tests. All diabetics were on different medications, mainly metformin, and they were not on special diets. Of 45 subjects, 1 diabetic patients had missing information thus data of the 44 participants were used.

Demographic characteristics like age, gender, marital status (married, divorced, and widowed), financial status (average annual income during the past three years), and occupational status as well as education level were obtained by questionnaire during a face to face interview. Anthropometric measurements, dietary and plasma TAC, and some biochemical parameters were evaluated. The subjects in both groups were matched according to socio-demographic features, sex and age.

Exclusion criteria were derived as follows: 1) individuals who were administered supplements during the 6 months prior to this study 2) patients with evidence of any diabetic complication and history of chronic conditions or diseases, including cardiovascular disease, certain cancers, and chronic obstructive pulmonary disease, 3) alcohol consumption and smoking 4) those who were on insulin treatment.

In accordance with the Declaration of Helsinki, after a clear explanation of the study protocol, all subjects gave written informed consent to participate in this study, which was approved by the ethics committee of Faculty of Medicine in Erciyes University (Kayseri, Turkey) (reference 2012/571).

Dietary assessment

All participants recorded their 24-hour food consumption for three consecutive days including at least one weekend day. Nutrient Database (BeBiS, Ebispro for Windows, Germany; Turkish Version/BeBiS 7) was used to determine energy and nutrient intake (21). Volumes and portion sizes were estimated with 2-dimensional food models and with a portion size picture

booklet including 120 photographs of food, each with 3–5 different portion sizes (22).

The calculation of total dietary TAC was based on previously published databases in which a modified version of the FRAP assay that allowed quantification of most water- and fat-soluble antioxidants (23) was considered for each food. This Antioxidant Food Database was used because the samples were procured from local stores and markets in Scandinavia, USA and Europe and from the African, Asian and South American continents. We calculated total dietary TAC of 3100 foods, and recorded them in the Nutrient Database (BeBiS, Ebispro for Windows, Germany; Turkish Version/BeBiS 7).

Anthropometric measurements

Anthropometric measurements were determined according to WHO criteria (24). Body weight and height were measured and BMI was calculated (BMI=body weight (kg)/height (m^2)). All subjects were weighed while wearing light clothing and without shoes, using a calibrated digital flat scale (Seca-803, USA). Standing height was measured without shoes to the nearest 0.5 cm using a measuring tape.

Assessment of biochemical parameters

Biochemical analysis

Following an 8 hour overnight fast, blood samples were collected between 08.30 and 10.30 am. Routine blood tests including serum fasting plasma glucose (FPG), triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), glycated hemoglobin (HbA1c), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and uric acid were analyzed in the Acıbadem Hospital laboratory.

Serum FPG, TG, TC, and HDL-C were determined with kits using Architect c16000 auto analyzer (Abbott Diagnostics, USA). The reported serum low-density lipoprotein cholesterol (LDL-C) data were calculated using the Friedewald equation as described elsewhere (25). HbA1c was measured by boronate affinity assay using the NycoCard HbA1c Kit (Axisshield, Oslo, Norway). Serum AST and ALT were determined using a commercial kit, Latrozyme TA-LQ (Dai Iatron Co, Tokyo, Japan). Serum uric acid was assessed by using colorimeter on a Konelab Autoanalyser (Thermo Scientific). The serum was separated by centrifugation and stored at 80°C then shipped on dry ice to the laboratory where TAC analysis was performed. TAC of plasma was determined based on the Trolox equivalent antioxidant capacity assay using a colorimetric commercial kit (Cayman Chemical Corporation, Ann Arbor, MI, USA).

Statistical analysis

Data were analyzed by SPSS, version 21.0 (Inc., Chicago, IL, USA). Normal distribution of data was determined with Shapiro-Wilk test. Chi-square analysis was used to compare the difference of variables between groups and Mann Whitney U test was used for non-normally distributed data by showing median and 25–75 percentiles. Spearman correlation was performed to determine the association of dietary and plasma TAC as well as energy and nutrient intake and biochemical parameters. $p<0.05$ was set as statistically significant.

Results

Mean ages of subjects were 55.86±5.69 years for diabetics, and 51.73±7.27 years for controls. 31.0% of diabetics (n=9) and 33.3% of controls (n=5) were male, while 69.0% of diabetics (n=20) and 66.7% of controls (n=10) were female. Socio-economic status, educational level, and occupation were similar in both groups. Median levels of body weight and height were higher in diabetics than in controls ($p<0.05$) while median level of BMI was not significantly different between groups ($p>0.05$) (Table 1). Dietary intakes of energy and nutrients were not significantly different between groups ($p>0.05$) (Table 2). Although statistically insignificant, dietary TAC in diabetics (3.09±1.81 mmol/day) was higher than in controls (2.97±1.76 mmol/day) (Table 2). Serum triglyceride ($p<0.05$), uric acid ($p<0.05$), and HbA1c ($p<0.01$) levels in diabetics were higher than controls whereas LDL-C were lower ($p>0.05$) (Table 3). Although statistically insignificant, plasma TAC level of controls (0.94±0.34 mmol) were lower than diabetics (1.00±0.42 mmol) ($p>0.05$) (Table 1). Negative and statistically significant correla-

Table 1. Anthropometric measurements and biochemical parameters of diabetics and controls

Parameters	Diabetics (n=29)	Controls (n=15)	p
	X±SD Median-25%-75%	X±SD Median-25%-75%	
Age (year)	55.86±5.69 57.00 (54.00- 60.00)	51.73±7.27 51.00 (45.00-58.00)	0.068
Weight (kg)	74.66±9.59 75.30 (68.90-81.00)	67.08±6.34 66.75 (61.10-69.91)	0.016
Height (cm)	163.10±8.17 164.00 (158.00-168.00)	156.17±6.28 156.00 (150.00-160.00)	0.012
BMI (kg/m²)	27.95±1.88 28.60 (27.50-29.45)	27.64±2.14 27.90 (26.80-29.51)	0.618
FPG (mg/dl)	112.19±64.09 97.00 (65.25-129.75)	81.75±54.58 64.00 (50.00-105.75)	0.073
TG (mg/dl)	205.07±112.25 196.00 (118.00-285.00)	138.53±83.23 118.00 (86.00-159.00)	0.032
TC (mg/dl)	219.79±37.10 224.00 (187.50-249.00)	238.93±38.62 234.00 (204.00-258.00)	0.117
HDL (mg/dl)	46.07±10.12 45.00 (39.50-53.50)	57.53±30.35 49.00 (42.00-58.00)	0.154
LDL (mg/dl)	134.31±35.47 129.00 (113.50-159.50)	156.93±30.75 150.00 (131.00-177.00)	0.042
AST (U/L)	17.93±8.88 17.00 (13.50-20.50)	18.77±7.19 16.00 (13.00-23.50)	0.795
ALT (U/L)	16.45±10.11 13.00 (10.50-17.00)	22.86±21.44 16.50 (10.25-29.50)	0.377
Uric acid (mg/dl)	5.29±1.69 5.20 (3.85-6.20)	4.13±0.85 3.90 (3.65-4.65)	0.018
HbA1c (%)	7.51±1.82 6.90 (6.30-9.00)	5.59±0.28 5.50 (5.40-5.80)	0.002
Plasma TAC (mmol)	1.00±0.42 1.00 (0.71-1.32)	0.94±0.34 0.96 (0.61-1.15)	0.626

tion was found between plasma TAC and HbA1c (r=-0.376, p=0.053) for diabetics, and LDL-C (r=-0.647, p=0.012) for controls (Table 3). When all participants were considered, a negative association was observed between plasma TAC and dietary intake of vitamin E in controls (r=-0.537, p=0.048). Also, a positive and statistically significant correlation was found between plasma TAC and dietary intake of niacin in diabetics (r=0.487, p=0.007). No remarkable differences were found between dietary TAC and plasma TAC in the groups (p>0.05) (Table 3). Negative and statistically significant correlation was observed between dietary TAC, HbA1c and fasting plasma glucose in diabetics (r=-0.531, p=0.004) (Table 4).

Discussion

To our knowledge, this is the first study designed to evaluate the dietary antioxidant intake and its association with plasma antioxidant status in adults with type 2 diabetes in Turkey.

Table 2. Energy and nutrients intakes of diabetics and controls

Parameters	Diabetics (n=29)	Controls (n=15)	p
	X±SD Median-25%-75%	X±SD Median-25%-75%	
Energy (kcal/d)	1660.56±588.73 1432.90 (1156.55-2095.30)	1696.59±485.25 1554.60 (1276.90-2173.00)	0.840
CHO (g/d)	193.76±73.68 181.70 (136.10-247.70)	190.89±48.97 179.30 (152.10-223.90)	0.893
CHO (%)	48.00±7.10 49.00 (42.00-53.00)	46.73±5.05 47.00 (43.00-49.00)	0.543
Protein (g)	57.12±19.74 53.00 (41.95-72.90)	65.17±22.56 65.20 (42.30-88.10)	0.229
Protein (%)	14.38±3.29 14.00 (12.00-16.00)	15.87±4.31 15.00 (12.00-18.00)	0.313
Fat (g/d)	70.85±29.20 66.40 (46.20-88.50)	72.58±26.79 66.50 (47.10-101.10)	0.849
Fat (%)	37.69±7.14 37.00 (33.00-44.00)	37.40±4.64 38.00 (35.00-41.00)	0.738
Fiber (g/d)	20.04±7.57 21.50 (12.85-26.40)	21.21±8.35 20.30 (14.40-25.50)	0.642
TC (mg)	237.04±125.80 237.30 (141.30-334.50)	274.71±99.96 234.80 (198.00-337.40)	0.321
Vitamin A (mcg)	1328.22±1103.31 1190.90 (606.65-1574.60)	1198.04±571.50 988.80 (861.20-1622.00)	0.853
Vitamin E (mg)	14.46±6.17 14.60 (9.80-18.60)	14.85±6.55 13.90 (9.00-19.60)	0.846
Thiamine (mg)	0.83±0.30 0.80 (0.65-1.05)	0.89±0.31 1.00 (0.60-1.10)	0.498
Riboflavin (mg)	1.37±0.55 1.30 (0.90-1.80)	1.46±0.59 1.60 (0.90-1.80)	0.601
Niacin (mg)	18.18±6.71 17.70 (13.25-21.45)	20.51±10.75 18.00 (10.10-28.50)	0.453
Vitamin C (mg)	135.68±87.23 129.60 (74.95-170.40)	154.89±84.31 140.80 (116.10-134.80)	0.488
Ca (mg)	652.99±273.81 592.80 (443.25-794.10)	675.49±343.58 690.40 (327.10-932.60)	0.814
Mg (mg)	248.57±107.16 249.90 (168.20-317.65)	241.85±97.47 266.60 (162.50-305.50)	0.840
Fe (mg)	29.38±99.77 10.80 (7.90-15.10)	11.10±3.72 10.30 (8.60-14.30)	0.931
Zn (mg)	8.05±2.94 7.40 (5.55-10.30)	9.63±3.84 9.40 (5.90-12.40)	0.193
Dietary TAC (mmol/day)	3.09±1.81 3.00 (1.45-4.80)	2.97±1.76 2.30 (1.60-4.00)	0.931

Table 3. Relationship between plasma and dietary TAC as well as dietary TAC and nutrient intakes and biochemical parameters in diabetics and controls

	Plasma TAC (mmol/L)			
	Diabetics (n=29)		Controls (n=15)	
	r	p	r	p
Dietary TAC (mmol/day)	0.293	0.123	0.482	0.081
Energy (Kkal)	0.239	0.214	-0.301	0.296
CHO (g)	0.254	0.184	0.204	0.485
CHO (%)	-0.053	0.786	0.520	0.056
Protein (g)	0.179	0.352	-0.314	0.274
Protein (%)	-0.067	0.730	-0.307	0.285
Fat (g)	0.248	0.194	-0.248	0.392
Fat (%)	0.007	0.972	-0.262	0.365
Fiber (g)	-0.101	0.601	0.156	0.594
TC (mg/dl)	0.123	0.524	0.321	0.263
Vitamin A (mcg)	0.017	0.929	0.253	0.383
Vitamin E (mg)	0.034	0.861	-0.537	0.048
Thiamine (mg)	0.163	0.398	0.115	0.695
Riboflavin (mg)	0.168	0.385	0.188	0.521
Niacin (mg)	0.487	0.007	-0.336	0.240
Vitamin C (mg)	0.193	0.316	-0.295	0.306
Ca (mg)	0.132	0.494	-0.002	0.994
Mg (mg)	0.116	0.550	0.090	0.759
Fe (mg)	0.009	0.962	0.235	0.418
Zn (mg)	0.155	0.423	0.279	0.334
FPG (mg/dl)	-0.158	0.440	0.336	0.312
AST (U/L)	0.002	0.990	-0.232	0.468
ALT (U/L)	-0.137	0.479	0.302	0.316
Uric Acid (mg/dl)	0.141	0.465	0.319	0.313
HbA1c (%)	-0.376	0.053	0.220	0.601
TG (mg/dl)	0.119	0.540	-0.029	0.923
TC (mg/dl)	-0.177	0.359	-0.427	0.128
HDL-C (mg/dl)	-0.020	0.918	-0.117	0.690
LDL-C (mg/dl)	-0.245	0.200	-0.647	0.012

The synergistic effect of antioxidants in human plasma is known to provide greater protection against free radical aggression than any single antioxidant alone (26). The current study measured the total antioxidant activity of plasma because of its established ability to withstand oxidative stress (27). Although statistically insignificant, plasma TAC level was higher in patients with diabetes compared with controls consistent with the results of Savu et al. as well as Kharroubi et al. (28, 29). As shown in limitations, similar plasma TAC levels may be due to insufficient individuals in both groups.

Table 4. Association between dietary total antioxidant capacity (TAC) and biochemical parameters in diabetics and controls

	Dietary TAC (mmol/day)			
	Diabetics (n=29)		Controls (n=30)	
	r	p	r	p
HbA1c (%)	-0.531	0.004	-0.247	0.522
FPG (mg/dL)	-0.406	0.036	0.112	0.577
TG (mg/dL)	-0.042	0.825	-0.364	0.052
HDL (mg/dL)	0.083	0.662	-0.225	0.240
AST (U/L)	-0.049	0.798	-0.464	0.034
ALT (U/L)	0.237	0.207	-0.173	0.429
Uric acid	0.073	0.703	0.493	0.011

Opara et al. (30) reported a decrease in antioxidant levels in diabetic subjects with complications while Srivatsan et al. (31) found an increase in antioxidant levels in diabetic subjects without complications. Most diabetic subjects in our study seem to have no obvious complications. This is consistent with the notion that the increase in free radicals seems to be associated first with an increase in antioxidant levels and with the progression of the disease the antioxidant levels decrease and complications eventually develop. The association between serum uric acid and diabetes mellitus and their findings are not consistent. Some studies reported that there is a positive association between high serum uric acid levels and diabetes (32-34), whereas other studies reported no association (35), or an inverse relationship (36, 37). Significantly increased plasma uric acid concentrations were found in patients with diabetes in the current study.

In type 2 diabetes, chronic exposure to hyperglycemia and insulin resistance has been implicated in altered oxidative metabolism. Several different mechanisms have been proposed to explain why oxidative stress is increased in diabetes mellitus; these mechanisms fall into two general categories: increased production of ROS and decreased antioxidant defences (38). In some previous studies (29,39), no or positive association was found between plasma TAC and HbA1c. However, in the present study, there was a negative correlation between HbA1c and plasma TAC (Table 3). This result was consistent with the results of Wahba et al. (40) and Peerapatdit et al. (41) which can be explained by the fact that hyperglycemia decreases antioxidant levels.

Nutrition is a potent tool in regulating glucose metabolism. Food, through fruit and vegetable consumption, can be a great source of antioxidants that protect the body against oxidative damage and insulin resistance (42). The results of the present work suggest that higher total dietary antioxidant intake is correlated with lower levels of HbA1c and fasting plasma glucose in diabetic individuals (Table 4). Niacin is one of the vitamins belonging to vitamin B complex. Niacin is found in both plant and animal foods. Due to the contribution of tryptophan, foods containing balanced protein may contribute to high niacin equivalent. Niacin compounds provide potential health benefits like combating cardiovascular disease, diabetes, osteoarthritis, neurological problems, and skin diseases (43). A positive and statistically significant correlation was found between plasma TAC and dietary intake of niacin in diabetics in this study. To our knowledge, there has not been a study designed to evaluate between the relationship dietary niacin intake and plasma TAC level in diabetics, thus further research is needed on this topic. Although, several observational studies showing a positive correlation between dietary and plasma TAC (15, 44-49), in our study, no remarkable differences were found between dietary and plasma TAC. FAO/WHO Expert Consultation on diet, nutrition and the prevention of chronic diseases recommends the intake of a minimum of 400 g of fruit and vegetables per day (50). According to Dietary Guidelines for Turkey, vegetable or fruit must be consumed at least 5 servings daily (51). Since the individuals did not consume the recommended amount of vegetables and fruits (diabetics=192 g, controls= 200 g), a significant relationship may have not been found between the diabetics and controls. This is consistent with results from a number of intervention studies (52-54). Studies that monitored dynamic change of plasma TAC immediately following consumption of tea, coffee, red wine, nuts, fruits, and vegetables found a significant increase of plasma TAC that reached its peak 1 or 2 hours after consumption (45,46,48,49). However, studies on the long-term effect of consuming TAC-rich foods on fasting plasma TAC levels reported inconclusive results (17, 52, 54). To date, few studies have examined whether dietary TAC modulates plasma TAC. Our study provides evidence that dietary TAC is not an important modulator of antioxidant status in diabetic subjects.

In conclusion no remarkable differences were found between dietary and plasma TAC in diabetic patients. But, it was shown that the increasing in TAC taken with foods can be effective in controlling HbA1c and fasting plasma glucose.

Limitations

This analysis was performed in a small sample. Longitudinal data on plasma total antioxidant and dietary antioxidant status among diabetic patients is needed.

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Original Article

Protective effects of camel milk and vitamin E against monosodium glutamate induced biochemical and testicular dysfunctions

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Summary. *Objective:* The current study was outlined to examine the protective effects of camel milk (CM) against the deleterious effects induced by monosodium glutamate (MSG). *Methods:* MSG was administered either alone or in combination with camel milk or vitamin E for 4 weeks. Serum and testicular tissues were examined using semiquantitative RT-PCR analysis. *Results:* CM normalized the decrease in serum levels of testosterone, luteinizing hormone (LH), sperm profiles and testicular antioxidant activities that were decreased by MSG. At the molecular levels, MSG down-regulated the mRNA expression of steroidogenesis related genes and receptors of androgen, LH and follicle stimulating hormone. MSG induced testicular apoptosis. All altered genes were normalized and upregulated in presence of CM when compared to the effect of Vit. E. *Conclusions:* The usage of camel milk as a therapy against MSG used in food industry is very indicative and protective.

Key words: monosodium glutamate, camel milk, protection, testicular dysfunction, gene expression, vitamin E.

Introduction

Monosodium glutamate (MSG) is the sodium salt of glutamic acid (1). About 78% of MSG is glutamic acid and 22% are sodium and water (2). Glutamate is one of the most common amino acids found in nature and is the main component of many proteins and peptides of most tissues (2). Glutamate plays an essential role in human metabolism. It is a major component of many protein-rich food products either in free or bound state of animal such as meat, fish, milk and cheese or vegetable origins such as mushroom and tomato (3). Moreover, MSG is widely used as a flavor enhancer especially in Chinese and Japanese foods and restaurants (4,5). MSG provides a flavoring function similar to the naturally occurring free glutamate which differ from sweet, sour, salty and bitter (6). Recently MSG was used as food additive as a flavoring or hydrolysed vegetable protein (7). MSG influences the appetite positively, and induces weight gain as it stimulates oro-sensory receptors and improves the palatability of meals. However, reports indicated that to a certain degree, MSG is toxic to human and experimental animals (7). MSG shows symptoms such as numbness, weakness, flushing, sweating, dizziness and headaches (8). In over doses, MSG exacerbate numerous conditions, including asthma, urticaria, atopic dermatitis, ventricular arrhythmia, neuropathy and abdominal discomfort (8). MSG induced neurotoxic effects that caused brain cell damage, retinal degeneration, endo-

crine disorder and some pathological conditions such as addiction, stroke, epilepsy, brain trauma, neuropathic pain, schizophrenia, anxiety, depression, Parkinson's disease, Al- zheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis (2). MSG is toxic for testis as it induced oligozoospermia and increased abnormal sperm morphology (9). It caused male infertility at histo-pathological level by causing testicular hemorrhage, alteration and degeneration of sperm cell count and morphology (10).

As known, vitamins are essential for all biochemical reactions. They act as antioxidants to protect tissues from oxidative stress due to their easy, effective and safe dietary administration in a large range of concentrations (11,12). Vitamin E (α- Tocopherol [(α-Toc]) is a membrane bound lipid-soluble, chain-breaking antioxidant that protects all cell membranes against lipid peroxidation (13,14). Vitamin E pretreatment has been reported to be beneficial in preventing formaldehyde-induced tissue damage in rats (15,16). The preventive effect of vitamin E on cypermethrin or endotoxin-induced oxidative stress in rat tissues is suggestive of its antioxidant activity (17). Vitamin E is the most known antioxidants that have a protective effect by either reducing or preventing oxidative damage (17). Vitamin E prevents lipid peroxidation by interfering with the propagation of lipid radicals (11). It is a non-enzymatic antioxidant (15,16). Vitamin E inhibits lipid peroxidation by both scavenging lipid peroxyl radicals (15,16), and inhibits oxidative damage induced by heavy metals and pesticides in experimental animals (15).

Camel's milk (CM) exhibits a wide range of biological activities including antimicrobial, antioxidative, antithrombotic, antihypertensive, and immunomodulatory effect (18). It was therapeutically used to treat jaundice, splenic problems, asthma, anemia, piles, and diabetes (19,20). Camel milk contains high levels of lactoferrin which is an iron-binding glycoprotein of the transferring family (21). Camel milk anticarcinogenic, anti-inflammatory and antioxidant activities were proposed to be mainly caused by lactoferrin (22). Camel's milk is effective in food allergy treatments due to its inflammation-inhibiting proteins, and hypoallergenic properties (23). Therapeutic potency of camel milk against several diseases is due to its newly identified nanobodies content (24).

From all established data, we can conclude that the deleterious effects of MSG on liver, kidney and testes were mainly at the histology and pathology levels (25, 26) but no studies about its harmful molecular effects and possible protection by camel milk were reported till now. Therefore, the current study aimed to examine the protective effects of camel milk on deleterious effects of MSG on hepatic and renal biomarkers and antioxidants levels compared to vitamin E action. Moreover, genes of testicular function and steroidogenesis were examined after MSG administration and protection with camel milk compared to vitamin E.

Materials and methods

Chemicals and kits

Monosodium glutamate, agarose and ethidium bromide were purchased from Sigma-Aldrich (St. Louis, MO, USA). The Wistar albino rats were purchased from King Fahd center for Scientific Research, King Abdel-Aziz University, Jeddah, Saudi Arabia. Kits for glutamate pyruvate transaminase (ALT), glutamate oxalacetate transaminase (AST), superoxide dismutase (SOD), malondialdehyde (MDA), catalase, glutathione reductase (GSH), creatinine and urea were purchased from Bio-diagnostic Co., Dokki, Giza, Egypt. The deoxyribonucleic acid (DNA) ladder was purchased from MBI, Fermentas, Thermo Fisher Scientific, USA. Qiazol for RNA extraction and oligo dT primer were purchased from QIAGEN (Valencia, CA, USA).

Animals handling and experimental design

Forty male Wistar rats, 8 weeks old, weighting 170–200 grams were given free access to food and water. Rats were maintained at 12h/12h day and light and at 25 ± 5°C. After 2 weeks of daily handling and acclimatization, rats were assigned for experimental procedures. Rats were allocated into 4 subgroups, with 10 rats per group. Control rats gained free access to food and water. MSG group was given a normal diet together with MSG (2 g/kg/day) for 4 weeks (27,28). MSG + Vit. E group that administered MSG orally (2 g/kg /day) for 4 weeks with Vit E at a dose of (20 mg/kg/day) (29). Camel milk (CM) + MSG group was given MSG for 4

weeks together with camel milk in a dose of 166.6 ml /24 hours / 10 rats according to Althnaian (30). After 4 weeks, all rats were anesthetized using diethyl ether after overnight fasting and blood was collected for serum extraction and biochemical assessments. Testicular tissues were taken on Qiazol for RNA extraction and gene expression (RT-PCR).

Serum separation and biochemistry analysis

Blood was collected from the eye using heparinized capillary tubes inserted into retro-orbital venous plexuses. Blood was left to clot at room temperature then in the refrigerator for 15 minutes and centrifuged for 10 minutes at 4°C and 5000 rpm, supernatant serum was taken and stored at -20 °C till assays. Fasting blood glucose levels were determined using spectrophotometric assay. Serum creatinine, urea, albumin, ALT, AST, triacylgycerol (TAG), total cholesterol and HDL were measured using commercial available kits that are based on spectrophotometric analysis.

Determination of testicular tissue antioxidant activity

For measurements of MDA, catalase and SOD activity, one gram of testicular tissues was homogenized in 5ml of cold buffer (50mM potassium phosphate buffer; PBS, pH 7.4). Cold buffer of catalase activity contains 1mM EDTA and 1mL/l Triton X-100. After centrifugation at 4000 *x g* for 15 minutes at 4°C, the supernatant was removed and stored frozen at -80°C until the time of analysis of catalase and SOD (U/g tissue) and MDA (nmol/g tissue). The activities of MDA, catalase and SOD were determined by ELISA reader (Absorbance Microplate Reader ELx 800TM BioTek®, Seattle, WA, USA). Results were calculated according to the manufacturer's instructions. The assays used for measurement of MDA, Catalase and SOD were based on previous studies (31-33) for MDA, catalase and SOD respectively.

Serum hormone assays

Changes in levels of testosterone and luteinizing hormone (LH) were measured using commercial kits (Testosterone ELISA Kit from abcam, Tokyo, Japan Cat # ab108666) that purchased from Clini Lab., Al-Manial, Cairo, Egypt. Serum LH levels were measured using LH SimpleStep ELISA™ Kit (ab108651)), Osaka, Japan. The instruction manual of each kite was followed as suggested by providers.

Sperm analysis (eosin-nigrosin stain)

The epididymal sperm was collected according to Blash et al (34) with some modification. The testes were removed from the scrotal sac within 5 to 10 minutes, placed in an insulator box and transported to laboratory and processed individually. The parietal tunic was removed leaving the tail of the epididymis exposed. A small lateral incision was made along the tail of the epididymis to open the convoluted tubules and put in petri dish with 3.025 g Tris, 1.7 g citric acid, 1.25 g fructose supplemented with 5.5 mg tylosin, 27.5 mg gentamycin, 16.5 mg lincospectin, and 330 mg spectinomycin per 100 ml. Spermatozoa were sedimented by gentle centrifugation at 800 X g (1200 rpm) for 5 min at 30°C and the pellet was washed twice with TFC medium to remove contaminating epididymal plasma. The cells were dispersed in the same medium and this preparation of spermatozoa was used for the experiments. Individual sperm motility was assessed by bright field microscopy. Diluted sperm was examined microscopically using adjusted hot stage microscope at 38°C. Individual sperm motility percent was determined on a subjective scale of 0–100% to the nearest 5% after examining several microscopic fields. The percentage of live and abnormal sperms was assayed by staining smears with eosin-nigrosin (35). A total of 200 sperm cells were examined randomly. Total sperm abnormalities and live percentage were recorded.

RNA Extraction, cDNA Synthesis and Semi-quantitative RT-PCR Analysis

Total RNA was extracted from testicular tissues in Qiazol reagent (50-100 mg per sample). RNA was extracted using chloroform-isopropanol extraction assay. RNA pellets were washed with 70% ethanol, briefly dry up, and then dissolved in Diethylpyrocarbonate (DEPC) water. For cDNA synthesis, a mixture of 2 µg total RNA and 0.5 ng oligo dT primer in a total volume of 11 µl sterilized DEPC water was incubated in the PeX 0.5 thermal Cycler (Thermo Electronic Corporation, Milford, Ma) at 65°C for 10 min for denaturation. Then, 4 µl of 5X RT-buffer, 2 µl of 10 mM dNTPs and 100 U Moloney Murine Leukemia

Virus (M-MuLV) Reverse Transcriptase (SibEnzyme Ltd. Ak, Novosibirsk, Russia) were added and the total volume was completed up to 20 µl by DEPC water. The mixture was then re-incubated in the thermal Cycler at 37°C for one hour, then at 90°C for 10 min to inactivate the enzyme.

For semi-quantitative RT-PCR analysis, specific primers for examined genes (Table 1) were designed using Oligo-4 computer program and synthesized by Macrogen (Macrogen Company, GAsa-dong, Geumcheon-gu. Korea). PCR was conducted in a final volume of 25 µl consisting of 1 µl cDNA, 1 µl of 10 pM of each primer (forward and reverse), and 12.5 µl PCR master mix (Promega Corporation, Madison, WI). The volume was brought up to 25 using sterilized, deionized water. PCR was carried out using Bio-Rad thermal Cycle with the cycle sequence at 94 ˚C for 5 minutes one cycle, followed by 26 cycles for examined genes and 23 cycles for the reference gene (glyceraldehyde-3-phosphate dehydrogenase; GAPDH). Each PCR cycle consists of denaturation at 94 ˚C for one minute, annealing at the specific temperature corresponding to each primer (Table 1) and extension at 72 ˚C for one minute with additional final extension at 72 ˚C for 7 minutes. Products of PCR were run on 1.5% agarose (Bio Basic INC. Konrad Cres, Markham Ontario) gel stained with ethidium bromide in TBE (Tris-Borate-EDTA) buffer and visualized under UV light and photographed using gel documentation system. The band intensities were densitometrically quantified and calculated using ImageJ software version 1.47 (http://imagej.en.softonic.com/).

Statistical analysis

The data are presented as the mean ± standard error of the mean. Analysis of variance and Fisher post-hoc descriptive test were used to analyze the data using SPSS software version 11.5 for Windows (SPSS, Inc., Chicago, IL, USA). Using the same software, regression analysis was performed. $P<0.05$ were considered to indicate a statistically significant difference.

Results

Serum biochemical, oxidative and antioxidative biomarkers

Administration of MSG constitutively for 4 week induced a decrease in urea, creatinine and increased ALT

Table 1. PCR conditions for examined genes in the testis.

Gene	Product size (bp)	Annealing (°C)	Direction	Sequence (5-3)
ABP	260	58	Sense Antisense	TCCGATACCACCAAGCACAAG TCAGGAAAGCTGGGAACACTG
LHR	272	52	Sense Antisense	AGAGTGATTCCCTGGAAAGGA TCATCCCTTGGAAAGCATTC
$P450_{c17}$	302	55	Sense Antisense	GACCAAGGGAAAGGCGT GCATCCACGATACCCTC
P53	547	55	Sense Antisense	ATCTGGACGACAGGCAGACT AGGCAGTGAAGGGACTAGCA
17β-HSD	653	55	Sense Antisense	TTCTGCAAGGCTTTACCAGG ACAAACTCATCGGCGGTCTT
AR	570	55	Sense Antisense	TTACGAAGTGGGCATGATGA ATCTTGTCCAGGACTCGGTG
FSHR	490	55	Sense Antisense	GAGTCATCCCGAAAGGATCA TAAAATGACTGGCCCAGAGG
Aromatse	389	58	Sense Antisense	GCCTGTCGTGGACTTGGT GGTAAATTCATTGGGCTTGG
β actin	457	60	Sense Antisense	ATGTACGTAGCCATCCAGGC TCCACACAGAGTACTTGCGC

Table 2. Protective effect of camel milk against MSG induced changes on renal and hepatic biomarkers.

Parameter	Urea (mg/dL)	Creatinine (mg/dL)	GOT (U/l)	GPT (U/l)
Control	36 ± 0.3	0.7 ± 0.01	82 ± 3.7	98.6 ± 7.5
MSG	33 ± 1.2*	0.3 ± 0.1*	182 ± 2.9*	186 ± 3.2*
MSG + Vit E	38 ± 1.8#	0.7 ± 0.1#	90.7± 12.8#	86.4 ± 4.1#
MSG + CM	39.9 ± 1.5#	0.67 ± 0.1#	93 ± 4.1#	103 ± 8.2#

Values are means ± standard error (SEM) for 10 different rats per each treatment.
*Values are statistically significant at *p<0.05 Vs. control and #p<0.05 Vs. MSG rats.*

and AST levels and co-administration of camel milk to MSG administered group protect from this significant decrease (Table 2). MSG decreased antioxidants levels. As seen in Table 3, MSG induced oxidative stress as indicated by the increase in MDA and decrease in SOD, GSH-R and catalase. Co-administration of CM with MSG counteracted this decrease and significantly normalized antioxidant levels. The effect of Vitamin E was parallel to the effects of camel milk

Testicular tissue oxidative and antioxidative biomarkers

To confirm the deleterious effects of MSG on testis, we measured testicular MDA levels as an oxidative stress marker and catalase and SOD as antioxidant markers. As shown in Table 4, MSG increased significantly MDA levels and decreased testicular catalase and SOD activities. Co-administration of either Vit. E or CM together with MSG inhibited this decrease and normalized it.

Sperm analysis

MSG induced significant decreases in sperm motility, percentage of live sperms, and an increase in sperm abnormalities. Camel milk supplementation into MSG group improved sperm and percentage of live sperms and decreased sperm abnormalities reported on MSG administered rats (Table 5). Parallel with changes in spermogram, a decrease in serum levels of testosterone and LH were reported in MSG administered rats that were normalized after camel milk sup-

Table 3. Protective effect of camel milk against MSG induced changes on antioxidants levels.

Parameter	MDA (nmol/ml)	SOD (U/ ml)	GSH-R (mg/ dL)	Catalase (U/ L)
Control	11.8 ± 2.6	227.9 ± 33	7.7± 1.1	166.7 ± 18.6
MSG	31.4 ± 3*	117.2 ± 2.2*	4.5± 0.4*	56.7 ± 11.4*
MSG + Vit E	18.3 ± 1#	256.6 ± 35#	6.2 ± 0.07#	169.7 ± 18.3#
MSG + CM	10.9 ± 1.4#	194.7 ± 9.6#	6.3 ± 0.2#	97.5 ± 2.8#

Values are means ± standard error (SEM) for 10 different rats per each treatment.
*Values are statistically significant at *p<0.05 Vs. control and #p<0.05 Vs. MSG rats.*

Table 4. Protective effect of camel milk on MSG induced changes in testicular antioxidant activity in Wistar rats.

	Control	MSG	MSG + Vit E	MSG + Camel milk
MDA (nmol/mg protein)	6.78±1.9	19.7±0.9	8.2 ± 0.6#	9.6 ± 0.3#
Catalase (U/ mg protein)	5.92±.1	3.7±0.2*	4.7 ± 0.04#	4.6 ± 0.06#
SOD (U/ mg protein)	4.3±0.09	2.7± 0.1*	3.9 ± 0.4#	3.6 ± 0.2#

Values are means ± standard error (SEM) for 10 different rats per each treatment.
*Values are statistically significant at *p<0.05 Vs. control and #p<0.05 Vs. MSG rats.*

Table 5. Camel milk protected MSG induced changes in sperm motility and abnormalities.

	Motility%	Live %	Abnormality %
Control	87.89 ± 2.2	83.44 ± 4.6	10.44 ± 2.8
MSG	54.22 ± 3.1*	46.44 ± 3.3*	27.33 ± 3.8*
MSG + Vit E	73.3± 4.1#	77.21± 4.6#	14.33 ± 2.8#
MSG + CM	66.1± 5.3#	66.8 ± 3.7#	14.9 ± 1.7#

Values are means ± standard error (SEM) for 10 different rats per each treatment.
*Values are statistically significant at *p<0.05 Vs. control and #p<0.05 Vs. MSG rats.*

plementation (Table 6). Of note the effect induced by CM is relatively the same reported for vitamin E administered group.

Testicular steroidogenesis and fertility related genes mRNA expression

Next, the effect of MSG on the testis activity at the mRNA expression level was checke. As seen in Figure 1, administration of MSG induced down regulation of genes of steroidogenesis as it decreased significantly the expression of androgen binding protein (ABP), 17 alpha hydroxylase ($P450_{c17}$), 17b-hydroxy steroid dehydrogenase (17β-HSD) and aromatase. Co-administration of CM or vitamin E to MSG groups normalized and even up-regulated the examined genes and retained their expression (Figure 1). In parallel, the genes of fertility markers as androgen receptor (AR), luteinizing hormone receptor (LHR), follicle stimulating hormone receptor (FSHR) and testicular apoptotic gene (P53) were greatly down-regulated in MSG administered rats. Administration of CM or vitamin E together with MSG normalized and up-regulated their expression compared to MSG administered group (Figure 2).

Table 6. Protective effect of camel milk against MSG induced changes in testosterone and LH levels

	Testosterone (ng/ml)	LH (ng/ml)
Control	3.5 ± 0.4	1.7 ± 0.2
MSG	2.1 ± 0.1*	1.0 ± 0.04*
MSG + Vit E	3.8 ± 0.2#	1.56 ± 0.1#
MSG + CM	4.7 ± 0.2#	1.48 ± 0.1#

Values are means ± standard error (SEM) for 10 different rats per each treatment.
*Values are statistically significant at *p<0.05 Vs. control and #p<0.05 Vs. MSG rats.*

Discussion

Monosodium glutamate (MSG) is a commonly-used food processing additive. Several studies showed that MSG is toxic to the various organs such as the

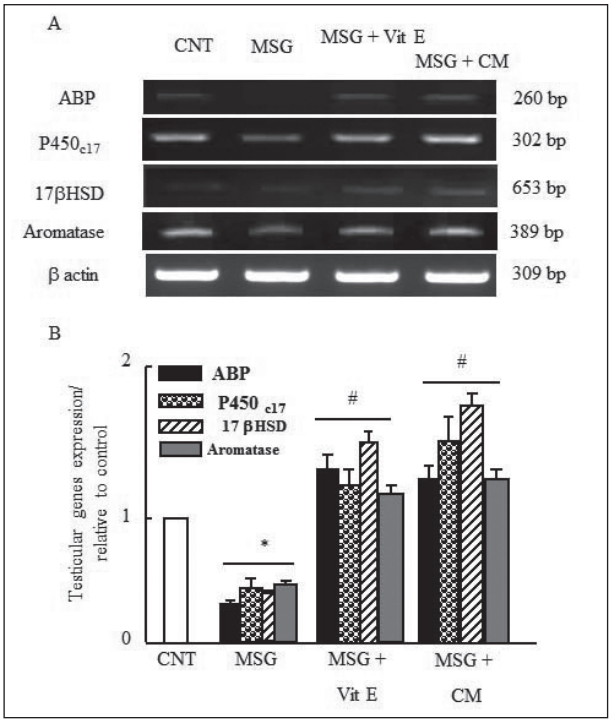

Figure 1. Effect of camel milk on steroidogenesis related gene expressions: Rats groups were treated with contol, MSG, camel milk plus MSG or Vit E plus MSG. Total RNA was extracted from testis tissues and the expressions of genes were analyzed by semi-quantitative RT-PCR analysis. Values are means ± SE of 10 rats. * P < 0.05 Vs control group, # P < 0.05 MSG group.

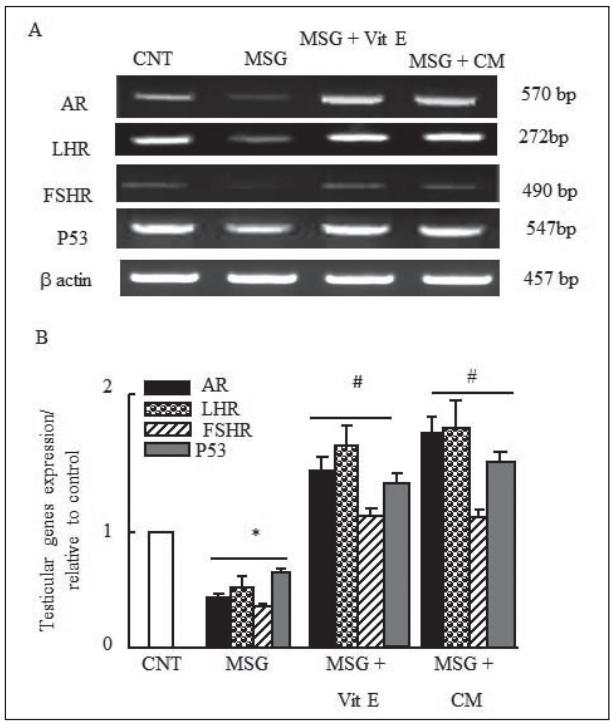

Figure 2. Effect of camel milk on fertility related genes and P53 mRNA expressions: Rats groups were treated with contol, MSG, camel milk plus MSG or Vit E plus MSG. Total RNA was extracted from testis tissues and the expressions of genes were analyzed by semi-quantitative RT-PCR analysis. Values are means ± SE of 10 rats. $^{*}P < 0.05$ Vs control group, $^{\#}P < 0.05$ MSG group.

liver, brain, thymus, and kidneys (36,37). This effect is attributed mainly to oxidative stress caused by MSG (38). Oxidative stress is caused by the excessive production or a decreased elimination of free radicals in cells, the majority of which are oxygen radicals and other reactive oxygen species (ROS) (39).

Camel milk is enriched with various protective proteins such as lysozyme, lactoferrin, lactoperoxidase, immunological properties, growth promotion activity and anti-tumor activity(40,41). Camel milk could play an important role in decreasing oxidative stress by alteration of antioxidant enzymes and nonenzymatic antioxidant molecules levels (23). Current findings confirmed that long term administration of MSG induced biochemical, renal, hepatic and testicular alterations. Moreover, MSG showed testicular dysfunction as presented by the decrease in its antioxidant activity and down regulation in all examined testicular genes.

Both ALT and AST are sensitive markers of liver damage (42,43). Therefore, the increase in the serum liver activity might perhaps be an indication of liver damage. MSG could dissociate easily to release free glutamate. The diminution of glutamate produces ammonium ion that could be toxic unless detoxified in the liver via the reactions of the urea cycle. Thus, the possible ammonium ion overload that may occur as a result of an increased level of glutamate following MSG intake could damage the liver (25).

Our findings are coincided with others at biochemical levels (25,26), as pre-treatment with vitamin E has been reported to confer protection against monosodium glutamate induced-hepatotoxicity and oxidative stress in rats (24, 42). In parallel, dietary antioxidants such as Vitamin C and Vitamin E has a modulator effects on MSG-induced serum urea oxidative damage in the liver and kidney of rats (29). The variation in the level of urea and creatinine are markers of renal dysfunction.

As known, the major indicator of oxidative damage in the body is the increase in lipid peroxidation that initiated by ROS and causes impairment of membrane function (44). The increase in MDA in this study may be attributed to the increase in generation of ROS results from MSG treatment. Similar observations have earlier been reported in studies involving other organs (27,29,36,45). A decrease in antioxidant status is a counter act mechanism by the tissues to restore their activity. A decrease in GSH, SOD and catalase levels in the serum and testis of our experimental animals, are similar to earlier observations that MSG induced oxidative stress in other tissues (36,45,46). The depletion in antioxidants levels reported here correlates with the increase in lipid peroxidation observed in the other tissues (27,29,36).

Antioxidants act as a direct radical scavenger and stabilize membrane structure through the removal of acyl peroxides formed during lipid peroxidation reaction (47). Glutathione depletion is a positive indicator of tissue degeneration and the magnitude of depletion parallels the severity of the damage (48). The increase in the activity of glutathione S-transferase (GST) following MSG administration might have contributed to the depletion of tissue glutathione. GST catalyzes both glutathione-dependent conjugation and reduc-

tion (49). It detoxifies endobiotic and xenobiotic compounds by covalently linking glutathione to a hydrophobic substrate, forming less reactive and more polar glutathione S-conjugate (50). The activities of superoxide dismutase (SOD) and catalase decreased significantly in MSG treated rats and that are coincided with other study (45). Camel milk administration returned this antioxidant activity by decreasing lipid peroxidation and increasing antioxidants levels. The antioxidant activity of CM is due to the presence of lactoferrin in its contents as described in another study (22) and the presence of camel a-lactalbumin (51).

Our results showed that MSG induced testicular oxidative stress. Possibly, the toxic effects of MSG on the sperms and testicular examined parameters might be related to the increased production of free radicals in the rat reproductive organs as decribed histologically in previous study (26). Our current investigation revealed that MSG caused significant decrease in SOD, CAT and GSH-R activities and these findings are greatly in accordance with Fabio et al (42). Here, CM ameliorated these testicular changes as reported for Vit. E and quercetin (29, 42), as CM has been shown to be with antioxidant properties (22,51).

Vit E showed protective effect against MSG. This effect may be due to impaired absorption of MSG in the gastrointestinal tract and/or its antioxidant effect (52). Vitamin E prevents oxidative damage to sensitive membrane lipids by destroying hydroperoxide formation, acting in conjunction with selenium, and protects cellular membranes and lipid containing organelles from peroxidative damage by oxidative stress (53). The recovery of sperm count and motility to the control levels in camel milk- treated rats can be attributed to antioxidant properties of camel milk as supported by serum findings listed in this study. It has been shown that camel milk inhibited oxidative stress by increasing the activities of SOD, catalase and glutathione reductase levels (45,55).

MSG down regulated the expression of steroidogenesis related genes, male fertility hormone receptors and apoptosis related gene (P53). In the testis, LH binds to Leydig cell receptors and initiates the activation of adenylate cyclase, resulting in an increase in cAMP production. Luo and his collaborators (56) reported that, StAR transfers cholesterol from the outer membrane to the inner mitochondrial membrane. Where, the enzyme cytochrome P450 side chain cleavage (P450scc) converts cholesterol into pregnenolone. Ultimately pregnenolone transferred to smooth endoplasmic reticulum. Where, the synthesis of testosterone takes place via the actions of 17β-hydroxysteroid dehydrogenase (17β-HSD). Moreover, StAR is considered to be the rate limiting step in testosterone biosynthesis and reduced StAR is always found in testicular dysfunction (57). The increase in testosterone can be changed to estrogen by aromatase. MSG decreased serum LH and testosterone levels that were confirmed by down regulation on AR and LHR as well as other examined testicular genes. MSG induced testicular oxidative stress and increased ROS production. Of interest, CM had the potential to prevent these changes and provided evidence for such beneficial protective effects at testicular levels. Probably CM interacted with MSG at the intestine and prevented MSG absorption. Moreover, it decreased free radical production, lipid peroxidation (53) as CM has a potential antioxidant activity (22).

In conclusion, CM supplementation had a protective effect against MSG induced oxidative stress and testicular dysfunction. Moreover, CM normalized genes of testis that were down regulated due to MSG administration. Therefore, usage of camel milk as a supplement therapy against toxic materials is very indicative at the testicular levels.

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Original article

Biological effect of calcium and vitamin D dietary supplements against osteoporosis in ovariectomized rats

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Summary. There is a direct association between the lack of estrogen and the progress of osteoporosis. This study was done to evaluate the biological effect of diet supplementation with calcium (Ca), and vitamin D (VD) on osteoporosis in ovariectomized (OVX) rats and to examine the possible potential mechanisms. Twenty-eight rats were randomly divided into 4 equal groups (n=7). To induce estrogen deficiency in rats, bilateral ovariectomy and sham (SHAM; negative control) surgery were done. In the basal diet, Ca and VD was supplemented with 210 mg/kg and 600 IU/kg, respectively, for 6 weeks. Alendronate as a standard anti-osteoporotic drug was used in a single weekly dose (3 mg/kg) for 6 weeks. After six weeks, serum markers of osteoporosis and bone femur status were evaluated. The results exposed that Ca and VD supplementation increased the body weight gain and diminished the uterine weight as a result of ovariectomy operation. These supplements significantly raised the serum Ca, bone-specific alkaline phosphatase, free thyroxin, and osteocalcin in OVX-rats, while the serum interleukin-1beta, interleukin-6, parathormone, and pyridinoline levels were significantly dropped. There were also significantly improved in femur bone mineral density and bone ash contents, mainly Ca and phosphorous. In conclusion, feeding of Ca and VD dietary supplements have an anti-osteoporotic activity in OVX rats due to improvement of bone formation and abolition of bone loss. The study recommends that intake of Ca and VD together may be beneficial for the inhibition of osteoporosis in postmenopausal women due to estrogen deficiency.

Key words: calcium, vitamin D, osteoporosis, bone mineral density

Introduction

Osteoporosis is a silently progressing disease of bones characterized by low bone mass and decreased bone mineral density (BMD) leading to high incidence of bone fragility and fractures (1). The mass of skeletal bone is controlled by a combination of some endogenous (genes, metabolic hormones) and exogenous (nutrition, exercise) factors (2). Osteoporosis represents a serious health problem that prevails among elderly women and younger postmenopausal women. Moreover, menopause drastically increases the risk of osteoporosis (3). Postmenopausal osteoporosis occurs due to imbalance between osteoblastic bone formation and osteoclastic bone resorption as a result of estrogen loss (4). Estrogen deficiency is the most potent initiator of osteoclastic bone loss and has been associated with osteoporosis (5). In addition to maintaining adequate Ca and VD intake and practicing exercise, the preventive measures against osteoporosis include avoiding

of smoking, excessive alcohol and caffeine intake (6). Estrogen, Ca, VD, calcitonin and several antioxidants help in the prevention of postmenopausal osteoporosis (7, 8). Estrogen replacement therapy (ERT) has been established as a regimen for prevention of postmenopausal bone loss, but long term ERT may be accompanied with severe adverse effects and increased risk of ovarian and endometrial cancers (9, 10).

Nutrition plays an important role in bone health, and there is an increasing interest in dietary nutrients which influence bone metabolism and health such as Ca and VD. Reduced dietary intake of Ca is associated with reduced bone mass and leads to osteoporosis. Chronic VD deficiency leads to osteomalacia (11). On the other side, adequate intake of Ca and VD is essential for bone health (2, 7, 11).

Oxidative stress, resulting from excessive formation of reactive oxygen species (ROS) or lowering of body antioxidant defense system, represents a main cause of postmenopausal bone loss (12). ROS are involved in bone resorption because of superoxide free radicals generate osteoclastic bone loss (13). Oxidative stress increased differentiation and function of osteoclasts, so increasing bone loss (14).

Therefore, the present study was undertaken to evaluate the effect of Ca and VD micronutrients on serum and bone biomarkers of osteoporosis in ovariectomized rat model, and to examine the potential mechanisms.

Materials and methods

Dietary supplements

Calcium carbonate was procured from El-Gomhoryia Company, Egypt, in the form of fine powder. Calcium carbonate is widely used as an inexpensive dietary Ca supplement. It was added to basal diet at 210 mg/kg according to Chen et al. (15). Vitamin D (Cholecalciferol, vitamin D_3) was obtained in the form of capsules and added to basal diet at 600 IU/kg according to Ghanizadeh et al. (16).

Alendronate drug

Alendronate (Fosamax®, Merck Sharp and Dohme Company, USA) is class of bisphosphonates that widely used for treatment of osteoporosis. It was obtained in the form of tablets each contains 70 mg Alendronate sodium. The dose of Alendronate 3 mg/kg body weight (b.wt)/week was orally given to rats according to Maria et al. (17).

Rats

Twenty-eight mature female Sprague Dawley rats (235-245 g b.wt and 10-12 weeks old) were used in this study. The rats were purchased from Laboratory Animal Colony, Helwan, Egypt. The animals were housed under hygienic conditions at room temperature of 24°C, relative humidity of 50% and 12 hr light/12 hr dark cycles. The rats were fed on either basal or experimental diets and water was provided as required. The experiment on rats was carried out according to the National regulations on animal welfare and Institutional Animal Ethical Committee.

Basal and experimental diets

The dietary supply of protein, fat, carbohydrates, vitamins and minerals was equivalent to the recommended dietary allowances for rats according to Reeves et al. (18). Basal diet consisted of 20% protein, 10% sucrose, 5% corn oil, 2% choline chloride, 1% vitamin mixture, 3.5% salt mixture and 5% fibers. The remainder was corn starch up to 100%. Experimental diets were basal diet supplemented with Ca (210 mg/kg) and VD (600 IU/kg).

Ovariectomy procedure

Under ether anesthesia, the bilateral ovariectomy was performed in rats by making two dorsolateral incisions using sharp dissecting scissors. The skin and dorsal muscles were then cut and the peritoneal cavity was thus reached. The uterine horn was picked out and the fatty tissue around the ovary was removed. The connection between the Fallopian tube and the uterine horn was clamped by artery forceps and cut was made under the clamped area to remove the ovary. Skin was closed bilaterally with one simple catgut suture. Tincture iodine solution (antiseptic) was applied locally on the skin at both sites of the operation. This technique was described by Lasota and Danowska-Klonowska (19). Similarly, sham (SHAM) operation was performed where the ovaries were exposed but not removed.

Experimental design

Twenty-eight rats were randomized into to 4 equal groups. Group 1 was sham-operated (SHAM) and fed on basal diet and the other 3 groups were ovariectomized (OVX) and left for 3 weeks post-operation to ensure almost complete clearance of their bodies from sex hormone residues. Group 2 was kept OVX (positive control) and fed on basal diet. Group 3 was fed on experimental diets supplemented with Ca + VD for 6 weeks. Group 4 was orally given Alendronate (standard anti-osteoporotic drug) in a single weekly dose (3 mg/kg) for 6 weeks. The initial and final body weights of rats were recorded and changes in weight gains were calculated. Blood samples were collected for biochemical analyses. The rats were then euthanized by prolonged exposure to ether anesthetic and uterine horns were dissected out and weighed. Femur bones were dissected out and prepared for bone analysis.

Biochemical analyses

Blood samples were withdrawn by cardiac puncture, left standing for 10 minutes to clot and centrifuged at 12000 rpm for 15 minutes to separate the serum which kept frozen at – 80°C till biochemical analyses. Serum concentrations of Ca (20) and phosphorus (21) were colorimetrically determined using specific diagnostic reagent kits (BioMérieux, France) and measured on UV spectrophotometer. Serum bone-specific alkaline phosphate (22) was estimated by colorimetric assay using specific enzyme kits (Sigma-Aldrich Chemical Co., USA). Serum measurements of osteocalcin (OC), interleukin-1 beta (IL-1beta), interleukin-6 (IL-6), pyridinoline (PYD), calcitonin (CT), and parathyroid hormone (PTH) concentrations were performed using quantitative noncompetitive sandwich ELISA assay kits (Market, San Jose, CA) as described by Norazlina et al. (23). Absorbance was read in at 490 and 540 nm according to manufacturer's instructions. Serum free thyroxin (T4) concentrations were measured using radioimmunoassay (RIA) method as described by Wang et al. (24).

Bone analysis

Both femur bones were dissected out and the soft tissues were removed. Both femur epiphyses were removed and the length of each femur was measured using Vernier caliper. Femur bone volume and BMD were calculated according to the principle of Archimedes (25). In brief, the femur was cut out at the mid diaphyses and bone marrow washed out. Each femur bone was placed in a vial filled with deionized water and the vial was placed in vacuum desiccator for 90 minutes. The femurs were removed from the vial, dried by blotted paper, weighed, and placed again in other vial containing deionized water. The bone was reweighed and bone volume was measured. Femur BMD was calculated using this formula: BMD = femur weight/femur volume. To obtain the ash, femur bones were dehydrated and defatted in acetone and anhydrous ether, dried for 6 hr in an oven at 700°C. The remaining ash was weighed, solubilized with 0.1 Mol/L HCl, transferred into volumetric flask and completed to 100 ml with 0.1Mol/L HCl according to Yang et al. (26). The final solution was used for estimation of calcium and phosphorus in the ash using colorimetric methods.

Statistical analysis

Data were presented as means ± standard error (SE). The statistical analysis was performed using computerized statistical package of social sciences (SPSS, version 20) program with one-way analysis of variance (ANOVA) followed by Duncan›s multiple range tests according to Snedecor and Cochran (27).

Results

The analysis of body weight revealed that OVX rats gained more body weight than SHAM negative control rats (Table 1). The body weight gain was 19.23% in OVX control group versus to 11.32% in SHAM negative control group. The ovariectomy in rats caused a significant ($p<0.05$) decrease in the uterine weight when compared with SHAM control group. The mean value of the uterine weight was 0.85±0.03g in OVX control rats versus to 1.80±0.04 g in SHAM control rats and 1.7±0.02g in standard group given Alendronate (anti-osteoporotic drug). Feeding of OVX rats on diets supplemented with Ca and VD significantly ($p<0.05$) decreased the body weight gain and increased the uterine weight when compared to the OVX positive control group.

Table 1. Effect of diets supplemented with calcium and vitamin D on body weight gain and uterine weight in ovariectomized rats.

Groups	Body weight (g)		Weight gain	Uterine weight
	Initial (Week 0)	Final (Week 6)	(%)	(g)
Group 1 SHAM control	265.0±3.3[a]	295.0±6.2[c]	11.32	1.80±0.04[a]
Group 2 OVX control	260.0±4.7[a]	310.0±9.1[a]	19.23	0.85±0.03[d]
Group 3 Ca + VD	262.0±3.2[a]	305.0±6.6[b]	16.41	1.22±0.02[c]
Group 4 Alendronate (Standard)	265.0±3.6[a]	296.0±7.5[b]	11.69	1.70±0.02[b]

Means±SE with different superscript letters in the same column are significant at p<0.05 using one-way ANOVA test.
n=7 rats in each group.

Bilateral ovariectomy in rats resulted in significant (p<0.05) increases in serum levels of Ca, P, b-ALP and OC when compared with the SHAM negative control group. Feeding of diets supplemented with Ca and VD significantly (p<0.05) lowered the elevated serum levels of Ca, P, b-ALP and OC in OVX rats when compared to the OVX positive control group. Aldereonate also markedly lowered the aforementioned serum markers of bone building in OVX rats as seen in Table 2.

Data in Table 3 showed that ovariectomy in rats significantly (p<0.05) increased serum levels of IL-1beta, IL-6 and PYD as compared to the SHAM control group. Diets supplemented with Ca and VD significantly (p<0.05) lowered the high serum IL-1beta, IL-6 and PYD when compared to the positive OVX group.

Table 4 exerts that the bilateral ovariectomy in rats exhibited significant decreases in serum levels of free thyroxin and calcitonin and an increase in para-

Table 2. Effect of diets supplemented with calcium and vitamin D on serum calcium, phosphorous, bone specific alkaline phosphatase and osteocalcin in ovariectomized rats.

Groups	Ca	P	b-ALP	OC
	(mg/dL)	(mg/dL)	(U/L)	(µg/L)
Group 1 SHAM control	10.90±0.3[b]	3.65±0.1[b]	125.0±4.7[d]	10.6±0.01[d]
Group 2 OVX control	13.20±0.6[a]	6.15±0.2[a]	179.5±7.2[a]	15.2±0.03[a]
Group 3 Ca + VD	11.50±0.3[b]	4.77±0.2[b]	158.5±7.4[b]	13.6±0.01[b]
Group 4 Alendronate (Standard)	10.65±0.2[b]	3.45±0.4[b]	135.6±8.5[c]	10.8±0.02[c]

Means±SE with different superscript letters in the same column are significant at p<0.05 using one-way ANOVA test.
n=7 rats in each group.

Table 3. Effect of diets supplemented with calcium and vitamin D on serum levels of interleukin-1 beta, interleukin-6 and pyridinoline in ovariectomized rats.

Groups	IL-1beta	IL-6	PYD
	(Pg/ml)	(Pg/ml)	(nmol/L)
Group 1 SHAM control	32.55±2.2[d]	110.0±6.2[e]	2.47±0.24[d]
Group 2 OVX control	63.66±6.7[a]	445.0±9.8[a]	6.22±0.19[a]
Group 3 Ca + VD	56.44±4.2[b]	385.0±8.5[b]	4.62±0.34[b]
Group 4 Alendronate (Standard)	40.65±3.6[c]	135.0±9.3[d]	2.73±0.61[c]

Means±SE with different superscript letters in the same column are significant at p<0.05 using one-way ANOVA test.
n=7 rats in each group.

Table 4. Effect of diets supplemented with calcium and vitamin D on serum levels of thyroxin and calcitonin and parathyroid hormone in ovariectomized rats.

Groups	T4	CT	PTH
	(ng/mL)	(ng/mL)	(pg/mL)
Group 1 SHAM control	18.55±0.2[a]	16.0±0.72[a]	22.47±0.24[d]
Group 2 OVX control	11.36±0.7[d]	10.0±0.18[d]	36.22±1.75[a]
Group 3 Ca + VD	15.44±0.5[c]	12.0±0.15[c]	30.00±0.90[b]
Group 4 Alendronate (Standard)	17.65±0.6[b]	15.0±0.13[b]	26.00±0.33[c]

Means±SE with different superscript letters in the same column are significant at p<0.05 using one-way ANOVA test.
n=7 rats in each group.

thyroid hormone as compared with the SHAM control group. Diets supplemented with Ca and VD significantly (p<0.05) normalized serum levels of T4, CT and PTH as compared to positive OVX rats.

Table 5 represents that the bilateral ovariectomy in rats induced significant (p<0.05) decreases in femur weight and BMD when compared to the SHAM control group. Feeding of OVX rats on diets fortified with Ca and VD significantly (p<0.05) restored the ovariectomy-induced decreases in femur weight and BMD when compared to the OVX control group. Alendronate drug increased femur weight and BMD when compared to the OVX control group.

The bilateral ovariectomy in rats produced significant (p<0.05) decreases in weights of femur ash, Ca and P levels in the ash when compared to the SHAM control group, as depicted in Table 6. Experimental diets supplemented with Ca and VD significantly

Table 5. Effect of diets fortified with calcium and vitamin D on femur weight, length, volume and bone mineral density in ovariectomized rats.

Groups	Femur Wt.	Femur L	Femur V	BMD
	(g)	(mm)	(cm^3)	(g/cm^3)
Group 1 SHAM control	1.65±0.01[a]	45.01±3.75[a]	0.68±0.02[a]	2.43±0.06[a]
Group 2 OVX control	0.88±0.03[d]	43.09±3.71[a]	0.67±0.03[a]	1.31±0.02[d]
Group 3 Ca + VD	1.30±0.01[c]	44.10±3.25[a]	0.66±0.01[a]	1.96±0.03[c]
Group 4 Alendronate (Standard)	1.55±0.01[b]	45.10±2.55[a]	0.68±0.01[a]	2.28±0.01[b]

Means±SE with different superscript letters in the same column are significant at p<0.05 using one-way ANOVA test.
n=7 rats in each group.

Table 6. Effect of diets fortified with calcium and vitamin D on femur ash weight and calcium and phosphorous ash levels in ovariectomized rats.

Groups	Ash Wt.	Ca	P
	(g)	(mg/g ash)	(mg/g ash)
Group 1 SHAM control	0.95±0.03[a]	12.5±0.02[a]	7.42±0.12[a]
Group 2 OVX control	0.60±0.01[d]	6.5±0.01[d]	4.41±0.13[d]
Group 3 Ca + VD	0.77±0.01[c]	8.2±0.03[c]	6.62±0.14[c]
Group 4 Alendronate (Standard)	0.85±0.03[b]	12.2±0.02[b]	7.24±0.10[b]

Means±SE with different superscript letters in the same column are significant at p<0.05 using one-way ANOVA test.
n=7 rats in each group.

($p<0.05$) normalized the femur weight, ash weight and Ca and P contents in the ash in OVX rats. Alendronate drug increased femur weight, ash weight and Ca and P contents in the ash when compared to the OVX control group.

Discussion

The mean Ca and VD intake in developed countries are considerably lower than USA and European countries (28). Therefore, the possible health consequences related to Ca and VD deficiencies in those countries are going to increase, mainly osteoporosis. The present study aimed to evaluate the protective effect of diets fortified with Ca and VD on serum and bone markers of osteoporosis in OVX rats.

Estrogen is the most potent inhibitor of osteoclastic bone resorption, so estrogen deficiency is a major risk factor in the pathogenesis of osteoporosis (29). The bilateral ovariectomy in rats caused dramatic decreases in the uterine weight, bone mineral content, density and biomechanical strength due to estrogen deficiency (30-33). Ovariectomy causes accumulation of ROS with subsequent oxidative stress and in turn promotes the production of cytokines, as IL-1beta and IL-6, which cause osteoclast generation so increasing bone loss (23). Postmenopausal osteoporosis is commonly treated by ERT and/or by some drugs such as Alendronate which inhibits osteoclast-mediated bone resorption (32). Moreover, inadequate intake of nutrients such as Ca and VD increases the risk of bone loss with subsequent incidence of osteoporosis, which are the most essential micronutrients for bone health D (7). However, Gennari (2) mentioned that adequate sunlight exposure may prevent and cure VD deficiency. The sunlight exposure or the ultraviolet irradiation is limited by concern about skin cancer and skin disease. The most rational approach to reducing VD insufficiency is dietary supplementation. Cholecalciferol-D_3, alfacalcidol, is a fat-soluble vitamin that helps the body to absorb calcium and phosphorus which are necessary for bone building (23).

Results of the present study showed that diets fortified with Ca and VD prevented ovariectomy-induced the increase in body weight gain and the decrease in the uterine weight and turned the changes in body and uterine weights to nearly normal weights of SHAM-operated rats. Moreover, estrogen was reported to increase the vascularity, growth and weight of the uterus in immature rats and mice (34). The decrease in the uterine weight induced by ovariectomy could be attributed to estrogen deficiency in OVX rats. This finding was previously also reported by Srikanta et al. (32) who found that bilateral ovariectomy in rats significantly increased the body weight gain and decreased the uterine weight.

Ca, VD, and PTH are critical regulators of bone remodeling (35). Ca and P are widely used as markers for bone formation as they have a vital role in bone mineralization (36, 37). In the present study, the bilateral ovariectomy decreased serum Ca and P levels as compared to sham-operated rats. In previous study, the decreased serum Ca and P levels were reported to be due to estrogen deficiency in ovariectomized rats (37).

Concerning serum biochemical analysis, the increases in serum levels of Ca, P, b-ALP and OC induced by ovariectomy in rats, as reported in this study, were similar to the previously reported by Tamir et al. (38), Coxam (31) and Srikanta et al. (32) who concluded that increases in body weight gain and serum b-ALP and OC are due to estrogen deficiency in OVX rats and mice. Serum calcium, phosphorus, b-ALP and OC are commonly used as biochemical markers of bone formation. Normalization of serum levels of these biochemical markers after feeding OVX rats on diets fortified with Ca and VD could be possibly due to an increased osteoblastic activity; consequently, enhancing bone formation (32). However, circulating osteocalcin hormone is a well-known marker for bone formation (39).

Regarding the metabolic hormones, the present results denoted that feeding OVX -rats on diet supplemented with Ca and VD significantly ($p<0.05$) elevated serum free T4 and CT as well as decreased PTH. These findings were partially in accordance with those reported by Norazlina et al. (23) and Dumic-Cule et al. (40). The later authors reported that intermittent administration of thyroid-stimulating hormone in a rat model with removed thyroid and parathyroid glands elevated free T4 and CT serum levels, so inhibiting calcium loss from bone into blood and stimulating calcium deposition into bone and improve bone health. On contrary, parathormone inhibits Ca depo-

sition into bone and increases urinary exertion of Ca causing hypocalcaemia (24).

The results of this study showed that feeding OVX rats on diet supplemented with Ca and VD significantly ($p<0.05$) increased in femur BMD and Ca and P contents in bone ash. These findings were similar to those reported by Chen et al. (15) who found that high-calcium plus vitamin D_3 diet plays a vital role in bone mineralization as it increases BMD and so can prevent osteoporosis. In addition, Suntar and Akkol (7) concluded that adequate intake of Ca and VD is essential for bone health. In addition, Agata et al. (41) suggested that a low Ca intake during periods of rapid bone loss caused by estrogen deficiency in ovariectomized rats might be one possible cause for bone loss. The mechanism of anti-osteoporotic activity of micronutrients Ca and VD could be due to enhancement of bone formation as high-calcium plus vitamin D_3 diet was reported to play a vital role in bone mineralization and so prevent osteoporosis (15).

In conclusion, the results denote that diet supplemented with Ca and VD micronutrients has an anti-osteoporotic effect in ovariectomized rats and these dietary supplements appear to be promising for the prevention of osteoporosis. In addition, the potential mechanisms of anti-osteoporotic activity of these dietary supplements appear to be though enhancing bone building and delaying bone loss. The study recommends that intake of adequate Ca and VD may be beneficial for the prevention of postmenopausal osteoporosis in women due to estrogen loss.

The limitations of the study are neither bone histopathology nor serum Ca and VD were determined.

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Original article

Effects of an exercise program with or without a diet on physical fitness in obese boys: a three-year follow-up

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Summary. Childhood obesity is a global epidemic, and understanding the relationship between physical fitness and various forms of intervention in obese children is essential to implementing effective exercise programs. The objective of the present study was to conduct a long-term follow-up (three years) of how an exercise program with or without diet affects the physical fitness components of obese boys. The participants were 18 boys, ages between 8 and 11, divided into two groups according to the program they followed. The exercise group (E group) followed a physical exercise program (three 90-minute sessions per week), and the exercise plus diet group (E+D group) this physical exercise program plus a low calorie diet. Physical fitness was assessed by the European physical fitness test battery including flamingo balance, plate tapping, sit-and-reach, handgrip strength, standing broad jump, sit-ups, bent-arm hang, 10×5-metre shuttle run, and 20-metre endurance shuttle run. Kruskal-Wallis test was applied to reveal overall intergroup differences (E and E+D group), and measurements showing significant differences were further analysed for differences between individual groups by the Mann-Whitney U-test. In both groups, changes were observed in various physical fitness parameters, especially limb speed, agility, aerobic fitness, and muscular strength in absolute and relative terms (which improved in more than one evaluation). Differences between the two programs were observed only in the short term. It was found that long-term longitudinal interventions based on exercise programs with or without diet produce improvements in obese children's physical fitness.

Key words: aerobic fitness, agility, balance, body mass index, strength

Introduction

The prevalence of childhood obesity is increasing (1). Associated with this pathology are numerous other disorders – cardiovascular, metabolic, gastrointestinal, pulmonary, orthopædic, neurological, psychological and social, among others. In addition, childhood obesity is a predictor of cardiovascular disease morbidity and mortality in adulthood (2). Recommendations regarding the treatment of childhood obesity focus typically on lifestyle changes, including the promotion of healthy eating habits and increased physical activity (PA) (3). PA plays an important role in the prevention of overweight and obesity in childhood and adolescence (4). However, the PA levels of youngsters are today very low (5). In this sense, research on overweight children's habitual PA patterns suggests that they are less active (6) and have poorer movement skills (run, vertical jump, throw, catch, kick, and strike) (7) than normal-weight chil-

dren. Other studies have shown that, compared with normal weight children, obese children had poorer performances on weight-bearing tasks, but not on all fitness components (8,9).

A sedentary lifestyle leads to poor physical fitness (PF), and this together with elevated body fatness is considered to be a strong predictor of cardiovascular disease in youth (10). Indeed, PF is considered a important marker of health throughout life (11). PF includes several components: cardiorespiratory fitness, muscular endurance, muscular strength, flexibility, coordination, and speed (12). Several studies have analyzed PF in obese children after an exercise program (13) or an exercise program plus diet (14,15). Most primarily looked at cardiorespiratory fitness through effort tests (13-15), although other PF parameters such as strength (13,14) and flexibility (14) have been included. These studies showed that exercise, both in isolation and in combination with diet, appeared to be beneficial by generating short/medium term (3-9 months) improvements of these parameters. In this regard, a recent meta-analysis of results on the obese pædiatric population has shown that programs based on aerobic exercise have a moderate positive effect on aerobic fitness (16). Often, the studies conduct interventions in the short to medium term, even though international recommendations propose longitudinal studies of the prevention and treatment of obesity (17,18). While there is a need to develop early interventions to improve PF in obese children (19), there have been no longitudinal studies to analyze the influence of exercise programs with or without diet on PF (17). Therefore, the aim of the present study was to track over the long term (three years – four evaluations) the influence of an exercise program with or without diet on the physical fitness components of obese boys.

Material and Methods

Participants

A total of 105 boys were invited to participate through the collaboration of various schools in the town of Caceres (Spain). The inclusion criteria were: (i) age between 8 and 11 years, and (ii) a body mass index (BMI) equal to or greater than the 97th percentile for the age and sex (male) of the subject as defined by Spanish population curves (20). Subjects were excluded if they were: (i) regularly practising PA, or following an exercise program or some other therapy (n=65); (ii) involved in any weight control program (n=18); (iii) were taking any medication (n=8); (iv) had any type of dysfunction limiting their PA (n=2); and other reasons (n=9). The final sample consisted of 18 Caucasian boys (10.7±0.9 years). They were divided into two groups (several subjects ate at the school's refectory, making it impossible to randomly assign membership to one or the other group): the exercise group (E group) who followed a multi-sports exercise program (n=8, 10.9±1.0 years), and the exercise plus diet group (E+D group) who followed a combination of two programs – the exercise program and a low calorie diet (n=10, 10.5±0.85 years). All the children's parents completed a prior informed consent form. The study was approved by the Bioethics and Biosecurity Committee of the University and respected the principles of the Declaration of Helsinki.

Interventions

Exercise program

The exercise program was carried out in a multi-sports hall, supervised by two PhD students in Sports Sciences (AGH, AMD) under the overall supervision of two PhD's in Sports Sciences (JMS, YE). The program design was based on previous studies (13) and on the more than 15 years experience in implementing this type of health-related exercise program of two of the authors (JMS, YE). The program was of three weekly 90-minute sessions. During the three years of the study, the participants carried out 230 session of 90 minutes each (20 700 minutes). Each session comprised a warm-up (15-20 min), a main part consisting of pre-sports and multi-sports games (soccer, basketball, baseball, hockey, among others) with a moderate to vigorous intensity aerobic component (60-65 min), and a cool-down (5-10 min). In the team games, the participants were asked to maintain the desired intensity throughout the activity. A progression was established to steadily ramp the subjects up to 60-65 minutes of moderate to vigorous intensity. The intensity of the session was monitored by accelerometry to ensure that all the subjects performed the activities with the

same intensity. A Caltrac accelerometer (Hemokinetics, Madison, WI, USA) was used to this end, programmed to function as a PA monitor (21). This uniaxial accelerometer contains a piezoelectric bender element which assesses the intensity of movement in the vertical plane. Its validity has been demonstrated as a method for estimating energy expenditure in children (22), and it has been used in other studies (21, 23). Although it is unable to monitor such activities as rowing or swimming, no activity of this type was used either in the exercise program or in the subjects' daily PA for the duration of the study. The intensity of exercise was also estimated by Rate of Perceived Exertion Scale (6-20 RPE Scale). Levels 9-11 were considered light activity, levels 13-15 were considered light to moderate activity, and levels 17-19 were considered vigorous activity (24). However, it must be noted that quantifying exercise intensity is one of the most complex aspects of the sport science in general (25), and of physical exercise and health especially.

Compliance was assessed as percentage of exercise sessions attended. Compliance with the exercise program was good, with the children attending more than 78% of the total exercise sessions. Quantifying the intensities of 13 of the sessions/year selected at random showed no significant differences between the E and the E+D groups in any session, with a mean of 79 and 81 motion counts per session, respectively (Figure 1). Not all the sessions were quantified since

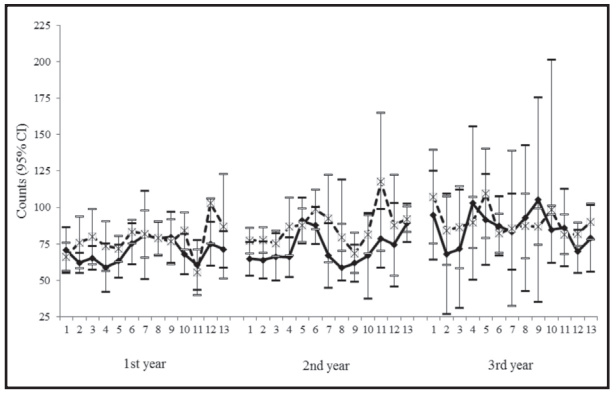

Figure 1. Representation of the means and confidence intervals of the intensity of the sessions of the physical exercise program as evaluated by accelerometry in the first, second, and third years. There were no differences between the E (solid line) and E+D (dashed line) groups

the programming and placement of the accelerometers meant taking time away from the physical exercise program. The use of accelerometers allows one to objectively quantify the subjects' PA, ensuring that the intensity was similar in the two groups. In developing treatment strategies for obesity, one requires quantitative information on PA to provide more effective goals (26).

Diet program

The low-calorie diet consisted of five balanced meals spread throughout the day, with an energy intake of 1500 kcal/day. In this sense, there have been studies that recommend diets of between 1500 and 1800 kcal/day in obese children who are still growing, since in this way their growth and development are not compromised (27). Thus the diet prescribed was of 1500 kcal/day, similar to that of other studies (28). The diet consisted of 57% carbohydrates, 17% proteins, and 26% fats. Foods were selected according to the subject's dietary habits. A series of general recommendations were established focused on basic healthy lifestyle eating: consume ≥ 5 servings of fruits and vegetables every day; minimize sugar-sweetened beverages such as soft drinks, sports drinks, and sugar-added fruit juices; have more meals prepared at home rather than purchasing take-away restaurant food, etc. Regular meetings were held with the children and their parents for the control and monitoring of the diet.

Measurements

Each subject was evaluated for the following parameters: eating habits, daily PA, pubertal status, kinanthropometry, and PF. The evaluations were made at the start (baseline), and at 7 (1st-year), 19 (2nd-year), and 31 (3rd-year) months into the program.

Eating habits

Nutrition was assessed with a self-reported 3-day food record (2 weekdays and 1 weekend day in succession – Thursday, Friday and Saturday) filled in by the parents. The weight of the food was estimated from the parents' records. The computerized database Nutriber was used to calculate the daily intake (29), with the program recording the average of the three days (kcal/day).

Daily PA

Daily PA was measured before, during, and after the intervention, using a validated uniaxial accelerometer (Caltrac), and covering a 3-day period (Thursday, Friday, and Saturday), except during bathing and swimming. During the intervention, the daily PA was evaluated once a month. All participants were instructed to record the amount of time spent cycling or swimming during the evaluation period. At the beginning and the end of the day, the children recorded the number of "motion counts" of the accelerometer, following previously published protocols (21). The data were collected by the children with the help of their parents. A once-a-term meeting was held with the parents to inform them of the program's evaluation. The final Caltrac score was recorded, as also was the average of the three days (motion counts per day).

Pubertal status and kinanthropometry

Pubertal stage was evaluated by a trained pædiatrician according to pubic hair development using the Tanner classification criteria (30). The kinanthropometric measurements followed the ISAK protocol (31): body height, body weight, and body fat percentage (bioimpedance). Standard equipment was used: a scale-mounted stadiometer (Seca, Berlin, Germany), a weight scale (Seca, Berlin, Germany), and a bioimpedance analyzer (Bodystat 1500, Bodystat Ltd, Douglas, Isle of Man, UK). BMI was calculated as weight divided by height squared (kg/m^2), and the BMI z-scores were determined (20).

Physical fitness

Physical fitness was assessed by the Eurofit Fitness Testing Battery (32). This standardized test battery was devised by the Council of Europe for children of school age, and it has been used in many European schools since 1988 and in literature studies on obese children (8). All tests were conducted according to standard procedures (32), in indoor sports facilities, by two PhD students (AGH, AMD). The tests evaluated were (with the better of two attempts being recorded): (i) 20-metre endurance shuttle run, measuring the maximum aerobic capacity of the subject, recording the number of shuttles completed (only one attempt); (ii) sit-ups, measuring trunk muscle strength by the number of sit-ups performed in 30 seconds; (iii) 10×5-metre speed shuttle run, measuring speed agility from the time taken in seconds; (iv) plate tapping, measuring limb speed in a task in which two 20-cm rubber discs were fixed horizontally onto an adjustable table, placed with edges 60 cm apart and a 10×20 cm rectangular plate equidistant between the discs, the subject was required to touch each disc alternately with a stylus until 25 cycles were completed and then repeated, the fastest 25 cycles being recorded as the score in seconds; (v) sit-and-reach, measuring flexibility according to the standard sit-and-reach test for range of movement, using equipment for the items of this test provided by Bodycare (Birmingham, United Kingdom); (vi) flamingo balance on a 50-cm-long beam, 4 cm in height and 3 cm wide, 4 cm off the floor, for 1 minute, recording the number of falls; (vii) handgrip test recorded on a grip dynamometer (Takei Kigi Kokyo, Tokyo, Japan), measuring the force of the grip in kilograms; and (viii) standing broad jump, measuring explosive power as the distance in centimetres that the subject jumped horizontally.

Finally, muscular strength parameters can be expressed in terms that are absolute (activities such as carry a suitcase, move a heavy object, handgrip strength test, etc.) or relative (activities in which the person has to lift, hold, or carry his/her own body weight, standing broad jump). In the analyses, the handgrip score was divided by the weight which implies a transformation from absolute strength to relative strength, and the standing broad jump score was multiplied by the weight so that it was transformed from relative strength to absolute strength (33). The bent-arm hang test from the originally planned battery could not be completed satisfactorily by a number of children. This item was therefore dropped from further consideration in the study.

Statistical analysis

All the variables satisfied the tests of homoskedasticity (Levene variance homogeneity test) and normality (Kolmogorov-Smirnov test) of their distributions. However, we used non-parametric tests as is recommended in the case of small sample sizes. The basic descriptive statistics (means and standard deviations) were calculated. The Kruskal-Wallis method was applied to test for overall intergroup differences (E and E+D group), and measurements showing significant

differences were further analyzed for differences between individual groups by the Mann-Whitney U-test (baseline, 1st-year, 2nd-year, and 3rd-year). Finally we performed a bivariate correlation (Pearson's P) analysis for each group at the end of the program to determine the possible effects of the kinanthropometric variables on the physical fitness. The level of significance for all statistical tests was set at $p \leq 0.05$. All calculations were performed using SPSS (version 16.0).

Results

The variables satisfied the tests of normality (Kolmogorov-Smirnov: $0.407 \leq z \leq 1.021$, $p>0.05$) and variance homogeneity (Levene: $0.008 \leq F \leq 4.361$, $p>0.05$). There were no intergroup differences in eating habits, daily PA, pubertal status, kinanthropometric, or PF parameters at the start of the program (Table 1).

Intra-group differences

Figures 2, 3, and 4 show the changes and effects of the treatment at different moments of evaluation (baseline [B], 1st-year [F], 2nd-year [S], and 3rd-year [T]). Figure 2 shows the evolution over the three years of intervention of the height, weight, body fat percentage, fat-free mass, BMI, and BMI z-score. No changes were observed in either daily PA or pubertal status. In both groups, however, changes were observed in the height and BMI z-score from the B to the T evaluations, and in the body fat percentage from the B to the F evaluations.

Figure 3 shows the evolution of each of the PF parameters. For the E group, there were differences between different moments of evaluation in the endurance shuttle run (B<T), the 10×5-metre shuttle run (B>T), and the plate tapping test (B>S,T; F>S,T). For the E+D group, there were differences between moments of evaluation in the endurance shuttle run (B<S,T), the 10×5-metre shuttle run (B>F,S,T), and the plate tapping test (B>S,T; F>T).

Table 1. Eating habits, daily PA, pubertal status, kinanthropometric, and physical fitness parameters of the study participants at baseline.

	E group (n=8)	E+D group (n=10)	Intergroup differences
	Mean ± SD	Mean ± SD	*p*
Eating habits			
Energy intake (kcal/day)	1952.4 ± 202.8	1928.6 ± 257.4	0.673
Daily PA			
3-day physical activity (counts/day)	156.2 ± 36.7	149.9 ± 36.3	0.914
Pubertal status			
Tanner stage (pubic hair)	1.62 ± 0.52	1.80 ± 0.63	0.937
Kinanthropometric			
Height (m)	1.49 ± 0.07	1.47 ± 0.09	0.235
Weight (kg)	62.4 ± 11.1	60.5 ± 11.8	0.815
Fat mass (%)	32.2 ± 3.77	33.0 ± 2.92	0.622
Fat-free mass (kg)	38.1 ± 7.45	39.3 ± 6.99	0.815
BMI (kg/m^2)	27.7 ± 2.95	27.9 ± 3.90	0.674
BMI z-score	4.00 ± 2.85	4.19 ± 2.81	0.256
Physical fitness			
Endurance shuttle run (n)	1.69 ± 1.41	2.20 ± 0.79	0.115
Trunk strength (n)	11.9 ± 4.55	12.2 ± 4.80	0.947
Agility run (s)	23.2 ± 1.39	23.9 ± 1.91	0.158
Limb speed (s)	14.9 ± 0.90	15.3 ± 3.83	0.072
Flexibility (cm)	-3.44 ± 6.94	3.40 ± 3.56	0.184
Balance (falls/min)	4.86 ± 1.68	6.00 ± 4.50	0.062
Handgrip strength (kg)	42.0 ± 6.55	37.5 ± 10.2	0.315
Standing broad jump (cm)	112.0 ± 18.8	108.3 ± 15.8	0.771

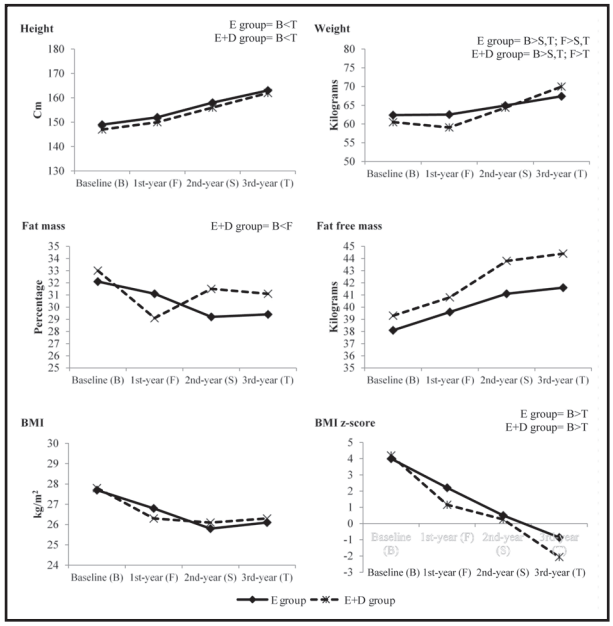

Figure 2. Changes in daily PA, pubertal stage, and kinanthropometric parameters at the baseline, first, second, and third year evaluations in obese boys; p<0.05.

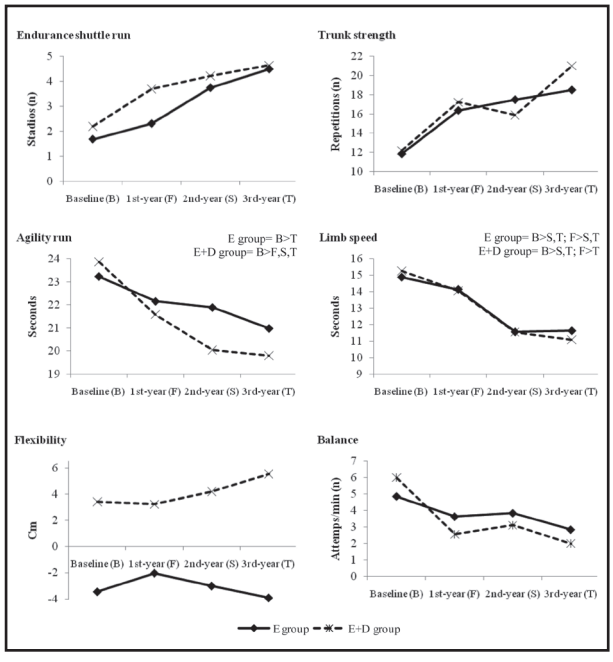

Figure 3. Changes in physical fitness parameters at the baseline, first, second, and third year evaluations in obese boys; p<0.05.

Regarding the relationship between the kinanthropometric variables and physical fitness, in the E group, relationships were found between height and handgrip strength (r=0.850, p=0.032), and between BMI and endurance shuttle run (r=-0.922, p=0.009), trunk strength (r=-0.878, p=0.022), and flexibility (r=-0.855, p=0.030). In the E+D group, relationships were found between height and endurance shuttle run (r=0.608, p=0.047), handgrip strength (r=0.713, p=0.014), and standing broad jump (r=0.606, p=0.048), between weight and handgrip strength (r=0.847, p=0.001), and between BMI and handgrip strength (r=0.640, p=0.034).

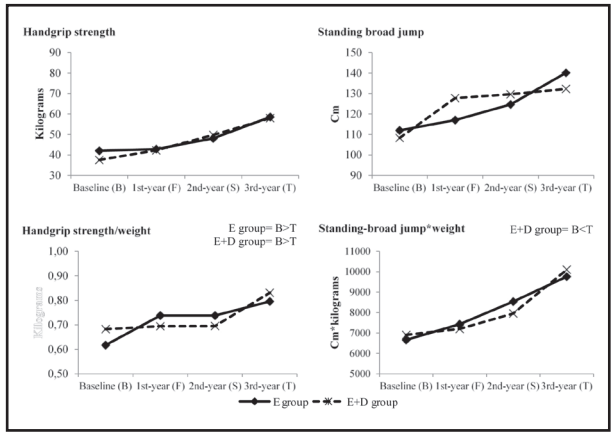

Figure 4. Changes in physical fitness parameters at the baseline, first, second, and third year evaluations in obese boys; p<0.05.

Figure 4 shows the evolution of each of the muscular strength PF parameters. For the E group, there were differences between several moments of evaluation in the handgrip strength and in handgrip/weight (B<T). For the E+D group, there were differences between moments of evaluation in the handgrip strength (B<T), in handgrip/weight (B<T), and in standing broad jump×weight (B<T).

Intergroup differences

Table 2 presents the intergroup differences. One observes differences in weight change (E group 1st year<E+D group 3rd year; E group 3rd year>E+D group 1st year), body fat percentage change (E group>E+D group, both 1st year; E group 3rd year>E+D group 1st year), 10×5-metre shuttle run change (E group 2nd year>E+D group 1st year, and E group 3rd year>E+D group 1st year), and standing broad jump (E group<E+D group, both 1st year).

Table 2. Differences between groups for the changes in kinanthropometric and physical fitness parameters

	1st year		2nd year		3rd year		ANOVA		Differences between groups
	E group	E+D group	E group	E+D group	E group	E+D group			
	A	a	B	b	C	c	F	p	
Δ Weight (kg)	0.16 ± 1.35	-1.42 ± 3.06	3.10 ± 1.83	5.12 ± 3.82	8.25 ± 4.94	8.69 ± 3.80	12.372	0.000	A<c; a<C
Δ BMI z-score	-1.79 ± 0.69	-3.03 ± 2.11	-1.56 ± 0.92	-0.74 ± 1.23	-1.36 ± 2.23	-1.84 ± 3.53	1.339	0.268	
Δ Body fat (%)	-1.07 ± 0.85	-3.04 ±1.94	-2.35 ± 3.54	-0.14 ± 1.07	0.38 ± 2.60	-0.93 ± 0.90	2.494	0.047	A>a; a<C
Δ Endurance s huttle run (n)	0.62 ± 0.52	1.50 ± 0.62	1.25 ± 1.13	1.17 ± 1.00	0.50 ± 0.63	0.25 ± 1.13	2.643	0.037	
Δ Trunk strength (n)	4.50 ± 3.50	3.30 ± 3.16	2.00 ± 2.28	2.33 ± 3.39	1.17 ± 2.99	3.62 ± 6.63	0.659	0.656	
Δ Agility run (s)	-1.08 ± 0.91	-2.44 ± 1.05	0.22 ± 1.56	-1.05 ± 1.51	0.44 ± 1.08	-0.13 ± 1.23	6.102	<0.001	a<B; a<C
Δ Limb speed (s)	-0.73 ± 1.43	-1.51 ± 2.55	-0.45 ± 0.63	-0.17 ± 1.04	0.32 ± 1.54	-0.01 ± 0.89	1.436	0.232	
Δ Flexibility (cm)	1.41 ± 3.71	-0.50 ± 3.94	-0.58 ± 2.90	1.49 ± 3.48	-1.15 ± 3.23	0.08 ± 1.60	0.837	0.532	
Δ Balance (falls/min)	-2.50 ± 4.75	-3.60 ± 4.57	0.00 ± 4.82	-0.56 ± 4.50	0.17 ± 3.49	-0.57 ± 2.07	1.078	0.387	
Δ Handgrip strength (kg)	1.43 ± 10.17	4.15 ± 3.21	2.58 ± 2.87	1.22 ± 3.19	5.40 ± 3.24	4.24 ± 5.20	1.554	0.197	
Δ Standing broad jump (cm)	5.00 ± 12.8	18.7 ± 4.90	4.50 ± 10.8	5.55 ± 12.0	7.17 ± 9.19	3.71 ± 8.60	2.989	0.022	A<a

Results expressed as mean ± S.D.; E, exercise; E+D, exercise plus diet.

Discussion

We have described a long-term follow-up study (three years – four evaluations) of the effects of an intervention based on exercise programs with or without diet on the physical fitness parameters of obese boys. The results indicate that such long-term longitudinal intervention consisting of exercise programs both with and without a diet improves the fitness of these obese subjects, with a possible need for adjustments to fit the specific needs of the subjects according to their weight status (34). Differences between the two groups (with and without diet) were observed only in the short term.

Intra-group differences

Both groups showed reductions in plate tapping time from the baseline to a later evaluation. Improvements in the two groups were similar, perhaps because performance in this test is not influenced by excess fatness (35). These improvements may have been due to the inclusion of such sports as tennis and padel in the exercise program, with their need for major arm mobility. On the other hand, there were no improvements in balance, sit-ups, or flexibility, contrary to the findings of a randomized controlled trial (7). Maybe this indicates that specific programs may generate specific changes. With respect to the 10×5-metre shuttle run test, we observed time reductions in both the E group (baseline > third evaluation) and the E+D group (baseline > first, second, and third evaluations). Thus, both intervention strategies appear to be beneficial in the short and long term at improving the obese subject's agility. The exercise program was initially focused on simple aerobics combined with strength work (5). As the body fat percentage levels decreased and the fitness levels improved, the intensity of the sessions was increased (Figure 1). By the end of the study period, we observed improved performances on the endur-

ance shuttle run test in both the E group (baseline > third evaluation) and the E+D group (baseline > second and third evaluations). These results are consistent with a meta-analysis and other studies indicating that both aerobic exercise-based intervention alone (4) and in combination with diet (14,15) lead to increased aerobic fitness. The improvement in this parameter is consistent with the reduction in body fat percentage since this reduction results in improved maximal aerobic power (36).

With respect to muscular strength parameters, the handgrip strength in absolute and relative terms increased in both groups from the baseline to the last evaluation (third year). In a similar study in the same line, but only with short term data (12 weeks), an exercise plus diet program produced improvements in the handgrip strength both intra-group (pre-test vs post-test, 9.4%) and inter-group (E+D vs D, 7.6%) (14). In the present study, the arm-specific activities and resistance weight-training incorporated into the exercise program could have favoured improvement in the handgrip strength (8). The improvements in this test, therefore, could be explained by changes in the neural mechanism and/or the quality of muscle contractile properties (37). This observation led us to introduce increased training of the upper body musculature (38). Finally, with respect to the standing broad jump relative to weight, this increased in the E+D group from the baseline to the last evaluation (third year). Such motor tasks as jumping which have to support the weight of the body's excess fat are probably among the most difficult exercises for obese people (39). For that reason, jumps or certain other activities involving sudden changes of direction were not implemented at the beginning of the program since obese young boys are limited in their ability to perform weight-bearing activities. Instead, the activities actually carried out were aimed at encouraging the continued participation of these obese individuals (40).

Intergroup differences

The results showed greater improvement in the E+D group than in the E group, especially in the short-term (Table 2). In particular, the E+D group improved their standing broad jump more than did the E group in the first year (1st year minus baseline in E group < 1st year minus baseline in E+D group; p=0.022). This could have been a reflection of the differences between the two groups in body fat percentage change recorded in the first evaluation period (1st year minus baseline in E group > 1st year minus baseline in E+D group; p=0.047) which confirmed that, in obese subjects, combined exercise plus diet programs generate greater improvements in body fat percentage than exercise alone. Similarly, since a higher BMI is associated with reduced performance in the standing broad jump (9), the downward trend shown by the BMI z-score would seem to favour improved performance on this test compared to the baseline. Furthermore, it is noteworthy that there was an increase in fat-free mass favoured by the exercise program, and this could have led to increased strength of these obese subjects (8). Finally, differences in favour of the E+D group were observed in the changes in the 10×5-metre run test (2nd year minus 1st year in the E group > 1st year minus baseline in the E+D group, p=0.002; and 3rd year minus 2nd year in the E group > 1st year minus baseline in the E+D group, p=0.001). This could be a consequence of a lack of experience in weight-bearing tasks, since obese young boys are limited in their ability to perform weight-bearing activities (41). In particular, although in both groups there was a downward trend as reflected in the long-term improvements in this parameter (Figure 3), the great improvement in the E+D group after the first year and the original scope for improvement in this group (at the baseline, they showed very little agility) could have favoured the differences with respect to the second and third evaluations of the E group (after the first year, the subjects were more agile, and there was less room for improvement).

Limitations

A number of limitations of this study need to be borne in mind. First, there was a lack of initial randomization of the groups. Several subjects ate at the school's refectory, or were unable to attend the exercise program, making it impossible to randomly assign membership to one or another group. Nonetheless, the homogeneity of the groups was verified by the absence of initial differences in any of the variables (Table 1). Second, the number of subjects in the study was small (n = 18), although the study's longitudinal character

could make this limitation of only relative importance. In long-term longitudinal studies, it is difficult to achieve the participants' adherence to the program or to reduce the number of drop-outs (42). Indeed, the numbers of subjects in other studies of much shorter duration (between 2 and 6 months versus the 31 months of the present study) are similar to ours (43) or just slightly greater in the case of the very short duration programs. Third, there was no group that only had the diet without doing the Physical Education classes. Despite these limitations, the existing scientific evidence for the effectiveness of Physical Education classes in improving physical fitness in obese children is inconclusive because of the small number of weekly sessions (usually just one or two) when at least three 60-minute sessions of physical exercise are needed to achieve improvements in aerobic condition (16). However, even a small frequency of Physical Education classes may help reduce sedentary habits (44).

Conclusions

A long-term intervention based on an exercise program with or without a diet program in obese boys improved their physical fitness parameters, especially those related to limb speed, agility, aerobic fitness, and muscular strength in relative and absolute terms. The combined intervention (exercise program plus diet) was the more effective in the short-term for some of the fitness parameters. There were no differences in effectiveness between the two interventions in the long term. The results further confirm the importance of physical exercise in increasing obese children's fitness.

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Effect of arginine supplementation on footballers' anaerobic performance and recovery

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Summary. *Background:* The use of supplements has increased in recent years. L-arginine is popular supplement in athletes and this supplement stimulates nitric oxide which purpose to increase sports performance. *Aims:* This study aims to determine the effects of L-arginine supplement on anaerobic performance and recovery. *Materials and Methods:* 28 male active football players who play in amateur leagues, get training regularly, between the ages of 18 and 30 participated the study. The subjects were randomly assigned to experimental and placebo group. During 14 days, experimental group consumed 6 grams of arginine and the placebo group consumed 6 grams of wheat bran. Both before and after the supplementation anthropometric, biochemical and anaerobic capacity levels were measured. In order to search recovery after the anaerobic test, the level of Lactic Acid (LA) and Heart Rate (HR) were observed up until the 10th minute of recovery. *Results:* The research results suggest that after supplementation, the experimental group's body mass index (BMI) decreased compared to pre-supplementation (Pre= 23,60±2,28kg/m^2 vs. Post= 23,39±2,12kg/m^2)($p<0.05$). On the other hand, the anaerobic performance measurements of both groups do not demonstrate any statistically significant difference before and after supplementation. The post supplementation recovery LA levels showed more rapid reduction from 5th min up to 10th min in experimental group. After the supplementation, 1st minute recovery HR levels were lower than pre in both groups but the experimental group experienced a higher decrease than placebo group. This suggests that suplementation of arginine helps to excrete LA from the body. The comparison of the HR values measured before and after the 14 day long supplementation period shows that both the experimental and the placebo groups experienced a decrease in the resting HR values as well as the HR values during the 1st minute of recovery. The experimental group experienced a higher decrease. After 14 day supplementation Aspartat Aminotransferaz (AST), Alanin Aminotransferaz ALT and Laktat Dehidrogenaz (LDH) (LDH; Pre= 229,41±47,23 vs. Post= 176.08±45.62) levels significantly decreased compared to the pre-supplementation in arginine group ($p<0.05$). *Conclusion:* Consequently, the findings suggest that supplementation of L-Arginine accelerates the excretion of lactic acid from the body and decreases the amount of fat in the body. It also rapidly recovers the muscle injuries caused by the decrease in LDH enzyme levels after training and has a positive impact on anaerobic performance. Finally, it accelerates recovery.

Key words: Footballer, L-Arginine, anaerobic performance, lactic acid, recovery

Introduction

In the last two decades an increasing interest can be observed for the matter of athlete's diet which is supported by research into biochemical and physiological aspects of training (1). Athlete cannot meet their increasing needs for nutrition through only organic diet. Therefore, natural supplements are assumed to satisfy their needs (2).

After strength training, active muscle fibers are damaged. It is proved, however, that a balanced and regular intake of protein reduces damage to muscles and it prevents muscle weakness which decreases physical power. It also improves recovery after strength training. Besides, supplementing a diet with protein is necessary for the improvement and recovery of muscles since protein has a key role in balancing anabolic hormones (3).

When training gets more intensive and it exceeds a certain limit, aerobic mechanism is unable to produce sufficient energy and anaerobic mechanism takes control (4). Anaerobic threshold is defined as the point at which lactate starts to accumulate in the blood stream (about 4 mmol) (5). Concentration of lactate in muscles and blood appears as a function of the intensity of training (6). The amount of lactate in the blood increases at an accelerating rate due to intense training (7). Since Lactic Acid (LA) is one of the metabolites that causes fatigue, the accumulation of it in muscles will decrease training performance (1). The body can recover by renewing the energy burnt and removing LA which has accumulated in the body (8).

One of the recent supplements; L-arginine triggers vasodilation because it increases the production of Nitric Oxide (NO) in muscles during training. When blood flow increases due to vasodilation, active tissues are supplied with a large amount of nutrition and oxygen. Similarly, increasing blood flow raises protein synthesis and eases the recovery of muscles (9). L-arginine is a semi-essential amino acid and it is a basic source for the production of NO (10). NO is a gas molecule which emits high signals and it is synthesized by NO synthase -a calcium dependent enzyme which has a low molecular weight- , L-arginine and L-citrulline. NO plays a crucial role in regulation of blood flow and blood pressure during training and in relaxing skeletal muscles (11,12). Among many roles of NO, two of them directly affect training performance; these are balancing vasodilation in cardiac and skeletal muscles and oxygen consumption (13). Little et al. (14) reported that L-Arginine is a supplement which improves training performance. It has been observed that it increases maximal strength and repeated sprint performance and improves physical endurance.

Effects of arginine on anaerobic performance and recovery were studied by different designs. But, this study is the only one investigating the impact of arjinin on anaerobic performance and recovery by a different test, which was Running Anaerobic Sprint Test (RAST). The aim of this study is to examine the effects of oral arginine supplement -which is given in daily amount of 6 grams during 14 days- on anaerobic performance and recovery.

Materials and Methods

Research group

The study was carried out with 28 male footballers between the ages of 18 and 30 who volunteered to take part in the study. They currently play football in amateur leagues. They are active football players who get training regularly. The footballers were assigned to one of two groups: experimental/arginine (n=14) and placebo (n=14). The subjects were chosen on condition that they were healthy, had no chronic diseases or movement disorder caused by any sort of reason. In order to increase the reliability of the study, it was conducted with only one football team and all the subjects were chosen from the same football team considering that different teams might have different training programs which would affect the study negatively. The study was approved and granted permission by Ondokuz Mayıs University, Clicnical Research Center, Ethical Foundation (Number: B.30.2.ODM.0.20.08/211).

Study Design

Before supplementation, the subjects' blood samples were analyzed to determine some biochemical parameters. Their height, weight and anaerobic capacity values were measured through RAST. Besides, in order to examine recovery after RAST, their LA levels

and Heart Rate (HR)s were measured. After these measurements, the experimental group consumed arginine, and the placebo group consumed wheat bran. After this 14 day period, the measurements made at the beginning of the study were repeated. In other words, each footballer who took part in the study were measured twice (before and after supplementation). The measurements were made under the same physical conditions.

Performance Testing and Biochemical Analyses

Height and Weight

In the study, subjects' height and weight were measured through a device (Seca) and their body mass indexes (BMI) were calculated.

Blood Measurements

Nurses drew five ml of venous blood from footballers and it was analysed by biochemical specialists. During the study, subjects' urea, creatinine, cholesterol, triglyceride, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), Gama Glutamil Transferaz (GGT), Alanin Aminotransferaz (ALT), Aspartat Aminotransferaz (AST), Alkaline Phosphatase (ALP) and Lactate Dehydrogenase (LDH) levels were analysed.

Running Anaerobic Sprint Test (RAST)

To measure the anaerobic capacity RAST test was done by using a New Test-Power Timer 1.9.5. (Newtest, Oulu, Finland). Footballers were asked to warm up before the test so that they can get mentally and physically ready and they were given 15 minutes to do so. During the RAST test, each footballer made six consecutive 35 meter sprints by giving 10 second breaks after each sprint. After a footballer started the test by making the first sprint and gave the first 10 second break, Power Timer device beeped and the footballer made the second sprint. The test was completed after six consecutive sprints were made in the same way.

Blood Lactate Test

For the lactate test, a lactate analyzer (Lactate Scout) was used. In order to meaure the LA level, blood samples were taken from ear lobe. Each time blood was taken for measurements, needles and test strips were changed. By measuring resting LA levels and LA levels in the 1st, 5th and 10th minutes after the RAST test, subjects' LA levels during recovery were determined.

Heart Rate (HR)

Polar watch (RS 800) to measure HR was used. A transmitter was placed in the chest and HR in the 1st, 3rd, 6th and the 9th minutes after the RAST test were determined.

Supplementation

One day after the pre-tests, 28 participants were randomly divided into two groups as arginine and placebo. The study was conducted as single blind.

The experimental group was given L-arginine supplement and the placebo group was given wheat bran during 14 days under the supervision of the researcher. They consumed three grams of supplement before training (one gram before breakfast and two grams 30 minutes before training) and three grams after training (two grams one hour after training and one gram before sleep). During the rest days, footballers were given three grams (two grams before breakfast and one gram before sleep) of their assigned supplements.

It was only known by the researcher and the person who made the measurements which group consumed which supplement. Therefore, psychological influences were eliminated and the study was carried out in a reliable atmosphere. Footballers were given explanations about their diets and asked to carry on with their daily routines and existing training programs.

One day after the 14 day period, biochemical tests, antropometric measurements and the RAST test were repeated.

Statistical Analyses

All values are expressed as means ± standard deviation. SPSS 19.0 version was used for the statistical analysis. Data sets were normally distributed. Values betwen pre and post supplementation were compared by paired sample t test.

Results

As a result of the study, we look at the statistical analysis of the results of tests and measurements, in terms of body weight, BMI, lactate, HR, biochemical test data and RAST values significant differences were found in some parameters, while no significant difference was brought out by some values.

Supplementation of arginine caused a decline in weight and BMI levels. Pre and post BMI levels showed statistically significant differences in arginine group ($p<0,05$). No such differences were recorded, however, in the BMI measurements of the placebo group ($p>0,05$).

The pre and post supplementation anaerobic performance values of neither group show any statistically significant differences ($p>0,05$).

In table 3, pre and post supplementation LA values were given. Both the resting lactate levels of placebo and arginine groups demonstrated a decrease after supplementation but only the placebo group showed a significant difference ($p<0,01$). Post-suplementation 1th min recovery lactate values were higher than pre-suplementation values in both groups ($p<0,05$).

Before suplementation of arginine, 5th min of recovery LA value was 10,67±2,83 mmol/L, and 10th min value was increased to 10,99±1,67mmol/L in experimental group. After 14 day-long supplementation of arginine, however, 5th min of recovery LA value was 12,07±2,92. Whereas, 10th min value was decreased to 11,23±1,97.

Before supplementation, the LA value in the 5th min was 9,66±3,38, it increased to 10,5±2,09 in the

Table 1. Physical features of subjects

			Arginine Group		Placebo Group	
Variable	**Period**	**n**	**Mean**	**Standard deviation**	**Mean**	**Standard deviation**
Weight (kg)	Pre- supplementation	14	71,85	9,34	74,71	10,10
	Post-supplementation	14	71,26	8,67	74,77	10,00
BMI (kg/m²)	Pre-supplementation	14	23,60 ♦ (p=0,031)	2,28	24,22	2,32
	Post-supplementation	14	23,39 ♦ (p=0,031)	2,12	24,31	2,40

p<0,05 ♦

Table 2. Anaerobic performance values before and after supplementation

RAST Values			**Arginine Group**		**Placebo Group**	
Analysis	**Period**	**n**	**Mean**	**Standard Deviation**	**Mean**	**Standard Deviation**
Peak Power (W)	Pre-supplementation	14	360,80	83,32	388,87	69,67
	Post-supplementation	14	375,04	68,82	403,95	48,99
Minimum Power (W)	Pre-supplementation	14	286,56	70,31	287,85	55,83
	Post-supplementation	14	285,88	65,58	294,80	44,64
Average Power (W)	Pre-supplementation	14	319,01	69,67	341,05	61,69
	Post-supplementation	14	333,95	60,84	348,12	43,56
Fatigue Index Average (W/s)	Pre-supplementation	14	40,65	2,97	38,40	3,58
	Post-supplementation	14	39,06	3,65	38,10	4,77

Table 3. Comparison of LA values between before and after supplementation

	ARGININE (n=14)				PLACEBO (n=14)			
		Recovery				Recovery		
LA(mmol/L)	Resting	Minute 1	Minute 5	Minute 10	Resting	Minute 1	Minute 5	Minute 10
Pre supplementation	2,32±,56	6,88±1,77	10,67±2,83	10,99±1,67	2,23±,71	5,53±2,37	9,66±3,38	10,5±2,09
Post supplementation	2,15±,93	8,95±3,42	12,07±2,92	11,23±1,97	1,72±,43 (p=,007)	8,28±3,07 (p=,046)	11,41±2,26	12,72±2,78
P	0.526	0.020 ♦	0.219	0.681	0.007 ♦♦	0.046 ♦	0.053	0.073

p<0,05 ♦ *p<0,01* ♦♦

10th min of recoveryin the placebo group. After 14 days, the LA value in the 5th min was 11,41±2,26 and it increased to 12,72±2,78 in the 10th min.

Table 4 shows the recovery HR values before and after 14 day-long supplementation. It is observed that the 1th min of recovery and resting HR levels of the arginine group decreased after supplementation (p<0,05 and p<0.01). Placebo group also showed a decrease after supplementation in their resting HR and HR level in the 1th min after training and a significant difference was recorded (p<0,05). Other HR findings of arginine and placebo groups did not show a significant difference (p>0,05).

AST, ALT and LDH values of the footballers who received arginine supplementation were found lower, Triglyceride value was found higher when compared with pre-supplementation values (p<0,05, p<0.01 and p<0.001). As for the placebo group, a decrease was found in the alkaline phosphatase values and a statistical difference was found (p<0,05). No statistically significant difference was found in other findings (p>0,05).

Discussion and Conclusions

The purpose of this study was to examine the effect of oral arginine supplementation on anaerobic performance and recovery. In the study, arginine supplementation caused a decrease in body weight and BMI values. L-arginine supplementation may also be effective on fat loss. No such difference was found in the placebo group. Researching the reason of this reduction in BMI, it is thought that arginine, which may have acute effect, provides the body fat's usage of energy resource by increasing the lipolysis level in metabolism. In their study with the footballers, Angeli et al. (15) gave daily 1 gram vitamine C supplementation and 3 gram arginine to the experimental group and 1 gram vitamine C to the control group. In the arginine group, at the end of 8 week-long weight training, the researchers found an increase in body weight and muscle mass and a decrease in body fat percentage. In their study with athletes, Burtscher et al. (16) gave L-arginine L-aspartate in saccharose to the experimental group and only saccharose to the placebo group during 3-weeks exercise. No physical feature difference was found between two groups at the end of the 3-weeks long supplementation. The results of Angeli et al. (15)'s study is similar to our study in terms of its results.

In our study, a significant difference was found in the 1st minute post-training lactate measurements of both groups; however, this difference was in the form of increase in the lactate levels just after exercise when compared with the pre-test findings. The reasons for this may be the content and intensity of the training before the second (post) test, too many anaerobic training methods in the exercises or the high difficulty level of the game played that week.

In our study, post exercise 1st, 5th and 10th minute LA levels were measured in order to track recovery in pre and post supplementation period. In the arginine group, LA levels of the 10th minute were higher than those of the 5th minute in the pre supplementation period while LA levels of the 10th minute were lower than those of the 5th minute in the post supplemen-

Table 4. Comparison of HR values between before and after supplementation

	ARGININE (n=14)					PLACEBO (n=14)				
		Recovery					Recovery			
HR values (heartbeat/min)	**Resting**	**Minute 1**	**Minute 3**	**Minute 6**	**Minute 9**	**Resting**	**Minute 1**	**Minute 3**	**Minute 6**	**Minute 9**
Before supplementation	74,33±9,31	176,50±6,47	104,16±10,70	99,83±9,54	96,58±8,99	79,33±11,73	173,22±11,44	112,55±10,80	106,11±12,11	104,44±12,99
After supplementation	67,66±12,35	169,25±10,73	104,41±8,07	98,00±8,22	97,66±8,40	74,77±12,44	167,44±13,00	108,55±13,21	100,00±13,84	100,33±13,67
P	0.037 ♦	0.008 ♦♦	0.920	0.448	0.553	0.036 ♦	0.025 ♦	0.241	0.083	0.315

p<0,05 ♦ *p<0,01* ♦♦

Table 5. Comparison of Biochemical values between before and after supplementation

ARGININE (n=14)											
Biochemical tests Mg/dL - U/L	**Urine**	**Creatinine**	**Cholesterol**	**Triglyceride**	**HDL Cholesterol**	**LDL Colesterol**	**AST**	**ALT**	**GGT**	**Alkaline Phosthapase**	**LDH**
Before supplementation	29,81±6,17	0,88±,09	159,41±37,19	65,08±21,26	47,58±11,75	98,81±29,76	36,66±13,29	24,25±8,75	17,83±6,43	84,25±30,86	229,41±47,23
After supplementation	27,58±7,09	0,88±,08	165,25±38,56	92,00±28,43	46,33±9,64	99,91±30,34	21,33±3,72	17,75±8,00	16,91±6,52	83,83±33,46	176,08±45,62
P	0.273	1.000	0.293	0.003 ♦♦	0.292	0.802	0.001 ♦♦♦	0.017 ♦	0.231	0.775	0.000 ♦♦♦
PLACEBO (n=14)											
Biochemical tests Mg/dL - U/L	**Urine**	**Creatinine**	**Cholesterol**	**Triglyceride**	**HDL Cholesterol**	**LDL Colesterol**	**AST**	**ALT**	**GGT**	**Alkaline Phosthapase**	**LDH**
Before supplementation	25,68±4,75	0,87±,09	143,55±18,35	77,88±17,57	41,44±4,53	86,53±16,88	25,66±5,36	25,22±11,34	17,11±3,88	107,77±51,67	184,33±27,05
After supplementation	25,55±4,38	0,93±,13	141,33±19,35	91,44±33,84	41,00±3,77	82,04±15,56	23,22±4,65	22,66±13,36	16,55±4,06	102,00±51,86	167,11±28,42
P	0.896	0.212	0.652	0.285	0.525	0.292	0.338	0.331	0.247	0.020 ♦	0.068

p<0,05 ♦ *p<0,01* ♦♦ *p<0,001* ♦♦♦

tation. However, this result was not the same for the placebo group. In the placebo group, LA levels of the 10th minute of recovery were higher than those of the 5th minute in both pre and post supplementation. In the placebo group, LA level was found to increase and thus, LA removal was found to be slow. In short, post arginine supplementation lactate levels of the experimental group were found to decrease faster. This result shows that arginine supplementation accelerates the removal of LA from the body and improves recovery.

In their study with 30 body builders, Imanipour et al. (17) gave arginine to the first of the experimental groups, BCAA (branched-chain amino acid) to the second experimental group and they did not give any substance to the control group. At the end of the 6-week long training, statistical test results showed that although basal levels of lactate in the arginine supplementation group had decreased slightly after 42 days supplementation, the difference was not statistically significant. Muazzezzaneh et al. (18) grouped male athletes in two as the experimental and placebo group. They gave daily 5 grams of arginine to the experimental group and daily 5 grams of wheat supplementation to the placebo group for 21 days. As a result of their study, they did not find a difference between the pre-training measurements of two group while the reduction of LA after exercise has been found to be more in arginine group than placebo. Wilkerson et al. (19) gave NG-nitro-L-arginine methyl ester (L-NAME) supplementation to the healthy male volunteers and although the blood lactate levels of the L-NAME supplementation group were found to be higher than those of the control group at the beginning of the exercise, there was no significant post exercise difference between them. In L-NAME group, blood lactate accumulation showed significant decrease. All these studies support the findings of our study and they also show that arginine supplementation accelerates the removal of LA.

In our study, HR levels were analyzed to track post anaerobic exercise recovery. Post supplementation rested and 1.min of recovery HR levels of both groups were found to decrease when compared with pre supplementation ($p<0,05$ and $p<0.01$). Similar characteristics of groups that have the same primary phase and the same training program bring to mind that arginine supplementation does not have any effect on HR. However, when the results were analyzed, it can be seen that the decrease in the post supplementation HRs of footballers who used arginine when compared with the pre supplementation were more obvious when compared with the placebo group; thus, recovery occured faster. It is thought that this decrease in the post arginine supplementation HR will also be effective on recovery. Willoughby et al. (20) gave daily 12 grams of arginine to 24 male athletes for 7 days. According to their results, post supplementation HRs of the experimental group just after exercise were found to be lower than those of the pre supplementation while an increase was found in the placebo group. In their study with 16 athletes, Burtscher et al. (16) gave L-arginine L-aspartate in saccharose (daily 3 grams) to the experimental group during 3-week exercise and only saccharose to the placebo group. As a result of their study, Burtscher et al. (16) found a decrease in the HRs of the L-arginine L-aspartate group and they found a statistically significant difference. These studies bring to mind the idea that L-arginine supplementation will improve performance and recovery.

Post arginine supplementation AST, ALT and LDH values were found lower, Triglyceride value was found higher when compared with pre-supplementation ($p<0,05$, $p<0.01$ and $p<0.001$). As for the placebo group, a decrease was found in the ALP values and a statistical difference was found ($p<0,05$). No statistically significant difference was found in other findings ($p>0,05$). AST enzyme, which was over the normal value range before supplementation in footballers who used arginine, got back to normal value range after supplementation. This result brings to mind that arginine use does not cause a disadvantage on liver enzymes and moreover it heals the enzymes. In addition, a decrease was seen in LDH -an intracellular enzyme responsible for the conversion of pyruvate to lactate- that increases as a result of muscle damage after intense and extreme exercise due to arginine supplementation. This decrease in LDH enzyme levels will accelerate recovery since it heals the muscle damage fast. LA causes intracellular acid and thus, the cell will be damaged. Thus, a fast decrease in LDH enzyme is important for recovery and renewal. Cells should become alkaline instead of acidic. These enzymes, which were critically high in

the arginine group before supplementation, decreased after supplementation and became normal. As a result of the decrease in LDH enzyme values caused by argininie supplementation, the muscle damage that occurs after intense exercise will be healed and accelerate recovery. In the placebo group, there was a decrease only in the ALP. The fact that all these differences did not occur in the placebo group brings to mind that arginine supplementation does not cause a harm to bodily biochemical values, arginine supplementation has positive effects on metabolism, performance and recovery and it is a reliable dietary supplementation. In their study with 12 athletes, Sales et al. (21) grouped these athletes in three groups as arginine, placebo and control and gave daily 4,5 g arginine supplementation to the arginine group. The researchers checked the pre and post supplementation urea and creatine levels of the athletes. According to their results, arginine supplementation did not cause a significant difference in urea and creatine values. Thus, their study supports the reliability of arginine.

In our study we analyse LDH one day after exercise, namely not right after the exercise. In the studies it was found increase because they analyse LDH 5 or 10 min after exercise or following acute exhaustive exercise. But they found less increase in LDH levels in arginine group than control. In the study of Taylor et al. (22) L-NAME hydrochloride appeared to lower LDH release independent of exercise. Exercise plus L-NAME resulted in less LDH release at both 5 and 10 min than exercise without L-NAME. In the study of Lin et al. (23), the activities of plasma Creatine Kinase (CK) and LDH were significantly decreased in Arginine supplemented plus exercised rats compared with exercised rats. These findings suggest that Arginine supplementation reduces the oxidative damage and inflammatory response on the myocardium caused by exhaustive exercise in rats. In the study of Gupta et al. (24) L-arginine administered orally 30 min prior to cold (5C)–hypoxia (428 mmHg)–restraint (C-H-R) exposure. The C-H-R exposure of control rats on attaining rectal temperature (Trec 23)C, resulted in a significant increase in LDH. On recovery (Trec 37) of control rats, there was an increase in LDH too. But L-Arginine supplementation resulted in a lower increase in LDH compared with controls (45.3 versus 58.5% and 21.5 versus 105.2%) on attaining Trec 23C during C-H-R exposure and on recovery to Trec 37C. The results suggested that L-arginine possesses potent anti-stress activity during C-H-R exposure and recovery from C-H-R-induced hypothermia. In the rats treated with L-arginine (100 mg/kg body weight), the increase in LDH levels both on attaining Trec 23C and on recovery of Trec 37C were less than in control rats. This suggested that L-arginine supplementation was acting at a cellular level in maintaining the energy-dependent process of membrane permeability. Their results supported the view that arginine supplementation will decrease the LDH levels and improve recovery. In the light of these findings, low levels of LDH bring to mind that it will improve performance and recovery.

As a conclusion, it was found that in footballers, L-argininie supplementation accelerated the removal of LA from the body, decreased the amount of fat in the body, healed the post training muscle damage caused by the decrease in LDH enzyme level, enabled muscle renewal accelerated recovery.

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Original article

Low protein diet score: a novel diet quality index and predictor of disease progression in patients with chronic kidney disease

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Summary. *Background and Aim:* Patients with chronic kidney disease (CKD) have been recommended to consume a low protein diet. However, there is no specific index for evaluating amounts of dietary protein and CKD progression in these patients. The objective of this study was to define a low protein diet score (LPDS) as a predictor of CKD progression and an index of diet quality in patients with CKD. *Methods:* For this cross-sectional study, two hundred twenty seven eligible subjects with CKD (stage 3 and 4) were selected. We used a validated food frequency questionnaire to assess dietary intake of patients. LPDS was defined based on the 3 cut of points: >2 gr/kg (score=1), 1.01-2 gr/kg (score=2) and ≤ 1 gr/kg (score=3). Renal function (i.e., blood urea nitrogen and serum creatinine) and cardiometabolic variables (i.e., low density lipoprotein, triglyceride, total cholesterol, fasting blood sugar and high sensitivity C-reactive protein) were measured by biochemical assessment. As dietary intakes of sodium, potassium, phosphorus, saturated fatty acid and cholesterol are important, we used intake of these nutrients to assess diet quality. *Results:* Patients who received higher scores, had better diet quality because they consumed lower amounts of phosphorus, potassium, saturated fatty acid and cholesterol (P<0.01 for all). Biochemical assessments showed that in comparison with the bottom LPDS, a marginally significant lower blood urea nitrogen was observed among subjects with higher scores (P=0.06). We did not observe any significant difference in other biochemical variables across the defined LPDS. After adjusting for all confounders, a significant decreasing trend for risk of CKD progression was revealed across LPDS (P for trend=0.04). *Conclusion:* The results of the present study showed that higher LPDS was associated with favorable nutrients intake (lower intakes of sodium, potassium, phosphorus, cholesterol and saturated fatty acid) among patients with CKD. Moreover, subjects who received higher LPDS had a marginally significant lower BUN. Also, we observed that LPDS was inversely related to the risk of being in the higher stage of CKD after adjusting for potential confounders. Therefore, it was a predictor of CKD progression.

Key words: chronic kidney disease, dietary protein, disease progression

Abbreviations

BUN: blood urea nitrogen, CKD: chronic kidney disease, eGFR: estimated glomerular filtration rate, FBS: fasting blood sugar, FFQ: food frequency questionnaire, hs-CRP: high sensitive C-reactive protein, LDL-C: low density lipoprotein cholesterol, LPDS: low protein diet score, SCr: serum creatinine, TC: total cholesterol, TG: triglyceride

Introduction

Chronic kidney disease (CKD) is a health concern in developed and developing countries. Reported results from NHANES data revealed that 13.1% of American population suffers from CKD (1). CKD is more prevalent among Iranian adults (18.9%) (2).

Dietary recommendations have an important role in medical management of CKD. Evidence showed that higher dietary protein intake in healthy subjects may increase the risk of renal hypertrophy, glomerular hyperfiltration and renal blood flow (3). Therefore, patients with CKD are recommended to lower protein intake (4). Observational studies revealed that CKD progression was associated with the amount of consumed dietary protein (5). Also, an inverse association between low protein diet intake and risk of death from renal disease was observed in subjects with CKD (6).

Patients with CKD were recommended to restrict dietary sodium, potassium and phosphorus because excess amounts of these minerals cannot be excreted (4). Therefore, dietitians prescribe a diet restricted in sources of mentioned minerals such as fruits, vegetables, legumes, dairies and whole grains (7). These restrictions are in contrast with recommended diets for healthy subjects. For instant, according to the HEI-2010, consumption of fruits, vegetables, whole grains and low fat dairies should be increased (8). Therefore, general dietary recommendations for healthy subjects cannot be used to assess diet quality of the subjects with CKD. Assessment of diet quality is important in individuals with CKD because most patients suffer from malnutrition (9). Unfortunately, a specific index has not yet been defined to assess diet quality of patients with CKD.

In 2008, carbohydrate scoring was suggested by Halton et al. (10). They score dietary carbohydrate by using percentage of energy as carbohydrate to assess the association between a low carbohydrate diet and risk of diabetes because dietary carbohydrate was critical for type 2 diabetic patients (10). In the management of CKD, dietary protein is the most important macronutrient. The findings of researches emphasized that protein intake should be controlled in dietary management of CKD (11). Evidence revealed that high dietary protein intake was directly related to CKD progression (12). Therefore, we hypothesized that a low protein diet score (LPDS) may predict the progression of CKD. Scoring the dietary factors is a common practice in nutritional research and studies reported that interpretation regarding the relation between diet and disease may be easier by macronutrient scoring (10, 13, 14). However, dietary protein was not scored previously. Moreover, there is no specific diet quality index for evaluating the quality of diet in subjects with CKD. Therefore, the aim of current study was to define a LPDS as a predictor of CKD progression and an index of diet quality in patients with CKD.

Methods

Subjects: This research was a cross-sectional study. Among patients who referred to clinics of nephrology, two hundred twenty one subjects with CKD were selected. Patients were chosen from both genders. We had no age restriction. A nephrologist calculated estimated glomerular filtration rate (eGFR) for each subject (15) and eGFR<60 mL/min/1.73m^2 was considered as CKD (16). CKD was categorized as stage 3 (30≤ eGFR ≤59 mL/min/1.73m^2), stage 4 (15≤ eGFR ≤29 mL/min/1.73m^2) and stage 5 (eGFR <15 mL/min/1.73m^2) (16). Written consent was signed by all patients.

Dietary assessment: Dietary intake of patients during the previous year was assessed by a validated (20, 21) food frequency questionnaire (FFQ) completed by trained assistants. This semi-quantitative FFQ covered 168 food items frequently consumed by Iranians. All reported consumed foods were converted to g/day by using household measures. The nutrient content of foods was calculated by Nutritionist IV software (N-Squared Computing, Salem, OR). Subjects who reported <800 or >4200 kcal/d were excluded.

Low protein diet score: Total protein intake was calculated by summing protein content of all consumed foods. The amount of protein intake for each individual was converted to gram per kilogram body weight (g/kg). As previous study used different amounts of dietary protein for patients with renal disease (17), LPDS was defined based on the 3 cut of points: >2gr/kg (score=1), 1.01-2 gr/kg (score=2), ≤ 1gr/kg (score=3).

For example, a patient who consumed 1.5gr/kg received a score of 2.

Biochemical measures: After 12-hour overnight fasting, a blood specimen was collected. Then samples were centrifuged at 3000×g for 10 min. The concentration of blood urea nitrogen (BUN) was measured by using urease enzyme. We assessed the concentration of serum creatinine (SCr) by standard spectrophotometric. Fasting blood sugar (FBS), triglyceride (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and high sensitivity C-reactive protein (hs-CRP) were measured as cardiometabolic variables. The level of FBS, TG and TC was analyzed by using enzymatic colorimetric tests. LDL-C concentration was measured by blocking and then enzymatic methods. Immunoturbidimetry assay was run to determine the level of hs-CRP. All kits were produced by Pars Azmoon Inc.

Other variables: General characteristics of patients were asked by oral questions. Socioeconomic status was evaluated by questions regarding income, occupation, education and region of residence. Physical activity was assessed by a one-day physical activity record.

Statistical Analysis: Normal distribution of variables were tested by Kolmogorov-smirnov test and checking the histogram curve. Qualitative variables included CKD stage, physical activity level (low, moderate and high), marital status, sex ratio and socioeconomic status (low, moderate and high) were presented as percentage frequency. Difference in these variables was assessed by Chi-square test. Quantitative variable (age, body mass index, biomarkers and dietary intakes of nutrients) reported as mean ± standard deviation. Biochemical variables were compared across the LPDS by analysis of variance (ANOVA). We used analysis of covariance (ANCOVA) to report energy-adjusted nutrient intakes across the scores of a low protein diet. Odds ratio and 95% confidence interval of CKD progression was calculated by logistic regression. The risk of being in the higher stage of CKD was presented in crude and 3 adjusted models. The firs model was adjusted for age, physical activity and body mass index. Further adjustment was performed for systolic blood pressure and diastolic blood pressure in model 2. The intake of carbohydrate, fat, potassium and phosphorus was included in the third model. We considered $P<0.05$ as significance level. Also, we used SPSS version 20 (IBM) to analyze this data.

Results

We have displayed demographic characteristics of the subjects with CKD across LPDS in Table 1. There was no significant difference in the age of subjects across LPDS (P=0.42). The percentage of male in the lower LPDS category was higher (P=0.04). Subjects with top score had higher body mass index (BMI) (P<0.01). We did not observe any significant comparison for other variables.

Energy adjusted dietary intake of selected unfavorable nutrients in renal insufficiency across LPDS is shown in Table 2. Patients who received higher score, consumed lower amounts of cholesterol, saturated fatty acid, phosphorus, potassium and sodium (P<0.01 for all).

Biochemical measurements of subjects with CKD across LPDS are displayed in Table 3. In comparison with the bottom LPDS, a marginally significant lower BUN was observed among subjects with higher score (P=0.06). We did not observe any significant comparison for other variables.

Table 4 shows the risk of CKD progression across LPDS. Odds ratios were reported in 4 different models. We did not observe any significant increased risk of higher stage of CKD in crude model (P for trend=0.84), model 1 (P for trend=0.22) and model 2 (P for trend=0.24). After adjusting for all potential confounders, a significant decreasing trend for risk of higher stage of CKD across LPDS was revealed (P for trend=0.04).

Discussion

The results of the present study showed that subjects with higher LPDS consumed lower amounts of unfavorable nutrients for subjects with CKD. Also, we found that adherence to a low protein diet could decrease the risk of CKD progression to the higher stage. To the best of our knowledge, this is the first study to introduce LPDS as an index for assessing diet quality and a predictor of CKD progression.

Table 1. Demographic characteristics of the subjects with chronic kidney disease across scores of the low protein diet

Variables	Low protein diet score			P value1
	1 (>2 gr/kg) N=26	2 (1.01-2 gr/kg) N=167	3 (≤ 1 gr/kg) N=34	
Age (y)	65.54±10.512	65.14±9.23	67.53±10.87	0.42
Male (%)	23	34	53	0.04
Socioeconomic status (%)				
Low	15.4	9.6	8.8	
Moderate	65.4	67	55.9	
High	19.2	23.4	35.3	0.75
Married (%)	100	98.2	97.1	0.69
Body mass index (kg/m^2)	21.75±3.04	23.58±3.34	25.64±3.03	<0.01
Physical activity level (%)				
Low	65.4	63.5	70.5	
Moderate	30.8	35.3	26.5	
High	3.8	1.2	2.9	0.43
CKD Stage (%)				
3	42.3	46.7	50	
4	57.7	53.3	50	0.84

[1] Calculated by chi-square test (for qualitative variables) or analysis of variance (for quantitative variables)
[2] Qualitative and quantitative variables are expressed as percentage and mean±SD, respectively.

Table 2. Energy adjusted dietary intake of selected unfavorable nutrients in renal insufficiency across scores of the low protein diet

Variables	Low protein diet score			P value1
	1 (>2 gr/kg) N=26	2 (1.01-2 gr/kg) N=167	3 (≤ 1 gr/kg) N=34	
Cholesterol (mg)	291.19±70.072	192.84±68.11	99.77±70.06	<0.01
Saturated Fatty acid (g)	33.87±13.97	22.73±13.54	12.63±13.98	<0.01
Potassium (mg)	4930.27±920.14	3317.81±893.71	1864.53±919.94	<0.01
Phosphorus (mg)	2407.77±378.01	1549.74±376.13	834.20±377.93	<0.01
Sodium (mg)	7778.73±4886.06	6183.35±6393.47	3997.60±2879.23	<0.01
Magnesium (mg)	443.89±79.61	313.81±77.27	171.51±79.63	<0.01

[1] Calculated by analysis of covariance
[2] Mean±SD. All values are adjusted for total calorie intake except for energy intake.

We observed an increased intake of sodium, phosphorus and potassium across LPDS. Legumes, meats and dairy are important sources of dietary protein. Legumes are rich in potassium and phosphorus (18). Moreover, previous studies reported an animal based diet resulted in higher sodium and phosphorus intake (19, 20). Also, vegetables (e.g., carrots, tomatoes, potatoes and stewed leafy vegetables) are consumed along with meats and legumes in Iran. Therefore, we observed higher potassium intake among those with higher protein intake. Usual diet quality indices focus on higher intakes of fruits, vegetables, legumes, whole grains and low-fat dairy (8). Therefore, we cannot use these diet indices to evaluate the quality of diet in patients with CKD because adherence to these recommendations results in higher intake of potassium, phosphorus

Table 3. Biochemical measurements of subjects with chronic kidney disease across the low protein diet score

Variables	Low protein diet score			P value1
	1 (>2 gr/kg) N=26	2 (1.01-2 gr/kg) N=167	3 (≤ 1 gr/kg) N=34	
BUN (mg/dl)	20.08±2.302	19.40±2.73	18.50±2.54	0.06
Creatinine (mg/dl)	1.91±0.28	1.97±0.24	1.96±0.29	0.52
GFR (mL/min/1.73m2)3	22.93±6.05	29.59±6.04	31.28±7.72	0.36
Total cholesterol (mg/dl)	271.73±24.68	271.13±21.79	272.32±17.45	0.95
Triglyceride (mg/dl)	246.92±32.75	253.17±29.91	253.15±29.81	0.61
LDL (mg/dl)	133.50±18.98	133.35±20.21	138.65±24.99	0.40
FBS (mg/dl)	130.58±14.76	129.20±14.44	129.79±13.49	0.89
hs-CRP (mg/L)	1.65±0.31	1.60±0.32	1.60±0.31	0.82

BUN: blood urea nitrogen, FBS: fasting blood sugar, GFR: glomerular filtration rate, hs-CRP: high sensitivity reactive protein, LDL: low density lipoprotein

[1] Calculated by analysis of variance; [2] mean±SD; [3] Calculated by MDRD equation

Table 4. Odds ratio (95% CI) for higher stage of chronic kidney disease across scores of the low protein diet

Variables	Low protein diet score			P value1
	1 (>2 gr/kg) N=26	2 (1.01-2 gr/kg) N=167	3 (≤ 1 gr/kg) N=34	
Crude	1 (Ref)	0.84 (0.36, 1.93)	0.73 (0.26, 2.05)	0.84
Model 1	1 (Ref)	0.67 (0.27, 1.71)	0.36 (0.11, 1.20)	0.22
Model 2	1 (Ref)	0.66 (0.26, 1.68)	0.37 (0.11, 1.23)	0.24
Model 3	1 (Ref)	0.39 (0.12, 1.30)	0.11 (0.02, 0.66)	0.04

Model 1: adjusted for age, physical activity and body mass index
Model 2: model 1 + systolic and diastolic blood pressure
Model 3: model 2 + dietary carbohydrate, fat, potassium and phosphorus

and protein intake. In contrast, our findings revealed that LPDS had a favorable association with important nutrients in CKD (protein, sodium, phosphorus and potassium) and it could be used as a diet quality index among patients with CKD.

We did not observe any significant comparison for biochemical variables (LDL, TG, TC, FBS and hs-CRP) except for BUN. It seems that these are more sensitive to the source of protein than its amount. Nutritional guidelines suggested lower red meat consumption to achieve reduction in LDL (21). Previous studies reported that higher red meat intake may be related to increased hs-CRP and TG (22, 23). In contrast, several studies reported that consumption of legumes have a beneficial effect on concentration of inflammatory markers and cholesterol (24, 25). In the designing of LPDS, we did not include the source of dietary protein and it may be the possible reason for non-significant observed result.

Although we did not find statistically significant odds ratio for higher stage of CKD in crude model, multivariate adjusted model (adjusted for dietary factors) showed a significant decreasing trend for risk of higher stage of CKD across LPDS. It shows that dietary factors had a confounding role in the association between LPDS and risk of higher stage of CKD. We did not include sex in multivariate adjusted model because it had been considered in MDRD equation (15). Biochemistrists suggested that adherence to a low protein diet could decrease the amounts of toxic waste products of protein metabolism not excreted due to insufficient renal function (26). Higher protein in-

take results in hyperfiltration, increased acid load and proteinuria (27). These factors may lead to CKD progression (27).

The source of dietary protein was not included in introducing the score and it was the most important limitation of this study. Our previous studies showed that soy as a source of dietary protein may have beneficial effect on metabolic factors in patients with renal diseases (28-31). Therefore, the source of dietary protein should be included in future researches. The design of the present study was cross-sectional and this dietary scoring method should be evaluated in prospective cohort studies. Also we did not measure other renal biochemical variables such as proteinuria and urine creatinine in this study.

We introduced a novel index of diet quality for patients with CKD and it was the major strength of the present study. As dietary recommendations for management of CKD emphasize on lower intake of fruits, vegetables, legumes, dairies and whole grains (32) we cannot assess diet quality of these patients by usual diet quality indices (e.g., HEI-2010 and DQIs). Therefore, the results of this study introduce a good diet quality index to evaluated quality of diet of individuals with CKD. Also, we measured cardiometabolic biochemical variables (i.e., LDL, TG, TG, FBS and hs-CRP) because their abnormal levels were prevalent among these patients (28, 30)

In conclusion, the results of the present study showed that higher LPDS was associated with favorable nutrients intake (lower intakes of sodium, potassium, phosphorus, cholesterol and saturated fatty acid) among patients with CKD. Moreover, subjects who received higher LPDS had a marginally significant lower BUN. Also, we observed that LPDS was inversely related to the risk of being in the higher stage of CKD after adjusting for potential confounders. Therefore, it was a predictor of CKD progression.

The results of the present study showed that subjects with higher LPDS consumed lower amounts of unfavorable nutrients for subjects with CKD. Also, we found that adherence to a low protein diet could decrease the risk of CKD progression to the higher stage.

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Original article

The effect of enteral nutrition support on muscle function capacity and pulmonary functions in malnourished patients with Chronic Obstructive Pulmonary Disease

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Summary. Patients with Chronic Obstructive Pulmonary Disease (COPD) usually have a decreased muscle function capacity and pulmonary functions. The purpose of this study was to determine the effect of enteral nutrition support given in addition to diet on muscle function capacity, forced expiratory volume in second (FEV1) and tiffeneau index (FEV1/FVC) in malnourished patient with COPD. Forty patients with body mass indexes (BMI) below 18.5 kg/m^2 were included in the study. Nutrition education was given to all patients then the patients were divided into two groups as treatment and control. Treatment group consumed two bottles of enteral nutrition product in addition to oral nutrition for three months. The muscle powers of the patients were measured with handgrip dynamometer and their FEV1 and FVC values were determined with pulmonary function tests, and tiffeneau indexes were calculated in the beginning and at the end of the study. In conclusion, enteral nutrition support increased hand grip strength and FEV1 without any significant effect on FEV1/FVC values in malnourished patients with COPD.

Key words: COPD, enteral nutrition support, FEV1, FEV1/FVC, hand grip strength

Introduction

Chronic Obstructive Pulmonary Disease (COPD) is a chronic inflammatory disorder of the lung and whole body caused mainly by tobacco smoking, and is characterized by progressive and persistent airflow obstruction (1). COPD is the fourth leading cause of death worldwide (2) whereas according to the results of the "Study of Disease Burden" conducted in Turkey in 2004, COPD is the third leading cause of death, and 26 thousand people die every year due to COPD in our country (3). It is estimated that the global burden of COPD will increase in the near future due to continuing exposure to risk factors related to COPD and the aging of the population (2).

The treatment of COPD requires a multidisciplinary approach including pharmacological, surgical and oxygen therapies along with physiotherapy, psychotherapy and nutritional therapy (4). Malnutrition is frequently observed in patients with COPD. Overall, 10-45% of patients with COPD are malnourished, and it is evident that malnutrition and under-nutrition are important prognostic factors for COPD patients (5). Chronic inflammatory processes, tissue hypoxia, muscle atrophy, anabolic hormone deficiency, and an increased resting metabolic rate all contribute physiologically to weight loss in patients with COPD (6). Malnutrition in COPD causes reduction in body fat and muscle mass through decreasing protein synthesis irrespective of lung function. Malnutrition can limit the exercise capacity by causing dysfunctions in diaphragm and other respiratory muscles and lead to emphysematous changes in parenchyma. In addition, it has been considered that malnutrition affects the re-

spiratory control center and reduces respiratory minute/volume and the ventilatory response to hypoxia and hypercapnia thus increases the incidence of acute respiratory failure in COPD patients (1,4,7).

Nutritional support is a critical part of the treatment plan for the patient with COPD. Proper care to prevent malnutrition will have a significant impact on quality of life and overall patient outcomes. The primary objective of nutrition support is to meet the calculated nutritional requirements thus prevent weight loss. Nutrition support shows positive effects on weight gain and provides functional recovery in the lungs by increasing energy intake (1,8,9,10). These patients may have increasing fatigue, dyspnea, and early satiety, which affect their ability to eat and consume enough calories. Therefore, under these circumstances, it is important to provide nutrition that has high calorie density. This will also help to minimize abdominal distention that may cause discomfort while eating (8). Nutritional intervention consists of oral supplementation and enteral nutrition to prevent weight loss and muscle mass depletion. Frequent small amounts of oral nutrition supplement ONS are preferred in order to avoid postprandial dyspnea and satiety as well as to improve compliance (6,11-13). Therefore, this study was performed to investigate the effects of enteral nutrition support in addition to diet on the muscle function capacity and some parameters of pulmonary function in COPD patients with malnutrition.

Materials and Methods

Participants and Study Design

This study was designed as a prospective, controlled, randomized trial to investigate the influence of enteral nutrition support along with the diet on muscle function capacity and pulmonary function in patients with COPD.

At the beginning, 63 patients were included in the study. However, 16 patients from the treatment group were excluded from the study because they did not consume the products regularly. Four patients from the control group and three patients from the treatment group expired without completing the study. The study was completed with 40 patients with 80% power (β = 0.20) and 95% confidence level (α = 0.05) by power analysis. Out of 40 patients with COPD, 29 patients hospitalized at Clinic of Chest Diseases and 11 outpatients admitted to the clinics of Pulmonary Disease in Trabzon Ahi Evren Thoracic and Cardiovascular Surgery Training and Research Hospital between January and November 2014 were enrolled in the study. The inclusion criteria were receiving a diagnosis of COPD by specialist doctors, having BMI below 18.5 kg/m^2, being over 18 years old, cognitively intact, not pregnant and lactating.

Patients were given verbal and written information about the study, and written informed consent was obtained from all individual participants. This study was initiated with the permission of the administration Trabzon Ahi Evren Thoracic and Cardiovascular Surgery Training Hospital dated 20/12/2013 and the decision of Erciyes University Medical Faculty Ethics Committee, Approval No: 2013/759.

Patients who were not confined to bed and not depending on respiratory devices and agreed to participate in the study were randomly distributed into control and treatment groups consisting of 20 patients in each after being informed about the investigation. Patients in treatment group received two packs/day of enteral nutrition support (Nutrivigor, Abbott) in addition to their diets for 12 weeks whereas control group had no nutritional support. Enteral nutrition product used in this study had a formula that provides an energy intensive complete and balanced nutrition for people who are malnourished or under risk of malnutrition and/or loss muscle mass. The product consists of 18 g protein, 11 g fat, 39 g carbohydrates, 1.2 g HMB, 1.7 g fructooligosaccharides, 352 mg calcium and 12 μg vitamin D in 220 ml volume.

Data Collection and Measurements

Demographic data (age, gender, education level, etc.) and information concerning the smoking, alcohol consumption, disease and treatment statuses of the patients participated in the study were collected through face to face interviews by researchers. Income levels were determined based on the patients' personal statements.

The body weight of the patients was determined with 0.1 kg precision digital scale (King, EB817). The height of individuals was measured by a stadiometer

with 10-200 kg±100 g and 60–200 cm±1 mm sensitivity (MK-150, Turkey) while the person in light clothing was standing without shoes, feet were together, and head was maintained in the Frankfort Horizontal Plane position (triangle of eyes in alignment with the upper side of auricle). The body mass index (BMI, kg/m^2) values were calculated through dividing the weight (kg) by height (m) square.

Nutritional statuses of patients were evaluated with NRS-2002 form proposed by The European Society for Clinical Nutrition and Metabolism (ESPEN) for the hospitalized patients. NRS-2002 form was composed of two parts. In the first part, the severity of the disease, food intake (presence of reduction) and weight loss within three months were questioned. If the answers to all of the questions were "no", no question from second part was asked, and weekly scans were continued. If the response to any of the questions was "yes", it was passed to the second part evaluating the malnutrition and disease severity. The severity of the disease was scored as absent (0 points), mild (1 point), moderate (2 points) and severe (3 points). Patients having a total score of three or above was considered as under risk of malnutrition (14).

A 24-hour food consumption of individuals was determined by the researchers using the backward reminder method. Daily energy, macro and micro nutrients intakes of individuals were determined by evaluating the food consumption data with BeBis (Pasifik Company, Türkiye).

The handgrip strength was measured with hydraulic hand dynamometer (Jamar, Lafayette Instrument Company, USA) while the patient was standing with a 45º angle between the arm and the body. The average of two measurements of the both hands was recorded as the result.

Pulmonary function tests were carried out with spirometer (ZAN 100, ZAN Messgerate Gmbh, Germany) by three measurements, and the best curve was used for the determination of forced expiratory volume in 1st second (FEV1), forced vital capacity (FVC) values. The measurements were performed in the beginning and at the end of the study to compare energy and nutrient intake, hand grip strength, FEV1, FVC and FEV^1 / FVC values obtained prior to and after the treatment.

Statistical Analysis

The analysis of the data was performed with Statistical Package for the Social Sciences (SPSS version 23). The frequency analysis for demographic characteristics and responses to questionnaires of individuals participated in the study were performed, and introductory statistics values were calculated. Kolmogorov-Smirnov test was used for normality testing of the data, and homogeneity of group variance was determined with Levene test. Student's t-test for independent groups (for the differences between control and treatment groups) and paired t-test for dependent groups (for the differences between the values obtained before and after the treatment) were used. Spearman rank correlation coefficient was calculated in order to determine the relationships between variables. Chi-square test was performed in order to examine the dependence between questions, and Pearson chi-square values were calculated, when the expected frequency was less than 5, Fisher's Exact or G-test (likelihood ratio) was performed. Statistical significance levels were considered as α = 5% for all calculations and interpretations.

Limitations of the Study

Because the nutritional habits, smoking and alcohol consumption, disease and treatment histories of the patients were recorded by the investigators with face to face interviews, the exaggerated or missing information may be given by the participants. Sixty three patients were included in the study during data collection process, but some elderly people in treatment group did not regularly consume enteral nutritional product or stopped consuming enteral nutritional product before the completion of the study. For this reason, 16 patients from the treatment group were excluded from the study. Younger patients were more aware and eager of using enteral nutrition support that resulted in a significantly lower mean age in the treatment group than the control group.

Results

The average age of the patients included in the study was 74.70 ± 10.31, the average duration of disease was found as 11.98 ± 1.08 years. The mean BMI of the patients was 17.18 ± 1.24 kg/m^2 which are below 18.5

kg/m^2 in accordance with the methodology of the study.

The 72.5% of the patients were hospitalized in clinic and the remainings (27.5%) were outpatient. Most of the patients (62.5 %) was retired because of their age thus 90% of patients had social security (social security institution, pension fund and bagkur) (p>0.05). The distribution of the demographic characteristics of the patients was shown in Table 1. According to the NRS-2002 score, the scores of all patients were above 3 which indicates malnutrition, and 50% of the patients stated that they lost weight in the last 3 months.

The history of smoking in 100%, alcohol consumption in 25% and other chronic diseases in 45% of the patients were recorded. According to the results questionnaires, 47.5% of the individuals skipped meals due to anorexia and slept late, and the skipped meal was lunch in 42.5% of the participants. It was determined that 32.5% of the subjects had no snack.

No significant differences were determined between control and treatment groups in regard to handgrip strength and pulmonary function test. There was no difference between individuals in the control group concerning the hand grip strength (p>0.05) whereas handgrip strength increased significantly (p<0.001) after the enteral nutrition support in COPD patients

Table 1. Distribution of some demographic characteristics of COPD patients

Variables	Treatment		Control		Total	
	n	%	n	%	n	%
Gender						
Male	20	100.0	19	95.0	39	97.5
Female	-	-	1	5.0	1	2.5
			-			
Age (year)						
55-64	5	25.0	4	20.0	9	22.5
65-74	6	30.0	4	20.0	10	25.0
>75	9	45.0	12	60.0	21	52.5
			χ^2=6.000; p=0.199			
Educational Background						
Illiterate	2	10.0	1	5.0	3	7.5
Primary school	12	60.0	17	85.0	29	72.5
Secondary school	2	10.0	1	5.0	3	7.5
High school/University	4	20.0	1	5.0	5	12.5
			χ^2=0.00092; p=0.222			
Monthly Income Level						
Lower than the minimum wage	13	65.0	16	80.0	29	72.5
Higher than the minimum wage	7	35.0	4	20.0	11	27.5
			χ^2=1.129; p=0.288			
Smoking						
Yes	0	0.0	1	5.0	1	2.5
Stopped	20	100.0	19	95.0	39	97.5
			-			

*Pearson Chi-square; p<0.05; ** Fisher's exact Chi-square; p<0.05

with low physical activity levels in treatment group (Table 2).

At the end of study, increases in FEV1 values were observed in control and treatment groups but the increase in FEV1 was significant ($p<0.05$) solely in patients supported with enteral nutrition support. There were slight but not significant ($p>0.05$) decreases in FEV1/FVC values in both groups at the end of the study compare to the initial values (Table 3).

Discussion

Malnutrition is frequently observed in patients with COPD. Overall, 10-45% of patients with COPD are malnourished, and it is evident that malnutrition and under-nutrition are important prognostic factors for COPD patients (4). The main reason for malnutrition is inability to meet increased energy requirements, depending on the hypermetabolism and increased breathing, with deteriorating food intake resulting from breathing difficulties (1,4,6,7).

Table 2. Effects of enteral nutrition support on hand grip strength of COPD patients

	Treatment	**Control**	***p*[a]**
	$\bar{X} \pm Sx$ **(Lower-upper limit)**	$\bar{X} \pm Sx$ **(Lower-upper limit)**	
Hand grip strength (kg) before treatment	26.09±7.67 (16.00-48.75)	25.70±6.36 (9.00-35.00)	0.863
Hand grip strength (kg) after treatment	26.80±7.51 (17.50-49.00)	25.56±5.73 (11.00-34.50)	0.561
p[b]	**0.000****	0.474	

** p<0.05; **Paired t-test; p<0.05; p[a]: Comparison of experimental and control groups; p[b]: The comparison of measurements performed before and after treatment*

Table 3. Effects of Enteral Nutrition Support on Pulmonary Function of COPD Patients

Variables	**Treatment**	**Control**	***p*[a]**
	$\bar{X} \pm Sx$ **(Lower-upper limit)**	$\bar{X} \pm Sx$ **(Lower-upper limit)**	
FEV1 (L/sn)			
Before treatment	39.60±22.53 (16.00-94.00)	44.30±17.92 (18.00-89.00)	0.470
After treatment	43.35±18.94 (19.00-95.00)	46.60±11.59 (22.00-63.00)	0.517
p[b]	**0.034****	0.472	
FEV1/FVC			
Before treatment	80.05±29.45 (43.00-136.00)	82.45±27.54 (53.00-136.00)	0.792
After treatment	73.65±21.41 (40.00-112.00)	75.85±19.58 (48.00-128.00)	0.736
p[b]	0.066	0.120	

** p<0.05; **Paired t-test; p<0.05; p[a]: Comparison of experimental and control groups; p[b]: The comparison of measurements performed before and after treatment*

COPD is generally seen in smokers, males, and elderly with low socioeconomic status (1, 9). In our study, 97.5% of the patients were male and the majority of the patients (77.5%) were over 75 years old. In the presented study, the mean age of the patients participating in the study were found as 74.70 ± 10.31 which is similar to results of the studies conducted in Turkey and indicating the COPD, a disease of advanced age, is seen more frequently in patients aged over 60 years (15,16).

The prevalence of COPD is higher in men than women due to higher smoking rate and more professional exposure. Thus the majority of COPD patients admitted to hospital were male which can be considered as the reason for higher proportion (97.5%) of men participated in the presented study (Table 1). Smoking is known as the most important cause of COPD, the risk of development of COPD in smokers is around 40-70% which significantly increases with age (17). The lungs of smokers are exposed to more oxidative damage and blood antioxidant levels have been found lower than non-smokers. The oxidant-antioxidant imbalance contributes to lung damage thus the risk of the development of COPD in smokers also increases. In studies related to the smoking history in patients with COPD, it was found that cigarette consumption increases the risk of COPD (18,19). In a study conducted on 276 patients with COPD in Japan, 75.3% of the patients had stopped smoking and 22.4% of them were still smoking (20). Similarly, all of the patients participated in the presented study used to smoke and only one patient was still smoking tobacco in control group.

Low level of education is one of the risk factors affecting the prevalence of COPD. In a study comparing the control group (without COPD) to patients with COPD, low education level was observed in patients with COPD (21). Similar results were found in our study indicating the vast majority of the COPD patients were illiterate, literate or primary school graduates (Table 1).

Low socioeconomic status is another risk factor affecting the prevalence of COPD. Intrauterine growth failure, poor living conditions, malnutrition, childhood respiratory infections and exposure to cigarette smoke have been reported to be associated with the development of COPD (22,23). In the present study, the majority of patients was on low-income and retired (Table 1) which is consistent with the study of Yılmaz (24) who reported that 100% of the COPD patients had lower income compare to their expenses.

Other diseases are commonly seen in patients with COPD and these diseases also have significant impacts on the prognosis of COPD. Some of the diseases evolve independently from COPD whereas some others have causal relationship with COPD (25). Comorbidity defines one or more concomitant diseases that are directly related to or not related to COPD. In all over the world, it is well known that at least two of the most common chronic diseases including COPD are present in 25% of the individuals aged over 65 years, and 10% of the elderly suffer from three or more concomitant diseases. Similarly, in this study, other diseases apart from COPD were determined in 45.0% of patients. Comorbid diseases commonly encountered in patients participating in the presented study were cardiovascular diseases, hypertension and benign prostatic hyperplasia (BPH).

In this study, BMI of the patients was lower than 18.5 kg/m^2 in accordance with the study design. Decreased body weight has been identified as a poor-prognostic factor in patients with COPD and the survival time is reported to be only 2 to 4 years in patients with severe disease who are lean and have a forced expiratory volume percentage in one second (FEV1%) of less than 50% (19). It has also been reported that COPD patients with a body mass index (BMI) of <20 kg/m^2 have a higher risk of acute exacerbations as compared to COPD patients with a BMI of 20 kg/m^2 or greater, and that patients exhibiting weight loss during a 1-year observation period are more prone to acute exacerbations than those who do not exhibit weight loss over the same period (26). Furthermore, body weight has been demonstrated to be positively correlated with the forced expiratory volume in one second (FEV1), exercise tolerance and diffusing capacity of the lung, even in patients with stable-phase COPD (5). Providing the daily energy requirements in COPD is of importance because insufficient energy intake causes destruction in muscle protein, weight loss and cachexia.

In our study, no significant difference was determined between COPD patients in control and

receiving enteral nutrition support considering the FEV1 measurements in both sampling times ($p>0.05$). However, a significant increase ($p<0.05$) was observed in FEV1 values after the enteral nutrition support whereas no statistically significant difference was detected in FEV1 / FVC (Table 3). It is still unclear that whether body weight loss is one of the causes of the severe deterioration in lung function or in severe lung disease. However underweight COPD patients more likely have tendency towards low diffusion capacity, air trapping and severe airway obstruction than overweight COPD patients (1,5). In a study performed on 79 patients with COPD, positive correlations have been reported between FEV1 and BMI and between lean body mass index (FFMI) and FEV1 (27). Lazarus et al. (28), suggested a positive relationship between lean body mass and lung function. In a study conducted on 32 patients with COPD who were obese and overweight, exercise capacity were significantly higher compared to patients with normal lean tissue mass and lower BMI (29).

Hand grip strength is considered as an objective measurement for evaluating the performance of the upper extremity. Hand grip strength is not only associated with upper limb muscle strength but also it is associated with overall body and pulmonary muscle strength. The reason for the decrease in lean body mass in patients with COPD is the enhanced muscle proteolysis. Glucocorticoids play a role in muscle proteolysis. Glucocorticoids, in one hand, enhance proteolysis and on the other hand, activate amino acid mobilization for gluconeogenesis by inhibiting transport of amino acids to the muscle and protein synthesis (30). In a study investigating the hand grip strength in patients with COPD, it has been claimed that hand grip strength of patients were significantly lower compared to a control group composed of healthy individuals and also associated with the deterioration of cardiac function in patients with COPD (31). In contrast, Heijdra et al. (30) who compared the muscle strength of COPD patients with healthy subjects, have determined that the patients participated in their study had normal lean body mass. These authors reported no significant difference between patients and healthy individuals concerning the grip strength and suggested that COPD patients with normal lean body mass index had not too severe peripheral muscle dysfunction. In the presented study, similar to the findings of Heijdra et al. (30) no significant difference was determined between control and treatment groups concerning the hand grip strength ($p>0.05$). The lack of difference in hand grip strength in this study may be due to presence of randomly selected COPD patients in control and treatment groups. However, enteral nutrition support significantly increased ($p<0.05$) hand grip strength of the patients whereas no significant difference ($p>0.05$) was observed between the hand grip strength measurements in the beginning and at the end of the study in patients who did not receive nutrition support (Table 2).

The results of this study have shown that enteral nutrition support in malnourished COPD patients increases FEV1 without any effect on FEV1/FVC ratio. Enteral nutrition support contributes to the treatment of disease by meeting the increasing energy demand as well as providing protein support. In some studies (6,7,12,13) functional recovery has been achieved with the increases in energy intake in the patients having enteral nutrition support. However, double-blind, controlled, long-term studies are needed in cachectic COPD patients receiving enteral nutrition support.

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Original article

Cell culture developing and the imaging of total protein product changing with SDS-PAGE in *Saccharomyces cerevisiae*

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Summary. Five groups were created in this work. i: Control group, ii: H_2O_2 group, iii: H_2O_2 + %10 Mulberry Juice (MBJ) group, iv: H_2O_2 + %15 MBJ, v: H_2O_2 + %25 MBJ group. After sterilization, H_2O_2 and fruit juice were inserted different concentration to *Saccharomyces cerevisiae* (*S. cerevisiae*) cultures and the cultures were developed at 30°C for 1h, 3h, 5h and 24 hours (overnight). *S. cerevisiae* cell growth was computed by spectrophotometer, total protein alteration was analysed by SDS-PAGE electrophoresis and reckoned with bradford method. our studies results indicated that; cell developing increased in MBJ groups in proportion to the positive control (H_2O_2) group at different growing times (1, 3, 5 and 24 hours) ($p<0,05$). As a result MBJ has a preservative role for reduce the oxidative damage and expanded cell developing and encourage protein synthesis in *S. cerevisiae*.

Key words: *S. cerevisiae*, mulberry juice, hidrogen peroxide, protein expression, SDS-PAGE

Introduction

Saccharomyces cerevisiae (*S. cerevisiae*) is important yeast and it has been employed recent researchs (1). The uptake of H_2O_2 by *S. cerevisiae* is to change the production of total protein and fatty acid in plasma membrane (1). ROS can oxidize protein, nucleic acid, fat and carbohydrates. for example, the oxidative abuse to proteins bring about to collapse of amino acid shacles diminishing the biologic activity (1, 3-5). Many works executed assert that unlike fruit content expands cellular growth in yeasts, supports protein expression and shows preservative properties towards oxidative stress (6-8). In reference to a study it has been detected that the intake of H_2O_2 at lower dose, lead to lethal stress in *S. cerevisiae* and bring on negative effect on the expression of significant proteins (1, 3-5). Native antimicrobials can be used with varied new conservation technologies to make easy the modification of conventional attitudes in food prevention (9). In the last years, new kind of fruit juice products, including pomegranate, strawberry, mulberry, grapefruit, lemon juice, etc. have be come very important for human health (10, 11). Fruit and vegetable juices are useful for the people live every time. Low sodium, cholesterol, fat; rich polyphenol, flavonoids and vitamin C acting essential roles in the salutary lives of people (12) in addition for example almond very distinguished for human health with regard to its protein and fatty acid contents (13, 14). Mulberry (MB) is one of the most consumed fruit in the world and it has a nice color, aroma and it leaves have been used as treatment of different illness. In addition, MB is also the source of quercetin, rutin, isoquercetin, and astragalin and significant phenolic compounds, this compounds has preservative effect against H_2O_2-induced oxidative damage, antidiabetic, anti-inflammatory activity and inhibit oxidative injury (15-18). In this work we studied the effect of MB on the rate of the cell developing, total protein expres-

sion and cell proliferation that the induced with H_2O_2 against to oxidative injury growing at 30˚C temperature of adding to MB in *S. cerevisiae* culture.

Material and Methods

Research groups and growth conditions

In this research five groups were composed. . i: Control group, ii: H_2O_2 group, iii: H_2O_2 + %10 Mulberry Juice (MBJ) group, iv: H_2O_2 + %15 MBJ, v: H_2O_2 + %25 MBJ group. After sterilization, H_2O_2 and fruit juice were inserted different concentration to *Saccharomyces cerevisiae* (*S. cerevisiae*) cultures and the cultures were developed at 30˚C for 1h, 3h, 5h and 24 hours (overnight). *S. cerevisiae* cell proliferation was calculated by spectrophotometer, total protein expression was indicated by SDS-PAGE electrophoresis and reckoned with bradford method for the developed and reproduce of yeast, YEPD (for 50 mL 1,5 g yeast extract, 1 g trypton, 1,5 g glucose) in addition, for the developing and reproduce of *S. cerevisiae*, mulberry fruit juices was added and cultivated. After sterilization, yeasts were cultured into media and the samples were incubated for 1h, 3h, 5h, 24 h (overnight, h: hour) at 30˚C (7).

Mulberry juice extract and H_2O_2 Chemical

Fruit (From center county of Elazığ city) was squashed in water and added in to *S. cerevisiae* media cultures and added 20% (v/v) ratio in at the reproducing for 30˚C. H_2O_2 was inserted in H_2O_2 and MBJ + H_2O_2 groups.

Cell concentration measurements

In these measurements, culture samples that were examined at 30˚C for 1, 3, 5 hours and overnight (24 hours) have been analyzed. The calculation has been accomplished using a spectrophotometer at 600 nm (OD_{600}).

SDS-PAGE (Sodium dodecyl sulfate polyacrylamide gel electrophoresis)

SDS-PAGE was made using BIO-RAD Mini-PROTEAN® 3 Cell gel electrophoresis system. The samples of *S. cerevisiae* cultures were organized for SDS-PAGE after which they were loaded to sample loading wells to be subject to electrical current and after this process, their images were taken and the intergroup protein bandings were used as data in the study (19).

Protein density measurements

The measurement has been accomplished using a spectrophotometer at 600 nm (OD_{600}) with regard to bradford method. BSA (bovine serum albumin) protein standards at different concentrations were obtained using BSA protein. Accordingly, the total protein amount in *S. cerevisiae* groups corresponding to this standard valuation was calculated (Figures 3, 4).

Statistical Analysis

SPSS 20.0 software was used. The comparison between experimental groups and the control group was made using one way ANOVA and Post Hoc Duncan and Games howell tests. Statistically important differentiation among groups have been stated as $p<0.05$ and the statistically non-significant differences have been specified as $p>0.05$. Standard deviations were point out as ±.

Results and Discussion

We think that the results of this study will provide important contributions to the present literature. The results of table 1 and figure 1 show that mulberry has essential effects on *S. cerevisiae* proliferation. It is indicated that mulberry juice (MBJ) maintains its live cell amount in spite of the growing hydrogen peroxide densities. A dissimilarity is detected between the yeast proliferation amounts for 1h in comparison with the control ($p<0.05$). It is observed that MBJ preserves the cell almost as much as the control opposite hydrogen peroxide which is the great radical origin in the 25% MBJ + H_2O_2 group and 15% MBJ + H_2O_2 group. When 3h values are investigated; it is obtained that MBJ has increased yeast development in the 25% MBJ + H_2O_2 group, in spite of the inverse effects of the hydrogen peroxide radical comparatively the control and H_2O_2 group ($p<0.05$). When the 5h values are investigated; it is obtained that MBJ has rised yeast improving at a maximum level in the 10% MBJ + H_2O_2 group despite the inverse effects of the hydrogen peroxide radical

Table 1. *Saccharomyces cerevisiae* cell growth in mulberry juices

OD_{600} 30°C	1h	3h	5h	Overnight
Control	1,488±0,00[b]	1,395±0,00[c]	1,755±0,00[c]	2,096±0,00[c]
H_2O_2	1,510±0,00[c]	1,413±0,00[d]	1,776±0,00[d]	1,876±0,00[a]
H_2O_2 + 10% mulberry	1,073±0,00[a]	1,286±0,00[a]	1,774±0,00[d]	2,092±0,00[c]
H_2O_2 + 15% mulberry	1,577±0,00[d]	1,364±0,00[b]	1,556±0,00[a]	2,102±0,00[d]
H_2O_2 + 25% mulberry	1,605±0,00[e]	1,464±0,00[e]	1,741±0,00[b]	2,071±0,00[b]

***a,b,c,d,e; among the groups which bearing of different letter are significant (p<0.05). one way ANOVA and Post Hoc Duncan and Games howell tests*

comparatively the control (p<0.05). When the overnight (24 h) values are investigated; it is obtained that MBJ has rised yeast growth in the 10% MBJ + H_2O_2, 15% MBJ + H_2O_2 and 25% MBJ + H_2O_2 groups, in spite of the opposite effects of the hydrogen peroxide radical in comparison with the control and H_2O_2 group; besides it can also be obtained that yeast growth has rised at a statistically significant degree in all other groups comparatively the control and H_2O_2 groups (p<0.05) (Table 1). Stinco et al (2015) indicated that orange juice activates the antioxidant defensive system towards free radicals for yeast development (20). Aslan et al (2014a) have indicated that pomegranate juice is protective against oxidative injury in *S. cerevisiae* (1). Again Aslan (2015) indicated that as a result of the work performed with several fruit juices and their mixtures that different fruit juices and their mixtures are preservative against oxidative injury in *S. cerevisiae* and that they rise yeast development (10). Tserennadmid et al (2011) have stated that apple juice has a protective role for growing in yeasts (21). Krivoruchko and Nielsen (2015) have stated that resveratrol and flavonoids act protective roles towards oxidative injury in bacteria and yeasts (22). Zhang et al (2017) showed that the mulberry extract has protective effect in human cell culture against oxidative stress (17). Rynko et al (2016) indicated that the leave of mulberry has antidiabetic activity, anticancer activity, antibacterial activity (15). Riche et al (2017) demonsrated that mulberry leaf extract decrease the human blood glucose level (18). Gregorio et al (2011) have stated that mulberry extract has antioxidant activity in *S. cerevisiae* (23). Chen et (2015) have put forth that mulberry fruit has antioxidant and hyperglycemic activity in vitro (16). When the SDS-PAGE results are investigated; it is obtained that protein band intensity rise in pellet gel images is greater in groups to which MBJ is applied

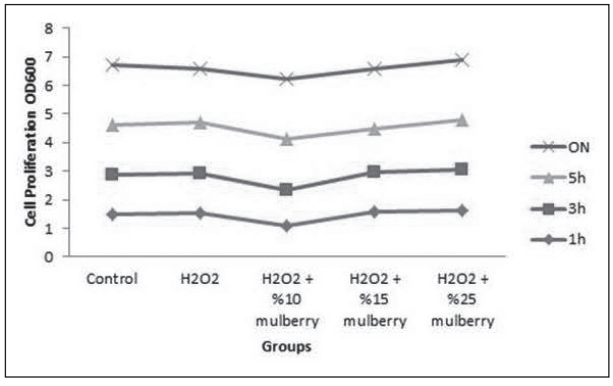

Figure 1. The growing of *Saccharomyces cerevisiae* in mulberry juices at different hours.

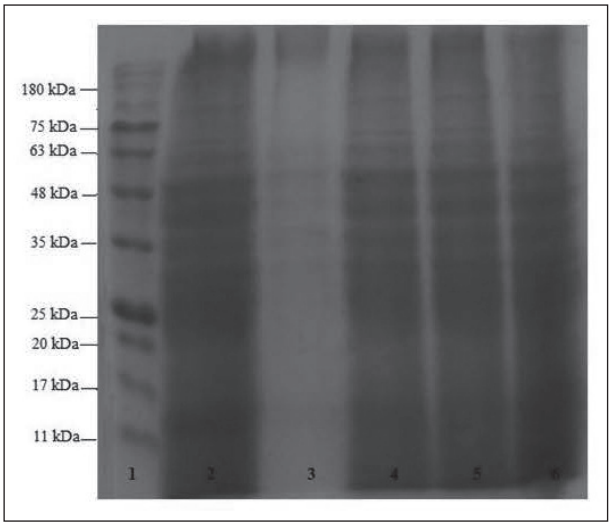

Figure 2. SDS-PAGE pelet total protein bands profiles for development at 30°C. Lanes 1: Marker; 2: Control; 3: H_2O_2; 4: H_2O_2 +10% MBJ; 5: H_2O_2 +15% MBJ; 6: H_2O_2 +25% MBJ

in comparison with the control (Figure 2). Aslan et al (2014b) have stated that pomegranate juice has a preservative effect in *S. cerevisiae* towards oxidative injury reasened by the applying of hydrogen peroxide and that protein band intensity rise is bigger in pomegranate applied groups comparatively hydrogen peroxide applied groups (3). When the bradford results in Figure 3 and figure 4 are analyzed; large protein quantity has been calculated in MBJ (H_2O_2+10% MBJ, H_2O_2+15% MBJ, H_2O_2+25% MBJ) applied groups comparatively to control and H_2O_2 groups (Figure 3,4). However, there are a lotof research in vivo on rat about fruit and vegetable mechanism. For example these, Aslan et al (2014c) and Aslan et al (2016a) have indicated that the milk thistle extract is preservative towards lung damage in rats (24, 25), Aslan and Can (2017a) have stated that lemon juice has a protective effect for diminish the oxidative injury, increased cell growing and protein synthesis in *S. cerevisiae* culture (26), Aslan et al (2016b) demonsrated that black cumin extract may be a drug for lung damage in rats (27), Aslan et al (2015) indicated that Nigella sativa extracts has a preservative effects against to rats lung damage (28), Ozsahin et al (2009) expressed that different sugar extracts induce fatty acid biosynthesis in the *S. cerevisiae* cell culture (29), Aslan et al (2017b) indicated that kiwi fruit juice has a protective effect against to hydrogen peroxide damage in *S. cerevisiae* (30).. With respect to these results, MBJ has a positive effect on *S. cerevisiae* cell proliferation and decreased the oxidative injury effect.

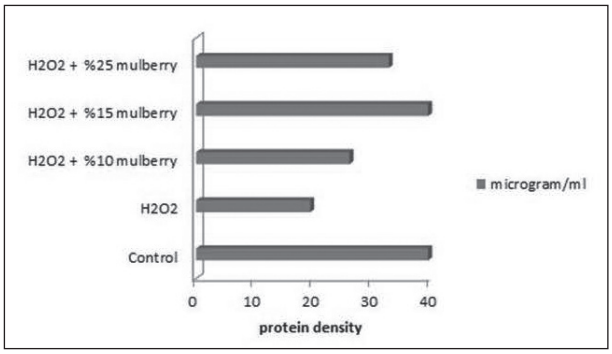

Figure 3. Protein density at between groups

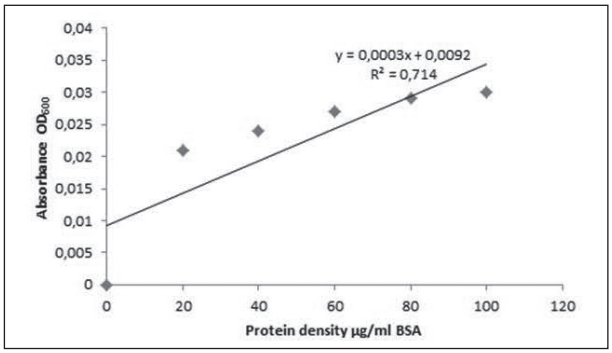

Figure 4. Bradford BSA (bovine serum albumin) standart graph

Conclusion

When these results are evaluated; we can said that MBJ is quite effective towards the hydrogen peroxide induced oxidative injury in *S. cerevisiae*, that it safeguards cell improve and even rises cell thrive; thus supporting protein expression in yeast cells. With respect to these findings, we expect that our study will support other studies to try MBJ in animal experiments and that in this respect MBJ will be digested more by people based on the positive results that will be gained.

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Original article

Postharvest preservation of citrus fruits (Kinnow) by gamma irradiation and its impact on physicochemical characteristics

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Summary. Citrus fruit (Kinnow) has several beneficial health and nutritive properties. Several techniques have been used to preserve postharvest quality of citrus fruits. Exposing foods to gamma radiation delays spoilage and increases protection by eliminating or lowering pathogenic bacteria. In the present study Kinnow fruit were exposed to radiation dose of 0.0, 0.25, 0.50, 0.75, 1.0, 1.5 and 2.0 kGy. Physicochemical and microbial analysis was performed on control and irradiated samples stored at both ambient and refrigerated temperature on weekly intervals. No significant change was observed in physicochemical properties of Kinnow at optimum dose, epiphytic microbial flora reduced in irradiated samples than control samples. The radiation dose of 1.5 kGy along with refrigerated storage extended the shelf-life of Kinnow for 1week without affecting sensory and physicochemical properties.

Key words: kinnow, physicochemical properties, nutritional quality

Introduction

Citrus fruits are among the highly consumed fruits not only for their sweet and energizing properties but also for their nutritional and medicinal importance. Citrus juices and citrus fruits have several nutritive properties which are very beneficial for health (1). Globally Pakistan stands in top 10 citrus producing countries (2). However, more than 95% of citrus fruit are produced in province Punjab and almost 70% of them are Kinnow (3). Citrus business of Pakistan is dominated by Kinnow (4) which is a prominent variety and contributes a lot more than 70% of citrus production in the country (5). Citrus species are vulnerable to several diseases caused by different kinds of pathogens like bacteria, fungi, viroids, viruses, nematodes, spiroplasmas and phytoplasmas (6). Postharvest decay is commonly the main issue limiting extended storage (7) and specifically fungal diseases are significant reason for decay and losses in harvested fresh fruit. Significant postharvest fungal diseases of citrus are blue molds, green molds, grey mold, sour rot, brown rot and *Alternaria* rot (8). Food and Agriculture organization of United Nations determined that 25% of all food product are wasted after harvest worldwide (9). The degree of post-harvest losses in vegetables and fruits vary from 35 -45% in Islamic Republic of Pakistan (10). Post-harvest treatment by using artificial chemicals is comparatively cheap method, it's simple to use and have therapeutic action against pre-existent or ongoing infections (11). However use of these chemicals typically leave chemical residue on fruit skin that can affect the health of human (12). There is a strong and increasing requisite to search and implement control techniques alternative to ancient antifungal agents for the control of post-harvest losses due to blue and green molds of citrus fruits. On the basis of their nature, three different decay management strategies are often used chemical, biological and physical. The major advantage of using physical treatments against fungus control is the complete absence of any deposit on treated products as well as insignificant impacts on environment

(13). Radiation has been utilized as a treatment to prevent fungal decay of fresh fruits (14). Gamma rays are electromagnetic waves that have high penetrating power they pass through materials without leaving any residue, an advantage comparing to other disinfection treatment. The impact of gamma rays on the activity of microbes has attracted considerable deal of attention (15). Joint expert committee on food irradiation after the assessment of nutritional, physical, chemical and toxicological characteristics of foods confirmed that food irradiated up to 10kGy is safe and nutritionally acceptable (16). Under smart working practices and ensuring correct handling of the products, irradiation eradicates harmful microorganisms that may cause fatal illness and food decay (17).

Materials and methods

Collection preparation and gamma irradiation of samples

Kinnows of nearly uniform size and shape were collected from local market of Lahore. The selected samples were divided in two categories control and experimental. The fruit samples were weighed and packed in labeled polythene bags. The experimental group was subjected to different gamma radiation doses at Pakistan Radiation Services (PARAS) Lahore, Pakistan using Cobalt-60 as the source at the dose rate of 60 Gy/hr. Harwell Amber 3042 dosimeter was used for dose measurement. After irradiation first day packet was examined and the remaining packs were put on storage at ambient and refrigerated temperature. Periodic evaluations were carried out on day 1, 7, 14, 21 and 28. Controls were also run parallel.

Sensory analysis

Color and texture of both control and irradiated of fruits were determined visually (18).

Determination of Microbial flora: For the determination of microbial flora serial dilution method was used. Each fruit sample was washed with 100 ml of 0.9% sterilized saline water. Different dilution (10^{4}–10^{7}) were made from this stock. 100µl of each dilution was transferred to different petriplates containing Nutrient agar (for non-fastidious bacterial isolation), MaCconkey agar (for gram negative bacterial isolation), Salmonella Shigella agar (for *Salmonella* and *Shigella* sp. isolation) and Potato dextrose agar (for fungi). Plates were then incubated at 37°C for 24 hour and at 30°C for 3-4 days for bacterial and fungal growth respectively (19). Total viable count, coliform count and fungal count was determined according to following formula (20).

$$\text{Colony forming unit / gram} = \frac{\text{No. of colonies}}{\text{Dilution factor} \times \text{amount plated}}$$

Colony morphology characteristics i.e. color, shape, texture, elevation, margins and optical characteristics (opaque/translucent) was noted. Gram and endospore staining, as well as motility and catalase test were also performed. The API-20E test kit was used for the identification of enteric bacteria. Fungal species were identified on the basis of micro and macroscopic characteristics (21).

Physicochemical analysis

Physiological loss in weight

Fruits were weighed periodically and percentage weight loss was calculated (22)

$$\text{Percentage physiological loss in weight} = \frac{Wi - Ws}{Wi} \times 100$$

Where Wi=Initial weight; Ws = Weight at sampling period

Moisture and Ash content: Moisture and ash content of both control and irradiated samples were estimated (23).

Percentage juice, Total soluble solid, Titratable acidity and Ascorbic acid content

Juice content: Fruits were cut into equal half and squeezed to extract all the juice by using manual juice presser. The extracted juice was filtered through strainer. The percentage juice content was calculated (24)

$$\text{Juice percentage} = \frac{\text{Total weight of juice (g) - Beaker weight (g)}}{\text{Total weight of fruit}} \times 100$$

Total soluble solid content

Total soluble solid was calculated by using Digital Brix Refractometer (Model: Atago PAL-3). Small amount of juice was placed on lens of refractometer and reading was noted (24).

Titratable acidity

Equal volume of juice and distilled water was taken. 2 - 3 drops of 1% phenolphthalein were added to observe the end point as indicated by change in color. The sample was titrated by using 0.1 N sodium hydroxide solution. Results were recoded as percent citric acid (24).

$$\text{Acidity \%} = \frac{\text{NaOH used} \times 0.0064}{\text{Volume or weight of sample used}} \times 100$$

Ascorbic acid (Vitamin C)

10 ml of freshly squeezed juice was poured in 250 ml measuring flask, 0.4% oxalic acid solution was added up to the mark. After filtration an aliquot about 5ml was taken in flask and titration was carried out using 2, 6-dichlorophenoindophenol dye until light pink color appeared which persisted for only few seconds (24).

Vitamin C content was calculated as:

$$\text{Vitamin C (mg/100ml juice)} = \frac{1 \times R_1 \times V}{R \times W \times V_1} \times 100$$

Where

R1 = dye used in titration of aliquot

R = dye used in titration of standard ascorbic acid solution

V1 = volume of juice used

V = volume of aliquot made by addition of 0.4% oxalic acid

W = volume of aliquot used for titration

Statistical analysis: The results obtained were analyzed by Costat version 6.4 using completely randomized block design and mean values were compared using Duncan's New Multiple Range test at $p \leq 0.05$ with five replicates. The mean square error of replicates from mean value was also calculated.

Results

Sensory evaluation: In the present study Kinnow fruit were irradiated at different doses (0.25-2.0 kGy). Radiations had no adverse effect on texture of Kinnow, except at higher doses (Table 1). No difference in color was observed between radiated and control fruits. Visual defects were only found in control and irradiated fruits at higher doses. No rind disorder was seen up to 1.5 kGy. Changes in color, texture and appearance was observed at 2.0 kGy. When irradiated at 2.0 kGy pitting was observed at the 14th day of storage. The firmness also reduced at 2.0kGy. In citrus fruits irradiation at higher doses causes peel injury the intensity of which is directly proportional to the radiation dose applied.

Microbial analysis: In present study Kinnow samples were exposed to gamma radiation doses of 0.25-2.0 kGy and effect of these radiation doses was evaluated on total viable, coliform and fungal count along with storage of refrigerated and ambient temperature (Table 2a and b, 3 and b, 4a and b, 5a and b). On refrigerated temperature microbial flora on Kinnow decreased at all applied doses. The control and irradiated samples decayed completely till 28th day except samples treated at 1.5 kGy. However, reduction in microflora was observed at 1.5 kGy. Microflora on fruit surface of untreated samples increased as the storage time increased when stored at both ambient and refrigerated temperature. Bacterial count was minimized in all irradiated samples as compared to control samples maximum reduction was observed at a radiation dose of 1.5 in Kinnow. In the current study irradiation also reduced total fungal count at all applied doses. The percentage mold development was less in irradiated samples of citrus fruits as compared to untreated samples in both samples kept at ambient and refrigerated temperature. Irradiation up to 2kGy could not completely inhibit the growth of yeast and mold during the entire storage period; however it was still lower than fungal count on control samples.

Analysis of Physicochemical properties

Impact of gamma radiations on physiological weight loss in Kinnow: Figure 1a and b depicted that weight of control and irradiated samples decreased as the storage time increased. Weight loss increased in irradiated samples than control samples. More weight loss was observed at higher doses.

Influence of gamma radiations on moisture content of Kinnow: In the present study effect of gamma radiations on the moisture contents of Kinnow (Fig. 2a and b) stored at refrigerated and ambient temperature was evaluated. The moisture content of control sample decreased as the storage time increased at

Table 1. Impact of gamma radiations on sensory properties of Kinnow

Radiation Doses (kGy)	**Storage period (Days)**				
	1	7	14	21	28
Texture					
Control	Smooth	Smooth	Smooth	Smooth	Decay
0.25	Smooth	Smooth	Smooth	Smooth	Decay
0.50	Smooth	Smooth	Smooth	Smooth	Decay
0.75	Smooth	Smooth	Smooth	Smooth	Decay
1.0	Smooth	Smooth	Smooth	Smooth	Decay
1.5	Smooth	Smooth	Smooth	Smooth	Smooth
2.0	Smooth	Rough	Rough	Rough	Decay
Color					
Control	Dark orange	No change	No change	No change	Decay
0.25	No change	No change	No change	No change	Decay
0.50	No change	No change	No change	No change	Decay
0.75	No change	No change	No change	No change	Decay
1.0	No change	No change	No change	No change	Decay
1.5	No change	No change	No change	No change	No change
2.0	No change	No change	No change	No change	Decay
Visual Defects					
Control	No defect	Light Scurfs	Light scurfs, shrunk	Dark scurfs, shrunk	Decay
0.25	No defect	Light silver scurfs	Light scurfs, shrunk	Dark scurfs, shrunk	Decay
0.50	No defect	Light silver scurfs	light scurfs, shrunk	Dark scurfs, shrunk	Decay
0.75	No defect	Light silver scurfs	Light scurfs, Shrunk	Light scurfs, shrunk	Decay
1.0	No defect	Light silver Scurfs	Light silver scurfs	Light scurfs, pitting	Decay
1.5	No defect	Light silver scurfs	Light silver scurfs	Light scurfs	Light silver scurfs
2.0	No defect	Light silver scurfs	Pitting, shrunk	Pitting, shrunk	Decay

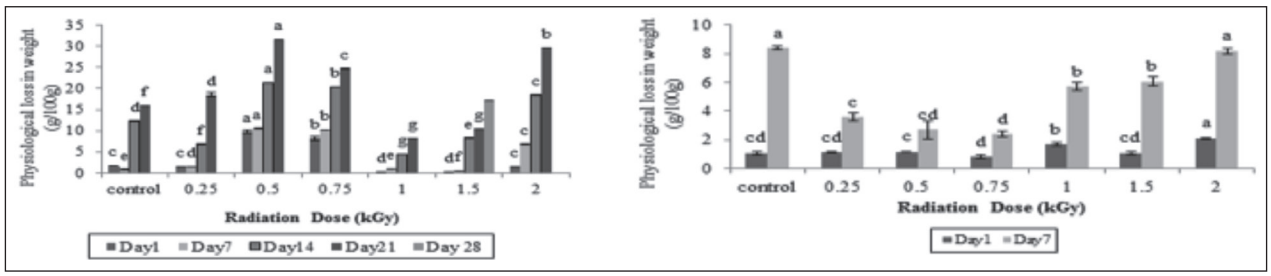

Figure 1. a) Effect of gamma radiations on physiological loss in weight of Kinnow stored at refrigerated temperature; b) Effect of gamma radiations on physiological loss in weight of Kinnow stored at ambient temperature

Table 2a. Effect of gamma radiations on total viable count of bacteria on Kinnow kept at refrigerated temperature using nutrient agar

Radiation Doses (kGy)	**Nutrient agar (cfu/ml)**				
	Storage Period (Days)				
	1	7	14	21	28
Control	$2.75\times10^{5}\pm0.022^{a}$	$2.82\times10^{5}\pm0.016^{a}$	$2.90\times10^{5}\pm0.024^{b}$	$2.96\times10^{5}\pm0.030^{c}$	Decayed
0.25	$2.63\times10^{5}\pm0.016^{b}$	$2.60\times10^{5}\pm0.018^{b}$	$1.59\times10^{5}\pm0.022^{d}$	$1.20\times10^{5}\pm0.025^{d}$	Decayed
0.50	$2.41\times10^{5}\pm0.016^{c}$	$2.39\times10^{5}\pm0.032^{c}$	$1.52\times10^{5}\pm0.030^{de}$	$1.12\times10^{5}\pm0.018^{d}$	Decayed
0.75	$2.04\times10^{5}\pm0.030^{d}$	$2.05\times10^{5}\pm0.028^{d}$	$1.98\times10^{5}\pm0.028^{c}$	$1.15\times10^{5}\pm0.024^{d}$	Decayed
1.0	$1.85\times10^{5}\pm0.020^{e}$	$1.97\times10^{5}\pm0.014^{e}$	$1.09\times10^{5}\pm0.023^{f}$	$1.05\times10^{5}\pm0.012^{d}$	Decayed
1.5	$1.65\times10^{5}\pm0.041^{f}$	$1.83\times10^{5}\pm0.018^{f}$	$1.43\times10^{5}\pm0.020^{e}$	$9.80\times10^{4}\pm0.282^{a}$	$1.60\times10^{4}\pm0.203$
2.0	$1.3\times10^{5}\pm0.023^{g}$	$1.62\times10^{5}\pm0.015^{g}$	$7.20\times10^{4}\pm0.089^{a}$	$6.3\times10^{4}\pm0.189^{b}$	Decayed

Each value is the mean obtained from five parallel replicates. ± indicates standard error among the replicates.
Different superscript in the same column indicates that the mean difference is significant at p≤0.05.

Table 2b. Effect of gamma radiations on total viable count of bacteria on Kinnow kept at ambient temperature using nutrient agar

Radiation Doses (kGy)	**Nutrient agar (cfu/ml)**				
	Storage Period (Days)				
	1	7	14	21	28
Control	$2.91\times10^{5}\pm0.069^{a}$	$3.00\times10^{5}\pm0.325^{a}$	Decayed	Decayed	Decayed
0.25	$2.68\times10^{5}\pm0.078^{b}$	$2.78\times10^{5}\pm0.056^{a}$	Decayed	Decayed	Decayed
0.50	$2.52\times10^{5}\pm0.051^{c}$	$2.38\times10^{5}\pm0.038^{b}$	Decayed	Decayed	Decayed
0.75	$2.48\times10^{5}\pm0.039^{c}$	$2.39\times10^{5}\pm0.052^{b}$	Decayed	Decayed	Decayed
1.0	$2.28\times10^{5}\pm0.032^{d}$	$1.88\times10^{5}\pm0.028^{c}$	Decayed	Decayed	Decayed
1.5	$1.96\times10^{5}\pm0.032^{c}$	$1.89\times10^{5}\pm0.031^{c}$	Decayed	Decayed	Decayed
2.0	$1.64\times10^{5}\pm0.028^{f}$	$1.59\times10^{5}\pm0.041^{c}$	Decayed	Decayed	Decayed

Each value is the mean obtained from five parallel replicates. ± indicates standard error among the replicates. Different superscripts indicate that the mean difference is significant at p≤0.05.

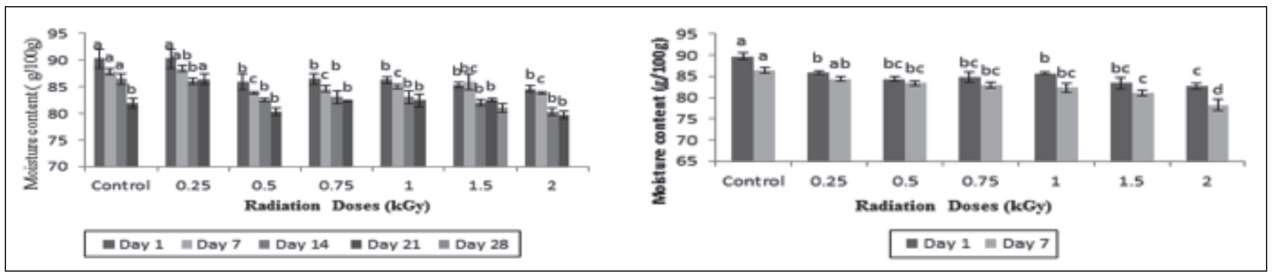

Figure 2. a) Impact of gamma radiations on moisture content of Kinnow stored at refrigerated temperature; b) Impact of gamma radiations on moisture content of Kinnow stored at ambient temperature

Table 3a. Effect of gamma radiations on total coliform count of Kinnow kept at refrigerated temperature using MacConkey agar

Radiation Doses (kGy)	MacConkey agar (cfu/ml)				
	Storage period (Days)				
	1	7	14	21	28
Control	$2.75\times10^{5}\pm0.022^{a}$	$2.59\times10^{5}\pm0.030^{a}$	$2.38\times10^{5}\pm0.034^{a}$	$2.37\times10^{5}\pm0.028^{a}$	Decayed
0.25	$2.37\times10^{5}\pm0.020^{b}$	$2.28\times10^{5}\pm0.022^{b}$	$1.58\times10^{5}\pm0.024^{b}$	$1.94\times10^{5}\pm0.020^{b}$	Decayed
0.50	$2.20\times10^{5}\pm0.015^{c}$	$1.32\times10^{5}\pm0.016^{d}$	$1.13\times10^{5}\pm0.016^{c}$	$1.39\times10^{5}\pm0.017^{c}$	Decayed
0.75	$1.75\times10^{5}\pm0.026^{d}$	$1.68\times10^{5}\pm0.034^{c}$	$1.09\times10^{5}\pm0.016^{c}$	$1.15\times10^{5}\pm0.015^{d}$	Decayed
1.0	$1.50\times10^{5}\pm0.023^{e}$	$9.90\times10^{4}\pm0.340^{e}$	$8.70\times10^{4}\pm0.357^{d}$	$3.90\times10^{4}\pm0.252^{e}$	Decayed
1.5	$1.50\times10^{5}\pm0.012^{e}$	$9.30\times10^{4}\pm0.291^{e}$	$6.40\times10^{4}\pm0.203^{e}$	$2.10\times10^{4}\pm0.089^{e}$	$9.00\times10^{3}\pm0.894$
2.0	$5.00\times10^{4}\pm0.172^{f}$	$4.00\times10^{4}\pm0.266^{f}$	$3.00\times10^{4}\pm0.152^{f}$	$3.50\times10^{4}\pm0.22^{ce}$	Decayed

Each value is the mean obtained from five parallel replicates. ± indicates standard error among the replicates. Different superscripts in the same column indicate that the mean difference is significant at p≤0.05.

Table 3b. Effect of gamma radiations on total coliform count of Kinnow kept at ambient temperature MacConkey agar

Radiation Doses (kGy)	MacConkey agar (cfu/ml)				
	Storage Period (Days)				
	1	7	14	21	28
Control	$2.68\times10^{5}\pm0.046^{a}$	$2.84\times10^{5}\pm0.038^{a}$	Decayed	Decayed	Decayed
0.25	$2.57\times10^{5}\pm0.045^{b}$	$2.60\times10^{5}\pm0.028^{b}$	Decayed	Decayed	Decayed
0.50	$2.43\times10^{5}\pm0.023^{c}$	$2.42\times10^{5}\pm0.037^{c}$	Decayed	Decayed	Decayed
0.75	$2.39\times10^{5}\pm0.022^{c}$	$2.21\times10^{5}\pm0.029^{d}$	Decayed	Decayed	Decayed
1.0	$2.08\times10^{5}\pm0.040^{d}$	$1.98\times10^{5}\pm0.049^{e}$	Decayed	Decayed	Decayed
1.5	$1.78\times10^{5}\pm0.017^{e}$	$1.61\times10^{5}\pm0.037^{f}$	Decayed	Decayed	Decayed
2.0	$1.59\times10^{5}\pm0.049^{f}$	$1.42\times10^{5}\pm0.018^{g}$	Decayed	Decayed	Decayed

Each value is the mean obtained from five parallel replicates. ± indicates standard error among the replicates. Different superscripts indicate that the mean difference is significant at p≤0.05.

both refrigerated and ambient temperature. Moisture content followed the same pattern in irradiated samples. The maximum reduction in moisture content was observed at dose 2.0 kGy. Highest value for moisture content was observed for control as compared to irradiated fruits kept at both ambient and refrigerated temperature. In irradiated samples kept at refrigerated temperature the lowest value of moisture contents obtained was79.67 g/100g at dose 2.0 kGy. Whereas in irradiated samples kept at room temperature the lowest value of moisture content obtained was 78.23 g/100g at 2.0 kGy. Irradiated samples were slightly lower in moisture content than control samples. However, decrease in moisture content was only significant at higher doses showing that by increasing the radiation dose moisture content decreases.

Influence of gamma radiations on ash content of Kinnow

Ash content of control samples of Kinnow was 0.64g/100g at first day of analysis. The ash content of control sample decreased as the storage time increased when kept at refrigerated and room temperature. Ash content followed the same pattern in irradiated sam-

Table 4a. Effect of gamma radiations on bacterial count of Kinnow kept at refrigerated temperature using Salmonella Shigella agar

Radiation Doses (kGy)	**Salmonella Shigella agar (cfu/ml)**				
	Storage period (Days)				
	1	7	14	21	28
Control	$6.9\times10^{4}\pm0.322^{a}$	$5.7\times10^{4}\pm0.200^{a}$	$3.4\times10^{4}\pm0.181^{a}$	$2.1\times10^{4}\pm0.152^{a}$	Decayed
0.25	$6.4\times10^{4}\pm0.141^{a}$	$4.3\times10^{4}\pm0.172^{b}$	$3.2\times10^{4}\pm0.241^{a}$	$2.0\times10^{4}\pm0.205^{a}$	Decayed
0.50	$5.3\times10^{4}\pm0.282^{b}$	$3.7\times10^{4}\pm0.144^{bc}$	$2.9\times10^{4}\pm0.228^{ab}$	$1.8\times10^{4}\pm0.164^{a}$	Decayed
0.75	$4.5\times10^{4}\pm0.278^{b}$	$3.1\times10^{4}\pm0.278^{cd}$	$2.5\times10^{4}\pm0.203^{bc}$	$1.3\times10^{4}\pm0.101^{b}$	Decayed
1.0	No Growth	$2.9\times10^{4}\pm0.200^{d}$	$2.0\times10^{4}\pm0.266^{c}$	$2.0\times10^{3}\pm0.282^{c}$	Decayed
1.5	No Growth	$2.0\times10^{4}\pm0.215^{e}$	$1.3\times10^{4}\pm0.101^{d}$	$1.2\times10^{4}\pm0.101^{b}$	No Growth
2.0	$2.2\times10^{4}\pm0.178^{c}$	$1.8\times10^{4}\pm0.200^{e}$	$1.0\times10^{4}\pm0.063^{d}$	$8.0\times10^{3}\pm0.894^{c}$	Decayed

Each value is the mean obtained from five parallel replicates. ± indicates standard error among the replicates. Different superscripts in the same column indicate that the mean difference is significant at p≤0.05.

Table 4a. Effect of gamma radiations on bacterial count of Kinnow kept at ambient temperature using Salmonella Shigella agar

Radiation Doses (kGy)	**Salmonella Shigella agar (cfu/ml)**				
	Storage Period (Days)				
	1	7	14	21	28
Control	$7.8\times10^{4}\pm0.382^{a}$	$7.9\times10^{4}\pm0.325^{a}$	Decayed	Decayed	Decayed
0.25	$6.7\times10^{4}\pm0.282^{b}$	$5.2\times10^{4}\pm0.272^{b}$	Decayed	Decayed	Decayed
0.50	$6.5\times10^{4}\pm0.272^{b}$	$4.9\times10^{4}\pm0.368^{b}$	Decayed	Decayed	Decayed
0.75	$5.3\times10^{4}\pm0.200^{c}$	$4.6\times10^{4}\pm0.340^{b}$	Decayed	Decayed	Decayed
1.0	$3.1\times10^{4}\pm0.342^{d}$	$2.8\times10^{4}\pm0.230^{c}$	Decayed	Decayed	Decayed
1.5	$2.6\times10^{4}\pm0.291^{d}$	$2.2\times10^{4}\pm0.170^{cd}$	Decayed	Decayed	Decayed
2.0	$2.4\times10^{4}\pm0.282^{d}$	$1.5\times10^{4}\pm0.202^{d}$	Decayed	Decayed	Decayed

Each value is the mean obtained from five parallel replicates. ± indicates standard error among the replicates. Different superscripts in the same column indicate that the mean difference is significant at p≤0.05.

ples. In irradiated samples the lowest value obtained was 0.39 g/100g at dose 2.0 kGy (Fig 3a) whereas at ambient temperature the lowest value obtained was 0.51 g/100g (Fig. 3b) irradiated at 2.0 kGy.

Impact of gamma radiations on juice content of Kinnow

The juice content of samples kept at ambient and refrigerated temperature was decreased as storage time and radiation dose increased. At day one the juice content of non-irradiated Kinnow was 26.70 g/100ml at refrigerated temperature whereas at the end of storage it was 21.23 g/100ml (Fig. 4a). For control samples stored at ambient temperature the juice content at day one was recorded to be 28.13 and 21.98 g/100ml at last (7th day) of storage period. In irradiated-refrigerated samples minimum amount of juice content on 28th day was 15.65 g/100ml at 2.0 kGy. Whereas at 7th day Kinnow irradiated and stored on ambient temperature had juice content of 18.31 g/ml at 2.0 kGy (Fig. 4b). Juice content in irradiated and control fruits decreased throughout the storage period at ambient and refrigerated temperature.

Table 5a. Effect of gamma radiations on fungal count of Kinnow kept at ambient temperature using potato dextrose agar

Radiation Doses (kGy)	**Potato Dextrose agar (cfu/ml)**				
	Storage period (Days)				
	1	7	14	21	28
Control	$1.9\times10^{4}\pm0.360^{a}$	$2.8\times10^{4}\pm0.170^{a}$	Decayed	Decayed	Decayed
0.25	$1.6\times10^{4}\pm0.130^{a}$	$2.6\times10^{4}\pm0.165^{ab}$	Decayed	Decayed	Decayed
0.50	$1.5\times10^{4}\pm0.228^{ab}$	$2.5\times10^{4}\pm0.223^{ab}$	Decayed	Decayed	Decayed
0.75	$1.3\times10^{4}\pm0.170^{abc}$	$2.5\times10^{4}\pm0.228^{ab}$	Decayed	Decayed	Decayed
1.0	$1.6\times10^{4}\pm0.212^{a}$	$2.1\times10^{4}\pm0.212^{b}$	Decayed	Decayed	Decayed
1.5	$9.0\times10^{3}\pm0.141^{bc}$	$1.1\times10^{4}\pm0.070^{c}$	Decayed	Decayed	Decayed
2.0	$8.0\times10^{3}\pm0.200^{c}$	$12.0\times10^{3}\pm0.013^{d}$	Decayed	Decayed	Decayed

Each value is the mean obtained from five parallel replicates. ± indicates standard error among the replicates. Different superscripts in the same column indicate that the mean difference is significant at p≤0.05.

Table 5a. Effect of gamma radiations on fungal count of Kinnow kept at refrigerated temperature using potato dextrose agar

Radiation Doses (kGy)	**Potato Dextrose agar (cfu/ml)**				
	Storage period (Days)				
	1	7	14	21	28
Control	$6.0\times10^{3}\pm0.894^{ab}$	$5.0\times10^{4}\pm1.264^{a}$	$3.1\times10^{4}\pm0.164^{ab}$	$5.3\times10^{4}\pm0.141^{a}$	Decayed
0.25	$5.0\times10^{3}\pm0.894^{bc}$	$2.4\times10^{4}\pm0.215^{b}$	$2.9\times10^{4}\pm0.322^{b}$	$3.8\times10^{4}\pm0.152^{b}$	Decayed
0.50	$2.0\times10^{3}\pm0.400^{c}$	$2.5\times10^{4}\pm0.203^{b}$	$3.0\times10^{4}\pm0.116^{ab}$	$3.2\times10^{4}\pm0.178^{b}$	Decayed
0.75	$4.0\times10^{3}\pm0.894^{bc}$	$2.0\times10^{4}\pm0.291^{b}$	$3.7\times10^{4}\pm0.189^{a}$	$3.8\times10^{4}\pm0.233^{b}$	Decayed
1.0	$7.0\times10^{3}\pm1.264^{ab}$	$1.8\times10^{4}\pm0.203^{b}$	$3.4\times10^{4}\pm0.268^{ab}$	$3.5\times10^{4}\pm0.291^{b}$	Decayed
1.5	No Growth	$2.7\times10^{4}\pm0.205^{b}$	$1.3\times10^{4}\pm0.101^{c}$	$2.1\times10^{4}\pm0.228^{c}$	$2.0\times10^{4}\pm0.266$
2.0	$9.0\times10^{3}\pm1.166^{a}$	$1.3\times10^{4}\pm0.089^{b}$	$1.8\times10^{4}\pm0.205^{c}$	$2.0\times10^{4}\pm0.189^{c}$	Decayed

Each value is the mean obtained from five parallel replicates. ± indicates standard error among the replicates. Different superscripts indicate that the mean difference is significant at p≤0.05.

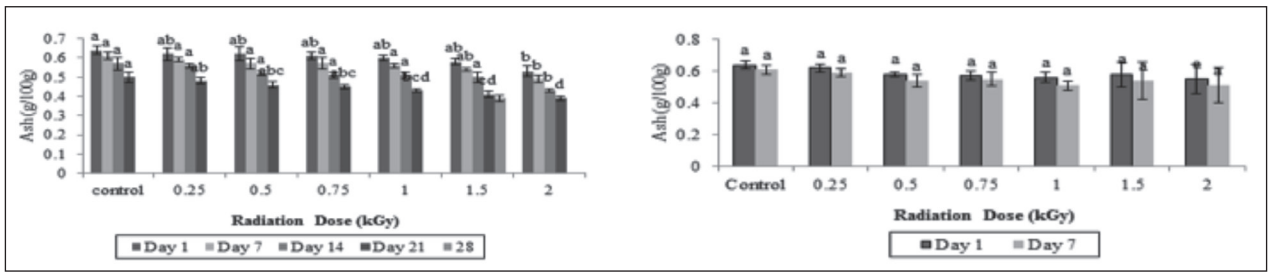

Figure 3. a) Influence of gamma radiations on ash content of Kinnow stored at refrigerated temperature; b) Influence of gamma radiations on ash content of Kinnow stored at ambient temperature

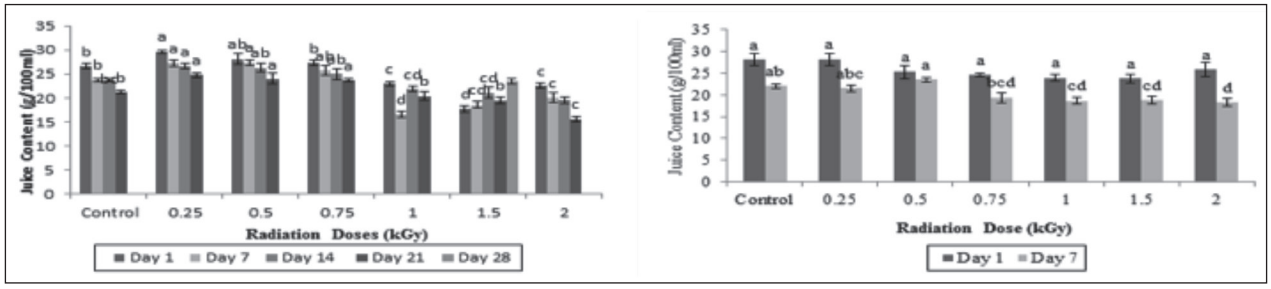

Figure 4. a) Effect of gamma radiations on juice content of Kinnow stored at refrigerated temperature; b) Effect of gamma radiations on juice content of Kinnow stored at ambient temperature

Influence of gamma radiations on total soluble solid (TSS) content of Kinnow

In the present study, effect of gamma radiations on total soluble solid of Kinnow stored at ambient and refrigerated temperature was evaluated (Fig 5a and b). As the storage period of control samples stored at ambient and refrigerator temperature was increased an increase in TSS was observed. Same pattern was followed in irradiated samples at all doses. At 28th day on refrigerated temperature TSS was 14.7Brix[a] for sample irradiate at 2.0 kGy. Whereas at ambient temperature the maximum value obtained was 13.2 Brix[a] for sample irradiated at 2.0 kGy.

Influence of gamma radiations on titratable acidity of Kinnow

As the storage period of control and irradiated samples kept at refrigerated and ambient temperature increased a decline in titratable acidity was observed (Fig 6a and b). At day one the titratable acidity of non-irradiated Kinnow was 0.42 g/100ml at refrigerated temperature whereas at 28th day it was 0.30 g/100ml. For control samples stored at ambient temperature the titratable acidity at day one was recorded to be 0.49 g/100ml and 0.39 g/100ml at the end of 28th day respectively. In irradiated refrigerated samples the lowest amount of titratable acidity at the end of storage was

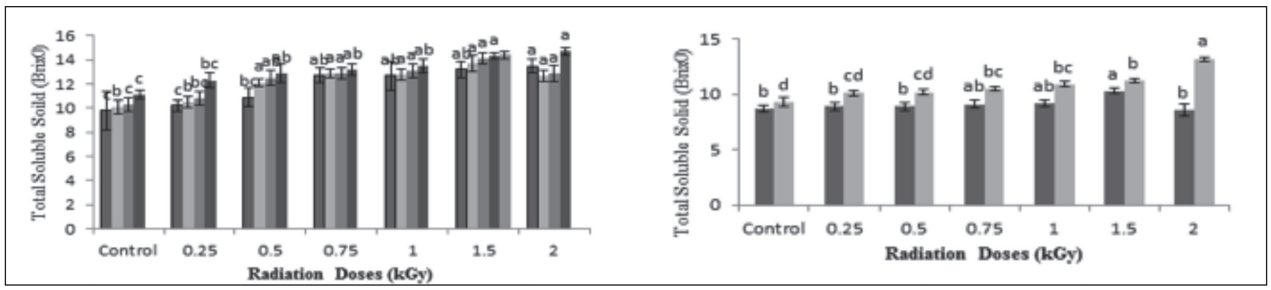

Figure 5. a) influence of gamma radiations on total soluble solid content of Kinnow stored at refrigerated temperature; b) Influence of gamma radiations on total soluble solid content of Kinnow stored at ambient temperature

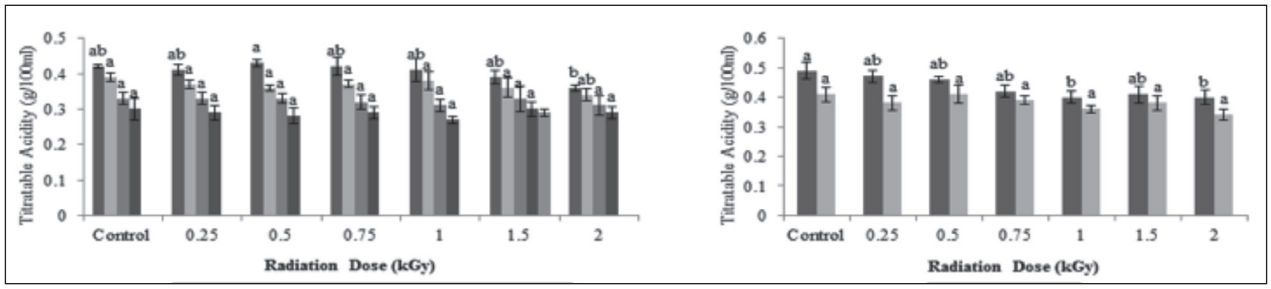

Figure 6. a) Effect of gamma radiations on titratable acidity of Kinnow stored at refrigerated temperature; b) Effect of gamma radiatens on titratable acidity of Kinnow stored at ambient temperature

0.29 g/100ml at 2.0 kGy. Whereas Kinnow irradiated and stored on ambient temperature had lowest titratable acidity of 0.34 g/100ml at 2.0 kGy.

Impact of gamma radiations on ascorbic acid of Kinnow

Decrease in ascorbic acid was observed as the storage time increased among untreated samples at both ambient and refrigerated temperature. Same pattern was followed in irradiated samples at all doses. However, maximum reduction was observed at higher doses. When stored under refrigerated temperature minimum ascorbic acid content was 26.8 mg/100ml at dose of 2.0 kGy (Fig.7 a). However, when stored at room temperature minimum ascorbic acid content was 33.9 mg/100ml at dose of 2.0kGy (Fig.7b).

Discussion

Exposing food to radiation treatment delays spoilage and enhance the safety of produce by eliminating or decreasing pathogenic microorganisms (25). In the present study Kinnows were irradiated at different doses (0.25-2.0 kGy). Radiations had no adverse effect on texture of Kinnow except at higher doses. Changes in color, texture and appearance was observed at 2.0 kGy. The change in color might be due to the decrease concentration of carotenoids in the peel (26). In citrus fruits irradiation at higher doses causes peel injury. This was probably due to the accumulation of substantial amounts of phenolic compounds in cells following irradiation which then lead to peel damage and cell necrosis (27). In this study no pre-treatment was given to fruits so the surface of untreated fruits was found to be highly contaminated with epiphytic microbial flora. This might be due to poor hygienic conditions in the local market and during transport, similar findings were observed by Gultie and Sahile (28). The predominant pathogens isolated from the surface of citrus fruits were *Staphylococcus aureus*, *Escherechia coli*, *pseudomonas aeroginosa* and *Shigella* sp. Presence of these coliforms on fruit surface indicated fecal contamination of fruit which can cause serious illness such as food poisoning and diarrhea so fresh fruit and vegetables can serve as a vehicle for the organism most likely to cause outbreaks. Gamma radiation worked remarkably to reduce the bacterial load. Bacterial count was minimized in all irradiated samples as compared to control samples and maximum reduction was observed at a radiation dose of 1.5 in Kinnow, perhaps it was due the reason that radiation breaks the bonds in the DNA molecules, of these microbes and cause defects in their genetic instructions or due to the biosynthesis of phenolic compounds following radiation treatment that extend the storage life of fruit and in some cases induce resistance against pathogens (29-30).

In the current study irradiation also reduced total fungal count at all applied doses. The percentage mold development was less in irradiated samples of citrus fruits as compared to untreated samples in both samples kept at ambient and refrigerated temperature. On the basis of macroscopic and microscopic analysis the dominant fungi identified on Kinnows samples were *Aspergillus flavus, Aspergillus niger, Alternaria Alternata, Penicillium*, *Fusarium, Mucor* and Yeast. Irradiation up to 2kGy could not completely inhibit the growth of yeast and mold during the entire storage period; however it was still lower than fungal count on control samples. It might be due to the reason that somewhat higher doses are required to have a fungicidal effect on these citrus damaging fungi (22-31).

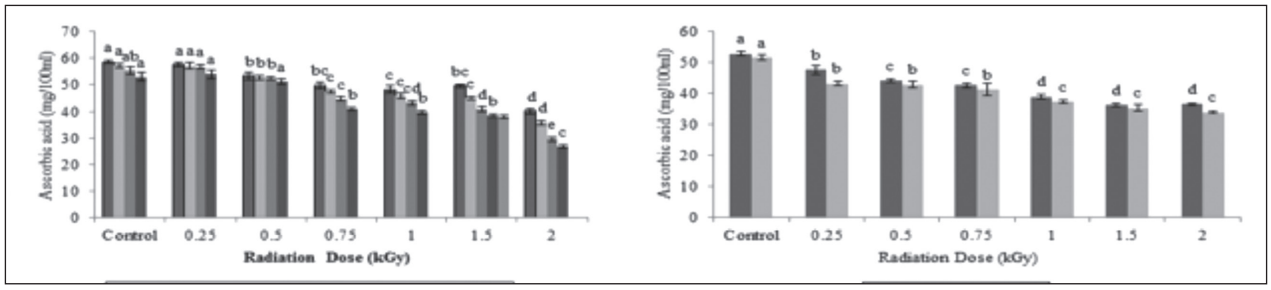

Figure 7. a) Effect of gamma radiations on scorbutic acid content of Kinnow at refrigerated temperature; b) Effect of gamma radiations on scorbutic acid content of minnow stored at ambient temperature

In the present study evaluation of physicochemical parameters of Kinnows stored at both ambient and refrigerated temperature showed that irradiation only at higher doses affect the nutritional aspects of fruit. Irradiation could not control the senescence of fruit, but along with refrigerated temperature, it caused the maximum retention of fruit quality attributes as it was also evident by the study made by Selles (32) and Srijaya*et al.* (22) who stated that irradiation at low doses along with refrigerated temperature did not cause any significant change in fruit quality characteristics. During the present work it was observed that weight of control and irradiated samples decreased as the storage time increased. More weight loss was observed in irradiated sample of Kinnow as compared to control samples with the passage of time. .Weight loss increased in irradiated samples than control samples of Kinnow. A more weight loss was observed at higher doses. Our result was in accordance to the result of Miller *et al.* (33). Decrease in fruit weight with the progression of storage period is also a natural phenomenon which might be due to transpiration occurring from fruit surface (34).

The moisture content of control and irradiated Kinnow was decreased as the storage time increased. Irradiated samples were slightly lower in moisture content than control samples. However, decrease in moisture content was only significant at higher doses showing that by increasing radiation dose moisture content decreases. These results were also similar to the results of Hajare *et al.* (35) who reported that increasing gamma radiation dose caused an increase in moisture loss of fruit. This was probably due to the ability of gamma radiation to target water molecules and breaking of hydrogen bonds, which ultimately lead to low moisture content in radiated fruits. Since high radiation dose caused increase moisture loss so the consequences were shrinkage and softening of the fruit at 2.0 kGy. The ash content is the amount of total mineral present in food. In this study the effect of gamma radiation on ash content showed that the ash content decreased following irradiation and ripening period of fruit at both refrigerated and ambient temperatures. Irradiated fruits had low ash content as compared to control. However, the effect was significant only at higher doses. The reason might be that the decrease was due the conversion of minerals into toxic substances with the time (36). Juice content in irradiated and control fruits decreased throughout the storage period at ambient and refrigerated temperature. This might be due to the reason that juice content increased towards maturity and then decreased once the maturity was over (18).

Total soluble solids content is an important parameter of fruit quality. In fruits total soluble salt consist of about 75-80% sucrose. In this study total soluble solids increased with the increase of storage period as well as radiation dose. Total soluble solids in irradiated samples were higher than control samples at both ambient and refrigerated temperature. Since, Total soluble solids consist of total dissolved solids and moisture content of the fruit, so the rise in TSS content may be due to low moisture content of the fruit (37).The effect of gamma radiations on Kinnow showed that increasing radiation dose caused decrease in titratable acidity of fruit at both ambient and refrigerated temperature. This reduction was also observed in control samples as the storage time increased. These observations were in line with observations made by Ahmad *et al.* (38) who stated decrease in titratable acidity of fruit occur with increasing radiation dose and storage period. The decrease in titratable acidity was might be due to the utilization of these constituent acids in the fruit respiratory process (34).Ascorbic acid content is an important constituent of citrus fruits. In the present study ascorbic acid content in Kinnow decreased with the increase in storage temperature and radiation dose. Same pattern was observed in control samples. These findings were similar to the findings made by Ahmad *et al.* (38) who reported reduction of ascorbic acid in oranges with the increase of storage temperature and radiation dose. The decrease in ascorbic acid content also might be due to the respiratory activity of fruit (39).

Conclusions

It was concluded that Kinnow irradiated at 1.5 kGy had no deleterious effects on quality and nutritional characteristics of fruit up to the storage period of 28 days along with refrigerated temperature. Gamma irradiation helped to improve the shelf life of Kinnow by 1week

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Original article

The effect of training provided for obese adolescents based on Health Promotion Model on their healthy lifestyle behaviors and life quality

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Summary. *Objectives:* The purpose of this study is to determine the effect of training provided for obese adolescents based on health promotion model on their healthy lifestyle behaviors and life quality. *Methods:* The research was conducted on the 114 obese adolescents in the form of intervention design with pretest- posttest control group between the dates September 2012 and January 2014. The experiment group was trained for three months within the Health Promotion Model which was constituted to decrease the risk factors of obesity. *Results:* With the training, monitoring and consultancy service provided for obese adolescents, it was found that BMI scores decreased significantly, their nutritional and physical activity habits were regulated, their sedentary life decreased, their general score averages for ALP and PedsQL scale were higher when compared with the pre-test and the difference between the experiment and control groups was found to be statistically significant ($p<0.05$). *Conclusion:* It was determined that the training obese students received which was based on health promotion model helped the students to develop healthy life style behaviors and to increase their life quality.

Key words: health promotion model, healthy life style behaviors, life quality, nursing, training

Introduction

Obesity is one of the most important health problems of our day and its frequency has been increasing gradually (1). According to World Health Organization (WHO)'s 2014 data, it has been reported that the rate of obesity has reached 600 million and an increase between 10-30% has been found in the prevalence of obesity around the world in the last decade (2). Studies in Turkey have reported 30% of individuals to be obese (3) and also 44% increase in the frequency of obesity in the last 12 years (4, 5).

While obesity can be seen in every age group, it shows an increase in the first years of life, between the ages of five and six and in the adolescence period. The fact that obesity develops before the age of five and after the age of 15 is a risk in terms of the continuation of obesity in adulthood (6-8). Although there are no national studies in Turkey examining the frequency of obesity in children and adolescents, there are various studies conducted locally and regionally. Studies have reported that obesity influences between 1.3 and 13.5% of children and adolescents and 50% of the children who enter the period of adolescence as being obese continue to be obese in their adulthood, too (9,10).

In addition to causing various health problems in the advanced periods of life, obesity in adolescents can also cause chronic diseases such as type 2 diabetes, hypertension and hyperlipidemia, which are seen in adults and the young ones (11-12). These chronic diseases, which are known to result from genetic pre-

disposition, lifestyle or environmental factors, are estimated to cause 35 million deaths each year, 80% of which are estimated to take place in low and middle income countries (8). As well as increasing the number of deaths with the diseases it causes, obesity also causes both individual and social problems since it is a chronic illness besides negatively affecting the economy of the country by causing a decrease in work power and an increase in health expenses (10).

Studies have shown weight management programs including diet, activity and behavior therapy and changes in the lifestyle to decrease BMI (13-21), while training which included only stress management was not found to decrease BMI (22). Web-based weight management programs (23), including stress management in the program (24), and at least three months long training and follow up have been shown to increase lifestyle behaviors and life quality (25-30) while other methods different from medication have been stated to be very successful in developing and improving mental health and life quality besides being cost-effective in terms of not having any risks and being inexpensive (25).

One of the most used models in giving health behaviors is Health Promotion Model (HPM) (31). According to HPM, healthy lifestyle behaviors are behaviors that individuals should have. According to WHO, healthy lifestyle behaviors, which are effective in promoting health, are gained and tested in adolescence period which is accepted as the age group of between 10 and 19 (32, 33). Middle (14-17 years of age) and late (17-21 years of age) stages, which are accepted as the developmental stages of adolescents, can be described as developmental periods which are risky in terms of starting negative health behaviors that can cause obesity. In addition, at the end of the adolescence period, anthropometric measurement values of adult life are reached (34).

A great number of researches on obese adolescents have been conducted in the world which have assessed the prevalence of obesity, risk factors, efficiency of healthy lifestyle programs and life quality (35-39). In Turkey, descriptive studies have been conducted such as prevalence studies, risk factors of obesity, and methods used in diagnosis and treatment (9, 13, 40-43). However, no studies have been found on the effect of model-based training for fighting obesity in middle and late adolescence periods on the healthy lifestyle behaviors and life quality of obese adolescents.

The purpose of this study is to research the effect of training based on health promotion model on the healthy lifestyle behaviors and life quality of obese adolescents.

Hypotheses of the Study:

H_1: The training given to students based on Health Promotion Model will decrease BMI.

H_2: The training given to students based on Health Promotion Model will decrease obesity related risk factors.

H_3: The training given to students based on Health Promotion Model will increase healthy lifestyle behaviors.

H_4: The training given to students based on Health Promotion Model will increase life quality.

Material and methods

Study population

The study was conducted with control group method and pretest-posttest design between September and January 2014, in four schools in which the highest number of obesity was determined and 136 obese adolescents between the ages of 14 and 18 with a BMI of ≥95 percentile (11, 43) were found as a result of obesity measurement conducted in 14 high schools in Rize. The experimental and control groups were determined with the method of drawing to provide randomization. The obese adolescents in 2 schools were taken in the experimental group, while the obese adolescents in the other 2 schools were taken in the control group. No sample was chosen. The research was completed with a total of 114 adolescents, 55 experimental and 59 controls who accepted to participate in the study and who met the selection criteria. Inclusion criteria were as follows:

- Not having any physical or mental disabilities preventing exercise,
- Not using any medication or seeing a doctor to lose weight,
- Not doing sports regularly
- Not having any chronic diseases except obesity.

Control Variables: The control variables of the study include Identifying properties of adolescents such as age, gender, monthly income of parents, education and working status of parents, family type, number of children, and presence of obese individuals in the family. When the students in the experimental and control groups were examined in terms of control variables, it was determined that there are not any statistically significant differences and both groups have a homogeneous distribution ($p>0.05$).

Approval was taken from Atatürk University Institute of Health Sciences Ethical Board and permission was taken from Rize provincial directorate for national education and the secondary education institution in which the research was conducted. In addition, the aim and method of the research was explained to the students constituting the research group, the study plan was given in writing and written permission was obtained from the students and their parents.

Data collection and variable definitions

For data collection, "Demographic Characteristics Form for Adolescents", "Obesity Related Risk Factors Form" "Adolescent Lifestyle Scale" and "Pediatric Quality of Life Inventory" were used.

Data Collection Tools

Demographic Characteristics Form for Adolescents: The form which was prepared by the researcher in line with the related literature (9, 20, 40, 42, 44) includes 10 questions about the age and gender of the adolescents, income of the family, parents' education and work status, type of family, number of children and whether there are obese individuals in the family.

Obesity Related Risk Factors Form: It is a form prepared by the researcher in line with the related literature (20, 33, 38, 40, 45). This form consists of 17 questions about BMI, dietary habits, status of sedentary life and physical activity which includes obesity related risk factors.

Adolescent Lifestyle Scale (ALS): The scale which was started to be developed by Pender was completed by Hendricks, Pender and Murdaugh in 2006 to measure the frequency of health promotion behaviors of early, middle and late stage adolescents (31, 46). Turkish validity and reliability studies of the scale were conducted by Ardıç in 2008 (47).

The scale which was based on HPM consists of a total of 44 questions and it has 7 subgroups (health responsibility, physical activity, diet, positive outlook on life, interpersonal relations, stress management and spiritual health) which can be used independently from one another (46-47).

The scale is a 4 likert type scale. 1 point is given for "never", 2 point for "sometimes", 3 point for "frequently" and 4 point for "always". The lowest score of ALS is 44, while the highest score is 176. The scale does not have a cut-off point, positive health behavior increases as the score increases (31, 46-47). Cronbach's alpha value of the Turkish form was found as 0.87. In this study, Cronbach's alpha value was found as 0.91. The scale used in this study was found to be suitable for the sample group.

Pediatric Quality of Life Inventory (PedsQL) (Adolescent 13-18 Years of age form): The inventory (48), which was developed by James W.Varni in 1999 to measure the health-related quality of life of children and adolescents between the ages of 2 and 18, was checked for Turkish validity and reliability for the ages of 13 and 18 by Memik et al. in 2005 (49). It is a life quality scale suitable for both healthy and sick children and adolescents in wide populations such as schools and hospitals (49).

PedsQL consists of a total of 23 items and it has four subscales as physical functioning, emotional functioning, social functioning and school functioning. Physical functioning has 8 items, emotional functioning has 5 items, social functioning has 5 items, and school functioning has 5 items. Scoring is made in three areas. First, physical health summary score (PHSS) (8 items) is measured, secondly psychosocial health summary score (PSHSS) (15 items) which is calculated by the measurement of item scores of emotional, social and school functioning is measured, thirdly total scale score (TSS) (23 items) is measured (48, 50-52). This inventory examines the last month of adolescents. The items in the inventory, which is 5-likert type, are scored between 0 and 100. 100 points are given for

the answer "never", 75 points for "rarely", 50 points for "sometimes" and 0 points for "almost always". The total score is obtained by adding up the points and dividing them by the number of answered items. In case of incomplete items, the scores of completed items are added up and divided into marked items. If more than 50% of the inventory is not completed, the inventory is not assessed. The higher total scale score of PedsQL means the better health-related quality of life (50-52). While the inventory's Cronbach's alpha coefficient was 0.88 for pediatric form (48), Cronbach's alpha value of the Turkish form was 0.82 in adolescent form (49). Cronbach's alpha value of this study was found to be 0.85.

Digital Stadiometer: F.Bosch digital measurement device, which could measure weight between 0 and 150 kg and height between 0 and 200 cm, was used to measure the weight and height of obese adolescents.

Nursing Intervention

The nursing intervention applied to obese students consists of health promotion training and monitoring process. The training structured according to SGM is a program that focuses on building health responsibility, decreasing sedentary behaviors and enhancing physical activity, healthy eating, personal support, healthy life benefits for stress management, and positive self-esteem in order to develop healthful lifestyle behaviors to reduce obesity-related health problems and improve quality of life. The training program given to the obese students was provided for the experimental group after the pre-test, in groups of 10-15 people with the training book named "Healthy Life Circle" structured according to SGM. The training, which includes the stages of teaching, convicting, performing, repeating, and practicing, was conducted by the researcher in the form of group training for ten weeks. It consisted of 30-40 minute interviews once a week. The training was conducted using oral expression, brain storming, discussion and demonstration methods and educational materials such as power point presentations, writing board and video demonstration. Follow-ups were continued for three months after the training as reminding consultancy through email and telephone once a month. No intervention was made on the control group. After the training of the adolescents in the experimental group finished, the training booklet was also given to the adolescents in the control group to comply with the "equality" principle.

Data Assessment

The data obtained from the research were transferred to computer with SPSS package program. In data assessment, Kolmogorov-Smirnov test was used to find out whether all the variables were normally distributed, and Chi-square, Mann-Whitney U Test, Wilcoxon Paired t Test and Friedman Test were used since they were not normally distributed.

Results

When the demographic characteristics of the adolescents in the experimental and control groups within the context of the research were compared, in the experimental group it was found that 47.3% of the adolescents were 16 years old, 51% were female, 60% had equal income and expenditure. It was found that mothers of 63.6% of the adolescents were primary school graduates, fathers of 32.7% of the adolescents were secondary school graduates, mothers of 85.5% were unemployed, while fathers of 89% were employed, 85.5% had nuclear family, families of 67.3% had 3 or more children, and no obese individuals were found in the families of 72.7%. In the control group, it was found that 47.5% of the adolescents were 16 years old, 55.9% were male, 57.7% had equal income and expenditure. It was found that mothers of 55.9% of the adolescents were primary school graduates, fathers of 42.4% of the adolescents were primary school graduates, mothers of 78.0% were unemployed, while fathers of 72.9% were employed, 79.7% had nuclear family, families of 57.6% had 3 or more children, and no obese individuals were found in the families of 55.9% (Table 1).

In the intra-group and intergroup comparisons of BMI pre-test and post-test values of obese adolescents in the experimental and control groups, it was found that the pre-test BMI values of the adolescents in the experimental group, which was 32.06±3.02, decreased (30.61±3.17) after training and the difference was found to be statistically significant ($p<0.05$). The dif-

Table 1. Comparison of Demographic Characteristics of the Adolescents in the Experimental and Control Groups

Demographic Characteristics	Experimental Group (n=55)		Control Group (n=59)		Test and p value
	S	%	S	%	
Age					
14-15 years of age	15	27.3	19	32.2	X^2=1.102
16	26	47.3	28	47.5	p=0.894
17-18 years of age	14	25.4	12	20.3	
Gender					
Female	28	50.9	26	44.1	X^2=0.534
Male	27	49.1	33	55.9	p=0.465
Monthly Income Status of the Family					
Income more than expenditure	12	21.8	14	23.7	X^2=0.076
Equal income and expenditure	33	60.0	34	57.7	p=0.963
Income less than expenditure	10	18.2	11	18.6	
Educational Status of the Family					
Literate	6	10.9	11	18.6	
Primary School	35	63.6	33	55.9	X^2=2.255
Secondary School	7	12.7	10	16.9	p=0.521
High School, University and higher	7	12.7	5	8.6	
Father's Educational Status					
Literate	4	7.3	5	8.5	
Primary School	17	30.9	25	42.4	X^2=2.097
Secondary School	18	32.7	17	28.8	p=0.552
High School and higher	16	29.1	12	20.3	
Mother's Employment Status					
Employed	8	14.5	13	22.0	X^2=1.062
Unemployed	47	85.5	46	78.0	p=0.303
Father's Employment Status					
Employed	49	89.1	43	72.9	X^2=4.802
Unemployed	6	10.9	16	27.1	p=0.028
Family Type					
Nucleus	47	85.5	47	79.7	X^2=0.723
Extended	8	14.5	12	20.3	p=0.697
Number of Children in the Family					
1-2	18	32.7	25	42.4	X^2=1.127
3 and more	37	67.3	34	57.6	p=0.288
Presence of obese individuals in the family					
Mother	4	7.3	9	15.3	
Father	5	9.1	7	11.8	X^2=4.189
Sibling	2	3.6	5	8.5	p=0.381
Mother and Father	4	7.3	5	8.5	
No obese	40	72.7	33	55.9	
Total	55	100.0	59	100.0	

ference between the pre-test and post-test BMI values of the adolescents in the control group was found to be statistically insignificant (p>0.05) (Table 2).

When the obesity related risk factors were compared between groups, it was found that there were no statistically significant differences between experimental and control group adolescents' pre-test obesity related risk factors except for the risk factor of eating too fast (p>0.05). In terms of post-test measurements, it was found that obesity related risk factors in experimental group adolescents had decreased when compared with the adolescents in the control groups and the difference was found to be statistically significant (p<0.05) (Table 3).

When the ALS scale average scores were compared between groups, the difference between the pre-test ALS scale average scores of experimental and control groups was statistically significant in all groups except the dimensions of health responsibility and spiritual health (p<0.05). In terms of post-test measurements, ALS scale average scores of experimental group adolescents who received health promotion training (135.9±14.2) were found to be higher than those of the adolescents in the control group (111.7±19.4) and the difference between groups was found to be statistically significant (p<0.05) (Table 4).

When the PedsQL and sub-dimensions average scores were compared between groups, it was found that there was a statistically significant difference between pre-test PedsQL average scores of experimental and control group adolescents in all sub-dimensions except social functioning and school functioning (p<0.05). In terms of post-test measurements, PedsQL average scores of experimental group adolescents who received health promotion training (86.07±10.9) were found to be higher than those of the adolescents in the control group (74.54±14.0) and the difference between groups was found to be statistically significant (p<0.05) (Table 5).

Discussion

It is thought that in case of obesity resulting from unhealthy lifestyle behaviors, HPM based trainings for adolescents will give them healthy lifestyle behaviors, help obese individuals to get back to normal levels of weight and to maintain their normal levels and positively affect life quality. The purpose of this study is to research the effect of training based on health promotion model on the healthy lifestyle behaviors and life quality of obese adolescents.

In the study, when the BMI pre-test and post-test values of obese adolescents in the experimental and control groups were examined, it was found that the pre-test BMI values of the adolescents in the experimental group decreased after health promotion training and the difference between two groups was found to be statistically significant (p<0.05) (Table 2).

In a study by Nemet et al (14) it was stated that a training which included diet, activity and behavior therapy and was applied 6 times within 3 months decreased BMI. In another study by Muller et al. (19) it was reported that a training to prevent obesity which included the subjects of healthy diet, physical activity and TV watching also decreased BMI.

Table 2. Intra-group and Inter-group comparisons of BMI pre-test and post-test values of obese adolescents in the experimental and control groups

Measurement	Experimental group X ± SS	Control Group X ± SS	Test and p value
Pre-test	32.06±3.02	32.24±3.54	t= 0.29 p= 0.771
Post-test	30.61±3.17	33.02±5.65	t= 2.78 p= 0.006
Test and p value	t=5.46 p= 0.000	t =1.63 p= 0.109	

Table 3. Intra-group and Inter-group comparisons of obesity related risk factors of obese adolescents in the experimental and control groups based on pre-test and post-test measurements

Obesity-related risk	Pre-				Inter-group Comparison Pre-		Post-				Inter –group Comparison Post		Intra-group Pre-Test- Post-
	Experimental		Control				Experimental		Control				
	Number	%	Number	%	X^2	p	Number	%	Number	%	X^2	p	
How the adolescent views his/her weight													**Experimental**
Overweight	15	27.3	11	18.6			16	29.1	6	10.2			X^2= 1.815; p= 0.178
Medium weight	17	30.9	31	52.5	5.465	0.065	29	52.7	33	55.9	8.006	0.018	**Control**
Normal weight	23	41.8	17	28.9			10	18.2	20	33.9			X^2= 2.130; p= 0.144
Number of daily meals													**Experimental**
1-2 meals	40	72.7	31	52.5			5	9.1	32	54.2			X^2= 31.410; p= 0.000
3-5 meals	15	27.3	26	44.1	5.959	0.051	17	30.9	24	40.7	45.814	0.000	**Control**
6 meals	0	0.0	2	3.4			33	60.0	3	5.1			X^2= 0.200; p= 0.655
Skipping meals/having snacks between meals													**Experimental**
Never	3	5.4	7	11.9			22	40.0	12	20.3			X^2= 30.857; p= 0.000
Sometimes	25	45.5	30	50.8	2.427	0.297	32	58.2	24	40.7	24.140	0.000	**Control**
Always	27	49.1	22	37.3			1	1.8	23	39.0			X^2= 1.316; p= 0.251
Having snack after													**Experimental**
Never	5	9.1	11	18.6			34	61.8	16	27.1			X^2= 37.356; p= 0.000
Sometimes	27	49.1	23	39.0	2.516	0.284	20	36.4	18	30.5	28.634	0.000	**Control**
Always	23	41.8	25	42.4			1	1.8	25	42.4			X^2= 0.273; p= 0.602
Having snack after waking up from nocturnal													**Experimental**
Never	11	20.0	16	27.1			52	94.5	20	33.9			X^2= 43.000; p= 0.000
Sometimes	31	56.4	37	62.7	3.899	0.142	3	5.5	33	55.9	45.137	0.000	**Control**
Always	13	23.6	6	10.2			0	0.0	6	10.2			X^2= 0.133; p= 0.715
Eating speed													**Experimental**
Fast	36	65.5	6	10.2			1	1.8	11	18.6			X^2= 51.000; p= 0.000
Middle	19	34.5	47	79.6	39.215	0.000	18	32.7	41	69.5	36.762	0.000	**Control**
Slow	0	0.0	6	10.2			36	65.5	7	11.9			X^2= 1.143; p= 0.285
Sitting at the dinner table for a long time													**Experimental**
Never	3	5.5	7	11.9			27	49.1	6	10.2			X^2= 35.372; p= 0.000
Sometimes	23	41.8	31	52.5	3.930	0.140	26	47.3	39	66.1	28.854	0.000	**Control**
Always	29	52.7	21	35.6			2	3.6	14	23.7			X^2= 0.333; p= 0.564

*Friedman

(continued)

Table 3. Intra-group and Inter-group comparisons of obesity related risk factors of obese adolescents in the experimental and control groups based on pre-test and post-test measurements *(continued)*

Obesity related risk factors	Pre-				Inter Group Comparison Pre		Post				Inter Group Comparison PostTest		Intra Group Pre Test-Post
	Experimental		Control				Experimental		Control				
	Number	%	Number	%	X^2	p	Number	%	Number	%	X^2	p	
State of doing another activity while eating													**Experimental**
Never	2	3.7	7	11.9			13	23.6	10	16.9			X^2= 26.561; p= 0.000
Sometimes	18	32.7	13	22.0	3.665	0.160	35	63.7	16	27.2	24.259	0.000	**Control**
Always	35	63.6	39	66.1			7	12.7	33	55.9			X^2= 2.462; p=
Frequency of fast food consumed in one week													**Experimental**
None	1	1.8	6	10.2			2	3.6	1	1.7			X^2= 25.326; p= 0.000
Once	8	14.5	6	10.2	5.058	0.168	29	52.7	10	16.9	23.192	0.000	**Control**
Twice	20	36.4	15	25.4			19	34.5	23	39.0			X^2= 5.765; p= 0.16
Three times and more	26	47.3	32	54.2			5	9.1	25	42.4			
State of overeating under stress													**Experimental**
Never	8	14.5	12	20.3			30	54.5	10	16.9			X^2= 28.900; p= 0.000
Sometimes	28	50.9	34	57.6	2.368	0.306	21	38.2	28	47.5	22.447	0.000	**Control**
Always	19	34.6	13	22.1			4	7.3	21	35.6			X^2= 1.286; p= 0.257
Time/day spent in front of Computer/TV													**Experimental**
None	0	0.0	3	5.1			18	32.7	8	13.6			X^2= 35.103; p= 0.000
About an hour	15	27.3	20	33.9	3.789	0.150	28	50.9	29	49.2	9.186	0.010	**Control**
More than two hours	40	72.7	36	61.0			9	16.4	22	37.2			X^2= 7.759; p= 0.05
Sport activity frequency/week													**Experimental**
None	38	69.1	29	49.2			3	5.4	35	59.3			X^2= 38.095; p= 0.000
2-3 times a week	16	29.1	27	45.7	4.889	0.087	32	58.2	22	37.3	43.440	0.000	**Control**
3-5 times a week	1	1.8	3	5.1			20	36.4	2	3.4			X^2= 0.806; p= 0.369
Time/week spent for sports activities													**Experimental**
None	38	69.1	30	50.8			1	1.8	27	45.8			X^2= 44.083; p= 0.000
Less than an hour	13	23.6	23	39.0	3.984	0.136	20	36.4	15	25.4	30.745	0.000	**Control**
1 – 2 hours	4	7.3	6	10.2			19	34.5	11	18.6			X^2= 1.125; p= 0.289
2 hours and more	0	0.0	0	0.0			15	27.3	6	10.2			

*Friedman

Table 4. Intra-group and Inter-group comparisons of ALS scale and sub-dimensions based on pre-test and post-test averages of experimental group and control group obese adolescents

ALS Scale and Sub dimensions		Experimental Group		Control Group		Inter-group Comparison Pre Test- Post Test	
		X ± SS	Intra-Group Comparison Pre Test-Post Test	X ± SS	Intra-Group Comparison Pre Test-Post Test	U	p
Health Responsibility	Pre Test	13.69±3.12	Z=6.164 p=0.000	14.31±3.53	Z=0.658 p=0.511	1471.50	0.389
	Post Test	19.35±3.37		14.63±4.07		633.0	0.000
Physical Activity	Pre Test	12.24±2.77	Z=6.464 p=0.000	14.10±2.96	Z=0.189 p=0.850	1007.00	0.000
	Post Test	18.58±2.79		14.22±3.38		517.0	0.000
Diet	Pre Test	15.09±2.89	Z=6.448 p=0.000	17.29±3.16	Z=1.672 p=0.095	932.0	0.000
	post Test	22.96±2.64		18.07±3.08		370.5	0.000
Positive Outlook on Life	Pre Test	15.96±3.33	Z=5.533 p=0.000	17.51±3.79	Z=0.912 p=0.362	1201.50	0.017
	Post Test	19.58±2.71		17.10±3.65		984.0	0.000
Interpersonal Relations	Pre Test	15.27±2.70	Z=5.675 p=0.000	16.71±3.33	Z=0.578 p=0.563	1172.00	0.010
	Post Test	18.85±2.43		16.85±3.45		1091.50	0.002
Stress Management	Pre Test	14.96±2.48	Z=5.890 p=0.000	15.95±2.83	Z=0.670 p=0.503	1207.50	0.018
	Post Test	18.93±2.37		15.61±3.33		682.0	0.000
Spiritual Health	Pre Test	14.40±2.79	Z=5.380 p=0.000	15.29±3.21	Z=0.259 p=0.796	1344.50	0.113
	Post Test	17.65±3.32		15.22±3.80		1066.50	0.002
ALS Scale Total	Pre Test	101.6±15.3	Z=6.444 p=0.000	111.2±16.7	Z=0.182 p=0.856	1027.0	0.001
	Post Test	135.9±14.2		111.7±19.4		504.0	0.000

Table 5. Intra-group and Inter-group comparisons of PedsQL scale and sub-dimensions based on pre-test and post-test averages of experimental group and control group obese adolescents

PedsQL and Sub Dimensions		Experimental Group		Control Group		Inter Group Comparison Pre Test- Post Test	
		X ± SS	Intra-Group Comparison Pre Test-Post Test	X ± SS	Intra-Group Comparison Pre Test-Post Test	U	p
Physical Health	Pre Test	59.49±15.9	Z=5.911 p=0.000	72.62±14.5	Z=0.021 p=0.983	861.00	0.000
	Post Test	86.59±13.5		71.93±16.6		758.50	0.000
Emotional Functioning	Pre Test	59.64±18.0	Z=5.835 p=0.000	69.83±17.5	Z=0.708 p=0.479	1151.50	0.007
	Post Test	81.64±14.5		72.29±20.0		1196.50	0.015
Social Functioning	Pre Test	81.45±13.7	Z=3.981 p=0.000	84.07±12.9	Z= 0.236 p=0.813	1426.00	0.261
	Post Test	91.09±13.0		84.58±14.7		1118.50	0.003
School Functioning	Pre Test	61.45±18.3	Z=5.806 p=0.000	68.14±15.7	Z=0.989 p=0.323	1326.00	0.091
	Post Test	84.64±12.7		70.93±17.4		859.50	0.000
Psychosocial Health	Pre Test	67.52±13.7	Z=6.051 p=0.000	74.01±11.9	Z=0.716 p=0.474	1202.50	0.017
	Post Test	85.79±11.1		75.93±14.9		984.50	0.000
Scale Total Score	Pre Test	64.72±12.3	Z=6.211 p=0.000	73.53±11.4	Z=0.538 p=0.590	931.00	0.000
	Post Test	86.07±10.9		74.54±14.0		816.00	0.000

In their study, Ağca et al. (13) stated that an hour of exercise twice a week for 10 weeks decreased BMI in adolescent girls , while Tucker (15) stated that diet restriction and exercise program on 4-18 age group decreased BMI and Berry et al. (26) stated that 6-week-long weight management training on 7-17 age group adolescent children decreased BMI. Dietary and physical activity regulation training in Kang et al.'s study (17), a training for decreasing the use of drinks with carbohydrate conducted twice in one academic year in

James et al.'s study (21), low calorie diet and exercise on obese adolescents for 12 weeks in Yosmaoğlu and Baltacı's study (18), and weight management program conducted as 7 training sessions in 2.5 months which was structured according to social cognitive theory in Törüner's study (20) were reported to decrease BMI.

The results of this study are in parallel with the results of other studies. However, in Katzer et al's study (22), although a positive change occurred in stress management 12 months after 10-week-long training for stress management in fighting obesity, no change was reported in BMI. In addition, 7-week-long individual training for fighting obesity in Potecha's study (44) and 12-week-long diet change and physical activity training in Caballero et al.'s study (53) did not report any changes in BMI. In this study, addressing issues such as regulation of dietary and exercise behaviors, decreasing the period of sedentary lifestyle and stress management and the follow-up and advisory intervention to make health promotion based trainings a lifestyle were found to cause significant changes in BMI. This result verifies the hypothesis that "The training given to students based on Health Promotion Model will decrease students' BMI".

When the obesity related risk factors were compared between groups, it was found that obesity related risk factors in experimental group adolescents had decreased after health promotion training when compared with the adolescents in the control groups and the difference between the two groups was found to be statistically significant ($p<0.05$) (Table 3).

In terms of obesity related risk factors, Frenn et al. (16) found that with a 4-hour-long training to decrease the rate of fat in the diets of 12-17 age group and to increase physical activity caused an increase in the duration of exercise. In his study, Törüner (20) aimed to give 4th graders changes in behaviors in subjects such as healthy diet, physical activity and sedentary lifestyle by using Social Cognitive theory. This weight management program based on school was applied as 7 training sessions in 2.5 months. Families were also trained to increase the efficiency of the intervention. At the end of the training, it was found that the children's level of information increased and BMI decreased. In their study, Nemet et al. (14) found that a 3-month-long training applied 6 times which included diet, activity and behavior therapy increased diet and activity levels, while Muller et al. (19) found that training for preventing obesity which included the subjects of healthy diet, physical activity and TV watching regulated the status of diet and activity and decreased sedentary life. In another study by Kang et al. (17), it was found that diet and physical activity training given to children caused an increase in children's levels of diet and activity. The results of this study are in parallel with the results of other studies.

Sedentary and/or active life style is effective on the physique and anthropometric measurements at least as much as diet (6, 54). Thus, in obesity programs it is quite important to increase the active exercise periods of children and adolescents, to decrease the duration of sedentary activity, to give the habit of regular exercise and to make these a way of life besides giving them a proper eating habit.

In this study, positive significant differences were found in the obesity related risk factors of dietary habits, sedentary life period and physical activity habits after the health promotion training and this change causes a significant decrease in the BMI of the adolescents in the experimental group. This result verifies the hypothesis that "The training given to students based on Health Promotion Model will decrease students' obesity related risk factors".

When the ALS scale pre-test and post-test average scores of experimental and control group obese adolescents were compared, it was found that the difference between the pre-test and pos-test ALS scale and sub-dimensions average scores of experimental group adolescents were found to be statistically significant ($p<0.05$) (Table 4).

In their study, Sousa et al. (55) reported that healthy lifestyle behaviors developed in adolescents of 12-18 age group after a 6-month-long intervention and 3-month-long follow up with HPM-structured web-based weight management program. In their study, Geçkil et al. (24) found that as a result of 8-week-long training of diet and coping with stress, adolescents' health behaviors about diet, stress management and exercise increased positively and the least behaviors adolescents showed were health responsibility, self-realization and interpersonal support. Berry et al. (26) reported that 3 months after a 6-week-long

weight management program training applied on obese children between the ages of 7 and 17, healthy lifestyle behaviors scale average scores increased and the highest change was in the sub-dimension of physical activity.

In this study, it was found that the highest changes in the sub-dimensions of ALS scale were in diet, health responsibility and physical activity, respectively. Diet, which is one of the sub-dimensions of ALS scale, determined the eating preferences and habits and meal choices of adolescents. Health responsibility influences individuals' levels of responsibility on their health, individuals' starting and maintaining health promotion behaviors. Physical activity determines adolescents' levels of regular exercise or activity (19). In this study, it was found that the training focused on developing health responsibility, decreasing sedentary behaviors, increasing physical activity and managing stress which was given to the adolescents in the experimental group to promote healthy lifestyle behaviors to decrease obesity related health problems and to increase life quality had significant influences on the ALS scale and sub-dimensions (Table 1). This result verifies the hypothesis that "The training given to students based on Health Promotion Model will increase students' healthy lifestyle behaviors".

When the Pediatric Quality of Life Inventory average scores of experimental and control group obese adolescents were examined, it was found that the difference between the pre-test and pos-test PedsQL inventory and sub-dimensions average scores of experimental group adolescents were found to be statistically significant ($p<0.05$) (Table 5).

In a study by Yackobovitch et al. (29), 12-week-long weight management training was found to cause an increase in PedsQL inventory and its sub-dimensions. Pratt et al. (56) found that life quality scores were increased in 8-18 years old children who were followed with obesity treatment program and Modi et al. (30) reported that medical weight management program was effective in increasing the life quality of 5-18 years old obese children. Alıcı and Pınar (25) reported that the 3-month-old training given to 18-65 years old obese individuals for six times developed and improved life quality. 12-week-long training including dietary and exercise recommendations by Poeta et al. (27) increased average scores of all sub-dimensions of PedsQL except school functioning. The highest change in pre-test and pos-test average scores was in physical health sub-dimension (pre-test 60.1-78.1, post-test 82.8-96.9), while the highest score in the pre-test and post-test sub-dimensions of the scale was in social functioning (pre-test 80.0-90.0, post-test 86.2-100.0). Similarly, in the present study, the highest change in pre-test and pos-test average scores of the experimental group in PedsQL and sub-dimensions was in physical health sub-dimension (pre-test=59.49±15.9, post-test=86.59±13.5), while the highest score in the pre-test and post-test sub-dimensions of the scale was in social functioning (pre-test=81.45±13.7, post-test =91.09±13.0) (Table 2).

Hofsteenge et al. (28) reported that a diet and physical activity training given to 11-18 years old obese adolescents for 7 times with time intervals of 2 weeks caused a significant development in the emotional and social functioning of the life quality scale and they commented that adolescents' attending school increased social functioning. The positive change observed in all the sub-dimensions of life quality scale in this study is thought to result from the fact that the 10-week-long training of diet, physical activity and sedentary life, which are accepted as the primary reasons of obesity, are given together. It can also be said that the group training caused peer influence and increased motivation.

In literature, it is recommended that nurses who give regular and programmed trainings about health and diseases should give information that positively affects physical, social and psychological dimensions of life quality especially in chronic diseases that influence life quality and the state of well-being (32). In this study, it was found that the training focused on developing health responsibility, decreasing sedentary behaviors, increasing physical activity and managing stress which was given to the adolescents in the experimental group to promote healthy lifestyle behaviors to decrease obesity related health problems and to increase life quality had significant influences on the PedsQL inventory and its sub-dimensions. This result verifies the hypothesis that "The training given to students based on Health Promotion Model will increase students' life quality".

Study limitation

This study was conducted in the schools in a city center of Turkey. Thus, the results of the study cannot be generalized to all obese adolescents.

Conclusion

The results of the study show that the training given to obese adolescents based on Health Promotion Model is an effective model in developing healthy lifestyle behaviors and increasing quality of life. In line with these results, it can be recommended that training should be given to increase awareness and protect individuals from obesity since it is an important cause of disease and death; adolescents should be followed by public health nurses and school health nurses to prevent obesity and problems that can be caused by obesity; intervention and consultancy services should be given and the society should be trained for awareness through mass media such as press and television.

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