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Review

Is a low concentration of Linoleic Acid related to the extended longevity of the Queen honeybee?

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Summary. Lifespan has been a topic of scientific interest for about a century and, in this regard, hundreds of theories have been expressed, calling into question numerous causes of aging, but none of which appears to be decisive. Different species can have hugely different species-specific life spans, but several eusocial species, e.g. honey bees, show very large differences in infra-specific longevity, that is, of individuals. One of the most widely accepted theory remains that of free radicals formulated by Dennis Harmann in 1956 in the historical work "**Aging: A Theory based on Free Radical and Radiation Chemistry**" (1**)** and in the following works, where the hypothesis foresees the existence of a strong metabolic activity in the production of free radicals, both on polyunsaturated fatty acids and on proteins with accumulation of oxidized products and damage at mitochondrial level. The effects of peroxidative damage seem to emerge as one of the factors most influencing lifespan. None of the works related to this longstanding problem, however, takes into consideration Linoleic Acid (C18: 2 n-6) as a central element among membrane fatty acids in the involvement of its peroxidation in relation to lifespan. In the examination of the main metabolic responsibilities of Linoleic Acid, attention is focused on the particular role that it, an essential fatty acid, could have as a determining element in the lifespan of worker honeybees with respect to the queen honeybee. **Highlights.** >Linoleic Acid is an essential fatty acid for mammalian life. >Linoleic Acid occupies a central position in the vital dynamics of animal and human organisms. >Linoleic Acid has the maximum "bulk" in the space within cellular membranes when compared to all the other polyunsaturated fatty acids. >High concentrations of Linoleic Acid can increase the risks of oxidation of Linoleic Acid itself by increasing the peroxidation index, more linked to Linoleic Acid rather than to other polyunsaturated fatty acids.

Key words: Royal bee, Worker bee, Lifespan, Linoleic Acid, Oxidation

Introduction

In trying understand the biological mechanisms at the basis of longevity, many animal models have been considered (2). Among these, the honeybee poses very difficult challenges to any aging theory. In general, social insects show a subdivision into fertile castes, females or queens and males or drones, and sterile one, or workers, and a division of tasks, in that the breeding caste focus exclusively on reproduction, while other tasks necessary for colony maintenance are performed by sterile caste.

Incidentally, evolution has produced a strong differentiation in longevity among the castes, reaching a lifespan of the queens by twice (ants) at tens of times longer than the workers (honeybees and termites). Worker honeybees, in addition, shift from age-dependent stages during their life, in which they develop relevant morphological changes in chronological succession and which are characterized by different aging

rates. Nurse bees, who develop the hypopharyngeal glands for the production of royal jelly from day 5 to day 17, show a slow aging rate while foragers, in the last two weeks of their life, age rapidly (3). However, under specific environmental conditions, e.g. a noticeable increase in the inflow of nourishment or after any demographic change of the worker population, a part of the workers can shift bidirectionally to another stage. In particular, even the oldest foragers can revert to the feeding stage by developing the hypopharyngeal glands again, thus demonstrating that the transition from slower to faster aging is not inevitably linked to age (3). A further complication is given by the time of the season when the workers are born: the workers born in spring and summer have a maximum lifespan of about 65 days (4), while workers born at the beginning of the unfavourable season, which will not have to feed the larvae for several months because the queen's oviposition stops during winter, enter a "diutinus" stage (5), with the characteristic of nurse workers, and a prolonged life of up to 280 days . Against this variable, but still short life span of the workers, the queen normally lives up to 5 years.

Over more than a century of scientific studies, more than 300 theories on aging have been produced (6), but many of these could hardly justify how such plasticity could have been selected during evolution.

Aging theories can be roughly brought together under two main strands of thought, namely that according to which aging would be a genetically programmed event, which would manifest itself through changes in the functioning of the nervous, immune and endocrine systems, and that of the progressive accumulation of damage caused by the influence of the environment (7).

In this article, we discuss the role of linoleic acid as a key factor among oxidizing substances able of accumulating harmful free radicals at the level of cell membranes, with reference to the honeybee.

Linoleic Acid between regulation of biological membranes and pathology

Living systems are highly complex, expressive of a continuous relationship between the parts, in the sign of what Spinoza in Ethica called *conatus sese conservandi*. In practice, a persistence, an effort, a tension to keep oneself in existence, which coincides with a continuous expansion of power, energy: *posse existere potentia est* (Ethica, I, XI, sec.). We exist as a point of force, because we have power, albeit limited; we are dynamic forces that, by nature, tend to expand.

All this is of surprising beauty (8).

Of course, this power to exist send back to the duration of life: a power is such with respect to the quality and quantity of its expansion. Here, Linoleic acid plays an essential role.

Linoleic acid, an essential substance in the life of animal organisms, was discovered by Burr & Burr in 1929 (9) and Holman encodes his needs, for humans, between 1 and 2% of total calories (10).

The scientific works on Linoleic Acid are numerous, however this fatty acid is mostly studied in relation to health effects rather than biochemical and molecular effects.

C18:2 n-6 plays a key role in the cell membrane. It can regulate the osmosis of the cell, as occurs, e.g. in the epidermis and has a close relationship with cholesterol and oxygen (11).

Cholesterol appears to be involved in the removal of reactive oxygen substances from membrane phospholipids in order to prevent a harmful peroxidation effect of Linoleic Acid (12, 13).

Always from the molecular point of view, Linoleic Acid is involved in the functioning of ion pumps and ion channels of cardiomyocytes (14) and in the regulation of temperature where its concentration changes with respect to changes of the same temperature as it also happens in insects such as Drosophila (15) and in animals that live in extreme temperatures (16, 17).

The prevalence of studies on linoleic acid, on the clinical side, concerns the possible link with cholesterol, its involvement in atherosclerosis, its participation as a promoter in carcinogenesis, etc.

A careful examination of the literature, however, did not provide direct and / or certain evidence of this involvement (18).

In recent years, thanks to the results obtained from a research on ischemic heart disease and psychopathology (Major Depression and Bipolar Disorder), it has been sought to identify the position of Linoleic

Acid in its role as a mediator and conditioner of pathological conditions, precisely, as those mentioned above (19-24).

Other aspects have been analysed such as, for example, the relationship between Linoleic Acid and Cholesterol (25), where it is clear that due to their chemical structure, Linoleic Acid and Cholesterol, in biological membranes, must regulate themselves synchronously. That is to say, that if the Linoleic Acid reduces its concentration also the Cholesterol must reduce its concentration to guarantee the functional balance of the membrane, maintaining the right conditions of mobility (viscosity and fluidity).

Again, the hypothesis that there is a connection between Linoleic Acid and psychopathology passing through the concept of "symmetry breaking" (26) has been proposed, further, that Linoleic Acid may be the possible key that unlocks the quantum dimension of the brain (27).

In conclusion, an attempt was made to demonstrate that Linoleic Acid occupies a central position in the vital dynamics of animal and human organisms (28-30).

Linoleic Acid and Oxidation

Linoleic Acid has the maximum "bulk" in the space in which it is found (e.g.: cellular membranes), compared to all the other polyunsaturated fatty acids: this characteristic allows the approach of other chemical species with pro-oxidant activity to react with LA.

Furthermore, the structure of Linoleic Acid indicates that the enlargement of the sheets of the cellular phospholipid membrane, in which it may be inserted, will more easily cause a relaxation of the membrane itself, increasing its fluidity and, consequently, modifying its functionality. In these chemical-physical conditions of the cell membrane, it is easy to foresee an easier access by the Reactive Oxygen Species (ROS), but also a "recall" of balancing molecules to restore a correct membrane function. The cholesterol, particularly suitable for compacting the membrane, is transported to the cells by the LDL with a mechanism that will obtain a double effect: of rebalancing the molecules for the functionality of the membranes and removing the quantity of already oxidized Linoleic acid (LDL oxidized).Therefore, linoleic acid appears to occupy a central position in the oxidation-reduction balance of the cell.

The above mechanisms, in conditions of high concentrations of Linoleic Acid, can increase the risks of oxidation of Linoleic Acid itself by increasing the peroxidation index, more linked to Linoleic Acid rather than to other polyunsaturated fatty acids.

The mechanism of the described actions is expected to remain in equilibrium since, at the same time, LDL and cholesterol decrease in the plasma, however LDLox will inevitably be produced.

Most food sources containing Linoleic Acid and alpha Linolenic Acid (i.e. non-long chain PUFAs) are particularly rich in Linoleic Acid and quite poor in alpha Linolenic Acid. This means that the increased risk of cellular oxidative damage is mainly linked to the Linoleic Acid (31-35).

It has been shown that the greater the number of double bonds and less radical are formed in a watery environment (36).

The excessive intake of Linoleic Acid from food sources, therefore, can be considered at high risk of oxidation and can be involved in the progression of cellular aging.

Linoleic Acid: Queen Bee and Worker Bee, Pollen and Royal Jelly

Regarding the composition of fatty acids, pollen and royal jelly have very different characteristics.

Examination of the literature shows a fatty acid composition of the lipids of royal jelly with respect to pollen, which demonstrates how Linoleic Acid is present in pollen in very high concentrations (37). In fact the food material intended for the larva and for the whole life of the queen bee (royal jelly) is obtained by enzymatic processing of the pollen and honey (collected) by the bees of the "court", does not contain Linoleic Acid, since this molecule is transformed into short-chain lipid components characteristic of royal jelly (38). Linoleic acid appears to be the fatty acid that essentially makes the difference between the two foods. In light of the previously reported biochemical-

molecular considerations, the aforementioned condition would seem to be of particular interest in order to avoid damage from peroxidation in the queen bee causing a dysfunction of its reproductive role (39).

Furthermore, the feeding of worker bees through the consumption of pollen and honey would justify the intake of Linoleic Acid in substantial concentrations (37).

Linoleic Acid and Royal Bee Lifespan: Hypothesis

The work of Haddad et al. opens to some interesting considerations about the relationship between the fatty acids composition of the queen bee compared to the working one as regards the possible cause of increase in the lifespan of the first compared to the second (40) and open an interesting discussion on the causes of aging of worker bees with respect to queens on the basis of the peroxidation index which characterizes the former with respect to the latter. This peroxidation index is three times higher in worker bees when compared to the queen bees. A careful reading of the data (fatty acids) contained in the aforementioned work also demonstrates that the fatty acid composition of the queens at birth and during aging is characterized by a substantial difference in concentration of Linoleic Acid in the queens compared to the workers and with respect to the alpha Linolenic Acid. The low concentration of Linoleic Acid in queen bee with respect to the worker bees is confirmed in all the districts (head, thorax and abdomen) analyzed (40). The very low concentration of Linoleic Acid, both, in royal jelly and in the body of the queen bee, seems to testify that the lack of Linoleic Acid protects the queen bee from the excess of peroxidation and that, consequently, increases the lifespan, unlike what happens in worker bees. In support of this hypothesis, it must be emphasized that the oxidation capacity of Linoleic Acid is greater than that of the alpha Linolenic acid, in an aqueous environment such as that of the cell membrane (41-43). If we observe the concentration of the alpha Linolenic Acid in the queen bees, it is higher than that of the Linoleic Acid, therefore it seems plausible the hypothesis that the oxidation of the alpha Linolenic Acid is indifferent with respect to the lifespan of the queen bee and that its lifespan depends mostly from the very low concentration of the more oxidizable Linoleic Acid. This observation, therefore, would justify the lower involvement of alpha Linolenic Acid in the determinism of the peroxidative phenomenon linked to aging. Further evidence regarding the Linoleic Acid is its concentration in the head (40) of the queen bee compared to the worker bee that is very similar to that found in the brain of mammal organisms (men and animals) living on earth, that is around 1% (44).

To better support our hypothesis, further studies are needed to verify if the trend pattern of the oleic acid content overlap significantly on the 3 female phenotypes characterized by as many physiological stages, namely the nurs, diutinus and forager workers (3). These stages are reversible between them and age-independent and therefore cannot be compared with workers schematically collected at a certain age (40) but need to be well characterized in the experiments.

It is also necessary to correlate the body's oleic acid (less susceptible to oxidation) (45) content of the different phenotypes with the relative amounts of royal jelly, pollen and honey fed to the different phenotypes. In fact, the relationship between the two foods in the daily diet of workers also varies in relation to the phenotypic stage.

Conclusion

In light of the acquisitions on the functional characteristics of Linoleic Acid and on its greater facility in oxidizing compared to all other polyunsaturated fatty acids, the consistent reduced concentration of Linoleic Acid in queen bee with respect to the worker bee, can allow the hypothesis of an increased oxidative phenomenon in the worker bee with consequent effect on the life span.

In the honey bee female eggs become workers or queens, depending on what they are fed, without any apparent difference in the common genome. If further research were to confirm there is a link between titer in linoleic acid and infraspecific longevity difference, and that this depends on differentiated composition of fatty acids in the nourishment, this would be a point in favor of the theory of progressive accumulation of

damage caused by the influence of the environment. However, considering that nutrition is differentiated starting from the egg stage, it is difficult to understand such an impressive effect in terms of physical and physiological changes without imagining that there are switches in the genome capable of turning on the expression of a particular program following a food stimulus (46).

For this reason, we believe that social insects, and in particular the honeybee, a farmed species, can be an excellent model for the study of the factors that guide the evolution of longevity in species.

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Review

The antioxidant properties of the medicinal fruits: a pivotal mechanism of their nutritional, pharmacological, and cardioprotective effects

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Summary. *Background and Purpose:* The pharmacological properties of the medicinal fruits are insufficiently elucidated and the mechanisms remain to be clarified. The purpose of this article is to describe the antioxidant properties of the medicinal fruits. *Materials and Methods:* Pertinent literature of the medicinal fruits, *Lycium barbarum*, mulberry, kiwifruit, and avocado were comprehensively screened for analysis. *Results:* The medicinal fruits are also rich in antioxidative components, especially the phenolic ingredients, with significant antioxidant capacities in addition to the nutrients contained. By decreasing the reactive oxygen species and promoting the levels of superoxide dismutase, catalase, and glutathione peroxidase, they play an important protective effect against deoxyribonucleic acid damage and myocardial, hepatic, and other organ damages. Based on the antioxidant mechanism, modifying the storage conditions with low O_2 and low temperature may prevent fruit ripening and control lipid oxidation. *Conclusions:* The medicinal fruits are not only nutritious foods, but also healthcare and medical agents as well. The underlying mechanisms responsible for the protective effects are via antioxidative properties.

Key Words: Antioxidants; fruit; myocardial ischemia.

Introduction

Reactive oxygen species is a crucial element triggering oxidative stress and eventually leading to cell apoptosis by breaking down matrix metalloproteinase. In contrast, the antioxidant defense enzymes, such as glutathione peroxidase (GSH-Px), catalase, and superoxide dismutase (SOD), are playing a protective part against oxidative stresses (1). It has been suggested that antioxidants might protect against oxidative stresses by reducing lipid peroxidation and promoting the activities of antioxidant defense enzymes (1).

Fruits and vegetables are rich in antioxidants, which may account for the beneficial effects of fruits on human health and help in lowering the incidence of degenerative diseases, such as cancer, arthritis, arteriosclerosis, heart disease, inflammations, brain dysfunction, and ageing process (2). Fruits and vegetables contain a lot of antioxidants that can reduce the level of deoxyribonucleic acid (DNA) oxidation, which to some extent can reduce the oxidative damage of DNA and enhance the repair ability of the damaged DNA. The most abundant antioxidants in fruits and vegetables are polyphenols and vitamin C, but vitamins A, B and E and carotenoids are less (2). The physiological functions of the natural products are attributed to the antioxidative property of the phenolic ingredients (3). Among fruits, berries carry a higher antioxidative capacity, which are considered to be due to the abundant contents of phenolics, anthocyanins, total flavonoids, and ascorbic acids (4). In spite of sporadic reports describing the antioxidant property of fruits, there is still a lack of comprehensive description of the characteristics of the medicinal fruits in relating to the antioxida-

tive capacities. This review aims to give a comprehensive description in this respect.

Lycium barbarum

Ingredients and mechanisms

Fruits of *Lycium barbarum*, also named *Fructus lycii* when dehydrated, are well-known in traditional Chinese medicine for longevity, vision, wellness, and headaches (2). *Lycium barbarum* is rich in nutrients, including *Lycium barbarum* polysaccharides, fats, proteins, amino acids, taurines, betaines, vitamins, and trace elements (iron, zinc, phosphorus, and calcium). These components can maintain normal cell development, improve the repair ability after gene damage and accelerate the reverse of aging process. *Lycium barbarum* has many physiological effects, such as enhancing immunity, reducing blood fat, protecting liver, anti-tumor, antiaging, and antistress effects and so on (5). *Lycium barbarum* polysaccharide is one of the most important active components. It has multiple biological and pharmacological properties, such as anticancer, antifatigue, neuroprotective, antioxidant, hypoglycemic, fertility-protective, and immunomodulating functions (6). In recent studies, it has been found that the flavonoids from the fruits of *Lycium barbarum* protect the blood cells and mitochondria against oxidative damages (7). In the experimental studies, *Lycium barbarum* has been shown to have antioxidant, immunoenhancing, radioprotective, and antiaging effects (2). The antioxidant activity was measured by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method showed that the antioxidant capacity of *Fructus lycii* was higher than that of the fresh fruits, in line with the results obtained for total phenolic contents (2). The antioxidant capacities detected by Folin-Ciocalteu, DPPH and Fenton methods showed similar results. However, the extracts of the non-defatted *Fructus lycii* had stronger antioxidant activities than that of the defatted under the same extraction condition (8).

Pharmacology

In patients taking fruits of *Lycium barbarum*, the DNA repair capacity in human lymphocytes and the activities of SOD and catalase were remarkably increased and the lipid peroxidase activity was significantly decreased (9). A study revealed that treatment with a longer duration and higher doses of *Lycium barbarum* polysaccharide (100 and 200 mg/kg) decreased the apoptotic rate fully and significantly (6). The effect of *Lycium barbarum* polysaccharide extracted from *Lycium barbarum* were also investigated on the proliferation rate, cell cycle distribution, and apoptosis in the human hepatoma QGY7703 cell line, by which it was discovered that *Lycium barbarum* polysaccharide treatment caused inhibition of QGY7703 cell growth with cycle arrest in the S phase and apoptosis induction (10). *Ginseng-Lycium barbarum* Decoction (formula: *Ginseng* 10 g, *Lycium barbarum* 10 g, longan pulp 10 g, and jujube 9 pieces) that were prepared by frying *Ginseng* for 20 minutes and boiling 10 minutes by adding other drugs showed satisfactory effects for the treatment of chronic heart failure. With one dose per day for a 5-day treatment course, the total effective rate was 95.8% for the experimental group and 87.6% for the control (11).

Food therapies

At present, there are at least 60 kinds of *Lycium barbarum* diet recipes, which are top grade tonifying substances for the liver, kidney, and lung, such as medlar broth (pork tenderloin 200 g and *Lycium barbarum* fruit 50 g), medlar chicken juice corn soup, and *Lycium barbarum*-Chinese yam milk soup, and *Ginseng-Lycium barbarum* wine, *etc*.

Product development

Lycium barbarum and its extract are suitable for the development of daily chemical and nursing products. They can be used as a supplement of hair cosmetics for preventing alopecia, maintaining the color and nutrition of hair and promoting hair melanin. As a supplement of facial cosmetics, they nourish the skins and keep facial skin smooth and delicate. They also serve as infant care products (12).

Mulberry

Ingredients and mechanisms

The mulberry belongs to the *Morus* genus of the *Moraceae* family. Mulberry contains nutrients necessary

for the human body, including protein, polysaccharides, alkaloids, cardiac glycosides, anthocyanins, lipids, free fatty acids, alcohols, volatile oils, tannins and cyanidin, essential amino acids, vitamins, and minerals (13). Mulberry fruits are also rich in organic acids, such as malic, citric and tartaric acids. The essential fatty acids, vitamins, and polyphenols that contain in the fruits are potent antioxidants (14). When the experimental diets of mulberry fruits were fed to Sprague-Dawley rats, hepatic GSH-Px, catalase, and 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase activities were increased in all experiemtal groups comparing with those with normal diets (15). The cytoprotective effects of cyanidin-3-glucoside (C3G) isolated from mulberry fruit against pancreatic β-cell apoptosis caused by hydrogen peroxide (H_2O_2)-induced oxidative stress showed that the C3G-treated cells were associated with a significant dose-dependent increase of intracellular reactive oxygen species-scavenging activity, 19.4% and 33.8% at 50 and 70 μg/mL C3G, respectively, and C3G exerted protective effects against oxidative stress-induced apoptosis in MIN6N β-cells by inhibiting DNA fragmentation, and protected pancreatic β-cells against cell death by inactivating caspase-3 and by regulating the phosphorylation of extracellular signal-regulated kinase (ERK) and p38-mitogen activated protein kinase (MAPK) (16). A polysaccharide purified from mulberry fruits was found to stimulate murine macrophages to release chemokine and proinflammatory cytokines (17). After hydrogen peroxide treatment, apoptosis-like morphologic changes, such as shrinkage, detachment, and cytoplasmic condensation, were observed, and were inhibited in the presence of mulberry extract (18). Mulberry polysaccharide treatment at appropriate concentrations significantly increased the proliferation of splenocytes via modulating the proapoptotic protein Bak and antiapoptotic protein B-cell lymphoma 2 (Bcl-2) expression ratios, suggesting that mulberry polysaccharide protects primary immune cells from apoptotic cell death. Polysaccharides isolated from strawberry and mulberry juice modulated Bak and Bcl-2 protein levels in murine primary macrophages. Furthermore, a negative correlation between the cytokine secretion levels and Bcl-2 protein levels suggested that proinflammatory interleukin (IL)-1β and IL-6 decreased Bcl-2 levels in the lipopolysaccharide-stimulated macrophages. The results showed that IL-4, -5, -10 and -12 and tumor necrosis factor-α levels secreted by the mulberry polysaccharide-treated splenocytes significantly increased in a concentration-dependent manner (19).

Pharmacology

1) Antioxidant: Mulberry polysaccharide can eliminate free radicals, playing antioxidant and antiaging roles. Mulberry polysaccharide has an obvious hypoglycemic activity, and it keeps blood sugar in normal range. The antiaging effect of mulberry is carried by mulberry anthocyanins, which show enhanced antioxidant capacity to Drosophila in inhibition of lipid peroxidation (13). Both *Panax Notogineng* suspension and mulberry extract improve activities of SOD and GSH-Px, and reduce malondialdehyde (MDA) content in the serum and liver of mice. The effect of mulberry extract was obvious on GSH-Px, while *Panax Notoginseng* suspension had more apparent effect on SOD. Therefore, both *Panax Notoginseng* and mulberry extract had antioxidant and antiaging functions (20). In D-galactose-induced aging mouse model, the serum and liver SOD, GSH-Px activities, and MDA content were investigated after oral mulberry extract of different doses (2, 4 and 6 g/kg/day) for 40 days. As a result, the mulberry extract could improve liver SOD and the GSH-Px activity, and reduce MDA content. Low-dose mulberry extract increased the serum SOD and GSH-Px activity, and MDA content decreased significantly (21).
2) Blood lipid regulation and anti-arteriosclerosis: The New Zealand white rabbit hyperlipoidemia model was given mulberry sarcocarp extract by intragastric administration at low-dose (1 g/kg), middle-dose (5 g/kg) and high-dose (10 g/kg). The serum lipoid was determined 0, 2, 4, 6 and 8 weeks after administration. Compared with the control animals, the serum levels of total cholesterol and triglyceride of the experimental group with extract from mulberry sarcocarp significantly decreased. The serum levels of low-density lipoprotein and apolipoprotein B of the middle- and high-dose mulberry sarcocarp extract groups also significantly decreased. The levels of high-density lipoprotein

and apolipoprotein A_1 of middle- and high-dose mulberry extract groups increased (22). An immunohistochemical study also revealed that with low- and middle-dose mulberry extract, expressions of intercellular cell adhesion molecule-1 (ICAM-1) were downregulated somewhat in the rabbit aorta and coronary artery, but weak positive staining in the high-dose rabbits, indicating the role of the extract in inhibiting atherosclerotic process (23).

3) Hematological functions: Pharmacological research confirmed that mulberry has immunoenhancing function by strenghthing the phagocytic function of animal macrophages, promoting T lymphocyte maturation, and participating in the killing effect of T cells on the target cells. It might decrease the sodium-potassium adenosine triphosphatase (Na^+-K^+-ATP) activity of erythrocyte membrane, promoting the growth of hematopoietic and granulocyte progenitor cells, promoting lymphocyte transformation, and increasing peripheral white blood cells (24). Mulberry xanthan gum showed good effects for the treatment of chronic idiopathic thrombocytopenic purpura (13).
4) Treatment of alopecia areata: By using the plum blossom needle combined with mulberry *Shengfa* Decoction for the treatment of alopecia areata, 42 patients were cured with significant effects (13).

Food therapies

The recipes of food products include: 1) Concentrated mulberry jam, which is good for *yin* deficiency, thirsty throat, dizziness, and constipation; 2) Mulberry honey cream, which is prepared by the use of mulberry and proper amount of honey, by which the mulberry decoction is simmered to boil cream, honey is then added and mixed, to drink, 10-15 g each time, 2-3 times a day. It is suitable for nourishing the blood, *yin* deficiency caused by premature graying, dizzy, women's menstruation, and amenorrhea; 3) Mulberry-*Linearstripe Rabdosia* granules, which is composed of mulberry, *Linearstripe Rabdosia*, *Herba Artemisiae Scopariae*, *Schisandra*, and other herbs, is effective for clearing away the *heat* and detoxification, and is indicated for patients with *dampness-heat*-induced hypochondriac pain, jaundice, acute and chronic hepatitis, and liver damage; 4) Mulberry *Cistanche* Decoction, which is prepared with dried mulberries 20 g, *Cistanche deserticola* 15 g, black sesame 10 g, and fried fructus 6 g, cooked together for 1 hour. To take it with proper amount twice daily, and it is good for nourishing the kidney and intestine and for the treatment of constipation; 5) Mulberry nut porridge, which is prepared with mulberry 75 g, raisins 50 g and *Semen Coicis* 50 g, cooked together into porridge, suitable for the treatment of chronic nephritis and cardiac edema; and 6) Mulberry consumptive thirst juice: Mulberry 15 g, and *Rehmannia glutinosa*, *Radix Scrophulariae*, and *Ophiopogon japonicus* 20 g each, are decocted for juice with supplement of crystal sugar, one dose daily. It can nourish *yin* and increase fluid, and suitable for the treatment of thirst due to insufficiency of body fluid, lung dryness due to *yin* deficiency, and consumptive thirst.

Product development

Mulberry vinegar drink green tea, one of the mulberry products, has been developed and it showed superior antioxidant DPPH-scavenging capacities (25). Mulberry can also be processed into other health care products, such as canned mulberry, mulberry beverage, mulberry wine, dried mulberry fruit, mulberry juice, mulberry jam, mulberry dew, mulberry pigment, mulberry jelly, and mulberry ice cream, *etc*. (26).

Mulberry extract can also be used in hair care cosmetics. The products include mulberry hair oil, mulberry mousse, mulberry shampoo, mulberry hair tonic, mulberry hair cream, mulberry hair conditioner, and mulberry dyeing shampoo, *etc*. (26).

Kiwifruit

Ingredients and mechanisms

Kiwifruits contain a variety of bioactive ingredients including ascorbic acid, carotenoids, dietary fiber, minerals, and phenolic compounds. The bioactive compounds contained in kiwifruits differ depending on cultivar, genotype, growing place, and degree of maturity of the fruits. The correlation coefficients between the total phenolics and the antioxidant capacity of kiwifruits measured by using the 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS), DPPH, and the oxygen radical absorption capacity

(ORAC) methods suggested that the phenolic compounds contribute more than flavonoids to antioxidant capacity. Among various ripe kiwifruits, 'Bidan' had the highest total phenolics and antioxidant capacity, but the lowest total flavonoids; whereas 'Chiak' had the highest level of total flavonoids, but the lowest antioxidant capacity (27). The methanolic extracts contain significantly lower amount of polyphenols than the hydrolyzed extract with the solvent with combined methanol and acid (28). The antioxidant activities varied among the kiwifruit samples as determined by various used assays: the highest was with ferric reducing antioxidant power (FRAP), intermediate with DPPH, and the lowest with ABTS (29). The results of the determined antioxidant activities of the investigated kiwifruit samples varied greatly. The data depended on the extraction procedure of the kiwifruit: solvent used (acetone, methanol, and water), duration, and the temperature of extraction (29).

Kiwifruit extracts have potent fruit-derived antioxidant capacities showing a preventive effect against certain cancers and cardiovascular disease. *In vitro* experiment revealed that aqueous, 70% ethanol, and linoleic acid emulsion kiwifruit extracts at 50 mg/mL showed antioxidant activities of 72.31%, 70.75%, and 96-98%, respectively. The inhibitory activity against angiogensin I-converting enzyme of kiwifruit extracts was 21-26% at 10 mg/mL, and was 46-49% at 50 mg/mL, and that against HMG-CoA reductase was 13-14% at 10 mg/mL and was 19-30% at 50 mg/mL, indicating that the cardiovascular protective effects of kiwifruit relied on the oxidative components, including vitamins, carotenoids, polyphenols, and flavonoids (30). Consumption of the fruit may decrease oxidative DNA damage in human cells owing to its potential antioxidant properties, and its effectiveness at decreasing oxidative DNA damage has been proved by in *ex vivo* and *in vitro* experiments, which proved it to be more potent than vitamin C in protecting DNA from damage (31).

Pharmacology

Kiwifruits are rich in vitamins, especially vitamin C. It also contains a variety of amino acids, actinidine, proteolytic enzymes, tannin, and trace elements. It contains cellulose and pectin, which promotes intestinal peristalsis. Researches revealed that kiwifruit can be used as an antidote to mercury, which reduces blood mercury and improves liver function (32). Kiwifruit showed protective mechanisms by antagonizing against chromium-induced cytotoxicity, cyclophosphamide-induced mutations and lipid peroxidation, reducing nitrite toxicity, and improving immune functions (33). Pharmacological studies have shown that kiwifruit and juice products can prevent carcinogens from forming in the body and lower blood lipids. In general, Kiwifruit has preventive and therapeutic effects on cancer, hypertension, hyperlipemia and coronary heart disease (32).

It has been reported that H_2O_2-induced DNA damage could be abated and the antioxidant capacity was significantly improved after taking a large amount of kiwifruits, thereby reducing the mutagenic effects in relation to carcinogenesis (34). Motohashi et al. (35) found five fractions of kiwifruit H_1, H_2 (hexane extract), A_1, A_2 (acetone extract), and M_2 (methanol extract) showed selective cytotoxic activity against human oral tumor cell lines. Kiwifruit polysaccharide has an anti-tumor effect, and it can inhibit the growth of cancer cells and induce apoptosis of cancer cells via the signaling pathways related to gastric cancer, including epidermal growth factor receptor/Ras/MAPK, protein kinase C, phosphatidylinositol 3-kinase, and transforming growth factor-β signaling pathways (36).

Kiwifruits showed obvious anti-myocardial ischemia effect. They could reduce the myocardial infarction area of the rat myocardial ischemia model made by coronary artery ligation. They could also increase the coronary blood flow in isolated guinea pig hearts and to decrease the heart rate and myocardial contractility (37). Experiments revealed kiwifruits contained arginine and glutamic acid salts, which were helpful in dilating the arterioles as what the vascular dilating agents did, thereby improving blood circulation and preventing thrombus formation. They could be used as a supplement to the human body for magnesium deficiency caused by cardiac diseases, such as myocardial infarction and hypertension.

Clinical observations revealed that *Actinidia chinensis* juice syrup (30 mL, three times daily, taken for 12 months) could prevent atherosclerosis and cardiovascular diseases by lowering blood cholesterol, triglyc-

eride, and low-density lipoprotein, and increase blood high-density lipoprotein level of patients with hyperlipidemia (38).

Health care

Nutritional effects: Kiwifruits contain a lot of vitamin C and minerals. Therefore, they are especially suitable for supplementing the electrolyte loss due to physical exercise. Meanwhile, kiwifruit juice contains 5% carbohydrate, which is conducive to keep the glucose level stable during strenuous exercise. The investigations showed that kiwifruit beverage could enhance the physical fitness of the athletes, increase blood cell density, maintain blood glucose within normal limit after a 2.5-hour exercise, and improve the insulin and vitamin C levels as well.

As a natural sugar alcohol, inositol contained in kiwifruit has a positive effect on glucose metabolism, and the supplement of inositol can improve the nerve conduction speed. As a second messenger in cell signal transduction, inositol serves as a regulator of hormones and nerve conduction in the cells.

Kiwifruits also contain healthful carotenoid (carotene, lutein, and yellow pigment), phenolic compounds (anthocyanins), and antioxidants, thereby capable of supplementing sufficient vitamin C for those working in a high temperature environment and in plateau pastoral areas, and for patients of extensive burn and vitamin deficiency. Kiwifruits are beneficial for the occupational diseases poisoned with lead, mercury and other materials, and also show preventive and curative effects for radioactive damages (39).

Product development

The pertinent products that have been under production include kiwifruit wine and low sugar preserved kiwifruit, *etc*.

Avocado

Ingredients and mechanisms

Avocado is a widely grown and consumed fruit. It is rich in nutrients but low in calories, sodium, and fats. Besides, it contains a large amount of potent antioxidants (monounsaturated fatty acids, fiber, vitamins B and E, and phytosterols), essential nutrients (monounsaturated, oleic acid, and polyunsaturated fats linoleic and linolenic acids), and potentially cancer-preventing phytochemicals (polyphenols, proanthocyanidins, tocopherols, carotenoids, β-sitosterol, β-carotene, lecithin, minerals, and vitamins A, C, D, and E).

Pharmacology

Medical and health care effects: Avocado oil contains a lot of unsaturated fatty acids, which can reduce blood lipids as evidenced by clinical observations. After the rat hyperlipidemia models were treated with avocado fruit pulp (2 mL/rat/day, orally) for 10 weeks, the serum hepatic enzymes, bilirubins, and liver and heart MDA levels were significantly reduced in a dose-dependent manner (40).

Avocado fruit is a high energy, low sugar food for people with diabetes. Avocado peel tea drinking plays a certain role in the remission of diabetes. The avocado fruits can also reduce blood lipid and is effective for scleroderma, periodontitis, and spinal epiphysis, *etc*. Professor Paul Spagnuolo from the University of Waterloo has discovered a lipid in avocados may eliminate the source of acute myeloid leukemia by targeting leukemia stem cells (41).

In the mesocarp tissues, C7 sugars and particularly mannoheptulose, playe a major antioxidant role. Cowan (42) proposed that C7 sugars have various important functions, among which is protection of certain key enzymes essential for fruit growth and development from damage by reactive oxygen species. Vinokur and Rodov (43) reported on the lipophylic versus hydrophilic radical scavenging activity of avocado. Certain C6 sugar alcohols (sorbitol and mannitol) act as antioxidants (44). *In vitro* and *in vivo* studies illustrated that the avocado nonsaponifiable fraction was a very potent antioxidant with 3-fold higher DPPH radical scavenging capacity and 20-fold higher ferric iron reducing ability, comparing with the fat soluble vitamin, α-tocopherol (45). When avocado extract (1 mL/kg body mass, for 30 days) was employed to the diethylnitrosamine-treated rats, the liver tumor necrosis factor-α, cyclooxygenase-2, lipoxygenase, caspase-3, DNA fragmentation, nitric oxide, MDA, and total protein decreased remarkably to the levels of the diethylnitrosamine-untreated control rats (46).

Low O_2 atmospheres could influence post-harvest physiology and quality of fruit. Avocados that were treated with combined 1-methylcyclopropene and low O_2, and stored under low temperature conditions showed lowered peroxide levels and increased iodine levels and SOD activities, by which lipid oxidation and ripening were effectively controlled (47). The control treatment with refrigeration maintenance showed increased antioxidant capacity and total phenolic compound content over the storage (48). However, the effects of 1-methylcyclopropene on oxidative features of avocado fruit were limited to a study of polyphenol oxidase and peroxidase activities associated with low temperature-induced mesocarp browning (49).

Food therapy

Avocado, a favorite food and fruit with high energy and low sugar, is an important raw material for food and beverage industry. Nutritional experts recommended that eating half an avocado fruit daily could enhance the short-term memory. In addition to be a fresh food, avocado can be made into salad, fruit juice, jam, and powder, *etc*. In the Canary Islands, Spain, there have been dozens of avocado food formulae, including 5 kinds of salads, 12 kinds of vegetable muds and jams, and 16 kinds of dishes. The formulae of guacamole amount up to 53, such as the Asian guacamole, *Asparagus officinalis L.* guacamole, broccoli guacamole, grilled onion guacamole, sweet pea guacamole, and bean curd guacamole. In South Africa, avocado pulp is also used as a baby food.

Product development

Avocado oil is a widely used high-quality raw material for cosmetic products owing to its ingredients of unsaturated fatty acids and vitamins, especially vitamin E and carotene, with strong ultraviolet radiation absorbing and sunscreen capacities. Avocado oil, because of its deep color, is not applicable for a direct use as cosmetics until proper decolorization. Avocado oil is non-toxic and non irritating to the skin. In addition to the general grease compositions, avocado oil also contains the effective components of plant sterol, ergosterol, folate, inositol, phosphoric acid, and lecithin, *etc*. Therefore, it has better lubricity, mildness, emulsification, and stability. The permeability of the skin by applying it is stronger than that by using lanolin. It also has a certain effect on inflammation and acne. At present, in the United States, Japan, and China, avocado oil is widely used for cosmetic product developments, such as skin cream, cleansing cream, nutrition cream, and sunscreen cream, *etc*. (50).

Conclusions

The medicinal fruits are rich in antioxidative components, especially the phenolic ingredients, with significant antioxidant capacities in addition to their high nutritional values. By decreasing the reactive oxygen species and promoting the levels of SOD, catalase, and GSH-Px, *etc*, they play an important protective effect against DNA damage, and myocardial, hepatic, and other organ damages. Based on the antioxidant mechanisms, modifying the storage conditions with low O_2 and low temperature may prevent fruit ripening and control lipid oxidation. The medicinal fruits are not only nutritious foods, but also healthcare and medical agents as well.

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Original article

The effect of coffee-enriched chlorogenic acid on insulin, GIP and GLP-1 levels in healthy humans: a systematic review

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Summary. *Background:* There are different supplements in the drug stores in order to prevent and treat some diseases. Chlorogenic acid is one of the polyphenolic compounds affected insulin, GIP and GIP-1 to animal study as the prevention factors for diabetes, while human studies show conflict results in this regard. As regard to the lack of systematic review investigated on this topic, the purpose of this study is to assess this issue. *Materials and Methods:* Systematic search was conducted in the databases of google scholar, Science direct, PubMed and web of knowledge by the end of Feb. of 2017. Keywords for the pub med database included: "Chlorogenic Acid", "Green coffee", "Coffee", "GIP", "GLP-1" and "Insulin". Also, the qualitative assessment of studies was done by the jadad table. *Results:* 1631 studies were found, after the searching based on research literature. Then, 8 studies were entered into the qualitative synthesis stage after classifying studies based on duplication and eligibility criteria. The results of studies showed no significant effect of the chlorogenic acid in the form of coffee extracts on blood insulin concentration. In additions, GIP was decreased in one study and GLP-1 was increased in another one study, due to the use of coffee extracts-enriched chlorogenic acid. *Conclusions:* Our findings demonstrated that the consumption of Chlorogenic acid may be has lowering effect on GIP, and increasing effect on GLP-1, while has not effect on insulin, but as regard to the lack of enough studies, it has needed further study in future.

Keywords: Chlorogenic Acid, Coffee, Insulin, GIP, GLP-1

Introduction

According to the recent studies, the prevalence of disease related to endocrine systems is increasing worldwide (1). Results of the recent studies show that there is a direct relationship between mortality rate and endocrine system disorders, like diabetic patients compared to the healthy subjects (2-4). Diabetes is known as one of the hormone-related disorders, so that four hundred and forty million adults are expected to be affected by this disease, in the end of the year 2030 (5,6). The risk of some diseases, like stroke, cardiovascular, macrovascular and microvascular diseases (nephropathy and retinopathy) is high in diabetic patients. Due to it, therapeutic problems are created and extreme costs are imposed to the healthcare system of societies (7-12).

The different types of herbal supplements are presented in the drug stores, due to the high prevalence of some diseases, and also the preventing and treating of them. Chlorogenic acid (CGA) is one of these compounds with the trade name of Svetol which affect insulin, GIP and GLP-1. Also, CGA is known as one of the polyphenol compounds obtained from the extraction of green beans coffee. The functional groups of CGA are hydroxycinnamic acids, p-coumaric acid, caffeic acid, quinic acid and ferulic acid (13-17). Overall, there is a high amount of CGA in drinks containing

coffee and is used as the main compound in the herbal medicine of china which has an important role in the treatment of some diseases like cardiovascular and viral diseases (18-21). In addition, CGA has antimicrobial, anticancer and antioxidant effects (22,23). According to the conducted studies, CGA affects the metabolism of insulin, GIP, and GLP-1 hormones through several important mechanisms. One of the mechanisms is the controlling Intestinal α-glucosidase enzyme, when this enzyme is prohibited, glucose absorption is decreased and the secretion of GIP/GLP-1 is increased. As a result, the rate of gastric emptying is decreased and insulin secretion is modulated (14,16,24). In additions, CGA regulates blood glucose level and insulin hormone by decreasing activity of the liver Glucose-6-phosphatase enzyme and the increasing transport of the glucose in muscles (GLUT 4) (25,26). Due to the lack of similar studies in this regard, our purpose in this study is the assessment of the effect of coffee-enriched CGA on insulin, GIP and GLP-1 concentration in healthy subjects.

Methods

We followed the preferred reporting items for systematic reviews and the current study recorded in the international prospective register of systematic reviews (CRD42017060785).

Search Strategy

In this study, the literature search was performed through Google scholar, Science direct, web of knowledge, and PubMed databases and it was complemented by publisher databases such as Wiley online, Elsevier and springer link until February 2017. Also, the reference list of suitable articles was reviewed for the supplementary data and no language was restricted in the literature search. The key words used during searching for PubMed database were: "Chlorogenic Acid", "Green coffee", "coffee", "caffeic acid", "green coffee bean extract", "Svetol", "hydroxycinnamic acid", "quinic acid", "p-coumaric acid", "Frulic acid", "prune", "blueberry", "calluna vulgaris" and "GIP", "gastric inhibitory polypeptide", "glucose-dependent insulinotropic polypeptide", "glucose indicator protein", "GLP-1", "glucagon-like peptide 1", "Insulin", "Insulin Receptor Substrate Proteins", "insulin resistance", "Insulin-Secreting Cells", "glycemic markers", "diabetes mellitus". The data terms were searched as mesh terms or abstract of studies.

Eligibility Criteria

In this systematic review, studies are considered by the assessment of the effect of CGA on insulin, GIP, and GLP-1 level. Inclusion criteria included:(1) studies conducted on the healthy human; (2) studies with complete data about the subject, method of study and characteristics of participants; (3) studies by randomized clinical trial design; 4) studies that have full text. Also, exclusion criteria included (1) studies without full-text; (2) studies without enough information about the subject, method of study and characteristics of participants 3) studies conducted on other than healthy humans; (4) studies with no randomized clinical trials design.

Data Extraction

We used the standard methods and detailed instruction manual for data extraction. Duplicated studies were excluded, after searching studies based on the literature search in databases. Then studies were sorted separately according to titles and abstracts by the searching about inclusion criteria and gained complete reports for all titles which looked to satisfy the inclusion criteria. In the next stage, studies sorted out the full-text reports and searched for additional information from studies to resolve the question about eligibility, where necessary. Data items were included demographic information, intervention detail such as dosage and trade name of the experimental intervention, duration of treatment, trial design, and trial sample size.

Method of Quality Assessment

The quality of studies was evaluated by the JADAD table which included the score of quality. This table included three items: randomization, blinding and dropouts in the study duration. The score of randomization and blinding is between zero and two that this depends on whether the method of randomization and blinding is clear descriptions or not. Also, the score of dropout is zero or one. Zero for studies with dropout and one for

studies by the lack of dropout. Anyway, JADAD scores are between zero for studies by low Quality and Five for studies by high quality (27).

Results

1631 articles were found and duplicate papers were omitted after the searching in databases. Then, 1564 papers remained which were classified based on relevance articles with the research title and having suitable abstract. So, 29 articles remained for the assessment of eligibility criteria, study design, presence or absence of the full-text and etc. In the final stage, due to the lack of eligibility criteria, 8 articles remained for qualitative synthesis and 21articles were omitted. Figure 1 shows search stages.

Quality Assessment

Based on the Jadad table, the qualitative assessment of studies showed that included studies were conducted randomly, but randomization method was explained in three of them. Also, seven papers were conducted by the blinding method, while this method was only explained in one of them (28-30). Finally, of 8 final papers, just three of them had dropouts while other studies participants had continued the study by the final stage. Johnston et al obtained the lowest score and Wedick et al had the highest score (14,30). The scores of Jadad table have been shown in Table 1.

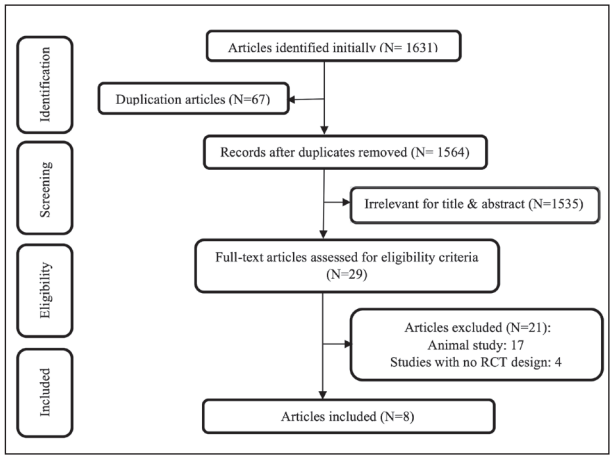

Figure 1: Flow chart of study selection

Study Characteristics

Overall, a total number of participants in all included studies were 136 individuals that all of them had the age range of 18-60. Men population (75%) were higher than the women (25%) in included studies. Studies were conducted in Japan (N=3) (31-33), China (N=1) (28) and USA (N=4) (14,29,30,34). Studies were published in the years 2003-2015. In the viewpoint of study design, 3 studies were conducted as randomized clinical trial by the parallel design and other studies were of the type cross over. Follow-up duration was between 2 hours to 6 hours in the conducted studies. Details of the studies are presented in Table 1.

CGA sources and isomers

Overall, four studies of included studies were used decaffeinated coffee (DCC) as one of the CGA groups, while other studies have used coffee polyphenol. Two studies were used GCA as intervention arms directly while one of the intervention arms was caffeinated coffee (CC) in Wedick et al (30). Also, four included studies were used the coffee bean as the source of CGA group, while other studies were used coffee granules as the source of it. CGA method extraction was hot water in three studies and other studies were not mentioned it. CGA isomers form tested were 3, 4, 5-caffeoylquinic acid; 3,4,5 feruloylquinic acid and 3,4/3,5/4,5 dicaffeoylquinic acid in the four studies while total isomers form tested were 3, 4, 5-caffeoylquinic acid among them. The isomers of CGA were measured by high-performance liquid chromatography from CGA sources in the studies cited. Table 1 show study characteristics.

Outcomes

One note for the analysis of data obtained from the review of studies entered into the qualitative synthesis shall be attended: some studies entered into the final stage of search have several intervention arms, so that each arm was considered as one independent intervention group for studying the final result. Table 1 show study characteristics.

Insulin: results of the studies entered into the final search stage showed that the level of blood insulin was not only measured in one study (28). Johnston et

Table 1: Characteristics of the studies included in the qualitative synthesis

study	Johnston (14)	Van Dijk (29)	Olthof (28)	Wedick (30)	Beam (34)	Ochiai (32)	Ochiai (31)	Jokura (33)
year	2003	2009	2011	2011	2015	2014	2015	2015
location	USA	USA	China	USA	USA	Japan	Japan	Japan
Target population	9 healthy subjects (4 male & 5 female)	15 healthy male	15 healthy male	41 healthy subjects (12male & 29female)	10 male cyclists	14 healthy male	13 healthy male	19 healthy male
Age (year)	22.8 – 29.2 Mean: 26	23.4 - 56.4 Mean: 39.9	23.4 - 56.4 Mean: 39.9	(27.5-53.7) Mean:40	21 – 31 Mean: 26	20 – 60 Mean: 40	30-60 Mean: 44.9	24 – 53 Mean: 38.1
BMI (kg/m2)	< 25	25 – 35	25 – 35	25 – 35	19.7-28.3	18.6– 26.5	NR	19.5– 24.1
Design	cross over	cross over	cross over	parallel	Parallel	cross over	cross over	parallel
Jadad score	2	3	3	5	3	3	3	3
Chlorogenic acid group	1) DCC + Glucose	1) DCC 2) CGA	1) DCC 2) CGA	1) DCC 2) CC	1) GCB + Dextrose	1) CPP + Glucose	1) CBPs	1) CPE
Chlorogenic acid dosage	1) 2.5 mmol	1) 264 mg 2) 1000 mg	1) 264 mg 2) 1000 mg	1) 264 mg 2) 302 mg	1) 350 mg	1) 600 mg	1) 600 mg	1) 355 mg
Chlorogenic acid total isomers	3-CQA , 4-CQA , 5-CQA	NR	NR	NR	NR	3,4,5 CQA 3,4,5 FQA 3,4/ 3,5 and 4,5-diCQA	3,4,5 CQA 3,4,5 FQA 3,4/ 3,5 and 4,5-diCQA	3,4,5 CQA 3,4,5 FQA 3,4/ 3,5 and 4,5-diCQA
Extraction method of isomers	HPLC	NR	NR	NR	NR	HPLC	HPLC	HPLC
Source and method of chlorogenic acid group extraction	coffee granules (no reported type of plant and method)	1) coffee granules (no reported type of plant and method) 2) NR	1) coffee granules (no reported type of plant and method) 2) NR	1&2) coffee (no reported type of plant and method)	green coffee bean (no reported method)	green coffee bean (hot water extraction method)	green coffee beans (hot water extraction method)	Roasted coffee beans (hot water extraction method)
Preparing method of intervention compounds	coffee were dissolved in 25 gr glucose into boiling water	Two supplements were dissolved in 270 mL of water	Two supplements were dissolved in 270 mL of water	Two supplements were dissolved in 177 mL of boiling water	GCB + 75 gr of dextrose mixed in 500 mL of water	CPP were used by 225 mL of a 75 gr Glucose - test solution	CBP beverage were dissolved in 100 mL of water	185 ml of CPE beverage were used directly
Control group	Glucose	Mannitol	Mannitol	Placebo beverage (No coffee)	Dextrose	Glucose	placebo beverage (No CBPs)	placebo beverage (No CPE)
Duration	180 min	120 min	120 min	120 min	120 min	120 min	360 min	240 min
Insulin (AUC)	No significant	1) No significant 2) No significant	NR	1) No significant 2) No significant	No significant	No significant	No significant	No significant
GIP (AUC)	lower significant	NR	1) No significant 2) No significant	NR	NR	No significant	NR	No significant
GLP-1 (AUC)	No significant	NR	1)No significant 2)No significant	NR	NR	No significant	NR	Higher significant

al demonstrated that consumption of DCC leads to the reduction in insulin concentration compared with control in the incremental AUC from 0 to 30 min (14). Also, we observed reduction in insulin level at 15 min after the start of experiment in CGA arm in Van Dijk et al only, and increase in insulin concentration with the use of CGA directly compared to baseline in Ochiai et al., but no significant effect of CGA on the level of blood insulin was observed in the each of studies, during total experimental period compared to control (29,32).

GIP: Overall, 4 studies have not been measured GIP while 4 studies were measured it as one of the outcomes. Johnston et al demonstrated that use of DCC leads to the reduction in GIP concentration compare with control (14), while the consumption of CGA in the form of coffee polyphenols led to significant increase in insulin level at 60 and 120 minutes of the Ochiai et al study duration, compared to baseline, but the intended intervention in this study had no effect on blood insulin compared to control (32).

GLP-1: Among all studies entered into the qualitative synthesis, 4 studies were measured GLP-1 as one of the outcomes. Jokura et al showed that CGA consumption increases level of GLP-1 (33). Also, the results of Johnson et al., and Olthof et al., showed that the consumption of CGA, in the form of DCC, led to increase the level of GLP-1 in the certain times of experimental period, but no significant effect on the level of this hormone was observed compared to the control, generally (14,28). GLP-1 concentration tended to be higher at 60 and 120 minutes of the Ochiai et al., duration compared to baseline but was not significant compare with control.

Discussion

Main Finding

Our finding showed that the consumption of CGA in the form of coffee extracts has no significant effect on insulin level while it was affected GIP (lowering effect) and GLP-1 (rising effect) in a few studies. So, several points may be contributing to these results:

Source of CGA groups was coffee granules in some included studies and others were used coffee polyphenol which can be included some polyphenols, such as DCC and CC. Given that the coffee is one of the main compounds extracted from the coffee bean, so it has different polyphenol compounds, and CGA is known as one of the functional substances (35, 36). Therefore, the effectiveness of CGA on insulin, GIP, and GLP-1 can be affected by other extract compounds and it can be caused a bias for studies included results. Also, as regards to the form of CGA in included studies (DCC, CC and coffee polyphenol extracts), assumptions listed above is honest related to CGA sources. One of the assumptions creates a challenge is the role of caffeine in impressment on included studies results. GIP was decreased in Johnston et al., due to the consumption of DCC, and coffee polyphenols-enriched CC could be increased GLP-1concentration in jokura et al. It is may be that CGA increased GLP-1 at low doses, in the form of CC, and causes the GIP reduction, in the form of DCC, at high doses in healthy humans. So, it has needed further studies. Table 1 show studies detail.

One of the important factors is the use of different CGA dosage in studies which may be affected results. As the evaluation of previous studies, there is no standard dosage of CGA for the affected insulin, GIP and GLP-1 in healthy humans. The range of CGA dosage was 355 to 1000 mg in included studies. GIP concentration was decreased in Johnston et al., due to the use of 2.5 mmol of CGA in DCC form by the coffee granules source, while GLP-1 was increased in Jokura et al., by the use of 355 mg of it in coffee polyphenol extract form with coffee bean source (14,33). However, other included studies were used the different dose of CGA, while they have obtained no significant effect on GIP, GLP-1, and insulin. This observation demonstrated that CGA by coffee granules source may be decreased GIP level, and CGA by coffee bean source may be increased GLP-1, but due to the low number of studies by human participants for this issue, the validity of this conclusion is poor and it have needed further studies.

Due to the assessment of preparing method of intervention compounds, Johnston et al., Beam et al., and Ochiai et al., had used glucose and dextrose in CGA groups. One of the mechanisms of CGA affected metabolism of the insulin, GIP and GLP-1 is the prohibiting of glucose absorption in the gut (14,16). So, the use

of glucose and dextrose can be affected insulin, GIP and GLP-1 with the synergistic effect of CGA.

The difference in sample size may be affected outcomes and which may be one of the reasons for reported different results in studies. Small sample size may have biased the study results (37). Johnston et al., and Jokura et al., with lowering effect in GIP level and increasing effect in GLP-1 concentration respectively, had 9 and 19 healthy subjects. Low sample size may be has affected the power of studies results.

Lack of the clear describing of randomization and allocation concealment approaches in most studies, compromises the interior validity of studies included qualitative synthesis, and subsequently limits the stability of conclusions that can be drawn on the effectiveness of CGA on GIP, GLP-1 and insulin (38).

Studies included qualitative synthesis had evaluated the acute effect of CGA on insulin, GIP, and GLP-1. Due to the lack of enough human studies about this subject, it is may be that CGA can affect these hormones in long duration and it have need added studies.

The total isomers of CGA which were used in some included studies were 3, 4, 5 caffeoylquinic acids (3, 4, 5 CQA), while there are four subtypes of CGA isomers included: caffeoylquinic acid, dicaffeoylquinic acids, feruloylquinic acids and p-coumaroylqunic acids. So, the range of the effect of each isomers on insulin, GIP, and GLP-1 may be different and led to different results.

Regarding that studies were conducted on healthy subjects in included studies, so there is a possibility to find different and opposite results for insulin, GIP, and GLP-1 concentration in other individuals, like diabetic patients compared to our findings, and subsequently more need is felt to conduct more exact clinical trials.

Due to the assessment of the previous animal studies, evidence showed similar results, compare with some included studies. For instance, the results of study conducted by Tunnicliffe et al., showed that the consumption of CGA has no effect on insulin and GLP-1 secretion in laboratory animals, but it can decrease the plasma response of GIP after eating, which is similar to Johnston et al., results (14,39). Overall, as regard to the lack of enough human studies, it is difficult to conclude the lowering effectiveness of CGA on GIP.

Limitations

Some studies were not reported outcome data completely which limited more comparisons of studies and performed meta-analysis on this issue. From the point of view of the qualitative assessment, one study acquire the highest score for describe method of randomization, blinding and dropouts while the other studies could not acquire the complete score of jadad items. Also, due to the assessment of included studies methods section, inadequate control for confounders may bias the results in the studies and affect our conclusions.

Conclusion

According to our findings in this systematic review, it was recognized that 1) the consumption of CGA in the form of DCC has lowering effect on GIP and has increasing effect on GLP-1 concentration in the form of coffee polyphenol extract in acute time, but this conclusion has low validity regards to low number of studies 2) consumption of CGA in the each form of coffee extract has no significant effect on the level of insulin. So, as regard to the lack of enough clinical human studies for this issue, so it is recommended to conduct further clinical trials in the future.

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Development of slow food awareness scale and examination of the effectiveness of slow food training

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Summary. *Aim:* In this research, it is aimed to develop a valid and reliable scale which can reveal the awareness of the students about slow food. Moreover, the aim of the study was to provide the students with training in slow food and to determine the effectiveness of the training given. *Materials and methods*: In the scale development phase of the research, descriptive model was used. On the other hand, experimental model was used to determine the effectiveness of the training. *Study group*: The study group consists of 210 students in Abant Izzet Baysal University, Gastronomy and Culinary Arts Department and 185 students in the Cookery Program of Mengen Vocational Higher School in 2016-2017 Academic Year in Bolu, Turkey. *Results and discussion:* In the scale development stage, exploratory factor analysis was performed according to the answers obtained from the study group and it was determined that the scale consisted of 22 items collected in three dimensions. The items in the scale explained 57.98% of the total variance. In addition to this, factor load values of scale items ranged between 0.55 and 0.79. In addition to the scale development study, 185 students were also included in the scale; pretest and posttest were applied. Slow food training was given to the students by using methods and tools such as power point presentation, question-answer, group discussion, demonstration, brain storming, dramas, games and storytelling. *Conclusions and suggestions:* As a result of the education of the students, it was found that there was a positive increase in the level of knowledge of both girls and boys.

Key words: Slow Food Training, Scale Development, Experimental Design

Introduction

Slow Food movement was initiated in 1986 in the town of Bra (Italy), as the Arci Gola. The founder of Arci Gola association was the journalist Carlo Petrini. Arci Gola became Slow Food in 1989, following a protest by Carlo Petrini organized against the opening of the first McDonald's restaurant in "Piazza di Spagna" in Rome. On November 9th, 1989, representatives from several countries signed the Slow Food manifesto at "Opera Comique" in Paris. Thus, Slow Food has become an eco-gastronomic nonprofit Organization; it was established as a response to contemporary life consumed in high speed, which led to the abandonment of the local gastronomic traditions and the decrease of people's interest in authentic food (1).

Having grown over the years, slow food movement has become an important part of a transition process to eating slowly and healthfully culture which aims to promote slow food culture instead of fast food culture, which means consuming unhealthy and harmful food quickly, by adopting the principle of consuming "clean, fair healthy food" in order to protect the producers and biodiversity in 132 countries (2). Slow food does not mean cooking food on low heat. This includes the communication between food producers and consumers, the communication between the food itself and the consumer and the communication among

the persons at the table (3). According to Petrini, food on the plate must be associated with the planet and it must be good, fair and clean because the main purpose of slow food is not only "to defend good food and gastronomic pleasure and thus to support slower life pace" but also "to defend biodiversity by preserving traditional dishes, main ingredients, cultivation and processing methods" (4). In this regard, the philosophy of "clean, fair, healthy food" which constitutes slow food movement is stated as follows:

"Slow Food in terms of the Concept of "Healthy"

Healthy: It is defined as a fresh, fragrant seasonal diet that is part of the local culture and satisfies the senses (5). Çakır et al (6) state that according to slow food movement, it is necessary for a nutrition or a beverage to be called "healthy" to be a traditional part of a local culture, have its own cooking method and have special materials and service codes. Işıkhan (7) in his work, suggests that slow food products have their own equipments and methods to be preserved and served. Keskin (8) points out that when it is examined in terms of slow food, the concept of "healthy food" can be defined as products with their own look, taste and smell and not being artificial.

Slow Food in terms of the concept of "Clean Food"

Clean: It is expressed as food production and consumption which does not endanger the environment, animals and human health (5). Çakır et al (6) state that with the concept of "clean food", the fact that nutrition and beverages should be produced and consumed in such a way as not to harm human beings and the other creatures in the world is stressed. This consumption concept which has been accelerated by industrialization has brought about a decrease in world resources and an increase in waste generated by production (9).

Slow Food in terms of the concept of "Fair Food"

Fair: It is defined as reasonable prices for consumers and fair conditions and fees for producers (5). Sağır (10) in his study put forwards that *"food should be fair. Food producers should take a fair response in their work in humanitarian conditions, while protecting and valuing their rights."* Çakır et al. (6), in his study, defines the concept of "fair food" as "*A food sector where consumers can pay the monetary value of the food they buy, where the farmers and producers are able to take the labor value of the food they produce and sell as monetary value and where the conditions are fair."* In addition, it is stated that the important point of fair food is to provide the wage and working conditions that the producers deserve, and to prevent the pressure and exploitation on them.

This study is important to gain knowledge of the philosophy of "clean, fair, healthy food" in slow food movement. Moreover, it is important that students and educators are trained in terms of slow food awareness and thus slow food culture is promoted in school settings. For students and educators it is important to give food education and to promote awareness. Furthermore, this study is significant because today, the field of gastronomy tourism shifts from the spiral of sea-sun-sand culture to culture tourism and slow food tourism.

In this study, it is aimed to develop a scale that reveals the knowledge level of the university students about slow food and do validity and reliability studies of the developed scale. At the same time, it is also aimed to provide a training to the students about slow food and determine the effectiveness of this training. In light of these aims, the following sub-problems were tried to be answered:

1. What are the validity and reliability results of the scale developed to measure the awareness of the students about slow food?
2. Is there a statistical difference between the average of slow food awareness of the students before and after the training?
3. Is there a statistically significant difference between the mean scores of food awareness of the students before the training according to their sex?
4. Is there a statistically significant difference between the mean scores of food awareness of the students after the training according to their sex?

Materials and Methods

Research Design

In the first phase of the research, descriptive design was used because of the scale development process (11). In the research, it was aimed to reveal the effectiveness

of the training given the students in the gastronomy and culinary arts department and the students in the cookery department about slow food. In light of this purpose, at the first stage, the developed Scale that have 22 items was applied to the students as pre-test in order to measure their knowledge level about slow food by the researchers. At the second stage, the training about slow food was given to the students by means of several various methods such power point presentation, question-answer, group discussion, demonstration, brain storming, dramas, games and storytelling. This training lasted two months (eight weeks) and was provided to the students once a week and 30 minutes a week. In the second phase of the training, visual materials were applied to the students during the application. After the training, the developed scale was applied to the students for the second time as post-test. In this research, single group pretest-posttest design of experimental research design was used to reveal the effectiveness of the training provided. In this design, the effect of the experimental process is tested on a single group (12).

Study Group

Two different study groups were included in this study. The study groups included the students studying at the department of Gastronomy and Culinary Arts and the Cookery Vocational Higher School in Abant İzzet Baysal University in 2017-2018 Academic Year in Mengen, Bolu.

Since it is possible to reveal the effectiveness of the method in the study, it is not necessary to select the sample from the population in experimental design studies (12). The number of the students who participated in the scale development process was 210 and the experimental design process was carried out according to the answers of 185 students.

When the distribution of the demographic characteristics of the students in the second study group was examined, it was seen that 55.1% were female and 44.9% were male. It was found that the students learned about slow food through press (30.8%), internet (32.4%) and the notice boards and courses at university (36.8%).

Data Collection Tools

The research data were collected by the measurement tool developed by the researchers. "Student Personal Information Form" and "Slow Food Awareness Scale" were used in the study. The data were collected from the students by the researchers.

The Slow Food Awareness Scale

In order to determine the awareness level of the students about slow food, a literature review was conducted in the first stage (3, 4, 6, 10, 13, 14, 15, 16). In light of the literature review, 30 items in three subdimensions were formed in the item pool. These 30 items in three subdimensions were examined according to the opinions of three experts in order to decide whether they were appropriate for the aim of the study and comprehensible. In this context, inappropriate and unclear items were removed. The draft form of the scale was applied to 25 students from gastronomy and culinary arts department and cookery school and whether the items were clear enough to understand was tested. Each item in the scale is in the 5-point Likert-type (1= I totally disagree; 5=I totally agree). The items in the scale were arranged as positive and negative, and the negative items were first reversed and then scored in the analysis.

In order to ensure the construct validity of the scale, a pilot application was made to 210 students studying at the gastronomy and culinary arts and cookery departments (7 times the number of items). Validity and reliability analysis were conducted on the data collected for the pilot application.

Data Analysis

The data collected in accordance with the purpose of the study were recorded in the SPSS-21 package program. In the scale development process, the data collected from 210 students in the first study group were used to conduct the validity and reliability analysis. For the construct validity of the scale, the exploratory factor analysis was made; for the internal validity of the items, total item correlation analysis was conducted and for the internal consistency reliability of the scale and its subdimensions, Cronbach Alpha reliability analysis was performed. In the experimental study, the scale developed by the researchers was re-applied to 185 students as pre-test and post-test. Skewness and kurtosis coefficients were calculated in order to examine the distribution of the scores ob-

tained from the students. The skewness and kurtosis coefficients was observed to vary in the -1 to +1 intervals. The skewness and kurtosis values of the students' pre-test and post-test scores from the scale and its subdimensions were given in Table 1.

In Table 3, it is seen that the skewness and kurtosis values which were examined to test the normality assumption vary between -1 and +1. It is stated that skewness and kurtosis coefficients can be accepted to be between -1 and +1 as a measure of normality assumption (17). In addition, histograms of each score were examined and it was found that the data sets did not show a deviation from the normal distribution. When the homogeneity of the test variance, namely the distribution of the Levene homogeneity test, was examined, it was concluded that the test variance of the distribution of points, according to Levene statistic with $p>0.05$, was distributed homogeneously. It was seen that the distribution of the points obtained from the scale is continuous data and it is at the level of interval scale. The fact that two samples (groups) were independent of each other, the dependent variables were measured at the interval scale or ratio scale level and the assumptions of normality and homogeneity were met showed that the parametric test assumptions were realized (18). Paired-Samples T-Test was used to analyze the differences between the students' pre-test and post-test scores they obtained from the scale and its subfactors. The differences between the students' pretest and posttest scores according to their sex were examined by Independent-Samples T-Test analysis.

Table 1. Skewness and kurtosis values regarding the normality of the scores from the scale and its subfactors

		Skewness	Kurtosis
Pre-Test	Healthy	-.558	-.153
	Clean	-.862	.305
	Fair	.514	-.075
	Overall	-.039	-.092
Post-Test	Healthy	-.339	.825
	Clean	-.194	.224
	Fair	-.081	-.854
	Overall	-.291	-.448

Results

1- What are the validity and reliability results of the scale developed to measure the awareness of the students about slow food?

When the results of the Kaiser-Meyer-Olkin (KMO) and Bartlett tests, which show the appropriateness of the data to the factor analysis, are examined, it is seen that the KMO fitness measure value is 0. 91. Kaiser (19) states that the calculated value is perfect as it gets closer to 1 and unacceptable when it is under 0.50 (perfect in 0.90, very good in 0.80, mediocre in 0.70 and 0. 60, unacceptable in 0.50). The calculated Bartlett Test of Sphericity was 2758.32 with a significance level of 0.01 (X^2_{231}= 2758.32). According to these values, the KMO value in the initial application reveals that the data set constitutes a perfect structure for factor analysis. A significant calculation of the Bartlett test shows that there are high correlations between variables, in other words, the data set is suitable for factor analysis (20). The eigenvalues and exploratory variances of the final version of the factor structure resulting from the exploratory factor analysis were given in Table 2.

As shown in Table 2, there are three factors with eigenvalues higher than 1.0. The variance that is explained by these three factors constitutes 57.98 % of total variance. When initial and after rotation eigenvalues and exploratory variances of the factors were compared, it can be seen that the eigenvalue of the first factor declined to 5.62 from 8.77 and the exploratory variance declined to 25.55 % from 39.87%. It is seen that the eigenvalue of the second factor increased from 2.84 to 4.24 and the exploratory variance increased from 12.91% to 19.27%. It is observed that the eigenvalue of the third factor increased from 1.14 to 2.90 and exploratory variance increased from 5.20% to 13.16%. The variance values explained by the factors

Table 2. Factor eigenvalues and exploratory variances

	Initial Eigenvalues			Total after rotation		
Factor	Total	Variance %	Cum%	Total	Variance %	Cum %
1	8.77	39.87	39.87	5.62	25.55	25.55
2	2.84	12.91	52.78	4.24	19.27	44.82
3	1.14	5.20	57.98	2.90	13.16	57.98

before and after the rotation were decreased in the first factor while increasing in the other factors.

As shown in Table 3, the items 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 have the highest factor load values in the first factor; the items 11, 12, 13, 14, 15, 16, and 17 in the second factor; the items 18, 19, 20, 21 and 22 in the third factor. It can be seen that in the first factor, the factor loads ranged from 0.55 to 0.78; in the second

Table 3. Factor load values, item total correlation values and reliability coefficients

	Factors			Item Total Correlation	Subfactor Reliability	Total Reliability
	Healthy	Clean	Fair			
1. The principle of "healthy" defends nutrition appealing to sense organs.	0.70			0.68*		
2. The principle of "healthy" offers nutrition with its own taste, smell, colour, shape, and tissue.	0.70			0.66*		
3. The principle of "healthy" protects local and traditional nutrition.	0.66			0.71*		
4. The principle of "healthy" stresses the importance of increasing the quality of the meals and sparing time for eating.	0.76			0.73*		
5. The principle of "healthy" leads to traditional meals instead of fast food.	0.78			0.74*	0.90	
6. The principle of "healthy" does not mean cooking meals on low heat.	0.62			0.58*		
7. The principle of "healthy" defends consuming local food growing only in the related region.	0.70			0.67*		
8. The principle of "healthy" works for passing the eating/feeding culture on to the next generations.	0.72			0.70*		
9. The principle of "healthy" is beneficial for health.	0.56			0.60*		
10. The principle of "healthy" appeals to culture tourism.	0.55			0.58*		
11. The principle of "clean" aims to protect nature and environment.		0.70		0.63*		
12. The principle of "clean" promotes consuming beneficial nutrition for human health.		0.64		0.64*		0.92
13. The principle of "clean" aims to protect all animal races and all kinds of fruit and vegetables.		0.60		0.66*	0.89	
14. The principle of "clean" includes nutrition produced organically.		0.64		0.71*		
15. Appropriateness to the principle of "clean" makes me feel healthier.		0.61		0.70*		
16. The principle of "clean" stresses that products should be recycled and alternative usage areas should exist.		0.77		0.71*		
17. Unlike Fast Food, the appropriate products in terms of the Slow Food principle of "clean" removes doubt.		0.79		0.74*		
18. The principle of "fair" defends that the chain between food producers and consumers should be short.			0.74	0.58*		
19. The principle of "fair" struggles for a food sector with fair conditions.			0.76	0.61*		
20. The principle of "fair" aims to stop the pressure and exploitation on the producers.			0.70	0.56*	0.79	
21. The principle of "fair" favors providing the producers with the best working conditions.			0.70	0.53*		
22. The principle of "fair" defends that the chain between food producers and consumers should be short.			0.73	0.56*		

p<.05

factor, the factor loads of the items ranged between 0.60 and 0.79 and the factor loads of the third factor ranged between 0.70 and 0.76. According to Tabachnick and Fidell (21), it was determined that the load value of item was mediocre when it is under 0.45 critical value. In this scope, the items with factor loads below 0. 45 were excluded from the scale. After the analysis of the remaining 22 items, the reporting process was started. The Scale was observed to have three subfactors and 22 items (10 items in the "Healthy" subfactor, 7 items in the "Clean" subfactors, 5 items in the "Fair" subfactors) and it was called "Slow Food Awareness Scale".

For the reliability of the scale, the Cronbach Alpha internal consistency Coefficients regarding the three subfactors of the scale were given in Table 3. The reliability coefficient of the "healthy" subfactor is 0.90, the reliability coefficient of the "Clean" subfactor is 0.89 and the reliability coefficient of the "Fair" subfactor is 0.79. The overall reliability coefficient of the scale is 0.92. Reliability coefficients for the subscales and the overall scale show that the scale has acceptable reliability levels. It is stated that the reliability coefficient of a Likert type scale should be close to 1 as much as possible in order for that scale to be accepted as a reliable scale (22). The reliability coefficients of 0.60 and over are regarded as acceptable reliability levels (20).

2- Is there a statistical difference between the average of slow food awareness of the students before and after the training?

In the research, it was aimed to determine the effect of the training about slow food on the students' awareness and information level about slow food. In this respect, the calculations regarding the scores the students obtained from the Slow Food Awareness Scale before and after the training were performed. The results are shown in Table 4.

In Table 4, it can be seen that there is a statistically significant difference ($t_{(184)}$=14.53, p<.05) between the pretest ($\bar{X}$ =41.08) and posttest ($\bar{X}$ =47.69) scores the students obtained from the "Healthy" subfactor of the scale. Similarly, a statistically significant difference ($t_{(184)}$=9.83, p<.05) is observed between the pretest ($\bar{X}$ =30.75) and posttest ($\bar{X}$ =33.58) scores of the students from "Clean" subfactor. Likewise, a statistically significant difference ($t_{(184)}$=9.73, p<.05) is seen between the pretest ($\bar{X}$ =12.68) and posttest ($\bar{X}$ =16.58) scores of the students from "Fair" subfactor. Furthermore, there is a statistically significant difference ($t_{(184)}$=16.84, p<.05) between the total pretest ($\bar{X}$ =84.50) and total posttest ($\bar{X}$ =97.85) scores the students obtained from the overall scale.

Table 4. Paired samples t-tests results regarding the differences between the students' pretest and posttest scores from the slow food awareness scale

	Test	n	$\bar{X}$	S	t	sd	p
Healthy	Pretest	185	41.08	5.71	14.53	184	.000*
	Posttest	185	47.69	3.22			
Clean	Pretest	185	30.75	3.71	9.83	184	.000*
	Posttest	185	33.58	2.00			
Fair	Pretest	185	12.68	4.61	9.73	184	.000*
	Posttest	185	16.58	5.18			
Total	Pretest	185	84.50	9.36	16.84	184	.000*
	Posttest	185	97.85	7.15			

*p<.05

3- Is there a statistically significant difference between the mean scores of food awareness of the students before the training according to their sex?

Independent Samples T-test was used to determine whether there is a significant difference in the awareness levels of the students about slow food according to their sex before the training and the results were shown in Table 5.

In Table 5, it can be seen that there is a statistically significant difference ($t_{(183)}$=1.99, p=<.05) between

Table 5. Independent samples t-test results regarding difference between the pretest scores of the students from the scale and its subscales according to their sex

	Sex	n	$\bar{X}$	S	t	sd	p
Healthy	Female	102	41.82	5.20	1.99	183	.048*
	Male	83	40.16	6.19			
Clean	Female	102	31.23	3.48	1.94	183	.054
	Male	83	30.17	3.91			
Fair	Female	102	11.82	4.13	2.84	183	.005*
	Male	83	13.72	4.97			
Total	Female	102	84.87	8.67	.60	183	.553
	Male	83	84.05	10.18			

*p<.05

the pretest scores of the female students ($\bar{X}$ =41.82) and the male students ($\bar{X}$ =40.16) in the "Healthy" subscale. On the other hand, no significant difference ($t_{(183)}$=1.94, p>.05) is observed between the pretest scores of the female students ($\bar{X}$=31.23) and male students ($\bar{X}$ =30.17) in the "Clean" subscale. However, in the "Fair" subscale, a statistically significant difference ($t_{(183)}$=2.84, p<.05) between the pretest scores of the female students ($\bar{X}$=11.82) and the male students ($\bar{X}$=13.72) is seen. In addition to these results, there is no statistically significant difference ($t_{(183)}$=.60, p>.05) between the total pretest scores of the female students ($\bar{X}$=84.87) and the male students ($\bar{X}$=84.05) from the Slow Food Awareness Scale.

4- Is there a statistically significant difference between the mean scores of food awareness of the students after the training according to their sex?

Independent Samples T-test was used to determine whether there is a significant difference in the awareness levels of the students about slow food according to their sex after the training and the results were shown in Table 6.

In Table 6, it can be seen that there is no statistically significant difference ($t_{(183)}$=1.87, p>.05) between the posttest scores of the female students ($\bar{X}$ =48.09) and the male students ($\bar{X}$ =47.20) in the "Healthy" subscale. Likewise, no significant difference ($t_{(183)}$=1.49, p>.05) is observed between the posttest scores of the female students ($\bar{X}$=33.77) and male students ($\bar{X}$=33.34) in the "Clean" subscale. Similarly, in the "Fair" subscale, no statistically significant difference ($t_{(183)}$=1.84, p>.05) between the posttest scores of the female students ($\bar{X}$=15.95) and the male students ($\bar{X}$=17.35) is seen. In addition to these results, there is no statistically significant difference ($t_{(183)}$=.07, p>.05) between the total posttest scores of the female students ($\bar{X}$=97.81) and the male students ($\bar{X}$=97.89) from the Slow Food Awareness Scale.

Table 6. Independent samples t-test results regarding difference between the posttest scores of the students from the scale and its subscales according to their sex

	Sex	n	$\bar{X}$	S	t	sd	p
Healthy	Female	102	48.09	3.01	1.87	183	.063
	Male	83	47.20	3.42			
Clean	Female	102	33.77	1.88	1.49	183	.139
	Male	83	33.34	2.11			
Fair	Female	102	15.95	4.79	1.84	183	.068
	Male	83	17.35	5.56			
Total	Female	102	97.81	6.82	.07	183	.941
	Male	83	97.89	7.57			

*p<.05

Discussion

In this study, the structure validity of the Slow Food Awareness Scale was determined through exploratory factor analysis. When the results of the factor analysis results regarding the validity of the scale are examined, it can be seen that the scale includes three subscales and 22 items (10 items in the Healthy subscale, 7 items in the Clean subscale and 5 items in the Fair subscale). When the reliability coefficients of the scale were examined, it was determined that it has acceptable reliability level. From these results, it can be understood that the Slow Food Awareness Scale can be used to measure the students' awareness towards slow food.

In the second phase of the study, the main aim was to determine the effectiveness of the training given to the students about slow food. It was understood that the students who participated in the study learn about slow food through mostly school notice boards and courses, internet and press. Clancy (23), on the other hand, puts forwards that the information about slow food is transferred through hosting guests and making home visits and these are effective in learning and spreading the awareness and culture about slow food movement.

In these research findings, it is seen that the students' awareness level towards slow food movement and philosophy in the "Healthy" subscale is lower before the training than their level after the training. It was determined that the training given to the students about this movement increased the students' level of awareness in the "Healthy" subdimension. Similarly, the results show that the students' awareness level about slow food in the "Clean" subdimension increased after the training. In this scope, it can be said that the training given to the students affected their aware-

ness level positively due to the fact that the training appealed to the students both visually and aurally. In parallel with the increase in the awareness of the students in the Healthy and Clean subdimensions after the training, the students' awareness level in the Fair subdimension increased after the training, as well. The results showed that the training became effective in increasing the students' awareness level about slow food movement in all three subdimensions that constitute the Slow Food. This increase in the subdimensions was observed in the overall scale results in the same way; that is, the training given to the students by using such methods as power point presentations, question- answer techniques, group discussions, demonstrations, brain storms, dramas, games and storytelling brought about a rise in their awareness level about slow food. In the related studies conducted, it is stated that educators in the slow food movement expressed that they tried to reach a greater level of awareness by providing food producers and consumers with necessary trainings (1,24). KIT (25), in a study, emphasized that increasing the awareness and promoting the change in the consumption trends could be realized through slow food trainings with focus on food with high biodiversity.

Nosi and Zanni (26) in their research on university students and instructors in gastronomy and culinary arts departments state that the principle that hedonic taste and slow food are cultural phenomenon can be promoted through educational activities, conferences and workshops related to sensory taste and food culture. Yurtseven (13) states that slow food movement is important to promote the palatal delight trainings. Aytimur (27) points out that social projects and educational activities about slow food movement are necessary to preserve and promote the local and traditional cooking methods.

In the study, in the Healthy subscale, the female students have higher awareness level than the male students before they got the training about slow food. This shows that at the very beginning of the training, the female students were more aware of the slow food than the male students. On the other hand, it is seen that before the training, both male and female students have similar awareness level in the "Clean" subdimension about the slow food. However, the male awareness level was higher than the female students in the "Fair" subscale of the Slow Food Awareness Scale at the beginning of the training. When overall scores that the students obtained from the Slow Food Awareness Scale were examined, the awareness level about the philosophy and movement of slow food was similar for both female and male students before the training. It can be said that this similarity in the female and male students' awareness level about slow food is due to the fact that slow food concept is related to the students's departments. Aytimur (27) suggests that the participant below 25 yers old have lack of awareness about slow food. Çatalca (28) states that the formation of Mother Earth finds its place in the aim of the slow food movement and thus studies and attempts have been initiated to increase the capacity of the local societies in order to provide "healthy", "clean" and "fair" food. In the KIT (25) Conference, it is emphasized that food biodiversity and unique traditional products in the slow food movement and philosophy should be taught to the students and adults in order to raise their awareness and protect the available food standarts about these unique products. In addition to this, it is stressed that the number of the products in the Taste Box has increased over the last five years. Moreover, it is stated that today, there are more than 4500 products that should be accepted to the Taste Box and should be in evaluation and under protection (30).

In a study conducted by Aytimur (27), it is stated that on the basis of the slow food movement is a healthier, cleaner, better and fairer producing and consuming food and a struggle should be realized against the national and international food companies in pursuit of easy and unfair benefits in the globalised developed world.

Another finding of the study is that there is no difference between the students' awareness scores in the healthy, clean and fair sub-dimensions of the Slow Food Awareness Scale and their total scores after the training according to the students' sex. At the end of the training process, it was determined that both female and male students were aware of the Slow Food Movement and Philosophy at similar levels. Especially it is seen that the difference in the awareness level about slow food between the female and male students before the training was removed through the

training. It is determined that the training eliminated the difference in the level of awareness arising from the sex variable, meaning that training is effective in equalizing the awareness levels of both girls and boys. KIT (25) states that it is necessary to conduct activities and workshops that will increase information and awareness about Slow Food. It is emphasized that girls should be educated more and more on this issue.

Kavas and Kavas (31) put forward that it is possible to make individuals follow a health diet by means of educational activities to protect local and traditional gastronomy culture in each country and in each region in each country, which constitutes the fundemental element of the slow food movement. Mother Earth (29) in another study, states that important national and international studies, conferences and workshops have been conducted in Turkey to promote the production of slow food. On the other hand, Sağır (10) emphasizes that slow food applications and movements fall short of feeding more than 10 billion people who have starvation problems in the world because although slow food movement is an alternative way, it can only be applied in the developed countries and it cannot be adapted to the developing countries.

Conclusions and suggestions

In this study, it was observed that the posttest scores the students obtained from the Slow Food Awareness Scale and its subscales were higher that their pretest scores and there is a statistically significant difference between these scores. At the end of the study, it was seen that the students' awareness level about slow food before the training was lower that their awareness level after the training.

In the awareness level according to the students' sex before the training, it was observed that while the female students have higher awareness level in "Healthy" subscale, the male students' awareness level was higher in the "Fair" subscale. The students' awareness level does not differed significantly in the "Clean" subscale and the overall scale.

In the awareness level according to the students' sex after the training, it was observed that the students get similar posttest scores in all three subscales and the overall scale. This results show that the training eliminated the difference caused by the sex variable between the female students and male students in their awareness scores.

In light of the results obtained at the end of this study, following recommendations can be made:

1. Through constant educational activities about the philosophy of slow food on social media websites, at schools and institutions an alternative way to the industrialized agricultural applications can be provided in order to deal with starvation in the future.
2. Experts on nutrition can organize training and education programs about slow food and thus people's awareness about lack of food, malnutrition and the danger of biodiversity extinction can be raised.
3. Further studies can be conducted with different and larger study groups to obtain various realiable and valid measurement tools and thus more comprehensible data can be obtained and clear comparisons can be made.
4. Further studies with more complex models such as structural equation modelling can be conducted by using larger samples and different variables.

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Assessment of vitamin D status in turkish adolescents: its relation to obesity, cardiometabolic risk factors and nutritional status

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Summary. Studies on effects of vitamin D on human body have indicated that vitamin D deficiency may contribute to an increased risk of obesity and cardiometabolic risk factors, during childhood; however, these relationships remain unclear. The aim of this study is to determine the influences of adiposity, dietary and environmental factors on vitamin D status and the relationship between 25(OH)D and cardiometabolic risk factors in adolescents. The research was carried out in 69 adolescents (60.9% female, 39.1% male) who applied to the Istanbul Marmara University Pendik Educational Research Hospital Pediatric Endocrinology Department, aged between 12 and 17 years. Data was collected in three stages; in the first stage, the questionnaire was developed including the demographic status, nutritional status and physical activities of those adolescents. In the second stage, anthropometric measurements were taken. In the third and final stage, biochemical analysis was measured. There were no statistical differences between groups (Vitamin D deficient and sufficient) in dietary and environmental factors that may have affected serum 25(OH)D. Serum 25(OH) D level was not inversely correlated with anthropometric measurements ($p>0.05$). While there were no significant differences between serum 25(OH)D levels and HOMA-IR; there was a positive correlation between serum calcium and HOMA-IR ($r=0.276$, $p<0.05$) independent of body adiposity. There was a positive correlation between serum 25(OH)D levels and amount of daily dietary protein ($r=0.344$) and fat intake ($r=0.286$) ($p<0.05$), but there was no correlation between serum 25(OH)D and dietary vitamin D and calcium intake ($r=-0.022$, $r=0.235$; respectively). Because foods are not primary sources of vitamin D, this correlation should be assessed with further research on population who consume fortificated food. The results from this study suggest the importance of vitamin D supplementation and food fortification in Turkish adolescents. This study didn't show relationship between vitamin D status and obesity, insulin resistance. Effects of vitamin D deficiency on chronic diseases need to be assessed with prospective studies.

Key words: Adolescent, Insulin Resistance, Obesity, Vitamin D

Introduction

The known task of vitamin D is to provide mineral homeostasis and skeletal function. There are two sources of vitamin D, cholecalciferol (vitamin D3) synthesized at the skin and ergocalciferol (vitamin D2) taken with food.[1]

Vitamin D deficiency, defined as a decrease in serum 25-hydroxyvitamin D level, is a common problem for all age groups.[2] Lack of exposure to direct sunlight, changes in the climate, clothing preferences and inadequate vitamin D content in the diet may result in a deficiency of vitamin D intake.[3] Vitamin D deficiency causes metabolic bone diseases and may increase the risk of chronic diseases such as type 2 diabetes and cardiovascular diseases.[2,3]

Obesity is defined by World Health Organization as abnormal or excessive fat accumulation that

may impair health. Centers for Disease Control and Prevention (CDC) assess pediatric obesity with age- and sex-specific growth charts.[4] Between 85th and 95th percent is considered at risk of overweight; 95th percentile is considered obese.[5] In obese children and adolescents, cardiovascular diseases such as hypercholesterolemia, hypertension, dyslipidemia and endocrine system diseases such as hyperinsulinemia, insulin resistance, impaired glucose tolerance, type 2 diabetes mellitus are common.[6]

The aim of this study was to determining the influences of gender, adiposity, dietary and environmental factors on vitamin D status and the relationship between 25(OH)D and cardio metabolic risk factors in adolescents.

Subjects and Methods

Study Population

The research was carried out in 69 adolescents who applied to participate in the study at İstanbul Marmara University Pendik Educational Research Hospital Pediatric Endocrinology Department aged between 12 and 17 years). Children with acute or chronic infections, genetic syndromes, cancers, auto immunologic diseases, hepatic or renal dysfunction, hormonal abnormalities or diabetes who are using any drug that may affect the blood and urine parameters (especially drugs including vitamin D and calcium and anticonvulsant therapy), and adolescents with eating disorders were excluded. The protocol of the study was approved by the Ethical Committee of the Marmara University.

Study Design

Data was collected in three stages. In the first stage, the questionnaire was developed including the demographic status, nutritional status and dietary habits as well as physical activities of adolescents under study. In the second stage, anthropometric measurements (weight, height, body mass index, waist circumference) were taken. In the third and final stage, biochemical analysis was measured. Insulin resistance was calculated using the homeostasis model assessment (HOMA-IR).

Anthropometric Measurements

Weight was measured provided that the subjects were lightly clothed and without any shoes using a digital scale to the nearest 0.1 kg, and height was measured with a calibrated wall-mounted stadiometer to the nearest 0.1 cm (Seca, Hamburg, Germany). Body mass index (BMI) [weight (kg)/height (m)^2] was calculated using gathered data. The minimal abdominal circumference between the xiphoid process and iliac crest was measured to determine the waist circumference.

Dietary assessment

Participants recorded the foods and beverages as well as the amount of consumption during a period of three days (Thursday, Friday, Saturday or Sunday, Monday, Tuesday). The nutrient composition of the food was computed by using a nutrition software (Ebispro for Windows, Stuttgart, Germany; Turkish version BeBiS, Version 6.1) which uses data source 97% the BLS (Bundeslebensmittelschluessel; German Food Code and Nutrient Data Base; Version II.3). The nutrient values for the remaining food items were taken from the US Department of Agriculture (USDA) data base.

Biochemical Analysis

Blood samples were taken in the morning (following an overnight fasting). Serum 25(OH)D, parathormone (PTH), calcium (Ca), phosphorous (P), hemoglobin A1C, insulin and glucose were analyzed by the Biochemistry laboratory of Faculty of Medicine in Marmara University.

Vitamin D insufficiency was defined as serum 25-hydroxyvitamin D concentrations of <20 ng/mL. Serum 25-hydroxyvitamin D levels of >20 ng/mL were considered optimal.[7] The homeostasis model was used for assessing insulin resistance (HOMA-IR) using the following formula: HOMA-IR = fasting blood glucose (mmol/L) ~ fasting insulin (μU/mL)/ 22.5.[8] For adolescents HOMA-IR ≥ 3.16 was considered insulin resistance.[9]

Statistical analysis

Statistical analysis was performed using the SPSS version 17.0. Data are expressed as mean or as percentage. Means and standard deviations were used to sum-

marize continuous variables that were normally distributed. Kolmogorov-Smirnov test was used to check for normality of distribution; $p < 0.05$ was considered evidence of abnormality. Student's t -test was used to compare mean values between men and women. The X^2 test was used to analyze both differences in proportion and distributions of obese and non-obese. Pearson or Spearman coefficient of correlation was used to assess linear correlation between 25(OH)D and other variables.

Results

The demographic characteristics based on serum vitamin D levels are summarized in Table 1. The mean age (range) of the adolescents was 13.3±1.5 years (12-17), 60.9% were female (n=42) and 39.1% (n=27) male. Participants were divided into broadly defined subsets based on serum vitamin D levels as vitamin D insufficient and sufficient. The mean BMI was 26.45±6.15 in the vitamin D insufficient group, 27.25±6.26 in the vitamin D sufficient group. There was no statistically difference between groups in term of BMI.

Serum levels of 25(OH)D did not differ according to gender, weight, and waist circumference. None of participants were taking vitamin D containing supplements. Prevalence of vitamin D deficiency in females is more common and there was no effect of wearing concealing on serum vitamin D level. Daily time spend watching TV was 2.37±1.29 hours in the vitamin D insufficient group while it is 2.09±1.2 hours in the vitamin D sufficient group however, there was no statistically difference between groups. Time spent in outdoor exercise was 3.15±2.24 hours a week in the vitamin D insufficient group whilst the quantity is 2.91±1.75 in the vitamin D sufficient group and did not differ on groups ($p>0.05$). Similarly average of duration of time spent under the sun did not differ on groups ($p>0.05$).

Biochemical variables are presented in Table 2. In this study, there were no differences in lipid metabolic profile, HbA1c, fasting blood glucose and HOMA-IR.

Serum vitamin 25(OH)D was positively correlated with dietary intake of proteins ($r= 0.344$, $p< 0.01$)

Table 1. Assessment of characteristics according to vitamin D status

Characteristic	Vitamin D insufficient (<20 ng/mL) (n=48)	Vitamin D sufficient (≥20 ng/mL) (n=21)	p Value
	Mean ± SD (median)	Mean ± SD (median)	
[a]Age	13.33±1.56	13.19±1.25	0.713
[b]Gender	n (%)	n (%)	
Female	32	10	0.221
Male	16	11	
[a]Body Mass Index	26.45±6.15	27.25±6.26	0.624
Waist Circumference (cm)	87.0±15.61	90.5±14.43	0.249
Waist to Hip Ratio	0.87±0.1	0.92±0.09	0.67
[d]Wearing Concealing Clothing			
Yes	3 (%12.5)	0	0.539
No	21 (%87.5)	10 (%100)	
[c]Time Spend Watching Tv (day)	2.37±1.29 (2)	2.09±1.22 (2)	0.527
Outdoor Exercise and Active Play in a Week (hours)	3.15±2.24	2.91±1.75	0.787
Taking Vitamin D Containing Supplements	n (%)	n (%)	
Yes	-	-	1.00
No	-	-	
Vitamin D Dietary Intake (μg)	1.45±1.21	1.34±0.58	0.672
[c]Average of Duration of Time Spent Under the Sun (min)	25.10±13.51 (20)	29.52±25.78 (20)	0.995

[a]Student t test; [b]Yates Continuity Correction; [c]Mann Whitney U test

Table 2. Comparison of biochemical parameters of vitamin D insufficient and vitamin D sufficient adolescents

Biochemichal Parameters	Vitamin D insufficient (<20 ng/mL) (n=48)	Vitamin D sufficient (≥20 ng/mL) (n=21)	[a]p Value
	Mean ± SD (median)	Mean ± SD (median)	
HbA1C %	5.04±0.43	5.22±0.33	0.109
[c]Triglyceride (mg/dl)	112.04±70.88	108.90±58.79	0.86
Total Cholesterol (mg/dl)	171.37±42.71	154.85±25.50	0.105
HDL (mg/dl)	52.56±15.02	46.62±11.45	0.111
LDL (mg/dl)	96.52±37.88	85.71±20.73	0.224
Serum Ca (mg/dl)	9.94±0.45	10.13±0.55	0.15
PTH (pg/ml)	50.57±16.38	44.27±17.59	0.155
Fasting Blood Glucose (mg/dl)	88.41±9.21	83.41±11.54	0.059
[c]Fasting Insulin	20.13±14.16 (16.5)	26.24±30.71 (14.6)	0.597
[c]Ca/Creatining (mg/g)	43.96±49.25 (30.96)	70.55±94.41 (50)	0.129
[c]HOMA-IR	4.46±3.48 (3.30)	5.16±5.97 (3.21)	0.527

[a]Student t test; [c]Mann Whitney U test

and fats (r= 0.286, p< 0.05), as listed in Table 3. There was no relation between serum 25(OH)D levels and fasting plasma glucose, HOMA index, serum PTH and insulin (p>0.05) (Table 3). Serum calcium level was positively correlated with HOMA index and insulin levels (r=0.247; p<0.05, r=0.267; p<0.05 respectively). Serum 25(OH)D, PTH, and calcium levels were not significantly correlated with any anthropometric measurements in adolescents (p>0.05) (Table 3).

Table 3. Correlation between 25(OH)D, PTH and serum Ca levels and various other variables

	PTH		Vitamin 25(OH)D		Serum Ca	
	r	*p*	r	*p*	r	*p*
Daily dietary nutrients						
Carbohydrate (g)	-0.163	**0.181**	0.188	**0.123**	0.008	**0.945**
Protein (g)	-0.275	**0.022***	0.344	**0.004****	0.227	**0.060**
Fat (g)	-0.135	**0.267**	0.286	**0.017***	0.108	**0.379**
Vitamin D (μg)	0.056	***0.648***	-0.022	***0.860***	0.195	***0.108***
Calcium (mg)	-0.235	***0.052***	0.235	***0.052***	0.110	***0.367***
Biochemichal Parameters						
Serum Ca	*-0.076*	***0.533***	*0.214*	***0.077***	*1*	***1***
PTH	*1*	***1***	*-0.167*	***0.170***	*-0.076*	***0.533***
Fasting Blood Glucose (mg/dl)	0.130	***0.289***	-0.101	***0.410***	0.221	***0.068***
[+]HOMA - IR	0.232	***0.055***	-0.013	***0.914***	0.247	***0.041****
25(OH)D	*-0.167*	***0.170***	*1*	***1***	*0.214*	***0.077***
[+]Insulin	0.203	***0.094***	-0.082	***0.504***	0.267	***0.026****
Anthropometric Measurement						
Weight						
BMI (kg/m^2)	0.094	***0.445***	-0.006	***0.962***	0.178	***0.144***
Waist Circumference (cm)	0.048	***0.694***	0.096	***0.430***	0.232	***0.055***

*r:Pearson Korelasyon; r:+Spearman's Correlation; *p<0.05; **p<0.01*

Discussion

Hypovitaminosis D is a problem in both adults and children.[10] In the United States, 27% of adolescents have vitamin D deficiency, which came to light from the result of the data in the National Health and Nutrition Examination Survey (NHANES).[11] Similarly, in the UK between the ages of 4 and 18 years, D vitamin insufficiency was found to be 35%.[12] According to the Endocrine Society Clinical Practice Guidelines, serum 25-hydroxyvitamin D level is the best indicator of vitamin D levels.[7] In our study vitamin D insufficiency was defined as plasma 25(OH)D concentration below 20 ng/ml.

Exposure to sunlight, measured on the basis of the time spent outdoors, the wearing of ultraviolet radiation-blocking clothing was also assessed in this study. As reported by Demirçeken, half of the girls in our country prefer concealing clothing and most of the adolescents' time spend outside are limited.[13] It is stated as the prevalence of low vitamin D in western-style women is 31%; 55% for hijab wearers; 83% in neqab wearers.[14] There are no differences between groups in terms of their clothing preference. Also, our study was conducted during winter, so results may be different in summer season.

Lack of physical activity can be independent risk factor for vitamin D deficiency because of limited exposure to sun light.[15] Children with deficiency of vitamin D have less physical activity (60.6%), compared with normal children (49%).[16] In a pediatric population aged 4-18 years, doing outdoor physical activity for less than half an hour a day or watching TV more than 2.5 hours a day increases vitamin D deficiency.[17] No differences in exposure to sunlight, outdoor activities under the sun, and physical activity among groups were found in our study ($p>0.05$).

Effects of vitamin D on obesity, cardiovascular disease and diabetes are contradictory in childhood.[18] Data obtained from the National Health and Nutrition Examination Survey (NHANES) indicate that serum 25(OH)D levels are lower in obese children.[19] Drincic et al showed that there is an inverse relationship between vitamin D levels and anthropometric measures.[20] In this study, serum levels of 25(OH)D did not differ in weight, BMI, and waist circumference and there was not a significant correlation among them.

There may be a link between low vitamin D levels and cardiovascular disease, type 2 diabetes.[21] Hypovitaminosis D has long been suspected as a risk factor for glucose intolerance. Ayesha showed that insulin secretion decreased significantly in vitamin D-deficient rats compared to control rats.[22] Zhang et al. found that serum 25 (OH) D levels were negatively correlated with insulin resistance in type 2 diabetic subjects.[23] Alemzedah et al. indicated that there is a relationship between serum vitamin D levels and insulin resistance, glucose intolerance in children and adolescents.[24] In a study conducted in high school in Turkey, no correlation was found between D vitamin deficiency and insulin measurements during oral glucose tolerance test.[25] Likewise, in this study there was no relationship could be established between serum 25(OH)D levels and fasting plasma glucose, HOMA-IR index and insulin ($p>0.05$).

Few studies have examined the relationship between 25(OH)D and cardiovascular risk factors in children and adolescents. Kumar et al., indicate that 25(OH)D deficiency was associated with elevated parathyroid hormone levels, lower serum calcium and high-density lipoprotein cholesterol levels in US pediatric population.[19] Liu et al., found that 25(OH)D levels are correlated with lower non-HDL cholesterol in children.[26] This finding is important because of the fact that non-HDL cholesterol is an important marker for cardiovascular disease risk. In our study there were no correlation between serum 25(OH)D levels and triglyceride ($r=0.062$), HDL-cholesterol ($r=-0.124$), LDL-cholesterol ($r=-0.093$), total cholesterol ($r=-0.105$ ($p>0.05$).

The main source of vitamin D is sunlight. Dietary intake alone doesn't meet vitamin D requirements to prevent deficiency states and associated problems.[27] Recommended daily allowances for vitamin D for adolescents aged 12-17 is 15 mcg.[28] Studies conducted with the children and adolescent implies that daily vitamin D intake is not adequate.[15,29,30] In this study daily dietary vitamin D intake did not meet RDA and there was no correlation between serum 25(OH) D and dietary vitamin D intake. Similarly milk and milk products, fish, and fortified foods are the main dietary sources of vitamin D.[31] Similar with Bezrati[32] et

al found, our results showed that dietary intake of proteins were positively correlated with serum vitamin D levels. Because foods are not primary sources of vitamin D, this correlation should be assessed with further research on population who consume fortificated food.

In conclusion, the present study highlighted the high prevalence of vitamin D insufficiency among Turkish adolescents. Because time spend outside is limited and the weather was winter in this study, it was difficult to assess the effect of sun light on serum vitamin D level. Nonetheless in Turkey, vitamin D fortification of food and preference is limited. Because of these reasons vitamin D supplementation should be recommended especially for this age group. On the other hand vitamin D fortification of food which preferred frequently could be helpful to maintain vitamin D levels. The another important result is about chronic diseases which could be related with vitamin D. This study didn't show relationship between vitamin D status and obesity, insulin resistance. Effects of vitamin D deficiency on chronic diseases need to be assessed with larger prospective studies.

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Practices, beliefs and attitudes about complementary feeding among turkish mothers: a qualitative study

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Summary. In this research the experiences, viewpoints and attitudes of mothers who have babies between 6 to 24 months old, related to transition to complementary feeding are explained. A qualitative investigation design was used. Research was carried out in 7 Family Health Centers (FHC) located in town of Ödemiş. Twenty mothers who had babies between 6 to 24-year old agreed to participate in the study. The experiences, viewpoints and attitudes of mothers having babies between 6 to 24-month old related to complementary feeding were analyzed in line with their knowledge, experiences, resources and cultural themes related to complementary feeding. Fourteen of mothers (70%) stated that they did not exactly know the right starting time of complementary feeding. Seven mothers (35%) reported that they had experiences babies' health problems such as constipation, growth-development failure, etc. after starting complementary feeding. Mothers' knowledge level about complementary feeding isn't enough. Primiparous mothers generally are to learn complementary feeding from their environment instead of health worker. Mothers should be encouraged to explain complementary feeding practices. Wrong practices should be corrected by health workers.

Key words. complementary feeding; nursing, qualitative research

Introduction

World Health Organization defines complementary feeding as giving mother's milk together with other foods or fluids from 6th month (1). Starting complementary feeding early before six months causes insufficient energy and food intake; whereas late start leads to ceasing or slowing down on the growth of infant (2,3). Therefore, starting the complementary feeding at the right time and with nutritional elements is highly important (4,5). However, it is well known that mothers do not wait for six months to initiate complementary feeding and generally start earlier (6-8). It was detected in investigations that mothers prefer solid foods (homemade cereals, ready-made cereals, etc.), baby formulas and fluid foods (anise, tea, fruit juice etc) when they start complementary feeding before six months and these applications varied according to communities and cultures (7-11).

Although children in our country are breastfed for a long time, complementary feeding is being started at the very early ages and 35.3 % of the 2-3 months old babies are fed only with mother's milk (12). It is reported that mothers' cultural beliefs, education levels, socioeconomic levels and social support systems affect babies' nourishment status (9) and applications and perceptions related to complementary feeding are extremely important in improving their feeding levels (2,5).

The fundamental strategy in improving the feeding of children is to develop knowledge and practices related to complementary feeding in families with 6-24 months old babies (1). Therefore, to evaluate moth-

ers' complementary feeding-related applications, experiences, viewpoints and attitudes is very important. While many quantitative studies have been carried out related to complementary feeding in Turkey, there are few qualitative studies on this topic. Therefore, the aim of this study is to evaluate mothers' experiences, viewpoints and attitudes related to transition to complementary feeding and the factors affecting them.

Method

A qualitative study design, phenomenological method was used to describe experiences, opinions and attitudes of mothers who have babies between 6 to 24 months old, related to transition to complementary feeding. A total of 20 mothers who are 18 years old and above, do not have verbal communication problem and have babies between 6 to 24 months old comprised the sampling of research. Participants' age varied between 18 and 38 years (mean age is 29.05 ± 5.51 years). Forty five percent of mothers are graduated from university and eighty percent of them are unemployed (Table 1). When their income levels were evaluated it was found that 60 % had income equal to expenses. Babies' age varied between 6 and 18 months (mean age is 10.1 ± 3.44 months).

Table 1. Mothers Demographics (n=20)

Demographics	Mothers N=20
Age	29.05±5.51
Education Status (%)	
Primary education	6 (30)
High School	5 (25)
Collage	9 (45)
İncome Situation **(%)**	
Equal to income	12 (60)
Over income	3 (15)
Less than income	5 (25)
Number of Children (%)	
One	8 (40)
Two	10 (50)
Three or ↑	2 (10)
Working Status (%)	
Yes	4 (20)
No	16 (80)

Data Collection

The study was conducted in 7 Family Health Centers (FHC) located in town of Ödemiş. Before the interviews started, the mothers were informed about the aim of the study and their consent was obtained. In-depth face-to-face interviews were held in a silent environment. The mothers who accepted to participate in the study were interviewed at their homes at predetermined time. The interviews were held by the one researcher and recorded with a voice recorder. Before the interviews, the participants were informed about the recorder and their permission was obtained. The interviews took 20-40 minutes and the participants were interviewed minimum once and maximum three times. Each participant was interviewed until new information could not be obtained. A semi-structured interview form including open-ended questions and developed by the researcher were used for data collection. The "Complementary Feeding Questionnaire and Semi-Structured Interview Form involves six questions developed to determine mothers' complementary feeding-related experiences, viewpoints and attitudes. Interviews were conducted by the researcher trained on this topic and a voice recorder was used.

Data Analysis

Inductive content analysis was used to analyze obtained data. Recorded data were verbatim transcribed, a categorization matrix was created based and all data were examined and coded according to categories created (Figure 1).

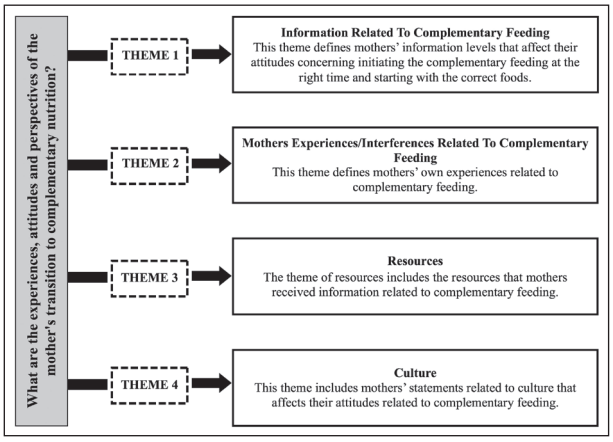

Figure 1. Categorization Matrix

Validity and Reliability

All interviews were recorded with a voice recorder and continued until satisfactory data were elicited and until the same concepts appeared (13). Questions eliciting details were used to collect in-depth data. All steps of the study were explained in detail, data were described in detail, emerging themes were exemplified by direct quotes and what the participants said were exactly described. Analyses of the data were evaluated by more than one researcher, subthemes of the categorization matrix were created by different researchers and a final version of the subthemes was agreed on. In other words, a researcher triangle was used to confirm that descriptions of data exactly reflected obtained data. Data collection tools and crude data are being kept for confirmation of original data.

Results

Four themes emerged following analysis of the interview data: information related to complementary feeding; mothers experiences/interferences related to complementary feeding; resources; culture (Table 2).

Table 2. Characteristics Related to Breastfeeding and Complementary Feeding

Characteristics	n	%
Breastfeeding Status		
Yes	15	75
No	5	25
Time To Start Complementary Feeding		
Before six months	11	55
Six Months	7	35
After six months	2	10
First Given Food		
Tarhana	8	40
Liquid foods (soda, anise, linden vb.)	4	20
Fruit puree (pear, banana, apple etc)	3	15
Formula	3	15
Yoghurt	2	10
Information Resources about Complementary Feeding		
Family members, relatives, neighbors	13	65
İnternet and books	3	15
Health Personnel	4	20

Theme 1. Information related to complementary feeding

This theme defines mothers' information levels that affect their attitudes concerning initiating the complementary feeding at the right time and starting with the correct foods. Fourteen of mothers (70%) stated that they did not exactly know the right starting time of complementary feeding. Only six mothers (30%) reported that they know the right starting time of complementary feeding. Mothers reported that they had experienced hard time about with which foods they should start as complementary feedings and what foods should be given on which months, that they used wrong applications and that most often they have felt themselves insufficient and expressed their concerns. Twelve mothers (60%) stated that they completely smoothed the foods. Eight mothers (40%) also reported that they used feeding bottle for liquid foods. Some mothers reported that they experienced perturbation about starting on egg (10% of mothers) and meat (20% of mothers).

"In fact, I did not know when I should start, because everyone was saying different things. Some said [you are too late], others said [it is too early to start]" (Mother 17, 21 years old, baby is 15 months old).

"They start having baby tasted some from everything when the baby completes 40 days. Therefore, I did so, I do not know if I did right" (Mother # 1, 20 years old, baby is 6 months old).

"First I started with yogurt; now I say thanks to god I did. But that time I have really wondered if my baby eats" (Mother # 18, 24 years old, baby is 11 months old).

"I did not know when and how to start on eggs. Therefore, I guess I was little late. I guess it was 9th month when I started; I did not know the boiling time of egg… first I had given the yolk of egg" (Mother # 5, 30 years old, baby is 11 months old).

"I started complementary foods on eight month with baby cookies and then I started adding bananas and other things. I quit cookies when my doctor riled at me, to my surprise it was not correct" (Mother # 13, 26 years old, baby is 14 months old).

Theme 2: Mothers experiences/interferences related to complementary feeding

This theme defines mothers' own experiences related to complementary feeding. Seven mothers (35%) reported that they had experiences babies' health prob-

lems such as constipation, growth-development failure, etc. after starting complementary feeding. Three mothers (15%) experienced constipation in their babies, two mothers (10%) experienced allergy problems, one mother (5%) experienced growth and development delay and one mother (5%) experienced anemia. Forty-two percent of mothers who had two or more child stated that they generally behaved according to the experiences they gained with first child. Seventy five percent of primiparous mothers stated that they generally determined the foods and starting time of complementary feeding by the comments they heard around them.

"I experienced too many problems with my first daughter on complementary foods; she became constipated and experienced bowel problems. When my second daughter was 2 months old, everybody suggested many things for baby but I did not listen to them" (Mother # 16, 28 years old, baby is 7 months old).

"I had hard time on feeding my daughter and still having hard time; therefore, when I start complementary foods on my son I have never insisted. The more he ate, the more I gave. I mean I did not run after him with spoon" " (Mother # 11, 37 years old, baby is 12 months old).

"Everybody says your baby is too weak, you do not take care of your baby good, your milk is not enough. They said this too much that I convinced finally and started giving baby some things" (Mother # 14, 32 years old, baby is 11 months old).

Two mothers stated that they started complementary feeding early due to the health problems experienced by their children.

"I think my baby was 2.5 or 2 months old. He got sick so many times that I thought my milk was not enough. I have started giving formula at the hospital and I continued when we came home, he got used to it and never sucked" (Mother # 2, 32 years old, baby is 7 months old).

"My baby was affected by jaundice; therefore we stayed at the hospital. I started formula when we were at the hospital and continued subsequently. He quit sucking completely" (Mother # 3, 30 years old, baby is 15 months old). Mothers also mentioned about the methods they used when preparing foods for their babies. Sixty percent of mothers stated that they completely smoothed the foods they have given their babies.

"I gave soups after I blended them in a food processor. Even I chopped fruits in the food processor but now my baby does not eat anything with grains. I add rice and noodles into soup, he does not even want. I do not know what to do. I wonder if he eats when starts teething?" (Mother # 4, 28 years old, baby is 10 months old).

Theme 3: Resources

The theme of resources includes the resources that mothers received information related to complementary feeding. Thirteen mothers (65%) stated that they received information related to complementary feeding from their own mothers, mothers in law and neighbors. Three mothers (15%) reported that they benefited from about books and internet. Four mothers (20%) stated that they received help from Family Health Centers.

"Nobody help me when I started complementary foods. Sometimes I review internet and seek for what to give and what to do" (Mother #9, 27 years old, baby is 9 months old).

"I have neighbors who have babies at the same age or older than mine. I talk and consult with them; I do similar things to what they have said and done. Of course all the babies are not same; for instance, my baby does not eat most of things they said but I am still trying" (Mother # 2, 32 years old, baby is 7 months old).

"I consulted with my nurse. I wish I had asked at the beginning, I think I am little late" (Mother # 10, 34 years old, baby is 8 months old).

Theme 4: Culture

This theme includes mothers' statements related to culture that affects their attitudes related to complementary feeding. There are many cultural applications that affect starting of complementary feeding and what foods will be provided on which months. Eight mothers (40%) said that they started complementary feeding with "Tarhana[1]". Other cultural practices expressed by mothers include: making sweet custard before baby sleep at night (seven mothers), inserting

1 Tarhana which consists of onion, tomato, pepper, yogurt, yeast, flour and spices, is traditional soup

into the baby's mouth after dipping his finger in the liquid food (five mothers), giving liquid foods such as soda, linden, anise before the sixth month (four mothers), giving solid food into the mouth of the baby after chewing in the mouth (three mothers).

"Because it was said that custard keeps baby satiated, I make custard by fixing rice flour, sugar and milk and give it at nights before bed time." (Mother # 14, 32 years old, baby is 7 months old).

"First I started with Tarhana. I asked my mother, she said they did the same thing. It is both nutritious and healthy." (Mother # 19, 32 years old, baby is 10 months old).

"In this area people dip a finger into the food on the table and give it to baby or they chew food in their mouths and give it to baby so that baby can eat little bit of everything" (Mother # 7, 34 years old, baby is 7 months old).

Discussion

In this study the experiences, viewpoints and attitudes of mothers having babies between 6 to 24-month old related to complementary feeding were analyzed in line with their knowledge, experiences, resources and cultural themes related to complementary feeding.

World Health Organization and United Nations Children's Fund recommend feeding babies only with mother's milk for first six months after birth and starting complementary feeding after then (15,16). In a study by Wasser et al. (2011) where feeding patterns of mothers who had low income and 3 to 18-month old babies were evaluated, it was discovered that mothers started solid foods and fruit juices after first month and the most widespread feeding behavior was formulas, solid foods and fruit juices (8). In a study by Wardle, De Domenico & Wenin (2014) Only 32% of the mothers were found to have begun solid foods after 6 months (14). In a study by Farhangi (2016) found that ratio of starting proper complementary feeding were 57.83. A study carried out in United Arab Emirates by Radwan (2013) it was established that 83.5 % of babies were given first solid foods before six months (7). In that study also mothers stated that they did not have sufficient information about the timing of complementary feeding. Fifty five percent of mothers stated that they initiated complementary feeding before six months and this situation has resulted from insufficient milk, babies getting sick and the directions of surrounding people.

The mothers stated that they had insufficient information related to with which foods they will start on complementary feeding and what foods will be given on which months and experienced perturbation about this topic. In a study by Garg & Chadha (2009), it was suggested that egg and meat consumption was considerably low in six 8-month old babies and this situation has originated from mothers considering that babies would not digest these foods (18). In a study carried out by Friel et al. (2010) with mothers having babies between 3 to 12 months old, meat was found to be the least consumed nutrition source, except for 12th month (6). On complementary feeding, solid foods, especially foods with animal-origin, should be given at appropriate amount and diversity (6). In a study by Rasheed et al. (2011), household stated that many animal-originated foods are rich in terms of nutrition, but they do not give children since they consider them inappropriate (5). In this study, 20% of mothers experienced problems during using animal products although they use broth.

Seventy five percent of the primiparous mothers stated they acted according to the information they obtained surroundings and mothers with two children stated that they behaved according to the experiences they gained with first child. Besides, mothers stated that initiating complementary feeding early has influenced by their sick children. In a study, Garg & Chadha (2009) discovered that the time saved by mother for child's care and complementary feeding practices decreases as the number of children increases and this situation affects children's feeding negatively (18). In a study carried out in India by Sinhababu et al. (2010) it was detected that the complementary feeding applications most often used by mothers were initiating complementary feeding late and infrequent and giving solid-semisolid goods insufficient (10).

In study carried out by Lindsay et al (2008) in Brazil, the most widespread problems were entrance of solid foods into feeding early and use of baby formulas (9). In studies the feeding style and the consistency of foods were found to be a great problem (19,20). In this study also mothers stated that they used bottle during

feeding (40%) their babies and preferred smooth and grainless foods (60%). As a complementary feeding, giving babies semisolid foods when they are six months old, rough foods smashed by the fork when they are seven months old and finger-foods when they are eight months old during are very important for improvement of the chewing skills. Therefore, foods should be given babies at the right time and consistency.

In the present study 65% of mothers stated that they received information related to complementary feeding from the senior members of the family and surrounding. In a compilation by Imdad et al. (2011) it was detected that approaches toward complementary feeding (feeding training) had a significant effect on weight gain (95 % CI 0.11-0.56) and increase in tallness (95 % CI 0.08-0.43) in 6-12 months old children (21). Cultural applications are used widespread in complementary feeding. Each country and culture has their own applications. In a study carried out in United Arab Emirates by Radwan (2013) it was found that 30 % of babies were given fluids other than milk, such as anise and tea before 3 months (7). In a study carried out in India by Sinhababu et al. (2010) it was observed that mothers have used inappropriate complementary feeding and giving water and other milks in the first six months where only mother's milk should be given, was widespread (10). In Rasheed et al.'s (2011) study, mothers stated that they tried to give babies the foods on the table in order to introduce them (5). In the same study, it was observed that information, skill, perception and social norms have played important roles in determination of complementary feeding applications (5). This situation has led shifting directly to adult foods after utilization of starchy custards or fluids at the early term (5). In this study also 20% of mothers used practices such as giving babies linden tea or soda before six months and traditionally forty percent of mothers initiated complementary feeding at the early period with Tarhana.

Conclusions

In this study 70% of the mothers stated that they did not completely know the correct timing of complementary feeding; they experienced hard time which food should be given first as complementary foods and what foods should be given on which months and they felt themselves inadequate on this subject. Forty-two percent of mothers who had two or more children stated that they generally behaved according to the experiences they gained with first child. Seventy-five percent of primiparous mothers stated that they generally determined the foods and starting time of complementary feeding by the comments they heard around them. Sixty five percent of the mothers stated that they have received information related to complementary feeding from their own mothers, mothers in law and neighbors. Fifteen percent of mothers obtained data related to complementary feeding from books and internet. In this case, utilization of health personnel as information resource seems insufficient. Mothers stated that their attitudes toward complementary feeding were influenced by cultural applications. This leads to the transmission of incorrect complementary feeding practices from generation to generation with cultural practices.

Consequently, mothers' knowledge level about complementary feeding isn't enough. Primiparous mothers generally are to learn complementary feeding from their environment instead of health worker. Specially, the first foods given to babies are affected by traditional practices. For this reason, it is very important for health worker to be knowledgeable and conscious, and to raise awareness of mother, as well as family members about complementary feeding and practices. Mothers should be encouraged to explain complementary feeding practices. Wrong practices should be corrected by health workers. Health workers should be aware of mothers' fear and anxiety for complementary feeding.

The most appropriate complementary nutrition is not only related to what a child is fed but also psychosocial care practices (mothers' feeding times are periods of learning and love, etc.) and sensitive nutrition (when, where and by whom a child is fed, etc.). These are important factors and positively affect the child's growth and psychological development. Health workers can be help mothers' by applying the principles of psychosocial care, proper and hygienic nutrition and develop sensitive nutrition practices.

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The relationship between quality of life and anthropometric measurements in premenopausal and postmenopausal among turkish women

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Summary. The aim of this work was to investigate whether there is a relationship between anthropometric measurements and quality of life scores during pre and postmenopouse period. A descriptive study was carried out on 1276 women (40–64 years). Demographic features, socioeconomic attributes and anthropometric measurements were considered using a validated instrument the Turkish version of the EUROHIS (WHOQOL-8.Tr) was performed. Significant body mass index (BMI), height, waist circumference, hip circumference, waist/ height ratio differences were determined by comparing pre and postmenopausal women ($p<0.05$). Significantly lower quality of life scores were observed in premenopausal women ($p<0.05$). BMI was determined as significant predictor for quality of life for each group. The number of pregnancy, number of live birth, number of stillbirth and waist/height ratio did not show significant association with quality of life. The age of first pregnancy was stated as significant predictor for quality of life just for premenopausal women. In our study, quality of life increased as the BMI decreased in pre and postmenopausal women. Significantly lower WHO-8 EUROHIS scores were observed in premenopausal women. The age of first pregnancy affected the life quality in positive way in just premenopausal women. As the age of first pregnancy increased, quality of life score increased in premenopausal women.

Key words: Menopause, Quality of life, BMI

Introduction

Menopause is a physiological phenomenon, defined as the final menstrual period and reflecting loss of the ovarian follicular function (1). In women from European countries, it occurs, on average, at the age of 50–52, in our country the mean of the age is between 48-52 years, but the age of menopause may differ from 40 to 58 (2). Menopause is an important event in a women's life, in the aspect of psychological and physical point of view. In this period, women experience biological, social and cultural changes (3). Most studies show that menopausal transformation in women leads to an increase of anthropometric measurements such as body weight, height, waist circumference, waist-height ratio and a change of adipose tissue distribution. The effect of the menopause transition on body fat distribution is unclear, but new studies suggest that the menopause transition is associated with an accumulation of central fat and, in particular, intra-abdominal fat (4,5). In the period of menopause, the changes in the hormonal milieu are associated with changes in body fat composition leading to abdominal obesity (6). In Turkey over 64.4% of women of more

than 51 years are overweight and obese (7). In another study, the prevalence of obesity in Turkey among pre and postmenopausal women is 43%, 64%, respectively (8). These transformations make a significant contribution to the shift of body pattern from the gynoid to the android pattern and hence, the type of body pattern might affect the quality of life. Poor quality of life was reported in especially abdominal obesity which is associated with multifactorial conditions such as metabolic, social, behavioral factors in menopause. Quality of life is a multidimensional health concept (9). Menopause symptoms affect the women's health and quality of life negatively. A different study shows that postmenopausal women evaluate quality of life as worse compared to premenopausal women (10). The aim of the study was to examine the relationship between anthropometric measurements and quality of life scores, using a validated instrument the Turkish version of the EUROHIS (WHOQOL-8.Tr) during pre/post menopause period (11).

Material and Methods

Participants

A total of 1276 Turkish volunteer women (459 premenopausal women, 817 postmenopausal women) participated in the study, in an urban area of Ankara, Turkey. The inclusion criteria of the participants were age between 40-64 years, diagnosis of any chronic diseases and ability to complete the questionnaire. This study was approved by Baskent University Institutional Review Board (Project no: 94603339/18.050.01.08.01-699).

Questionnaire

A questionnaire form was administered by face to face interview method. Pre and postmenopausal women's demographic features and socioeconomic attributes, including age, marital status, education and employment status were recorded. Menopausal status was categorized by the declaration of the participants.

Anthropometric Measurements

Body weight, height and waist circumferences, hip and thigh circumferences, waist/height ratio were measured and BMI was calculated. Anthropometric measurements on individuals wearing light clothing and without shoes were conducted by well-trained examiners. Height was measured to the nearest 0.1cm using the portable stadiometer. Weight was measured in the upright position to the nearest 0.1 kg using a calibrated balance beam scale. BMI was calculated by dividing weight (kg) by height squared (m^2). The participants were grouped into four categories according to World Health Organization (WHO) standards; underweight, normal weight, overweight and obese in accordance with the cut-off points of <18.5 kg/m^2, 18.5 to 24.9 kg/m^2, 25.0 to 29.9 kg/m^2 and >30 kg/m^2, respectively (12). Waist circumference measurements were taken at the end of normal expiration to the nearest 0.1 cm, measuring from the narrowest point between the lower borders of the rib cage and the iliac crest. Waist/height ratio was calculated by dividing waist circumference (cm) by height (cm). It has been proposed that a cut-off value of 0.5 for both men and women (13). Hip circumference was measured at the level of the widest circumference over the buttocks. Thigh circumference was measured on the left leg directly below the gluteal fold.

Quality Of Life

The quality of life level of the pre and postmenopausal women was examined by WHO-8 EUROHIS Quality Of Life. WHO-8 EUROHIS index is composed of eight items (overall QOL, general health, energy, daily life activities, esteem, relationships, finances, and home). All answer scales have a 5-point response format on a Likert scale, ranging for instance from 'not at all' to 'completely' (11,14).

Statistical Analysis

Data were presented as mean and standard deviation (SD) or frequency (f) and percent (%). Continuous data were tested for normality using the One Sample Kolmogorov–Smirnov test. Comparisons between the groups were made using the independent groups (Student t) test. Chi-square test was used for categorical variables. The Pearson's correlation coefficient was used to evaluate the strength of linearity between variables. Multiple linear regressions were used to explore risk factors for quality of life, which were the outcome

variables in the regression. Data were analyzed using SPSS statistics software version 17.0 (SPSS Inc.). All statistical tests were two-sided and p<0.05 was considered statistically significant.

Results

Data were obtained from 1276 Turkish women in this study. More than half of the women were postmenopausal (64%) and the rest were premenopausal (36%). The mean age of the study population was 52.3±7.30 years. Demographic characteristics; life style, age group and BMI status were shown categorized by menopausal status in Table 1. Significant differences were determined comparing pre and post menopause among marital status, educational background, age groups, age (year), working status, and BMI status (p<0.05).

Anthropometric measurements showed a number of differences between pre and postmenopausal women (Table 2). The mean BMI was 29.4±5.41 kg/m^2 in postmenopausal women. Postmenopausal women had highly significant differences than premenopausal women (p=0.000). Similarly, significant height, waist circumference, hip circumference, waist/ height ratio differences were determined comparing pre and postmenopausal women (p<0.05). Significantly lower WHO-8 EUROHIS scores were observed in premenopausal women (p<0.05). According to items analysis, postmenopausal women's score were higher than premenopausal women's score in last four items that have been shown in Table 2 (p<0.05).

The WHO-8 EUROHIS score showed strong association with the age of first pregnancy, the number of pregnancy, the number of live birth, weight, waist circumference, waist/height ratio and BMI for each group (p<0.05). Nevertheless, there was a negative significant correlation between the WHO-8 EUROHIS score and the number of stillbirth just in premenopausal women (p<0.05). Also, the age of menopause and the number of abortion were determined to have moderate association with quality of life. No associations were observed for age of first menstruation (Table 3).

Using the WHO-8 EUROHIS as the dependent variable, age of first pregnancy was identified as significant predictor quality of life among premenopausal women. However, BMI was determined as significant predictor quality of life for each groups. The number of pregnancy, number of live birth, number of stillbirth and waist/height ratio did not show any significant association with quality of life. Table 4 below summarized the predictors quality of life in pre and postmenopausal women. The partial regression coefficient of BMI (β

Table 1. Demographic and other characteristics of the study population according to menopausal status

Demographic Characteristics	Premenopausal Women (n=459)		Postmenopausal Women (n=817)		p
	f	%	f	%	
Marital status					
Married	375	81.7	594	72.7	**0.000***
Single	34	7.4	52	6.4	
Divorced	35	7.6	60	7.3	
Widowed	15	3.3	111	13.6	
Educational Background					
Illiterate	12	2.6	26	3.2	**0.021***
Literate	3	0.7	29	3.5	
Primary school	134	29.2	225	27.5	
Secondary school or equivalent	39	8.5	65	8.0	
High school or equivalent	104	22.6	210	25.7	
University and higher	167	36.4	262	32.1	
Age Groups (years)					
40-50	381	83.0	91	11.1	**0.000***
50-60	71	15.5	469	57.4	
60-64	7	1.5	257	31.5	
Age (year) ($\bar{C}$±SD)	45.4±4.59		56.3±5.36		**0.000***
Working Status					
Retired	45	9.8	250	30.6	**0.000***
Housewife	238	51.9	422	51.7	
Self-employment	37	8.1	36	4.4	
Officer	98	21.4	87	10.6	
Worker	41	8.8	22	2.7	
BMI Status					
Underweight	1	0.2	2	0.2	**0.000***
Normal	145	31.5	178	21.7	
Overweight	170	37.1	295	36.2	
Obese	143	31.2	342	41.9	

Chi-square test; *p<0.05

Table 2. Mean and standard deviations of anthropometric measurements and WHO-8 EUROHIS score by menopausal status

	Premenopausal Women (n=459)	Postmenopausal Women (n=817)	Total (n=1276)	p
	C±SD	C±SD	C±SD	
Weight (kg)	72.7±13.57	73.9±13.34	73.5±13.43	0.124
Height (cm)	161.1±6.29	158.9±6.23	159.7±6.34	**0.000***
Waist circumference (cm)	94.2±13.43	98.3±13.32	96.8±13.49	**0.000***
Hip circumference (cm)	107.9±10.94	109.8±11.00	109.1±11.01	**0.003***
Thigh circumference (cm)	37.8±3.98	37.8±3.97	37.8±3.97	0.739
BMI (kg/m^2)	28.1±5.43	29.4±5.41	28.9±5.45	**0.000***
Waist /height ratio	0.5±0.09	0.6±0.09	0.6±0.09	**0.000***
WHO-8 EUROHIS	28.7±4.43	29.2±4.37	29.0±4.40	**0.048***
1 How would you rate your quality of life	3.4±0.76	3.5±0.74	3.5±0.75	0.090
2 How satisfied are you with your health	3.4±0.87	3.4±0.91	3.4±0.89	0.553
3 Do you have enough energy for everyday life	3.5±0.86	3.5±0.86	3.5±0.86	0.799
4 How satisfied are you with your ability to perform your daily activities	3.6±0.84	3.6±0.84	3.6±0.84	0.952
5 How satisfied are you with yourself	3.6±0.95	3.7±0.91	3.7±0.93	**0.032***
6 How satisfied are you with your personal relationships	4.1±0.74	4.2±0.72	4.2±0.73	**0.004***
7 Have you enough money to meet your needs	3.1±0.85	3.2±0.86	3.2±0.86	**0.046***
8 How satisfied are you with the conditions of your living place	3.6±0.81	3.7±0.83	3.7±0.82	**0.005***

Independent Groups t Test; *p<0.05

Table 3. Correlations between WHO-8 EUROHIS score with some parameters in pre and postmenopausal women

	Premenopausal Women (n=459)		Postmenopausal Women (n=817)	
Parameters	WHO-8 EUROHIS (0-40)		WHO-8 EUROHIS (0-40)	
	r	p	r	p
Parity				
Age of first menstruation	0.001	0.988	0.031	0.370
Age of first pregnancy	0.198	**0.000***	0.095	**0.010***
Age of menopause	0.095	0.816	0.074	**0.036***
Number of pregnancy	-0.172	**0.000***	-0.150	**0.000***
Number of live birth	-0.180	**0.000***	-0.129	**0.000***
Number of stillbirth	-0.111	**0.018***	-0.046	0.195
Number of abortion	-0.061	0.195	-0.089	**0.012***
Anthropometric Measurements				
Weight (kg)	-0.298	**0.000***	-0.221	**0.000***
Waist circumference (cm)	-0.308	**0.000***	-0.254	**0.000***
Waist / height ratio	-0.288	**0.000***	-0.231	**0.000***
BMI (kg/m^2)	-0.307	**0.000***	-0.250	**0.000***

*p<0.05

Table 4. Multiple linear regressions predicting quality of life in premenopausal and postmenopausal women (WHO-8 EUROHIS)

Parameters	Premenopausal Women (n=459)					Postmenopausal Women (n=817)				
	β§	Std. Error	95%CI for β Lower Bound	95%CI for β Upper Bound	p	β§	Std. Error	95%CI for β Lower Bound	95%CI for β Upper Bound	p
Age of first pregnancy	0.112	0.050	0.013	0.211	**0.027***	-0.011	0.038	-0.085	0.064	0.778
Number of pregnancy	0.053	0.161	-0.264	0.370	0.742	-0.140	0.089	-0.315	0.035	0.117
Number of live birth	-0.027	0.304	-0.625	0.572	0.930	-0.071	0.140	-0.346	0.204	0.613
Number of stillbirth	-0.853	0.731	-2.291	0.585	0.244	-0.026	0.249	-0.514	0.463	0.918
BMI (kg/m^2)	-0.200	0.073	-0.344	-0.056	**0.007***	-0.158	0.047	-0.251	-0.066	**0.001***
Waist / height ratio	-2.055	4.463	-10.829	6.720	0.645	-3.420	2.849	-9.014	2.173	0.230

*p<0.05; §: β is the partial regression coefficient

in pre and postmenopausal woman has been found as -0.20 and -0.251, respectively. The coefficients indicate that women's quality of life scores 0.20 (premenopausal) and 0.251 (postmenopausal) points decrease is caused for every increase one unit (kg/m^2) in BMI while holding other predictors in the model constant. These results were statistically significant (p <0.05).

Discussion

The mean age of pre and postmenopausal women were 45.4±4.59, 56.3±5.36 years, respectively. In a Chinese study, the mean age of each group was determined similarly (46.502 ± 3.503, 55.15 ± 2.961 respectively) (10). In our study there was a significant association between the level of education and marital status depending on menopausal status alike the survey that was conducted in Arabian Qatari women (15). Obesity is known as a multi-factor metabolic change of epidemic proportions. Shobeiri et al. (16) reported that 41.3% of the menopausal women were overweight. Similarly, in the study of Ghorbani et al. (17) showed that 44.8% were overweight and 35.3% were obese among postmenopausal women. However, in our study we found that 36.2% of the postmenopausal women were overweight.

Obesity is a public health problem, with overweight individuals representing approximately 20% of the adult world population (18). Quality of life is in a relation with obesity (9). In our study, population, weight, waist circumference, waist/height ratio and BMI were significantly related to scores in quality of life. Likewise, a survey of women in Spain found that obese respondents had a self-reported health-related quality of life lower than that of women of normal weight (19). Central adiposity in postmenopausal women has been recognized as an independent risk for developing metabolic syndrome, dyslipidemia, and cardiovascular diseases (20). In a cross-sectional study, the mean waist circumference of participants was 91.70±13.19 cm (21). In another study, postmenopausal women's waist circumference was 92.9+11.4 cm (22). In our study we determined that the mean waist circumference was 98.3±13.32 cm in postmenopausal women. The reason of this variation might be ethnicity and the population size which we observed. The waist/height ratio is an effective index for assessing central fat distribution. In this study, waist/height ratio was 0.6±0.09 in postmenopausal women. In a Spanish study, visceral fat area was evaluated in postmenopausal women and waist/height ratio was stated 0.56±0.1 alike our study (23).

Analysis of the results revealed that scores in quality of life were worse for premenopausal women than for postmenopausal women by contrast with Fuh et al. (24) who contradicted this outcome. In our study, the mean of WHO-8 EUROHIS score in pre and postmenopausal women were 28.7±4.43 and 29.2±4.37, respectively (p<0.05). However, in the other survey in Greece, it was found that there is no effect of menopause on the quality of life (25). Also, another study on Taiwanese women confirmed no significant effect of menopausal transition on quality of life (26). The hot flushes, joint pain, sleep disorder, depressive mood, irritability, fatigue and libido decrease as the most common

symptoms may arise and can prevail until post menopause. These symptoms affect the quality of life. In a cross-sectional population-based study has shown that earlier age at first childbirth and higher parity are risk factors for obesity in later life (27). In this study, the key predictors associated with quality of life among each group of women were age of first pregnancy, number of pregnancy, and number of live birth. When the data was analyzed using multiple linear regressions; the age of first pregnancy was determined as significant predictors' in quality of life for premenopausal women. A cohort study findings suggested that younger maternal age at first delivery was independently associated with a higher risk of central obesity and metabolic syndrome in postmenopausal women (28). Another study highlighted that young mothers, and particularly teenage mothers, are a vulnerable group at high risk of poor mental health outcomes compared to mothers aged 25 years and above (29). A significant reduction in menopausal quality of life as a result of high BMI levels has been reported in several studies (31,31). Also, in our study, WHO-8 EUROHIS score were negatively correlated with BMI for each groups ($p<0.05$).

As a conclusion, quality of life increased as the BMI decreased in pre and postmenopausal women in this study. But, the age of first pregnancy affected the quality of life in positive way in just premenopausal women. Number of pregnancy, number of live birth, number of stillbirth and waist/height ratio were not associated with changes in WHO-8 EUROHIS in both groups. Obesity is frequent in postmenopausal period. Especially abdominal obesity and related health problems can occur in this period. And these conditions may lead to a decrease in quality of life of women. Additional research about quality of life and anthropometric measurements in status of menopause with a longitudinal design seems necessary to confirm our results.

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Effect of vitamin D receptor fokI gene polymorphism on chronic renal disease

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Summary. Many factors play a role in the progression of Chronic renal disease. Many polymorphisms have been detected in vitamin D receptor gene. FokI polymorphism is one of these. We aimed to present the role played by FokI gene polymorphism in CRD have been examined. Three study groups were formed as group 1 with a total of 60 patients with RRT need with ages ranging between 59,43±12,58, group 2 with a total of 60 patients with GFR decrease of >4 ml/min/year and ages ranging between 59,73±13,14 and group 3 with a total of 60 patients with stable GFR level during follow up period with ages ranging between 63,50±12,21. A total of 77 individuals without CRD and with ages ranging between 41.78±14,28 were included as the control group of the study during polyclinic controls. The frequency of FokI polymorphism FF, Ff and ff genotypes were determined as 51.7%, 35.0% and 13.3% respectively in group 1, as 50.0%, 41.7% and 8.3% respectively in group 2 and as 45.0%, 48.3% and 6.7% respectively in group 3. Whereas the frequency of FF, Ff and ff genotypes were determined respectively as %51.9, %39.0 and %9.1. Prevalence of CRD in all genotypes was determined to be statistically significant for all groups (p>0.05). No relationship was determined between FokI polymorphism and risk factors. In conclusion, it was observed that FokI polymorphism is not related with CRD and risk factors.

Keywords Chronic renal disease, fokI polymorphism, renal replacement therapy, rapid progression

Introduction

Chronic renal disease (CRD) is a syndrome characterized by the progressive loss of kidney functions due to various reasons. Glomerular filtration rate (GFR) generally reduces over the months or years and this reduction varies significantly according to the underlying reason (1). The prevalence of chronic renal disease has increased rapidly during the last decade. It is estimated that the glomerular filtration rate of 13 % of the adult population in the United States of America is lower than 60 ml/min/1.73 m^2. Prevalence in individuals aged 65 and above was determined to vary between 38-44 % (2, 3).

Vitamin D and its active metabolite of 1,25-dihidroksi vitamin D3 (1,25OHD, calcitriol) is one of the well known regulators of cell growth and differentiation. It has been reported in recent studies that vitamin D is related with bone and calcium metabolism control in addition to many biological processes such as immune response formation, metastasis, angiogenesis and apoptosis. In addition, it also known that 1,25O HD stimulates the expression of the oxidative enzymes which are cytochrome P450 family members (4).

Vitamin D shows its effect by interacting with vitamin D receptor (VDR) which is a member of the nuclear receptor gene family. VDR has been coded by a large gene (>100 kb) on chromosome 2q12-14

(5). Allele variations of VDR differing between ethnic groups and races have been defined (6). First studies on VDR polymorphisms were carried out using bone metabolism parameters and especially osteoporosis (7). The relationship between VDR gene polymorphisms and other diseases including cancer and immune system diseases have been put forth in later studies (8). In general, majority of the VDR gene polymorphisms have been determined in regulator regions such as 5' promoter region and 3' UTR region instead of exon coding region.

(T/C) polymorphism is found in the first potential start region. This polymorphism synthesizes a longer VDR protein comprised of 427 amino acids by way of producing an additional start codon. This polymorphism is named as f allele. The existence of f allele is related with less transcriptional activity (5).

The FokI polymorphism on Exon 2 is a functional polymorphism. It is considered as normal when the transcription starts from the first ATG sequence at the restriction allele (f), if there is no restricton (F allele) transcription starts from the next ATG sequence (transcript) and a shorter but functional allele formation takes place. ATG is transformed into ACG as a result of the T C transformation in the start codon of ATG and translation starts from the second ATG. As a result, the 424 aminoacid long VDR protein is synthesized (F allele). Translation starts from the first ATG in cases when there is no T C transformation and the VDR protein that is 3 aminoacids longer (427 aminoacids, f allele) is synthesized (9).

The purpose of this study was to examine the genetic role of VDR FokI polymorphism along with demographic, clinical and laboratory variables on rapid progression with conservative therapy and RRT requirement in chronic renal disease stage 3-5 patients in the Turkish society.

Patients and methods

Formation of the Patient and Control Groups

In this study, patients aged between 18-80 with GFR <60 ml/dk/1.73m^2 who have been included in the chronic renal disease training program after application to the Antalya Education and Research Hospital Nephrology polyclinic have been scanned retrospectively. The patients were classified into groups according CRD staging based on the GFR values. Demographic, clinical and laboratory data on the patients were acquired from the follow-up folders. Three study groups were formed as group 1 with a total of 60 patients with RRT need during follow-up with ages ranging between 59,43±12,58, group 2 with a total of 60 patients with GFR decrease of >4 ml/min/year and ages ranging between 59,73±13,14 and group 3 with a total of 60 patients with stable GFR level during follow up period with ages ranging between 63,50±12,21. Patients followed up for a period of less than 3 months were not included in the study. A total of 77 individuals without CRD and with ages ranging between 41.78±14,28 were included as the control group of the study during polyclinic controls. Approval from the local ethics council was obtained prior to starting the study and written approvals were taken from all individuals in both the control and patient groups after explaining the purpose and procedure of the study (No: 4/6).

DNA isolation

Two milliliter blood samples were drawn from the individuals who participated in the study and placed in tubes containing K_3-EDTA. The blood samples were stored at -80 °C until DNA isolation. Afterwards, the frozen samples were defroested at room temperature and DNA isolation was carried out via QIAquick PCR Purification Kit (250) (Cat no:28106) (Hilden, Germany) with the standard method. The isolated samples were spectrophotometrically measured via NanoDrop and DNA quantitation was made.

Amplification of DNA polymerase chain reaction

The primers used in the study have been designed using the ExonPrimer (http://ihg.gsf.de/ihg/ExonPrimer.html) software. The primers were synthesized by the Sentromer (Istanbul, Turkey) company as 20 pmol.

FOK1-rs222857-F	AGCTGGCCCTGGCACTGACTC
FOK1-rs222857-R	ATGGAAACACCTTGCTTCTTCTCCCTC

The following PCR cycle has been used in the study.

At 95°C	15 Minutes		
At 95°C	45 Seconds		
At 58°C	45 Seconds	⇒	40 repetitions
At 72°C	1 Minute		
At 72°C	10 Minutes		
At 4°C	infinite		

Exonuclease1 and Shrimpalkalen phosphase enzyme mixtures were used to inactivate the excess primers and dNTPs remaining after PCR. Sequencing reaction was carried out using the final products processed via Exo1-SAPIT and BigD Terminator v3.1 and standard protocols.

Sequencing clean up was carried out using Sephadex and Qiagene Sequencing cleanup columns. 3,5 gr Sephadex (Merck G-50 medium) was dissolved in 50 ml water and 600 µl solution was placed in each column. Water was removed via centrifugation at 2500 g for 2 minutes. Products subject to sequencing reaction were placed on the remaining sephadex on the column and the products were cleaned via centrifugation at 2500 g for 5 minutes. The product that is about 30 µl was transferred onto the optical plate and sequencing reaction was carried out by loading to ABI 3130 device. Seq Scape2.5 software was used for analyzing the sequencing reactions.

Statistical evaluation

SPSS 16.0 package software was used for the biostatistical evaluation of the data obtained as a result of the study. Multivariate analysis of variance (ANOVA) was used for evaluating the variables between the control and patient groups and the genotypes for biochemical parameters, Tukey HSD multiple comparisons test was used for comparing the factors. c^2 test was used for the nonparametrik tests, the results were given as average ± standard deviation and $p<0.05$ were accepted as statistically significant.

Results

Characteristic properties for the individuals in CRD groups have been given in Table 1. Similar values were observed in all three groups with regard to age averages and gender difference. Patient diagnosis distribution among the groups was also similar and no statistically significant difference was observed between the groups. Whereas no statistically significant difference was observed between the groups with regard to systolic blood pressure a statistically significant difference was observed between the diastolic pressure values of the 1st and 2nd Groups in comparison with the 3rd Group (respectively, $p<0.05$ and $p<0.001$). The 1st group was observed to be different at a statistically significant level in comparison with the other two groups with regard to basal creatinine and basal GFR ($p<0.001$). No statistically significant difference was determined between groups 2 and 3 with regard to the basal creatinine values, however basal GFR values were observed to be higher in group 2 at a statistically significant level ($p<0.05$). It was observed upon comparing groups 2 and 3 with group 1 that the basal calcium values of group 1 (respectively, $p<0.05$ and $p<0.01$), basal phosphorous, parathormon and hemoglobin values were lower at a statistically significant level ($p<0.001$). On the other hand, no statistically significant difference was observed between groups 2 and 3 as a result of these measurements. Basal proteinuria value was observed to be higher in group 1 at a statistically significant level in comparison with other groups (respectively, $p<0.05$ and $p<0.001$) while the values of group 2 were observed to be higher at statistically significant levels when compared with those of group 3 ($p<0.01$). It was observed when the nonparametric tests were examined that ACE/ARB use was low in Group 1 contrary to the other two groups ($p<0.01$), whereas eritropoetin use was determined to be high at a statistically significant level ($p<0.001$). No statistically significant differences were observed between the groups with regard to the use of vitamin D, atorvastatin and antiacidose. Average follow up times in the groups actualized as 9, 18 and 16 months respectively.

Genotype and allele frequencies for vitamin D receptor and FokI gene of the patient and control groups have been given in Table 2. It was determined that the frequency of FF, Ff and ff genotypes of individuals with chronic renal disease were 51.7 % (n=31), 35.0 % (n=21) and 13.3 % (n=8) respectively for Group 1, 50.0 % (n=30), 41.7 % (n=25) and 8.3 % (n=5) respectively for Group 2 and 45.0 % (n=27), 48.3 % (n=29) and 6.7 % (n=4) respectively for Group 3 (Table 2). Whereas the FF, Ff and ff genotype frequencies for

Table 1 Characteristic properties of the study groups

Patient characteristics	1. Group	2. Group	3. Group
n	60	60	60
Age (years)	59,43 ± 12,58	59,73 ± 13,14	63,50 ± 12,21
Gender (F/M)	22/38	20/40	25/35
Diagnosis (n)			
Hypertension	15	13	20
Diabetes	25	19	21
KGN	2	10	5
KPN	3	6	4
PCRD	4	5	3
Unknown	11	7	7
Systolic Pressure (mmHg)	144,67 ± 22,80	140,50 ± 28,00	136,50 ± 22,30
Diastolic Pressure (mmHg)	87,58 ± 11,25 [a,b]	82,66 ± 12,73	79,83 ± 10,81
Basal Creatinine (mg/dl)	3,23 ± 0,85 [b,c]	2,00 ± 0,49	2,24 ± 0,67
Basal GFR (ml/dk)	20,66 ± 7,04 [b,c]	35,59 ± 10,21 [d]	31,08 ± 11,15
Basal Calcium (mg/dl)	9,03 ± 0,62 [a,d]	9,25 ± 0,38	9,30 ± 0,48
Basal Phosphor (mg/dl)	4,25 ± 0,67 [b,c]	3,53 ± 0,66	3,62 ± 0,63
Basal Parathormone (pg/ml)	192,50 ± 142,16 [b,c]	119,60 ± 68,46	102, 56 ± 61,94
Basal Hemoglobin (g/dl)	10,86 ± 1,43 [b,c]	12,19 ± 1,63	12,26 ± 1,71
Basal Proteinuria (g/g)	2,98 ± 3,17 [a,b]	1,92 ± 2,29 [d]	0,70 ± 0,68
ACEi/ARB (%)	25,00 [e,f]	46,70	51,70
Eritropoetine (%)	45,00 [b,c]	18,30	13,30
Vitamin D (%)	63,30	68,30	48,30
Atorvastatine (%)	31,70	30,00	16,70
Antiacidose (%)	41,70	35,00	21,70
Follow up period (months)	9,88 ± 6,26	18,06 ± 8,90	16,40 ± 8,64

[a] p<0,05 when compared with Group 2; [b] p<0,001 when compared with Group 3; [c]p<0,001 when compared with Group 2; [d]p<0,05 when compared with Group 3, [e]p<0,01 when compared with Group 3; [f]p<0,01 when compared with Group 2

the control group were determined respectively as 51.9 % (n=40), 39.0 % (n=30) and 9.1 % (n=7). It was observed as a result of the evaluation of the prevalence of all three genotypes between the groups that the result is not statistically significant (p>0.05). It was determined when the allele frequencies for the Fok I gene were examined that the F allele frequency was 69% for Groups 1 and 3 and 71% for Group 2 and the control group (Table 2). Whereas the f allele frequency was determined as 31% for Groups 1 and 3 and as 29% for

Table 2 Genotype and allele frequency for vitamin D receptor FokI gene in chronic renal disease and control group

Genotype frequency	Group 1	Group 2	Group 3	Control
FF	31 (%51,7)	30 (%50,0)	27 (%45,0)	40 (%51,9)
Ff	21 (%35,0)	25 (%41,7)	29 (%48,3)	30 (%39,0)
ff	8 (%13,3)	5 (%8,3)	4 (%6,7)	7 (%9,1)
Allele frequency	Group 1	Group 2	Group 3	Control
F	69%	71%	69%	71 %
f	31%	29%	31%	29%

Group 2 and the control group (Table 2). No statistically significant difference was observed between the groups with regard to allele frequency.

The relationship between the demographic characteristics of the groups, their parametric and nonparametric properties and vitamin D receptors Fok I genotypes have been given in Table 3.

No statistically significant difference was observed between the age average of the patients in all groups and the prevalence of FF, Ff abd ff genotypes. Even though FF genotype is observed more frequently in males in all 3 groups, it was observed that the ff genotype is observed equally in both females and males in Group 1 and more frequently in males at ratios of 1/4 and 0/4 in Groups 2 and 3. However, this difference was not statistically significant (Table 3). No statistically significant relationship could be established between the patient diagnoses and genotype relationship. No statistically significant difference was observed between the groups with regard to ACEi/ARB, EPO, vitamin D, atorvastatine and antiacidose use. A statistically significant relationship could not be observed between the basal and final follow-up biochemical values and VDR FokI genotypes. However, basal creatinine values were measured lower in the ff genotype in all 3 groups. Similarly, basal GFR was determined to be higher in ff genotype individuals in these groups in comparison with the other genotypes. Basal proteinuria levels in all 3 groups were determined to be lower than the ff genotype. However, this difference was not statistically significant (Table 3).

Discussion

Chronic renal disease is becoming more frequent globally (10). Its complications including cardiovascular diseases are starting to become a major public health issue thereby resulting in economic impacts (11).

There was no statistically significant difference in our study between the age average of the groups (Group 1=59,4 years, Group 2=59,7 years and Group 3=63,5 years). No impact was observed on kidney failure progression or RRT requirment. No relationship was determined between advanced age and CRD progression in a study for which age average was calculated as 66,8 years (12). Young age was determined to be related with progression in this study. In another study examining the impacts of age on CRD progression (13), GFR rate of decrease was determined as 2,61 ml/min on average for the elderly population at the end of a monitoring period of 5 years and as an interesting paradox, a greater GFR decrease was detected in the group with normal serum creatinin levels in comparison with the lower Basal GFR. The impact of age and gender on CRD progression was examined in another study (14) in which patients diagnosed with stage 3 CRD were monitored for a period of 10 years and GFR decrease was observed in 73% of these patients. In addition, female gender was determined to be related with a slower decrease in GFR, better patient and kidney survival. While no difference with regard to female/male ratio was determined between the groups in our study even though CRD is observed more frequently in the male population. It is a fact that CRD is observed more in the elderly population in comparison with the young population. In general, accompanying diseases that are observed frequently in the population such as arteriosclerosis, cardiac failure, high blood pressure, diabetes should be taken into consideration as phenomena that may affect CRD progression rather than male gender and physiological process due to age.

The relationship between blood pressure and kidney function is questionable in patients with chronic renal disease (15). The relationship between blood pressure and progression in nondiabetic stage 3 CRD adults was examined during a study in which an average annual GFR decrease of >2.5 ml/min was accepted as fast progression and annual GFR change was determined using kidney function change cystatin C, creatinine and their combination (14). In this study, blood pressure was determined to be related with progression only for the patient group for which cystatin C based or combined equation was used for systolic blood pressure >140 mmHg or diastolic blood pressure 90 >mmHg. No statistically significant relationship was determined in the group for which creatinine based GFR measurement was made. The difference of our study is that fast progression is accepted as an annual average GFR decrease of >4 ml/min and that GFR measurement is made via modification of diet in renal disease (MDRD). The relationship put forth between systolic blood pressure and fast progression and RRT time has been determined in

Table 3 Relationship between the FokI genotypes and the study group characteristics

	Group 1			Group 2			Group 3		
Patient characteristics	**FF**	**Ff**	**ff**	**FF**	**Ff**	**ff**	**FF**	**Ff**	**ff**
n	31	21	8	30	25	5	27	29	4
Age (years)	59,41 ± 2,47	60,76 ± 3,01	56,00 ± 4,88	56,56 ± 2,52	62,04 ± 2,76	67,20 ± 6,17	63,33 ± 2,65	62,51 ± 2,56	71,75 ± 6,90
Gender (F/M)	10/21	8/13	4/4	13/17	6/19	1/4	10/17	15/14	0/4
Diagnosis (n)									
Hypertension	10	4	1	8	5	0	8	9	3
Diabetes	12	10	3	7	10	2	10	10	1
KGN	0	1	1	5	3	2	3	2	0
KPN	1	2	0	5	1	0	1	3	0
PCRD	3	0	1	2	2	1	1	2	0
Unknown	5	4	2	3	4	0	4	3	0
Basal Creatinine (mg/dl)	3,25 ± 0,74	3,29 ± 1,01	2,95 ± 0,87	2,12 ± 0,52	1,90 ± 0,45	1,78 ± 0,33	2,20 ± 0,68	2,30 ± 0,66	2,10 ± 0,87
Basal GFR (ml/dk)	20,03± 6,95	20,97 ± 7,12	22,32 ± 7,81	32,90 ± 11,43	37,90 ± 7,99	40,24 ± 9,66	32,07 ± 10,26	29,41 ± 11,72	36,55 ± 13,29
Basal Calcium (mg/dl)	9,00 ± 0,69	9,19 ± 0,52	8,72 ± 0,48	9,30 ± 0,31	9,20 ± 0,46	9,20± 0,32	9,28± 0,59	9,30± 0,40	9,42 ± 0,29
Basal Phosphor (mg/dl)	4,32 ± 0,76	4,18 ± 0,54	4,15 ± 0,66	3,69 ± 0,76	3,37 ± 0,61	3,36 ± 0,25	3,64 ± 0,72	3,64 ± 0,51	3,40 ± 0,77
Basal Parathormone (pg/ml)	170,19 ± 122,01	189,62 ± 131,13	286,50 ± 212,30	101,43 ± 54,22	138,08 ± 76,67	136,20 ± 87,14	106,33 ± 56,89	102,10 ± 69,96	80,50 ± 30,98
Basal Hemoglobin (g/dl)	10,80 ± 1,44	10,95± 1,08	10,86 ± 2,24	11,77 ± 1,67	12,66 ± 1,51	12,40 ± 1,66	12,49 ± 1,50	11,91± 1,91	13,25 ± 0,90
Basal proteinuria (g/g)	3,36 ± 3,52	2,60 ± 2,81	2,53 ± 2,80	2,53 ± 2,68	1,38 ± 1,71	0,94± 1,32	0,71 ± 0,69	0,75 ± 0,71	0,20 ± 0,20
Final Creatinine (mg/dl)	4,95 ± 1,40	5,40 ± 1,55	5,27 ± 0,91	3,27 ± 1,54	2,55± 0,76	2,24 ± 0,64	2,00 ± 0,72	2,19 ± 0,79	2,25 ± 1,38
Final GFR (ml/dk)	11,57 ± 3,96	10,64 ± 3,51	11,17 ± 2,64	22,68 ± 11,04	28,08 ± 8,57	31,60 ± 10,73	36,79 ± 12,90	31,94 ± 12,76	36,75 ± 18,78
Final Calcium (mg/dl)	8,93 ± 0,50	8,84 ± 0,77	9,15± 1,04	9,40± 0,91	9,28 ± 0,39	9,78± 1,11	9,36 ± 0,48	9,31 ± 0,49	9,65 ± 0,67
Final Phosphor (mg/dl)	5,01 ± 0,15	4,99 ± 0,18	4,58 ± 0,30	4,31 ± 0,15	3,60 ± 0,17	3,94 ± 0,38	3,63 ± 0,16	3,66 ± 0,15	2,82 ± 0,42
Final Parathormone (pg/ml)	183,04 ± 20,09	218,28 ± 24,42	183,50 ± 39,56	116,60 ± 20,43	115,52 ± 22,38	120,20 ± 50,04	113,29 ± 21,53	115,17 ± 20,78	118,75 ± 55,95
Final Hemoglobin (g/dl)	10,87 ± 1,34	10,20 ± 1,31	11,10 ± 1,38	11,44 ± 1,88	12,51 ± 1,55	12,16 ± 1,24	12,99± 1,46	12,15 ± 1,38	13,32 ± 2,23
Final Proteinuria (g/g)	3,79 ± 3,68	2,95 ± 2,96	5,88 ± 6,76	1,97 ± 2,31	1,57 ± 2,68	0,52 ± 0,30	1,28 ± 1,79	0,93 ± 1,10	0,35 ± 0,50
ACEi/ARB (n)	7	6	2	12	13	3	15	14	2
Eritropoetin (n)	14	10	3	9	2	0	3	5	0
Vitamin D (n)	17	15	6	20	19	2	14	13	2
Atorvastatine (n)	13	3	3	6	10	2	6	4	0
Antiacidose (n)	10	12	3	13	7	1	4	8	1

our study (12). On the other hand, similar results have been obtained in another study in which a similar method was used with our study during which 211 adult individuals with stage 3-5 CRD diagnosis with an average monitoring period of 56,6 months were retrospectively reviewed (16). These researchers could not determine a statistically significant relationship between the initial systolic and diastolic blood pressures and predialytic CRD progression. Similarly, the non-existent relationship between CRD progression and the blood pressure level at the time of application was also put forth in an observational study carried out on 1094 Afro-American patients (17) and another study carried out on patients with polycystic renal disease (18).

In addition to the expected contribution of age and CRD stage, it has been set forth that there is a correlation between the progression of renal disease and proteinuria and that the progress is slowed down with the use of angiotensin converting enzyme inhibitor (ACEi) and angiotensin receptor blockers (ARB) (19-21). Our results are in compliance with the results of these studies. These researchers have examined the relationship between proteinuria and ACEi/ARB use on predialysis monitored patients and decrease in renal functions and/or RRT onset. A total of 547 stage 4-5 CRD predialysis patients were monitored for a period of about 7 years in the study as a result of which a decrease of 0,35 ml/min/1.73 m2/month on average was observed in renal functions in patients with light proteinuria (beteen >0,3 and ≤1,0 g/24 h). When patients undergoing RRT were compared with patients without proteinuria (≤0,3 g/24 hour) a higher decrease in renal functions was observed. When the level of proteinuria was taken into consideration (between >1,0 and ≤3,0 arası, >3,0 and ≤6,0 and >6,0 g/24 hour), it was observed that early RRT requirement was correlated with increasing proteinuria and increased progression. On the other hand, a lower RRT start rate was determined when patients using ACEi/ARB at the start (n=16) or during follow-up (n=133) were compared with the patients who do not use ACEi/ARB (n=152). In conclusion, they were of the opinion that there is no proof regarding the harm of using ACEi/ARB in predialysis monitored patients and that proteinuria may be used as an indicator of CRD progression risk. It was determined in another study (21) that the rates of ACEi/ARB use were 31%, 46% and 51% respectively for patient groups with GFR <15 ml/min, GFR 15-24 ml/min and GFR 25-29 ml/min. These findings support our results. In addition, findings indicating a relationship between proteinuria above 1 g/g and fast progression and RRT requirement are also in accordance with the findings of our study.

The issue on the relationship between statin use and low mortality as well as its impact on decreasing RRT requirement and renal failure progression is a matter of debate (22-25). No statistically significant difference was determined in our study between statin use among groups. The reason for the conflicting results between renal progression and statin use may be the fact that majority of the data have been acquired from studies on cardiovascular primary endpoints.

Metabolic acidosis is traditionally defined as a decrease in blood pH related with a decrease in serum bicarbonate (HCO_3) concentration and is related with progressive CRD (26). There is a correlation between HCO_3 decrease due to nephron loss and impairment in ammoniac metabolism and GFR decrease. This may be related with the progression of metabolic acidose renal disease and it has been put forth that CRD progression may be prevented if it is improved (27). Low and high serum bicarbonate levels were determined in 13.9 % (n=5796) and 1.6 % (n=652) of the patients respectively during a study carried out on 41749 patient individuals (28) and a statistically significant relationship was determined between low serum bicarbonate and mortality related with all causes after the related co-variables were adjusted. Even though this relationship is not statistically significant between stage 4 CRD and diabetes patients, the importance of the relationship between mortality and decrease in bicarbonate level from normal to low levels has been indicated. High serum bicarbonate levels were determined to be related with mortality regardless of renal function level. However, the fact that serum bicarbonate levels have not been taken into consideration in our study was a limiting factor in establishing this relationship. The fact that sodium bicarbonate use is higher in the Group with RRT requirement (41,70 %) and the Group with progression (35 %) in comparison with the stable GFR group (21,70 %) indirectly indicates the probable contribution of acidose in renal failure process even though the difference is not statistically significant.

It has been reported that bringing hemoglobin levels to normal for patients with chronic renal disease prevents tubular damage and interstitial fibrosis development by improving oxygen flow to the kidneys thereby reducing renal disease progression. In addition, it has also been set forth that the use of EPO prevents oxidative stress and apoptosis and may have direct protective impacts on tubular cells (29, 30). Low hemoglobin levels in the group with RRT requirement in our study leads us to think that anemia is observed more frequently in this group with further decrease in GFR and that it may be effective in progression to end-stage renal disease (ESRD).

Accumulation and combination of genetic and environmental factors may play a role on the onset and progression of ESRD in CRD which is accepted as a multifactorial disease. For example, there may be genetic impacts on the development of hyperparathyroidism secondary to renal failure and VDR gene from among these genes may play a role in progression. Vitamin D plays a role in the regulation of independent biological processes such as the endochrine system, bone metabolism, immune response from birth, cell proliferation and differentiaton (5). Vitamin D response takes place with VDR function. VDR gene is present in many tissues and its activation may modulate more than one target gene expression. Various polymorphisms have been determined in the VDR gene and their functional importance along with potential impacts on disease predisposition have been examined (31, 32). Significant allele variations of the VDR gene in different populations have attracted attention in these studies. FokI polymorphism is one of these allele variations.

Individual allele frequency varies between different ethnic or geographical populations. F allele is observed more frequently than f allele in many societies including our own. For instance, F and f allele distribution was observed as 71,5 % and 28,5 % in a study carried out on healthy population in Northern India [32] which was in accordance with the results of our study. When F/f allele frequency is examined, it can be observed that F allele is more frequent in Finland (60/40), England (69/31), Australia (61/39), Japan (68/32), Taiwan (61/39), White Massachusets (59/41) and Black Pensylvania (78/22) [33]. Allele frequencies that are similar to our results (73 % F and 27 % f) were obtained as a result of a previous study (34) carried out on healthy Turkish population. FokI polymorphism differs among different populations with regard to homozygote and heterozygote mutations. For example, even though the FF genotype ratios are higher in England and Black Pensylvania in accordance with our results (48 % and 63 % respectively), Ff genotype was observed at higher ratios in Northern India (49 %), Finlan (58 %), Australia (48 %), Japan (51 %), Taiwan (49 %) and White Massacusets (45 %) societies. Whereas the ff genotype was determined as the genotype with the lowest frequency even though it is observed at various rates in all societies. However, its ratios were determined more in Finland (14 %), Australia (15 %) and White Massachusetts (18 %) populations in comparison with other societies (34).

The number of studies on the impact of FokI polymorphism on CRD progression is limited (35). The impact of VDR gene ApaI, TaqI, FokI and BsmI polymorphisms has been examined on 258 ESRD patients and 569 healthy control group in the Northern India population as a result of which a statistically significant difference was observed in the FokI ff ($p=0.001$) genotype frequency (35). It was put forth as a result of the haplotype analyses that individuals with a/t/F/b haplotypes are prone to 11,0 times greater risk. In conclusion, they have asserted that VDR gene FokI polymorphism is related with ESRD (35). Nevertheless, a relationship could not be determined in our study between FokI polymorphism and renal progression and RRT requirement. A statistically significant difference could not be observed between the healthy control group and patient groups with regard to FokI gene polymorphism. However, it was determined in all 3 groups that the basal creatinine values were lower in the ff genotype and thus the Basal GFR levels were higher. Even though the difference is not statistically significant, these results have brought about the opinion regarding the need for future studies related with the positive impact of ff genotype on renal progression.

Similar results have been obtained in another study as well (36). These researchers have examined the relationship between FokI polymorphism and 1,25 OHD vitamin levels and CRD stages during a study carried out with 410 type 2 diabetic CRD patients as a result of which no relationship was determined between FokI

polymorphism and CRD which is in accordance with our results. Moreover, they observed an interaction between ff genotype and 1,25 OHD vitamin (p=0,008). They determined that the negative relationship between 1,25 OHD and CRD stages was repressed more in FokI ff genotype rather than FokI FF genotype.

The relationship between VDR start codon polymorphism (FokI) and PTH levels, calcidiol and calcium was examined in another study carried out on 64 Spanish CRD patients [37] as a result of which the genotype frequencies were determined in the patient population as 54,7 % FF, 28,1 % Ff and 17,2 % ff; whereas the values for the healthy control population were determined as 46,7 % FF, 43,3 % Ff, 10 % ff. The difference between the patient and control groups was determined to be statistically significant ($p<0.01$). Serum PTH levels in the FF genotype group patient population (159,77 +/- 25,69 pg/ml) to be higher at a statistically significant level in comparison with both Ff and ff groups (106.67 +/- 19,07 and 77.55 +/- 15,85 pg/ml, respectively, $p <0.05$). However, no statistically significant difference was observed between the genotypes with regard to calcidiol or calcium levels (37). Contrary to this, a statistically significant relationship was determined in our study between PTH levels and FokI polymorphism. No statistically significant difference was observed between the calcium levels. The reason for this may be due to the fact that a certain polymorphism most likely does not manifest itself among ethnic groups even though ethnic differences are observed between VDR gene polymorphisms. Because there is the opinion that the physiological role of vitamin D endocrine system is the same in all ethnic groups (5). These results seem insufficient in determining the impact of polymorphism on progression and PTH response in CRD patients. In addition, vitamin D deficiency and vitamin D resistance in ESRD depends on many different factors. Therefore, there is a need for more comprehensive randomized controlled studies depending on vitamin D plasma level measurement or vitamin D treatment status.

On the other hand, ethnic variations of the VDR gene may yield beneficial results in genetic studies due to the fact that VDR may play a role in the progression of various chronic inflammatory and degenerative diseases and due to its polymorphic content. Significant differences in FokI allele frequency will be beneficial for establishing a bond between the individuals in different societies. These studies may provide a foresight for determining the sensitivity towards certain diseases and the clinical management of patients in the long run.

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Compliance with ethical standards
Conflict of interest
The authors declare no conflict of interest.

Ethical approval
All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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Morin controls high-cholesterol diet-induced inflammatory cardiac dysfunction through the regulation of nitric oxide synthesized enzymes and P65 NF-ϰB gene

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Summary. Morin is a natural yellow compound that is scientifically proven to have hypoglycemic, anti-inflammatory and anti-oxidant properties. The ameliorative effect of morin on high-cholesterol-diet (HCD) induced cardiac damage has not yet been assessed. Hence, in the present study, we evaluated the ability of the compound to controls HCD induced cardiac inflammation and oxidative damage through the regulation of nitric oxide synthesized enzymes and nuclear factor kappa B (P65 NF-κB) gene. HCD rats exhibited an increased activity of markers of cardiac enzyme in serum such as lactate dehydrogenase (LDH), creatine kinase-MB (CK-MB) and creatine kinase (CK). Administration of HCD to the rats was fond to elevate the serum and cardiac lipids profiles. Moreover, cardiac pro-inflammatory and cardiac oxidative markers were raised and the antioxidant markers were lowered. However, in the morin treated to HCD rats, the above markers for biochemical, inflammatory, antioxidants, and oxidative changes were reverted to near normal food consumed control rats. The myocardial protein expressions of neuronal nitric oxide, inducible nitric oxide, and endothelial nitric oxide synthases, as well as P65 NF-κB were significantly increased in rats supplemented with HCD. When treated with morin, these protein levels were lowered and were comparable to those of normal food consuming rats. Such an alleviated inflammatory myocardial dysfunction upon morin administration has been proven by the improvement of histological features. The results of this study suggest that morin administration combats HCD induced myocardial inflammation and dysfunction through the regulation of nitric oxide synthesized enzymes and P65 NF-κB gene. These results vouch for benefits of dietary morin against HCD induced cardiac dysfunction.

Key words. hypercholesterolemia, morin, oxidative stress, inflammation process

Introduction

Hyperlipidemia associated oxidative inflammation plays an important function in atherosclerosis and cardiovascular diseases (CD). CD, particularly coronary heart disease (CHD), is a well-known growing public health issue worldwide. Hypercholesterolemia is extensively known as a lipoprotein metabolic disorder characterized by accentuated lipid profiles and is the most important risk issue for CD (1-3). Obesity is an established causative factor for the expansion of atherosclerosis and CHD, and is known to produce systemic and cardiac inflammation finally resulting in a failure of cardiac cell function (4). A prolonged consumption of high cholesterol food is the predominant reason for obesity as well as an increase in systemic and cardiac inflammatory markers such as tumor necrosis factor (TNF-α,), interleukin-1 beta (IL-1β), interleu-

kin (IL-6) and caspase-3. Earlier studies have shown that increased levels of proinflammatory markers, including TNF-α, IL-6 and IL-1β occur in heart failure conditions (5-7). Cytokines are known to play a protective role in certain developed and developing diseases, including heart failure (8). Therefore, cytokine-based preventive drugs without prominent side effects are urgently needed for treating high cholesterol food induced heart failure patients.

Chronic hypercholesterolemia has been found to induce oxidative organ damage, which is an important factor for raising the risk of CD (9). Earlier studies have clearly indicated that hypercholesterolemia is causally associated with a significant increase in reactive oxygen species (ROS) and a concomitant lowering of the cardiac tissue antioxidant capacity (10, 11). High circulating cholesterol levels attributed to HCD consumption may activate endothelial cells and lead to increased production of ROS (12, 13). This mechanism induces vascular function impairment, cell proliferation, cell death, and cardiac remodeling (14, 15). Certainly, HCD supplementation leads to oxidative stress, impulsive arterial vasoconstriction, and systemic hypertension (16). Goncalves and his colleagues (17) reported that the Western diet is high in fat and induces biventricular cardiomyocyte hypertrophy, increased stiffness, and impaired relaxation in rats. However, the effects of HCD on bioenergetics and oxidative stress, and impairment of cardiac function are only partially understood and no treatment has demonstrated compelling effectiveness.

Currently, the use of dietary flavonoids, known as polyphenolic compounds, is gaining the attention of researcher. The compound is abundant in plant-derived beverages such as red wine and tea, as well as in many fruits, green vegetables, and traditional medicinal plants. Flavonoid display anti-inflammatory properties as it brings about detoxification of free radical, metal chelation, antioxidant enzyme modulation, and inflammatory cytokine regulation (18, 19). Recently, it has been shown that flavonoids can also improve intracellular bioenergetics (20, 21). Several reports have concluded that these compounds can exert cardio-protective effects due to their ability to attenuate oxidative stress. Morin, a yellow colored bioflavonoid possesses an extensive assortment of pharmaceutical and biological properties including antioxidant, antiviral, anti-carcinogenic, and anti-inflammatory properties (19, 22, 23). Morin also decreases oxidative damage in the fibroblast cells of the lungs (24), cardiovascular cells (25) and hepatocytes (26, 27). The effectual characteristics of morin against high-HCD-induced cardiac damage have not yet been explored. Hence, in this study, we attempted to check whether the compound controls HCD induced inflammatory cardiac dysfunction through the regulation of nitric oxide synthesized enzymes and P65 NF-κB gene.

Materials and Methods

Animals

140-160 g adequate numbers of Wistar albino rats were attained from Pharmacy College Animal Care Center at King Saud University. All received animals were acclimatized for 10 days prior to start the experiments. All rats were sustained in standard conditions such as 22 ± 1 °C temperature, 50-55% humidity, and equal 12 h day/night cycles. All the experimental protocol such as euthanasia procedure, blood sampling and final sacrifice were followed by National Institute of Health guide care policy (NIH, 1996) and this animal study was approved (647-EACC-2017 dated 02-01-2017) by Pharmacy College Animal Care Center Ethical Committee.

Food composition for normal food and high cholesterol food

High cholesterol diet in pellet form was prepared by adding 1% cholesterol + 0.5% cholic acid with normal cholesterol rat chow (NCRC) powder. Six rats were fed on NCRC (content: protein 20%, fat 4%, fiber 3.5%, ash 6%, total energy 2850 Kcal/kg) and twenty-four rats were fed HCD for 6 weeks. Water and food were allowed to free access in this whole experiential duration.

Chemicals

Morin, cholesterol and cholic acid were purchased from Riedel-del Haen, Germany, Alpha Chemika, India and Fluka, Switzerland, respectively. The diagnostic kits of total cholesterol (TC), triglyceride (TG), low-density lipoprotein-cholesterol (LDL-C),

high-density lipoprotein-cholesterol (HDL-C), CK-MB, LDH and CK were acquired from Human Diagnostics, Wiesbaden, Germany. The diagnostic kits of IL-1β, TNF-α, caspase-3, IL-6, Superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST) and glutathione peroxidase (Gpx) were acquired from standard R&D company from USA. The diagnostic kit of Glutathione (GSH) and thiobarbituric acid-reactive substance (TBARS) were obtained from Cayman Chemical, USA.

Study design:

Ten days of environmental espoused animals were administered HCD or NCRC for 6 weeks. After 6 weeks the rats were randomly divided in to five groups by taking six rats in each group: 1) NCRC control, 2) HCD control 3) HCD + Morin (25 mg/kg/day), 4) HCD + Morin (50 mg/kg/day) and 5) HCD + Morin (100 mg/kg/day). Morin was treated orally for four consecutive weeks and this period HCD was continued until end of the experiment. The body weight and health conditions of animals were checked carefully by weekly once. Blood were gathered by cardiac puncture under total anesthesia state. The serum was suppurated by 4,000 rpm centrifugation of sample for 10 minutes and stored at -20 ºC prior to analysis. Finally, animals were decapitated and heart tissues were dissected, weighed, immediately small cross section of each heart tissue and dipped into liquid nitrogen for 1 min. This heart section was stored at -80 º C until analysis. Another cross sectioned heart was preserved in 10% formaldehyde for histopathological evaluations.

Estimations of lipid levels in serum

TC, TG, LDL and HDL levels were estimated by commercially existing kits.

Estimations of cardiac enzymes in serum

CK-MB, CK and LDH were estimated by commercially existing diagnostic kits.

Estimations of lipid profile in cardiac tissues

Heart lipids were extorted by standardized Folch et al (28) method and used in chloroform–methanol mixture (2:1 v/v). Briefly, tissues were homogenized with 0.74% potassium chloride (1:1 w/v) and suspended in 2 ml of chloroform andmethanol mixture for 2min and then centrifuged.The chloroform layer was dried and the remaining cardiac lipid contents were used for analysis. The tissue phospholipid (PL) was ascertained by ideal method of Zilversmit and Davis (29). FFA was ascertained by ideal method of Falholt et al. (30). TC and TG were ascertained by kits which are available in commercially. Results were articulated as mg/g of tissue.

Estimations of inflammatory biomarkers in cardiac tissue

IL-1β, TNF-α, IL-6 and caspase-3 were ascertained by ELISA kits which are available in commercially.

Western blot analysis

Myocardial total protein was assayed in each sample by using assay kit (BioRAD, Hercules, CA). The protein bonds were separated by using SDS-PAGE. The obtained protein bonds were transferred from gel to PVDF membrane at 25 volt. The membrane was incubated with primary antibody solution by overnight at 4°C. After the membrane was washed tree to five times with TBST buffer and then incubated with the secondary antibody (HRP-conjugated solution) for one h at room temperature. Finally, the membrane was washed for three to five times by using TBST buffer. The chemiluminescent substrate was used for bonds development and the bands were seen and captured by CCD camera-based ChemiDoc TM imager (Bio-Rad Laboratories, Inc, 2000 Alfred Nobel Drive, Hercules, California 94547, USA). The band intensity of target protein was calculated by image analysis software.

Estimations of oxidative stress parameters in cardiac tissues

TBARS and GSH levels were ascertained by kits which are available in commercially. SOD, CAT, GPx and GST were ascertained by using commercially available ELISA kits.

Histological assessments procedure

A collected portion of a heart tissue from each group was conserved in 10% formalin. Each sample was implanted separately in paraffin blocks and then 5 mm section was removed by using rotary microtome.

The section was stained with haematoxylin and eosin. Finally, the histology was captured by microscope and evaluated the changes of histology.

Statistical Analysis

Result was conveyed as mean ± standard error. Statistical variations from used groups were analysed using one-way analysis of variance (ANOVA) and Student-Newman-Keuls multiple comparisons test. Different letter and symbol are represents as statistically different if the p value was less than 0.05, 0.01 and 0.001.

Results

The enzymes of CK, CK-MB and LDH are considered the cardiac markers and these were estimated and shown in Figure 1. In HCD administered rats, the serum enzymes of CK, CK-MB and LDH were shown to increases (P<0.001) compared to NCRC control group. 100 g of morin treatment (100 mg/kg/day) markedly inhibited these enzymes changes in HCD fed animals. Moreover, the lower and higher dose of morin (25 and 50 mg/kg/day) also inhibited these activity of enzymes but not statistically significant.

Serum TC, TG and LDL-C were increased significantly (P<0.001) while HDL-C was shown unchanged level in HCD fed animals compared to NCRC control rats. The elevated TC and LDL-C levels were found in markedly decreased (P<0.05 and P<0.01) by morin (50 and 100 mg/kg/day) treatment as compared to HCD fed control group. However, morin treatment significantly and dose dependently inhibited the increased TG levels in serum of HCD fed rats while compared to untreated HCD fed animals. These interesting serum lipid profiles results are shown in Figure 2.

Myocardial lipids including TC, TG, PL and FFA were estimated and presented in Figure 3. Cardiac TC, TG and FFA levels were significantly (P<0.001) increased in HCD fed rats when compared to that of NCRC control group. Cardiac PL was markedly (P<0.01) inhibited in HCD consumed rats when compared to NCRC control rats. Treatment of morin in HCD consumed rats, these TC, TG and FFA levels were reduced and PL level was increased (P<0.05) when compared to HCD consumed control rats. These

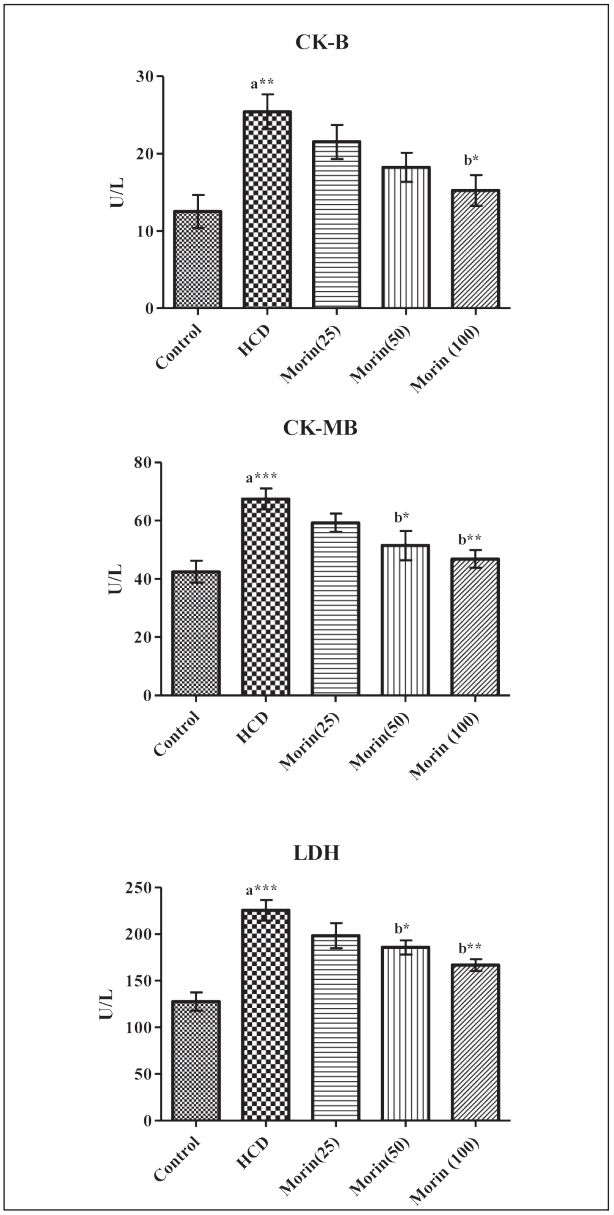

Figure 1. Effect of morin on HCD-induced changes in serum CK, CK-MB and LDH levels.

cardiac lipids results are shown in Figure 3.

TNF-α, IL-1β, IL-6 and caspase-3 are the markers of cellular inflammation and these were found in increases significantly (P<0.001) in cardiac tissue of HCD consumed rats when compared to NCRC rats. Morin treatment to HCD fed rats these cellular inflammatory markers were inhibited significantly when compared to HCD control group. These cardiac cellular inflammatory markers results are shown in Figure 4.

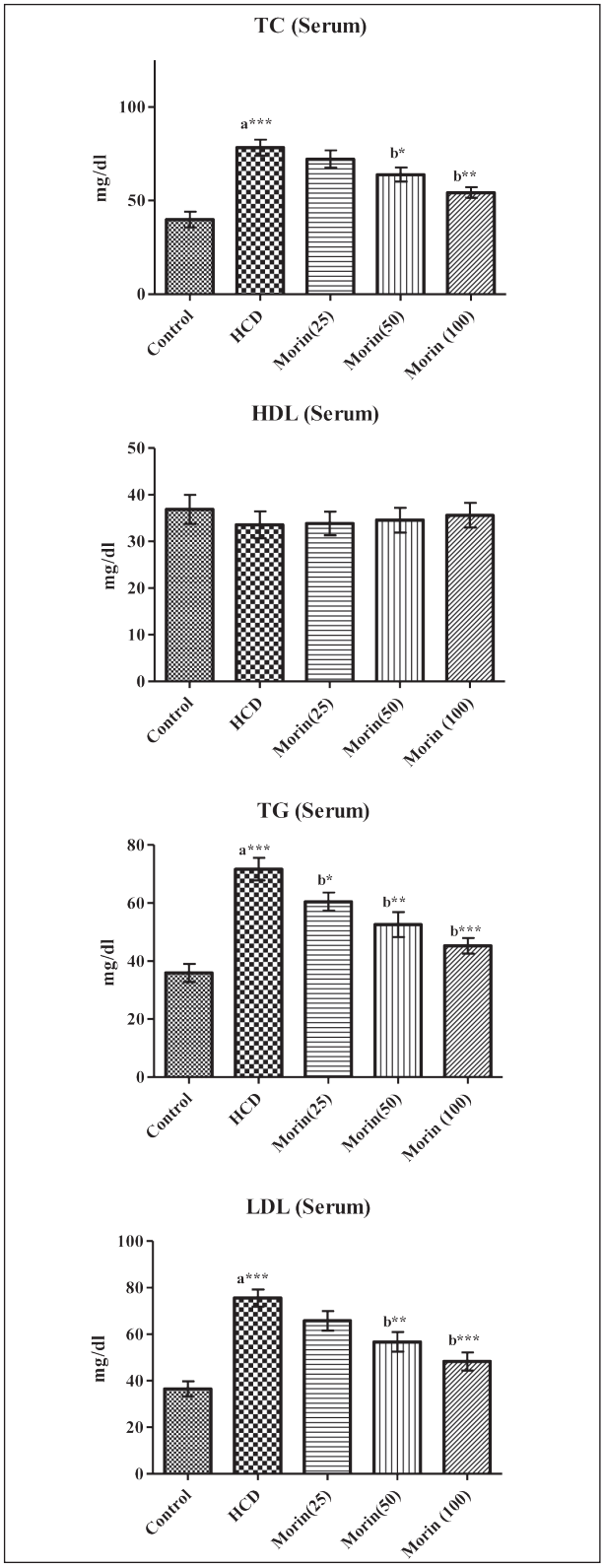

Figure 2. Effect of morin on HCD-induced changes in serum lipid levels of TC, TG, HDL-C and LDL-C in rats.

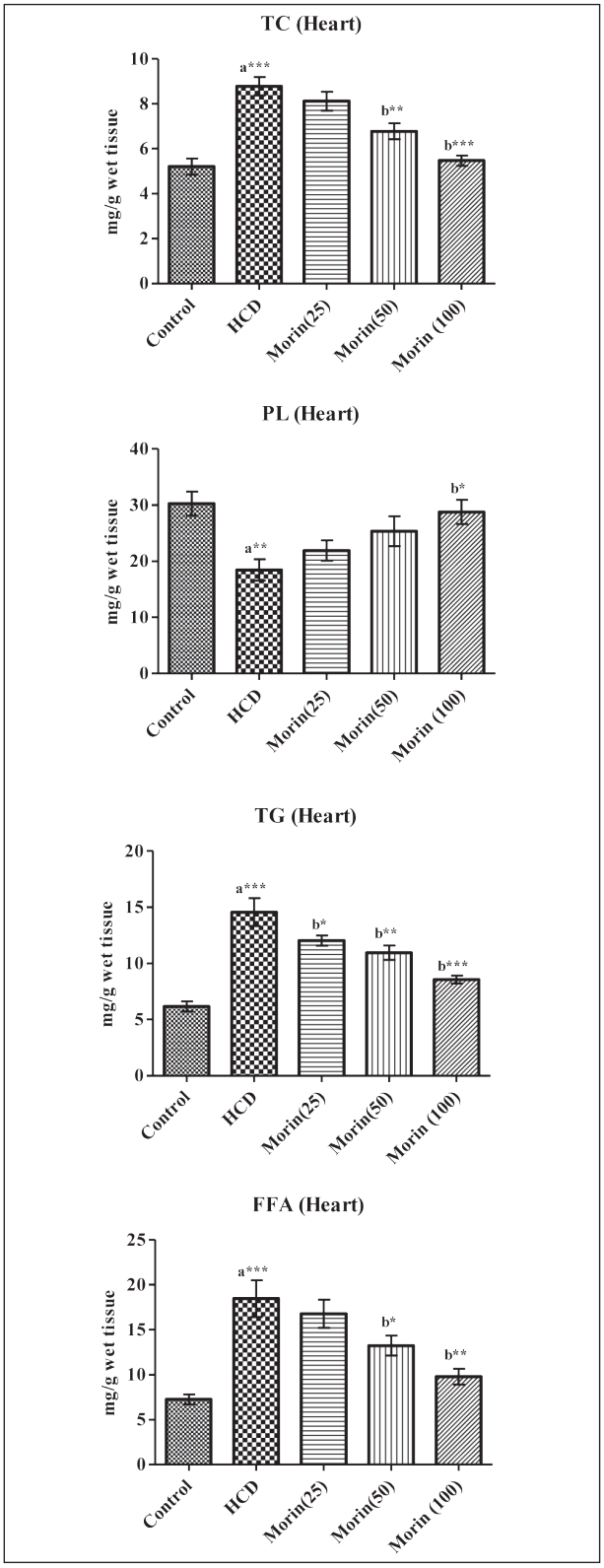

Figure 3. Effect of morin on HCD-induced changes in cardiac lipid levels of TC, TG, PL and FFA in rats.

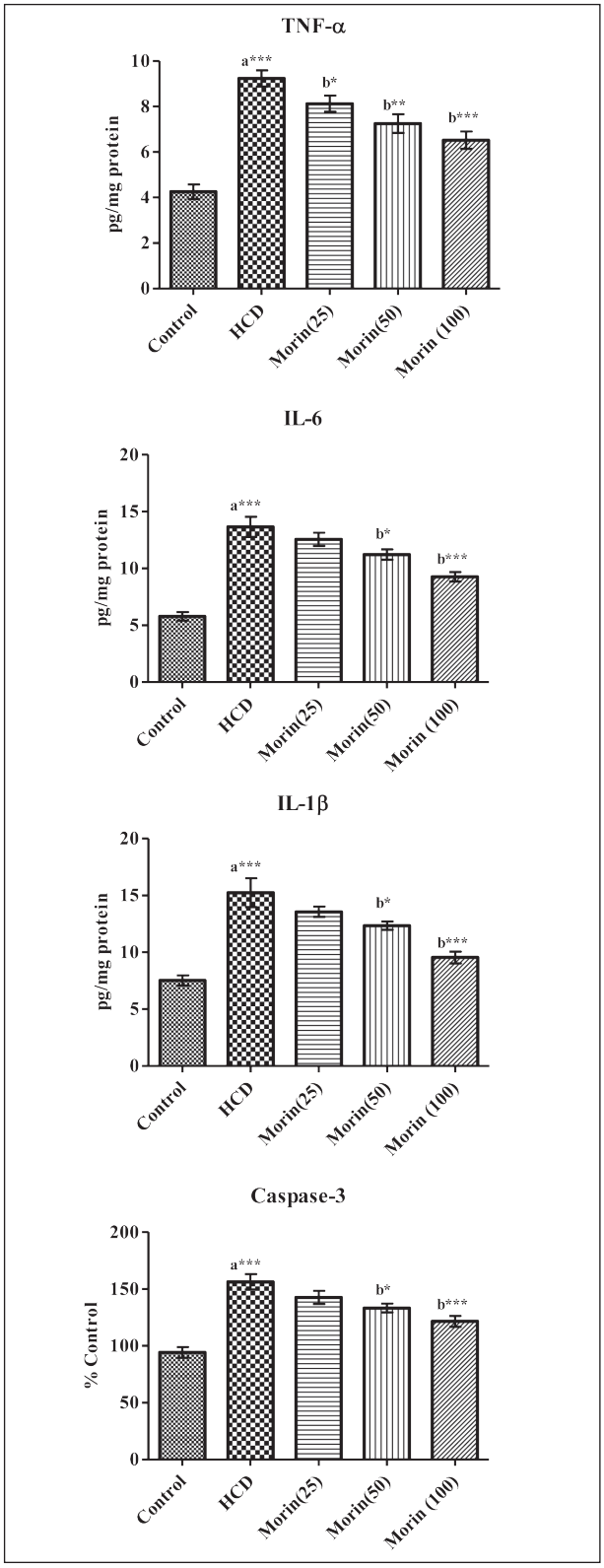

Figure 4. Effect of morin on HCD-induced changes in cardiac pro-inflammatory biomarkers including TNF-α, IL-6, IL-1β, and caspase-3 levels.

Myocardial protein expressions of inducible (iNOS), neuronal (nNOS), and endothelial (eNOS) nitric oxide synthases, and NF-kBp65 were significantly (P<0.001) increased in HCD consumed rats when compared to that of NCRC control animals. Morin treatment markedly decreased these protein expressions in dose dependent manner and these results are shown in Figure 5.

Oxidative stress in cardiac tissue was seen in HCD supplemented rats (Figure 6). TBARS level was in high significantly (P<0.001) while GSH level was reduced (P<0.001) in cardiac cells of HCD fed rats compared to NCRC control animals. Morin treatment (50 and 100 mg/kg/day) for 4 weeks to HCD fed rats, the TBARS was reduced markedly (P<0.05 and P<0.001, respectively) and the GSH was increased (P<0.05 and P<0.01, respectively) when compared to HCD supplemented

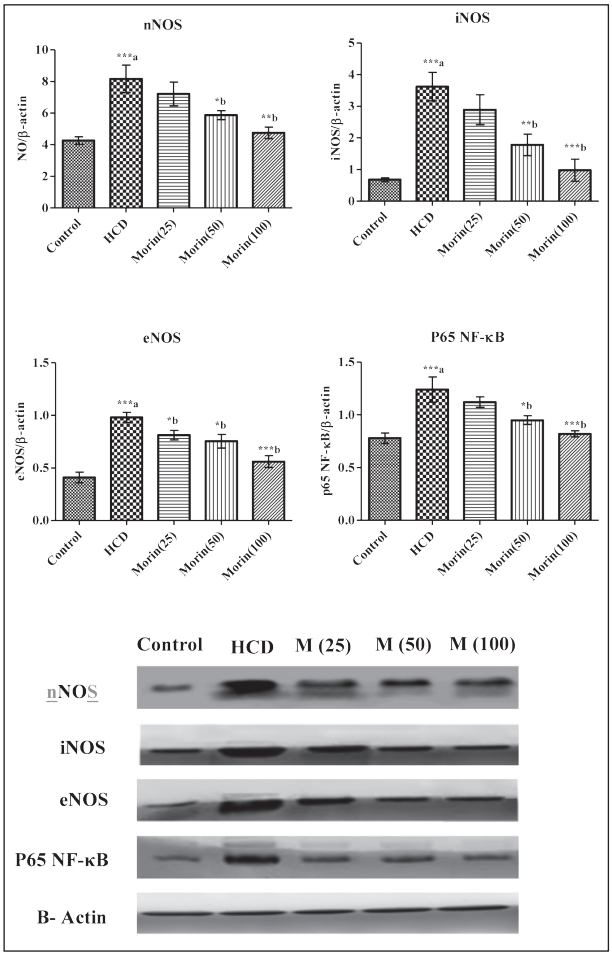

Figure 5. Effect of morin on HCD-induced nitric oxide synthase and P65 NF-κB gene changes in heart.

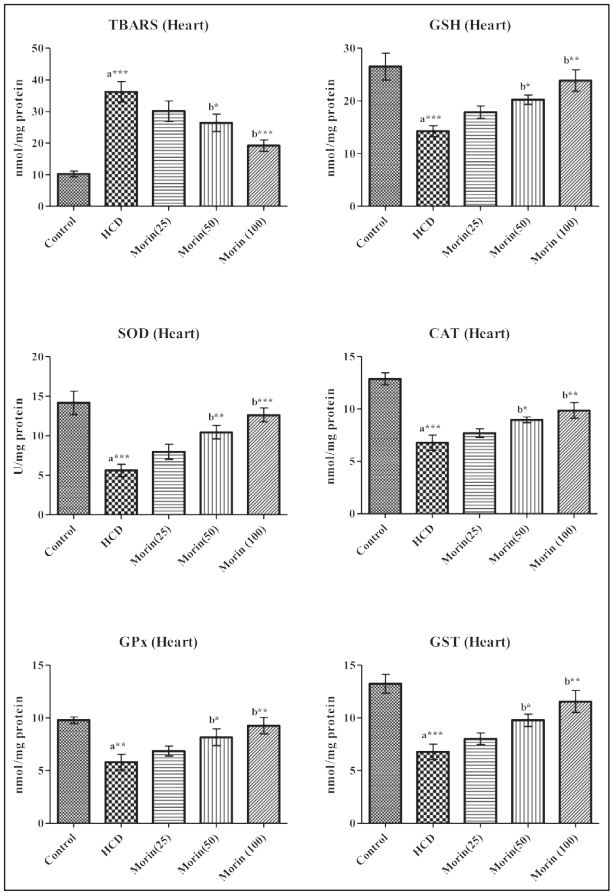

Figure 6. Effect of morin on HCD-induced antioxidant systems markers in heart

control rats. Enzymatic cardiac antioxidants of SOD, CAT, GPx and GST were found to reduces ($P<0.001$) in HCD fed rats compared to NCRC control group. Morin higher dose markedly enhanced the enzymatic cardiac antioxidants when compared to HCD control group and these results are sown in Figure 6.

Histopathological changes are reported in Figure 7. NCRC control rats [Picture A] showed normal myocardial tissue with normal blood vessels. In HCD fed rats [Picture B] revealed blocked vessels due to deposition of cholesterol on inner wall of the vessels (plaque), and then the arthrosclerosis was developed. In the middle dose (50 mg/kg/day) of morin treated group showed reduced cholesterol deposition on inner wall of the vessels [Picture C]. The higher dose (100 mg/kg/day) of morin treated group showed normal blood vessels and the cholesterol deposition was seen in minimal [Picture D].

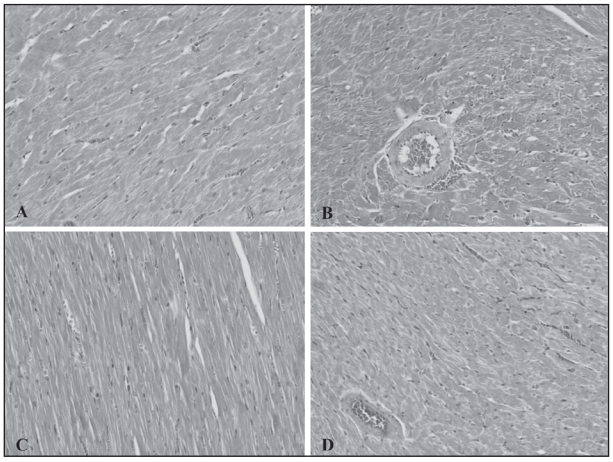

Figure 7. Effect of morin on HCD-induced changes in cardiac tissue where [A] Control [B] HCD, [C] morin (50 mg/kg/day) treated to HCD fed rats and [D] morin (100 mg/kg/day) treated to HCD fed rats

Discussion

Recently, the prevalence of nutrition associated diseases such as over weight, obesity, diabetes and cardiovascular problems have been increased worldwide. Lipids are very energetic source in heart health but, currently, studies revealed that excess lipids in human body associated with much kind of diseases including cardiac cell dysfunction. However, earlier studies also reported that HCD supplementation induces excess lipids in blood and organs including heart that is characterized by increasing in TG, FFA, TC, LDL-C, CK, CK-MB and LDH in blood and heart (31-33). The CK-MB, LDH and CK are definite markers of myocardial injury and these showed peak release at 5 min after the reperfusion (33). Hypercholesterolemia, a foremost threat factors for heart disease development. It was suggested that the LDL peroxidation is foremost factor for the enlargement of atherosclerosis (34-36). Therefore, the cholesterol lowering successful compounds without any harmful effect are urgently needed in current society (37-39). In our study, the HCD supplemented rats showed increased serum TC, TG, LDL-C, CK, LDH and CK-MB levels. Moreover, in this study, the cardiac TC, TG and total FFA increased while the PL decreased in HCD supplemented rats when compared to NCRC control rats. Morin treatment improved these cardiac markers and lipids changes to nearby NCRC control rats which clearly showed that the potential

successful role of morin against HCD-induced cardiac toxicity in rats. Earlier studies proved that many phenolic compounds contains protective role against the metabolic diseases and atherosclerosis due to their mechanism of inhibiting LDL-C, oxidation of lipids, and enhanced cellular inflammatory signaling pathways (40-42). In our study, morin supplementation ameliorated the HCD-induced cardiac toxicity in rats due to lowering of TC, TG, and LDL-C. Earlier study also showed that morin decreases lipid profile against the hypercholesterolemic rats (43). Recently, Naowaboot and his colleagues (44) reported hypolipidemic effect of morin against obese mice.

It is well documented that the inflammatory biomarkers are linked with the cardiovascular threatening factors (45). Earlier studies proved that the improvement of myocardial infarction and atherosclerosis are linked with the peripheral and cardiac tissues inflammation (46-48). TNF-α, IL-6, and IL-1β cytokines are regulated by several biological progressions and participates in inflammation, host defense against organ disorders, and others (49). Similar results were reported for a clinical study, wherein IL-6 and TNF-α levels increased in fatty liver disease patients (50). In an experimental study, HCD-fed rats presented with significant increases in cardiac TNF-α and IL-6 levels (51, 52). It has been proposed that inflammation and immune system anomalies are associated with atherosclerosis. This hypothesis was reinforced by the detection of plaques composed of pro-inflammatory cytokines including TNF-α (53) and IL-1β (54). These were activated by NF-κB. Activated TNF- α /CD95 interacts with at least one cell surface receptor and triggers caspase activation and cytochrome c release. Caspase-3-mediated P21 cleavage and subsequent upregulation of cyclin A/Cdk2 activity are important cell death mechanisms. Our results align with those in a recent report which showed that HCD upregulate caspase-3 expression in cardiac cells. In our study, TNF-α, IL-1β, caspase-3, and IL-6 were found to enhances in circulatory and heart of HCD supplemented rats and upon treated with morin were inhibited these cytokines production to nearby NCRC control rats. It has been reported that morin has several pharmacological functions including protective role of oxidation of cellular lipids (55, 25) and cellular inflammation (56). It has also proved that morin reduces inflammation of liver by downregulating SphK1 activity, blocking NF-kB nuclear translocation, and inhibiting IL-1β, IL-6, and TNF-α secretion by hepatocytes (57). Furthermore, Lee et al., (58) demonstrated that morin pretreatment protected mice from hepatic damage by reducing NF-kB activation.

An earlier study suggested that hypercholesterolemia-induced organ damage is probably associated with ROS accumulation (59). Other studies suggested that the association between the hypercholesterolemia-induced tissue damage and ROS overproduction and the results enhance the lipid peroxidation, damage DNA, degrade proteins, and deplete antioxidant defense systems (60). In this study, HCD supplemented rats showed enhanced levels of oxidative stress biomarker like TBARS and decreased the antioxidant systems biomarkers like SOD, GSH, GPx, CAT and GST compared to NCRC control rats. Montilla et al. was reported similar results in HCD fed animals (61, 62). Administration of morin significantly improved the cellular antioxidant systems due to reducing effect of cellular lipids and inflammation. Flavonoids are natural antioxidants (63). Morin, a yellow colored flavonoid, protects against nephrotoxicity, hepatotoxicity and ischemia-reperfusion through the anti-inflammatory and anti-oxidant properties (24, 25, 64, 65).

Nitric oxide (NO) act as a gaseous cellular messenger and this synthesized from L-arginin by the nitric oxide synthase (NOS) enzyme which is in three isoforms including iNOS, nNOS, and eNOS. NO plays a crucial role for the NF-kB causative effect of cardiac diseases. Enhanced nitric oxide free radical bioavailability has shown in hypercholesterolemia condition and this is plays a decisive role in cardiac cell dysfunction and apoptosis in high cholesterol associated diseases (66, 67). The DNA binding protein of p65 NF-kB is playing a central role for the pathophysiology of cardiac dysfunction by the transcription of pro-inflammatory cytokines. Increased level of reactive oxygen species including nitric oxide may represent an initial step in the signal cascade of NF-kB activation (68, 69). In this study, the protein level of nNOS, iNOS, eNOS, and NF-kB (p65) were increased in HCD supplemented rats and these were reverted by administration of morin. In our experiment, such an

alleviated inflammatory myocardial dysfunction upon morin administration due to the inhibitory effect of nitric oxide synthesized enzymes and p65 NF-kB gene activation. The detailed mechanism should be studied in future. Present study concluded that the morin treatment has improved the cardiac markers and lipids changes which clearly proved that the potential role of morin against HCD-induced cardiac toxicity in rats. This protective role of myocardial inflammatory dysfunction might be due to the regulation of nitric oxide synthesized enzymes and p65 NF-κB gene. Such an alleviated inflammatory myocardial dysfunction upon morin administration has been proven by the improvement of histological features. These results vouch for benefits of dietary morin against HCD induced cardiac dysfunction.

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The effect of nutrition theraphy on oxidative stress, inflammation, glycemic control in type 2 diabetes patients

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Summary. *Aims:* Type 2 diabetes mellitus is a metabolism disease which is seen frequently among adult population in Turkey. The aim of this study was to determine the medical nutrition theraphy effect on oxidative stress, inflammation, glycemic control in type 2 diabetes patients. *Methods:* An interventional study was carried on 35 type 2 diabetes ages between 20-65 years old at the Department of Endocrinology of Başkent University İstanbul Hospital in 2015. In 3 month period a personal nutrition theraphy was applied. Biochemical parameters, anthropometric measurements and body analysis were also determined. The three day food consumption and biochemical parameters were requested at the beginning and at the end of the study. *Results:* When the impact of the blood values of the new medical nutrition theraphy which the patients practiced during the first visit and the follow up visit were compared; it was seen that there was a significant decrease on fasting plasma glucose, fasting insulin, HbA1c, CRP, TG and MDA values ($p<0.05$). The mean diabetic age of the patients was 7.63±6.22 years. When diabetic age of the patients was increased, there was a positive correlation between the fasting plasma glucose and HbA1c values ($p<0.05$). *Conclusions:* Type 2 diabetes patients were evaluated 3 month of medical nutrition theraphy and it was seen that the personal medical nutritional theraphy contributed to providing glisemic control and decreasing oxidative stress and inflammation.

Key words: glycemic control, inflammation, oxidative stress, medical nutrition therapy, Type 2 diabetes

Introduction

Type 2 diabetes mellitus is a metabolism disease which is seen frequently among adult population in Turkey and especially in developing countries in particular. Type 2 diabetes is seen between the ages of 40 and 59 and it consists of the 90% percent of the whole diabetic occasions. Type 2 diabetes is the most increasing disease. According to the datas of World Health Organization (WHO), there were 171 millions of diabetic patients in the year of 2000 and it is expected that this will be 366 millions in 2030. International Diabetes Federation (IDF) expects that there are 425 millions of diabetic adult patients around the world. It is also expected that the prevalance of diabetics will reach 592 millions by 2035. So, 8.03% of the world population have diabetics and 6.9% have impaired glucose tolerance (1).

According to the Turkey datas of IDF, in 2012 the prevalance of diabetic disease was found as 8.3%. The epidemiologic studies identifying the prevalance of type 2 diabetes in Turkey started in 1990. According to the The Turkish Diabetes Epidemiology *Study* I and II (TURDEP-I and TURDEP-II) studies which was practiced by TURDEP on 25.000 ≥19-year-old people in 1997 and 2010, the prevalance of type 2 diabetes disease was increased from 7.2 to 13.7%, which comes up to 95%, in 12 years period of time. It was determined that the prevalance of diabetic disease was 14.6% among men and 12.4% among women. In our country, the number of the type 2 diabetics patients

are 10.3 millions. In an attempt to determine the heart disease and the risk factors in parallel with the disease in adults, in the Turkish Adult Risk Factor Study (TEKHARF), the prevalance of type 2 diabetic disease was specified as 11.3% among over 35-year-old people in the years of 1997 and 2005, and this number comes up to 3.3 millions of people (2).

By establishing a decent glisemic control on type 2 diabetes patients, developing diabetic microvascular and macrovascular complications can be kept under control. The gold standard of evaluating glisemic control among diabetes is HbA1c. However, especially postprandrial hiperglisemi peaks are accepted as the sign of cardiovascular risk increase. To provide a strict glisemic control, it is necessary to bring fasting and postprandial plasma glucose degrees to the condition of near-normal degrees. UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Turkish Diabetes Foundation determined that, as a glisemic control aim, fasting blood sugar glucose should be 120 mg, HbA1c value should be less than 7%, young non-cardiovascular risk patients should be less than 6.5% and postpradial blood glucose should be less than 140mg/dL (3).

Nutritional theraphy and lifestyle modification including exercise are the bases of the treatment of type 2 diabetes disease. The importance of nutritional theraphy for diabetic patients has long been recognized for many years and has been in use since the 1550s. Although the nutritional treatments that have applied since the very beginning times have quite changed, the main aim is to keep the hyperglisemia of the patients under control. Besides, another aim is to provide optimal body weight, keep the hyperlipidemia under control and avoid akute and chronic complication development. Thanks to this review, it was observed that with the medical nutrition theraphy applied on type 2 diabetic patients, the development of mikro and makrovascular complications were regressed by means of decreasing HbA1c level. Different nutrition programs are being studied for optimal medical nutrition therapy for patients with type 2 diabetes (4). American Diabetic Association suggests that 45%-65% of the total energy comes from carbohydrates, 20%-35% from fat and 10%-20% from total protein when nutritional theraphy is planned in diabetic patients. Low carbonhydrate nutrition theraphy provides normaglisemia, but less than 130 grams of carbonhydrate intake is not suggested Daily (5). When the recommendations of guidelines are reviewed, it appears that the vitamin mineral requirements for diabetics are not very different from those of healthy individuals (6).

Oxidative stress is caused by the imbalanced in free radical production so that mitochondrial dysfunction increases and antioxidant defence decreases. According to the conducted researches, elevated blood glucose levels lead to vascular complications and lipid peroxidation. The experimental and clinical researches show that oxidative stress had a decisive role on the pathogenesis of diabetes. Free radicals are found more in diabetes patients (7,8). The amount of cytokine in their circulation increased because of the inflammation in type 2 diabetes patients. The increased cytokine level is the risk factor for atherosclerosis and cardiovascular diseases. Some of the cytokines are released from liver and some from the adipose tissue. CRP is produced in the liver and associated with diseases such as obesity, coronary heart disease and insulin resistance (9).

Chronic low level inflammation and increased oxidative stress are seen in Type 2 diabetes patients. Inflammation and oxidative stress can be reduced with the help of nutritional theraphy practiced on patients. In this study, it was aimed to evaluate the influence of on medical nutrition theraphy on oxidative stress, inflammation and providing glisemic control on type 2 diabetic patients.

Materials and Methods

This interventional study was carried on among 45 type 2 diabetic patients at the age of 20 and 65 who applied for Başkent University İstanbul Hospital the department of endocrinology between february and december 2015. All the patients participating in the study were given an informed consent form and all of them had written approval. Patients who want to leave at the beginning of the study or in any part of it have been removed without study. People who are under 20 and over

65 years old, smokers, pregnants and lactatings, those using antioxidant vitamins and mineral supplementations, having acute infections, using stomach pills and diuretics are not included in the research. Four of the patients who joined the research left the research because of not coming to the control meeting, and 6 of them left the research because of not practising the planned medical nutrition programme. The study was completed with 35 patients with type 2 diabetic patients. For this study, the ethic commitee approval was taken by Başkent University Clinical investigations ethic commitee the number for 15/25 and the date for 2015, february 18th.

At the beginning of the study, a general questionnaire consisting of 23 questions, an anthropometric measurement questionnaire, a three-day nutrition consumption registration form (three consecutive days which includes a weekend) and a physical activity assesment form were applied to patients. The height, weight, BMI, waist to hip ratio, neck circumference and body fat analyse of patiens were measured. At the end of the assessment, a medical nutrition treatment programme was prepared to protect their weights for normal weight patients, while to lose the 5%-7% of their initial weight for mildly obese and obese patients.

The evaluation of nutrition status

To identify the dietary habits of the patients, food consumption frequency form was applied. To determine the dietary status of the patients, a three-day food consumption form was taken at the beginning of the study and three months later nutritional education was given. 24-hour food consumption form was determined through the recordkeeping technique. By the researcher, the consumption forms were given to the patiens who were provided portion education and it was required to record the food and drinks they consumpted for three days, which includes a weekend and two weekdays, in the morning, afternoon and evening with the detail of the place and time they consumpted on the form. The portion education was provided to the people who joined this study by using food and nutrition photo catalog (10).

Determining the level of physical activity

To determine the level of the physical activity of the patients taking part in the study; in the beginning and after three months of the study, during 3 days it was needed to save the physical activities they did in details on the form. These activities were made by the daily physical activity level (PAL) (11).

Assessment of anthropometric measurements

The height measurement was done with the height meter, the brand of Seca-206 (Hamburg, Germany). While the height measurement was being done, the standing position and head at Frankfurt plane were paid attention. The body analyse of the patients was done with Tanita BC-418 analyzer (Tokyo, Japan). BMI can be measured with the weight/height equation (12). While evaluating the results of BMI, the BMI classification of WHO was used (13). The waist circumference of the first and last coming of the patients, the smallest waist circumference between bottom kosta and prosessus spina ilaca anterior superior were measured and saved with a measure parallel to the ground from navel by the researcher.

Biochemical measurements

Hemoglobin, HDL-C, LDL-C, TG, fasting plasma glucose, HbA1c, ALT, AST, CRP, and additionally MDA rates of the patients were practiced biochemical tests were done in Başkent University İstanbul Hospital the main labratory. The bloods were taken after 12-14 hours fasting in the morning. The insulin resistance was evaluated by HOMA-IR thanks to being practical. If the value calculated by HOMA-IR was over 2.5 mg, it is supported that the patient had an insulin resistance (14).

Statistical evaluation

The data of the research was evaluated by SPSS version 22.0 for Windows programme (SPSS Inc, Chicago, IL). The identifying statistics were denoted as average ± standard deviation, minimum and maximum, frequency distribution (n) and percent (%). The normal distribution suitability of the variables was analysed by using visual and analitical methods (Shapiro-Wilk Test). Paired Sample T Test was used for the normally-distributed variables between two dependent groups, and Student's T Test was used between two independent groups. Wilcoxon Signed Ranks Test was used for the non-normally-distributed variables between two dependent groups and Mann-Whitney

U Test between two independent groups as a statistical method. The relation between the variables were evaluated by the Spearman Test. The statistical significance level was accepted as $p<0.05$.

Results

The demographic characteristics of the study participants are shown in Table 1. The difference of the educational level between men and women is considered as something significant statistically. It was determined that the 80% of men and 35% of women work income-generating business. This difference between men and women was considered as something important statistically.

The biochemical finding of the patients who took part in the study is shown in Table 2 months after the beginning. With The blood samples which were taken in the beginning and at the end of the study, it was seen that there was a significant difference between serum hemoglobin, fasting plasma glucose, fasting insulin, HOMA-IR, HbA1c, CRP, TG and MDA values statistically. After practicing the nutrition programme for 3 months, it was determined that the values of hemoglobin, fasting plasma glucose, fasting insulin, HOMA-IR, HbA1c, CRP, TG and MDA of the patients reduced significantly compared to the beginning values. It was also determined that the values of HDL-C, LDL-C, ALT and AST were similar compared to the beginning and 3 months afterwards.

Table 1. The distribution of demographical qualities of the patients

Demographical Qualities	Male (n=15)		Female (n=20)		Total (n=35)	
	n	%	n	%	n	%
Age (years)						
≤50	6	40.0	4	20.0	10	28.6
51-60	4	26.7	6	30.0	10	28.6
>60	5	33.3	10	50.0	15	42.8
±SD **(Min-Max)**	**54.53±8.79**		**58.20±7.50**		**56.63±8.16 (37-65)**	
Marital Status						
Married	13	86.6	15	75.0	28	80.0
Single	1	6.7	2	10.0	3	8.6
Widow	1	6.7	3	15.0	4	11.4
Educational Status*						
Primary school	1	6.7	1	5.0	2	5.7
Secondary school	-	-	8	40.0	8	22.9
High school	2	13.3	5	25.0	7	20.0
University	12	80.0	6	30.0	18	51.4
Occupation						
Government official	3	20.0	-	-	3	8.6
Employee	2	13.3	4	20.0	6	17.1
Housewife	-	-	12	60.0	13	37.1
Retired	3	20.0	1	5.0	3	8.6
Self-employed	7	46.7	3	15.0	10	28.6
Income-Generating Business *						
Not-working	3	20.0	13	65.0	16	45.7
Working	12	80.0	7	35.0	19	54.3

* $p<0.05$

Table 2. The average of the biochemical values of the beginning and 3 months afterwards of the patients

Biochemical Values	Total (n=35)				p
	Beginning		Three months afterwards		
	±SD	Median (Min-Max)	±SD	Median (Min-Max)	
Hemoglobin (mg/dL)	14.1±1.6	14.2 (9.0-16.9)	13.8±1.3	13.8 (10-16.5)	0.011*
Fasting blood glucose (mg/dL)	144.8±39.5	137 (90-240)	134.2±49.8	120 (83-336)	0.013*
Fasting İnsülin (µU/mL)	18.8±17.8	12 (4-90)	17.4±17.9	10.2 (2.3-84.7)	0.010*
HOMA-IR	6.93±6.94	4.65 (1.38-28.89)	6.08±6.97	2.96 (1.09-29. 49)	0.007*
HbA1c (%)	7.0±1.6	6.5 (4.9-12.4)	6.7±2.1	6.2 (4.3-13.6)	0.001*
CRP (mg/L)	6.2±9.8	2.85 (0.20-43.12)	3.8±5.7	1.39 (0.20-23.54)	0.023*
HDL-C (mg/dL)	40.8±10.3	41 (21-66)	41.6±9.9	42 (24-73)	0.160
LDL-C (mg/dL)	134.7±40.1	130 (48-215)	125.9±32.4	126 (62-184)	0.124
TG (mg/dL)	218.1±231.9	178 (61-1420)	172.3±102.3	144 (60-605)	0.002*
MDA (ng/mL)	36.8±14.6	33.16 (16.05-69.53)	28.7±8.1	28.79 (16.47-50.58)	0.006*
ALT (U/L)	25.5±11.6	22 (14-65)	24.5±10.9	21 (13-65)	0.187
AST (U/L)	23.9±10.2	21 (8-60)	22.8±8.1	21 (8-44)	0.425

* $p<0.05$

The relation between the biochemical parameters and the nutrition programme practiced by the patients at the end of three months was shown in Table 3. There is a negative statistically significant relation between the energies that the patients took with their diets which they practiced at the end of threee months and MDA, and positive statistically significant relation between fasting plasma glucose, HbA1c, LDL-C, TG and CRP values. There is a positive nonsignificant relation between fasting plasma glucose, HbA1c and MDA with the percent that comes from carbonhydrates of the diets, and a negative nonsignificant relation between LDL-C, TG and CRP. It was determined that there is a negative statistically significant relation between fasting plasma glucose and the percent coming from the protein of the diet energy. It is also determined that there is a positive statistically nonsignificant relation between CRP and fasting plasma glucose with the percent coming from fat that is taken, and a negative statistically nonsignificant relation between HbA1c, MDA, LDL-C and TG. It was found that there is a positive statistically significant relation between polyunsaturated fatty acids and fasting plasma glucose.

Discussion

The medical nutrition therapy practiced on diabetic patients is a support treatment for the medical treatment. The aim of the nutrition programme suitable for the diabetics is to provide weight management, a better glisemic control and blood pressure. It is known that in diabetics, in the antioxidant protective mechanism the balance is damaged against the oxidant stress and cell damage is increased. In these patients, providing the control reduces the oxidative stress and inflammation. Therefore, the density of the microvascular and macrovascular complications of the diabet is reduced. In patients who cannot provide glisemic control for a long time, nonenzimatik glication, the metabolic stress caused by the changes in the energy metabolism, the activity of the sorbitol pathway, hipoksi and iskemia-reperfüzyon cause to increase the free radical production of tissue damage resulting from hipoksi and iskemi reperfüzyon and to change the antioxidant defence system, as a result cause to increase the density of diabetic complications in diabetics (15).

Lifesytle modification and associated weight loss in Type 2 diabetes patients are important in reducing insulin resistance, providing glycemic control, reducing lipidemia and blood pressure, reducing cardiovas-

Tablo 3. The relation between biochemical parameters and dietary factors at the end of 3 months

Dietary factors		Fasting blood glucose	HbA1c	MDA	LDL-C	TG	CRP
Energy (kkal)	r	0.218	0.229	-0.033	0.134	0.154	0.182
	p	0.209	0.186	0.849	0.442	0.379	0.296
Carbohydrate (total energy %)	r	0.104	0.123	0.173	-0.003	-0.110	-0.021
	p	0.553	0.482	0.321	0.988	0.528	0.904
Protein (total energy %)	r	-0.367	-0.276	0.067	0.179	0.197	-0.140
	p	0.030*	0.108	0.702	0.305	0.258	0.423
Fat (total energy %)	r	0.026	-0.050	-0.178	-0.144	-0.009	0.066
	p	0.882	0.774	0.305	0.409	0.958	0.707
Glucose (g)	r	0.060	0.266	0.013	0.050	-0.122	0.075
	p	0.732	0.123	0.940	0.774	0.486	0.667
Fructose (g)	r	0.117	0.312	0.090	0.073	-0.118	0.001
	p	0.503	0.068	0.605	0.677	0.499	0.994
Sucrose (g)	r	0.264	0.292	0.026	-0.052	-0.026	0.325
	p	0.125	0.088	0.882	0.766	0.881	0.056
Vitamin A (RE)	r	0.204	0.125	-0.050	-0.114	0.015	0.179
	p	0.240	0.473	0.776	0.515	0.930	0.304
Vitamin C (mg)	r	0.186	0.312	-0.019	0.037	0.012	0.180
	p	0.286	0.068	0.914	0.835	0.944	0.300
Vitamin E (mg)	r	0.345	0.205	-0.105	-0.109	0.290	0.158
	p	0.042	0.236	0.549	0.535	0.091	0.366
Protein (g)	r	-0.002	0.020	0.129	0.196	0.296	0.002
	p	0.993	0.910	0.461	0.259	0.085	0.989
Fiber (g)	r	-0.105	0.104	0.110	0.106	-0.150	-0.089
	p	0.549	0.551	0.531	0.545	0.390	0.612
Total cholesterol (mg)	r	0.265	0.099	-0.120	-0.113	0.142	0.265
	p	0.125	0.573	0.492	0.519	0.416	0.123
Saturated fatty acid (g)	r	0.169	0.174	-0.156	-0.025	0.046	0.171
	p	0.333	0.319	0.372	0.887	0.793	0.326
Monounsaturated fatty acid (g)	r	0.079	0.076	-0.244	-0.044	-0.055	0.049
	p	0.654	0.666	0.158	0.803	0.753	0.779
Polyunsaturated fatty acid (g)	r	0.336	0.267	-0.134	-0.010	0.234	0.126
	p	0.049*	0.122	0.444	0.956	0.176	0.472

* $p<0.05$

cular risk factor, inflammation and oxidative stress. In the look aheas study which was practiced with multicenter randomized conntrolled trial of 5.145 diabetics, the lifestyle intervention was made to the patients and in the end the patients lost the 8.6% of their initial weight. The levels of serum HbA1c reduced from 7.3% to 6.6%, and it was determined that there was a decrease in hypertension and hyperlipidemia, also a decrease in taking lipid lowering drugs (16). In another study, the nutrion treatment was practiced on the patients who had 9-year-diabet, not-provided a good glisemic control and a 7% HbA1c level, and at the end of 6 months it was seen that there was a 0.5% decrease in their HbA1c level (17). These studies are to show that which nutrition programme is useful to provide a glisemic control, and which is more useful for hiper-

lipidemia and hypertension control. In these studies the blinding factor is the loss of weight. It should be evaluated well that how much contribution a practiced nutrition programme type and loss weight provides to the change.

The medical nutrition theraphy practiced on Type 2 diabetes patients aims to control hyperglisemia and to provide loss weight in a long term. In the studies done, with the medical nutrition theraphy practiced on type 2 diabetes patients, it was seen that there was a 0.5%-2% decrease in the level of HbA1c and a regression of composing a micro and macrovascular complications (4). In the ukpds study which was done with over 5100 participants with the Type 2 diabetes, the effect of the glisemic control on micro and macrovascular complications provided on type 2 diabetes patients. It was seen that the 0.9% decrease in the level of HbA1c caused a significant decrease on micro and macrovascular complications ($p<0.05$) [18]. At the beginning of this study, the average of the HbA1c level was 7.0-1.6%, but at the end of the study after the individually planned medical nutrition programme for the patients, the average of the HbA1c level was determined as 6.7-2.1% and the difference between these values was considered as something important statistically ($p=0.001$).

Malondialdehid is the last product of the polyunsaturated lipid peroxidation used to determine the level of oxidative damage. MDA shows a significant correlation with the degree of lipid peroxidation. It can be looked in blood and urine (19). Its uprising causes an increase in lipid peroxidation. The MDA level in diabetics indicates the destruction of pancrease caused by oxidative stress. Soliman and et al. in a study thay they practiced with 80 diabetics, they determined that hyperglisemia increased oxidative stress and caused decreasing of antioxidant capacity in 2008 (20). In this study, the level of MDA in their blood was analyzed. The decrease happened on the MDA level was considered as statistically important ($p<0.05$).

In a prospective study, the levels of proinflammatory cytokines such as serum CRP are also elevated in type 2 diabetic individuals, but because of being economic, CRP is used more frequently. Serum CRP levels are identified as nonlipid cardiovascular risk indicator. It was found that people having more than 2.4 mg/L serum CRP level have a heart disease risk twice more than those having 1mg/L. In the Hoorn study, it was determined that it is a mortality indicator having a high serum CRP level after the 5-7 years of process for the type 2 diabetics (21).

Refined grains trigger proinflammatory sitokin production as they cause akut hyperglisemia. Instead of them, whole grain production usage causes a decrease of circulation of free radicals and proinflammatory sitokins. High CRP level, which is an indicator of systematic inflammation, can be reduced with lifestyle changes. These changes are loss weight, quitting smoke, doing exercise, reducing saturated fat intake, increasing the consumption of vegetable, and fruit and whole grain productions. In the woman health study practiced with type 2 diabetic women, it was seen that whole grain products and low glisemic index diet decrease CRP levels (22). In the meta-analysis study of Steckhan and et al. low fat (less than 30% of the amount of total energy coming from fat) and saturated fat reduction diets caused a decrease in serum CRP level, low carbonhydrated diets accelerated loss weight by causing a decrease in the insulin level, loss weight caused a significant decrease in proinflammatory sitokin release (23). At the end of this study, after 3 months period of time, it was determined that the decrease on CRP level was statistically important.

Compared to the healthy people, the balance between oxidant and antioxidant systems were impaired in Type 2 diabetes diabetics. In an oxidative stress parameters comparation study that 59 Type 2 diabetes diabetics and 48 healthy people were compared, there was a positive correlation between serum MDA levels and HbA1c levels of the participants, and it was determined that chronic hyperglisemia caused a significant increase in the oxidative stress indicators of the patients (24). At the end of this study, after 3 months diet, it was determined that there was a reduce in fasting plasma glucose, fasting insulin, HOMA-IR, HbA1c and MDA levels of patients compared to the initial study and this reduce was statistically significant.

Conclusion

Diabetes is accepted as a pandemi by WHO and United Nations, and its a disease that has a high mor-

tality and morbidity rate, which is rapidly increasing in the population. Every year 4.9 millions of people die because of diabet, and 50% of them have cardiovascular origins. Diabetes and the treatment of the diseases it causes are difficult and costly, so it is one of the diseases that are frequently observed in our country and in the world.

Diabetes is a chronic disease which can cause complications in a long term. Providing and maintaining a good glisemic control on patients can provide the development of the complication. At the beginning of the diabetes, a lifestyle change which will be practiced with a nutritional theraphy can reduce the possibility of diabetic complication, mortality and morbidity, also contributes to the economy of the country.

Altough many different studies about the nutrition treatments given to diabetics were done, the number of the studies identifiying the contribution of the inflammation and oxidative stress levels which cause the diabetes complications are limited. In the studies, in spite of seeing that different diet practices avail, it is known that individually prepared and practiced nutrition programme is effective by taking the nutrition habits and socioeconomic status of the patients into consideration. Therefore, a nutrition programme was prepared for the patients who took part in the study considering the individual features and suggestions of the guides.

The educations practiced on diabetics and these educations being put into practice by the patients in their daily lives have a siginificant place in the course of the disease. Educations being repeated in the suitable frequency and level for each patient by taking the suggestions of the guides into consideration, sparing time for the educations of patients as long as possible and planning these educations by using a comprehensible language suitable for everyone are quite important to establish a good glisemic control. There is a need to plan the studies that contain new methods and suggestions about the subject and to evaluate the contribution for the course of the disease.

Despite the fact that the time of the study and the number of the samples are limited, the results obtained emphasize the importance of the nutrition treatment for the diabetic patients. It is thought that it will be a significant source for the studies being done in a long time and with more samples.

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The effect of parents' nutritional knowledge and attitudes on their children's eating habits

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Summary. The aim of this study was to explore the effect of parents' nutritional knowledge and attitudes on their children's eating habits. Nutritional knowledge and dietary behavior of Kuwait University undergraduate students and their parents were evaluated using paper-based questionnaires to assess lifestyle and nutrition behavioral changes. A total sample of 700 female and male students was recruited for the study. A week positive relationship was found between parents' nutritional knowledge and children's eating habits (r=0.229). The findings suggest that parents' nutritional knowledge can slightly assist in adopting healthier eating habits in children, which may reduce nutrition-related diseases. However, future studies should emphasize on exploring other factors affecting dietary behavior such as taste, availability, food cost or security, cultural or religious beliefs and perceptions about food and health.

Key words: Nutritional Knowledge; parents; dietary behaviours; adolescents

Introduction

Assessments of the WHO reveal that non-communicable diseases will be the leading cause of about 75% of total death in the developed world by 2020. Nowadays, adopting an unhealthy lifestyle and diet, which may lead to the development of obesity, could be the main health problem (1, 2). An estimation of 80% of existing chronic diseases is mainly due to dietary and lifestyle factors according to the World Health Organization (3). Consequently, obesity, diabetes, hypertension, cardiovascular diseases and some types of cancer, are attributable to unhealthy and inadequate diet, eating behaviors, and insufficient physical activity, especially if adopted during early adulthood (4, 5). The nutritional transition, in which westernized diet is substituting healthy eating patterns, has increased the diet-related disease burden mostly among university students (4). Based on the reports of the Behavioral Risk Factor Surveillance System (BRFSS; 1991-1998), young adults between the ages of 18 and 29 have the highest increases in obesity (5).

Lifestyle has been recognized to be the main factor in sustaining health and preventing non-communicable diseases. The primary reasons for changing one's lifestyle are the dietary patterns of individuals (1, 2).

One study conducted by Demosthenes et al. indicated and that dietary patterns rich in whole grains, legumes, fruits, vegetables, and fish had significant effects in reducing the unfavorable symptoms of metabolic syndrome, whereas, dietary patterns characterized by high intake of alcohol and meat increased these indicators (6). Another study conducted in Iran by Epuru et al. showed similar results, in which healthy eating patterns defined by high consumption of fruits, vegetables, fish, dried fruits and low fat dairy products, were accompanied with lower levels of systolic blood pressure, while western patterns which comprise foods rich in meat, fat, and sweets were related to higher triglyceride, fasting blood sugar and insulin concentrations (7).

Much work has been done to explore the link between the traditional Mediterranean diet pattern and adiposity (8, 9). One research conducted in Lebanon by Al-Isa et al. supports a protective effect of the

Mediterranean diet on adiposity and weight gain (8). Furthermore, evidence from epidemiological studies correlates this dietary pattern to decreased incidence of type 2 diabetes, coronary diseases, and cancer, as well as to increased life expectancy (9).

In Kuwait, obesity and type II diabetes prevalence is continuously increasing. The obesity rate in adults has reached 36% for men and 38% for women, while the overweight rate in adults has reached 74% for men and 77% for women (8). Also in Kuwait, as per the 2017 statistics, diabetes prevalence in adults has reached 15.1% (10). Studies have proposed that improved dietary patterns can, even with merely minimal weight loss, prevent or assist in controlling a number of costly, chronic conditions like hypertension, cardiovascular disease, diabetes, and even some types of cancer (11).

The period of emerging adulthood, which is defined as the period of 18-25 years of age, and mostly spent at colleges or universities, is being understudied, as the incidence of unhealthy dietary patterns and physical activity practices increases, and therefore prevalence of obesity increases in this life period (12, 13).

College is a crucial life period as various lifestyle behaviors are shaped and may continue throughout life (5, 14). Researchers have observed that dietary habits worsen during college age, leading to an increased risk of chronic diseases as they grow older (15, 16). In their reports titled "Nutritional Prevention Strategy/ Health Eating" and "The Surgeon General's Vision for a Healthy and Fit Nation", the U.S. Surgeon General highlighted colleges and schools as locations for spreading knowledge and teaching students about nutrition to assist them in applying the "Dietary Guidelines for Americans" recommendations to lessen the coronary diseases risk (17).

Available evidence suggests that majority of college students might not follow the dietary and physical activity guidelines intended to minimize the risks associated with non-communicable diseases. A typical dietary intake of a college student could be characterized by low consumption of fruits and vegetables and high intake of fast, junk and convenience foods containing fat, sodium and sugar, along with frequent intake of calorie-dense snacks (2, 14, 15, 16). As discussed earlier, unhealthy eating habits in the period of emerging adulthood have been associated with a number of long-lasting critical health consequences such as osteoporosis, obesity, hyperlipidemia, and diabetes (16). A Greek study concluded that college students had a considerably increased intake of saturated and total fat and a lower intake of polyunsaturated and monounsaturated fat, folate, vitamin E and fiber (18). Another study by Butler et al. stated that female students in a university located in the Midwest of USA had a significantly increased daily fat consumption as well as a significantly decreased intake of bread and vegetables in their first year of college (19). Another study by Morse and Driskell has described the college students' consumption of fast food as a lifestyle due to convenience, taste, cost, social environment and location (15, 20). A review of college students' physical activity indicated that a limited percentage of students (35%) exercise on a regular basis with an increased rate for males (40%) vs. females (32%) (21). College-aged students are deemed to be the most susceptible age group to gain weight. It is reported that American first-year college students gain approximately 1 to 3 kg of weight (22).

Investigators revealed that fast food consumption is positively correlated with weight gain in the long-term. Evidence from The 1994-1996, 1998 continuing Survey of Food Intakes by Individuals (CSFII) suggested that young adults aged between 20-39 years have the highest fast food consumption among different age groups. Moreover, these findings showed that 52% of young adults ate fast food on one or both days of that survey since dietary data were collected via 24-hour dietary recalls in two nonconsecutive days (23). In a similar vein, these data concluded that young adults (19-39 years old) occupy first place in soft drink intake (24). Racette and colleagues stated in their study that more than 50% of college students ate at high-fat fast food restaurants at least thrice a week (25), whereas, Niemeier et al. found that fast food restaurants use and breakfast skipping increased dramatically throughout the period of adulthood transition (26). A recent Australian report discovered the prevalence of convenience meal consumption and its relationship with diet quality. It assumed that convenience meals were consumed as a main meal at least once per week by 30% of the students. In addition, it showed a substantial diet quality as

a result of commercially prepared meal intake (27).

Despite the WHO, United Kingdom, and Canada's dietary guidelines to consume at least five to seven portions of fruits and vegetables daily, it has been demonstrated that college students failed to meet their fruit and vegetable requirements, and their daily consumption varied between 2.2 and 2.8 portions every day (28, 29, 30). Likewise, Dodd et al. concluded that 66% of university students eat less than the five servings of fruits and vegetables per day (31). In their fruit and vegetable consumption study, Silliman and colleagues reported that fruit and vegetable intake was less than 1 serving a day in 64% and 58% of the students respectively, and those who consumed vegetables twice or thrice a day were only 14% of the total study population. As for fruit intake, it was observed that 25% of female and 11% of male students consumed fruits twice or thrice a day (32). In the Kingdom of Saudi Arabia (KSA), only 17.2% of female university students adhered to the fruit and vegetable intake recommendations (33). Additionally, evidence from recent Saudi study in Al-Hasaindicated concluded that 78% of female university students did not consume at least 5 servings of fruits and vegetables (34). Likewise, in another study done in Hail University in KSA, Epuru et al. revealed that more than 70% of female students were not following WHO recommendations (7). Findings from other Gulf countries showed parallel results. For example, it was noted that approximately 25% of Bahraini students consumed the recommended daily amount of fruits and vegetables (35). Al-Rethaiaa et al. summarized the dietary behaviors of 357 male students aged 18-24 years from College of Health Sciences at Rass, Qassim University. These included low fruit and vegetable consumption, frequent snacking, high intake of fried food, and having two meals per day (36). In spite of the dietary recommendations that energy coming from solid fat and added sugars should only constitute 20% of total daily energy, it has been found that consumption of such foods by men aged between 19 and 30 represented 42% (37).

In a pilot study implemented at Kuwait University to report food habits of nutrition students, it was determined that about 87% of students did not adhere to the WHO recommendations. On the one hand, their intake of sweets, fast food, soft drinks and fatty foods was high. On the other hand, their consumption of vegetables, healthy fats and water was low. A small percentage of students met their fruit requirements (38).

Parents have an imminent responsibility in shaping their children's eating habits and preferences by acting as role models. Children's attitudes and dietary behaviors rely heavily on what type of foods their parents allow into the household (39, 40). Enhancing children's nutritional status is highly dependent on the level of their parent's dietary knowledge. A researcher observed that Ethiopian mothers do not provide vegetables to their infants because they assumed that it may cause stomach illnesses and indigestion. Another study found that Ethiopian mothers did not feed their toddlers any animal-origin foods believing that their bodies were not able to process such foods. Accordingly, parents could restrict certain types of food from their children once it's perceived as being harmful (41).

A number of elements may influence a mother's dietary behaviors including socioeconomic status, educational level, age, working position and level of nutritional knowledge. A vast amount of research shows that the child's nutritional status is positively correlated with the mother's nutritional knowledge level. Mothers who had a higher level of nutritional knowledge provided their children with healthier food such as vegetables, fruits, legumes and restricted them from having unhealthy food options such as soft drinks, fast food, food containing artificial flavors and colors (40).

Evidence from an Omani study indicated a positive correlation between children's eating habits scores and the mothers' nutritional knowledge scores (42). Another study conducted in the late nineties, which discussed the effect of mothers' knowledge and children's dietary behavior found that intakes of total and saturated fat, cholesterol, and sodium in preschool children were negatively associated with maternal knowledge (43). Previous reports also suggest that children diets are associated with their parents' food (21, 32). Beydoun et al. discovered a moderate positive association between parents and children dietary intake, with higher associations for younger children (44). Weak to moderate associations of parent-child dietary or nutrient intakes have been found in other studies (21, 32). Still, researchers have still not come

to a conclusion as to whether these findings are applicable for overweight/obese mothers and their children. Additionally, researchers assumed that socioeconomic status might influence parent-child food choices and availability (45).

Understanding the parents-child relationship in lifestyle-related risk factors of overweight and obesity among Kuwaiti adolescents is crucial for devising public health policies and effective strategies to prevent and treat childhood obesity. Despite these major considerations, there have been no systematic studies among Kuwaiti adolescent children to characterize their eating habits, and their parents' nutritional knowledge and attitude simultaneously. Therefore, the objective of this study is to investigate the effect of parents' nutritional knowledge and attitudes on their children's eating habits

Methods

Sampling

Kuwait University undergraduate students of both sexes attending different colleges of all majors (n= 16) with a mean age of≈21years were recruited for this study [no. of subjects 700 individuals (87% females and 13% males), equal to 1.9% of the entire population (37,000students)]. The population of Kuwait University is equal to 0.93% of Kuwait population (4 million) (Kuwait University, 2017) and (Kuwait Central Statistical Bureau). Students were informed of the study and some of them were recruited to participate through a series of announcements that were made before or at the end of usual lecture times. Students were selected from classrooms in agreement with professors, cafeterias, lounges, campus squares, and lobbies. Participation was voluntary and anonymous. All students were surveyed over four months, from October to the end of February 2017. A consent form and an information sheet about the purpose of the study were included in the questionnaire. Students represented both theoretical (non-science) and practical (science) colleges. From a total of seven hundred questionnaires, six hundred and ninety were returned; males (n= 87) and females (n= 603). The response rate was 98.6%, since 1.4% of the students either filled out the questionnaire incorrectly or left more than half of the questionnaire incomplete; therefore, they were excluded from the sample.

Eligibility Criteria

Undergraduate Kuwaiti and non-Kuwaiti students of both sexes aged 17 years and older with children or not, were included in the study. Graduate and pregnant students, as well as college personnel were excluded from the study.

Data Collection

Questionnaire

We used a dietary questionnaire established earlier and tested regarding its reliability (46) as well as confirmed for content validity by two professors in the Food Science and Nutrition Department, and it was self-administered during college time. We obtained the written consent from Giovanna Turconi, the author of this questionnaire, to use it in our study. All questions of the questionnaire were translated into Arabic in order to make it easier for students to respond. Some questions, especially in Section 3 (eating habits) of the questionnaire were localized to meet Kuwaiti culture and remove ambiguity of untraditional cuisines, while some of them were omitted, as they are culturally and religiously unacceptable. These questions were about drinking wine and beer at meals, and eating alcohol-containing foods. The questionnaire included nine main sections.

Section 1- Socio-demographics, contained information on personal data and socio-demographics and were collected by means of ten questions. The socio-demographic section covered questions about age, gender, nationality (Kuwaiti and non-Kuwaiti), residential area, social status, year and college of study. Anthropometric measurements including students' weight and height were also collected. These measurements were self-reported by the students. The BMI cut-off points used were based on the National Institute of Health guidelines, which classified students' weight status into four categories: underweight (BMI ≤ 18.5), normal weight (BMI between 18.5 – 24.9), overweight (BMI between 25–29.9), and obese (BMI ≥ 30) (USDDH, NHLBI, 2003). Data about family monthly income was collected and divided into three categories low

(<1,000 KD), medium (1,000–3,000 KD), and high (>3,000 KD). The other sections contained 90 items overall. Appendix 1 contains the questionnaire, which consists of the various topics described below.

Section 2- Food frequency questionnaire, contained 19 questions and has been validated for use in the Kuwaiti population. The aim of this section was to discover students' daily average, frequency of consumption of typical food and beverages such as bread, rice, cereal products, fruits and vegetables, milk, tea, coffee and weekly consumption of other foods such as meat and meat products, fish, eggs, cheese, legumes, etc. Visual aids about the quantities of food items were included in the questionnaire in order to help students predict their portion sizes.

Section 3 - Eating habits questionnaire, consisted of 13 questions. This section was designated to explore the food habits of college students, particularly students with regard to breakfast contents, the number of meals per day, daily fruit and vegetable intake, as well as the consumption of both soft and energy beverages. Seven questions under this section had the following response categories: always, often, sometimes, never; whereas the other six had four categorical responses to assess eating habits. A 0 to 3 score range was assigned to each answer, with the highest score given to the healthiest response and the minimum score to the least healthy response. The total score of this section was 39.

Section 4 - Nutritional knowledge: contained 10 questions; each question had four answers out of which one is correct. Corrects answers were given 1 score, whereas, the incorrect answers were given 0 score. The purpose of this section was to assess the student's nutritional knowledge from different aspects. The total score of this section was 10.

Section 5- Reflection of parents' nutritional knowledge on child's nutrition: This section was added to the dietary questionnaire of Turconi. We used a previously constructed questionnaire (40). It contained 20 questions, which had the following response categories: always, often, sometimes, never. This section was completed only by students who have children and aimed at exploring the impact of parents' nutritional knowledge on their children's eating habits. The score ranged from 0 to 3; with the maximum score assigned to the healthiest one and the minimum score to the least healthy one. The total score of this section was 60.

The total score of each section was divided into tertiles, with the lowest tertile assigned to the worst assessment category and the highest to the best assessment category. Before distributing the questionnaire, we explained the aim of the research to the students and asked for permission to participate in this study. In order to decrease the probability of bias, the dietitian or the observer who supervised the questionnaire was well instructed on the process and was guided to give a standardized explanations in case any of the students' had questions; without providing any answers to the questionnaire items. The questionnaire was self-administered which enabled the gathering of a relatively larger set of data from different locations simultaneously in a cost effective manner as compared to personal interviews. On the other hand, a self-administered questionnaire makes it more challenging to validate response truthfulness (47).

Data Analysis

Data of all questionnaire items were entered manually and were analyzed using a Statistical Package for Social Sciences (SPSS), version 22. The scores obtained in each section were expressed as mean ± standard deviation. The percentage distribution of students in each tertile score was also calculated by using SPSS. Student-t test was calculated to investigate differences in scores obtained by males and females, normal and overweight plus obese subjects. Pearson-Product Moment correlation coefficients were computed to analyze the relationship between BMI and the investigated variables and between various questionnaire sections to test our hypotheses. In addition, Chi-Square test was calculated for the relationship between children's dietary behaviors and their parents' nutritional knowledge.

Reference Standards

According to guidelines stated by the National Institute of Health, weight status is classified into four categories: underweight (BMI ≤ 18.5), normal weight (BMI between 18.5 – 24.9), overweight (BMI between 25–29.9), and obese (BMI ≥ 30)(USDDH, NHLBI, 2003).

Results and Discussion

Characteristics of the sample are presented in Table 1. Most of the respondents were women (87.4%). The age of students ranged from 18 to 37 years with statistically significant gender differences. Males mean age was 21.7 ± 3.1 years while 20.7 ± 2.5 years for females. The majority of students were Kuwaitis (80.7%), while non-Kuwaitis represented 19.3% of the sample. Table 2 reports the demographic characteristics of college students who participated in this study. Most of the students were seniors (45.4%), while juniors, sophomores, and freshman represented 22.2%, 21.1% and 11.3% of the sample, respectively. More than half of the students (54.3%) studied at practical colleges (e.g., engineering, pharmacy, science, etc.), while 26.1% studied at theoretical colleges (e.g., arts, commerce, law, etc.) and 19.6% were not specialized. Geographically, Kuwait consists of six governorates, where some of them are defined as urban and others as semi-urban. In our study, more than one half (64.6%) of the sample live in urban areas. About 17.7% belonged to families with high monthly income (+3,000KD). In addition, BMI mean value was 27.1 ± 10 kg/m2 for males and 24.2 ± 5.2 kg/m2 for females, with statistically significant differences between both genders (p= 0.000). According to the guidelines of the National Institute of Health cut-off points' reference standard for BMI (48), 2.3% of males and 5.8% of females were underweight, 23% of males and 23.4% of females were overweight, and 20.7% of males and 10.6% of females were obese. The age distrubutrion for obese students was slightly higher for both males and fesmales as compared to the age distribution for normal weight males and females. Only 6.2% of our sample had children.

According to the BMI of students, the high prevalence of overweight was almost the same in both sexes, while the prevalence of obesity was two times higher in males than females. These results are consistent with the finding of Al-Isa et al. who revealed that the prevalence of obesity among Kuwaiti college students is higher in males than females (8). However, most of the college students were in the normal range of values according to guidelines of the National Institute of Health (48).

Food Frequency of Food Intake

Table 3 presents students' responses to food frequency intake of favorable food types. Results indicate that approximately two-thirds of students reported

Table 1. Sample Characteristics

Variables	Males (n=87)	Females (n=603)
Age (years)	21.7 ± 3.1	20.7 ± 2.5
Weight (Kg)	82.9 ± 32.1	61.5 ± 14
Height (m)	1.7 ± 0.07	1.6 ± 0.06
BMI (kg/m2)	27.1 ± 10	24.2 ± 5.2
Underweight subjects' BMI (kg/m2)	18.2 ± 0.3 (2.3%)a	17.4 ± 0.8 (5.8%)a
Overweight subjects' BMI (kg/m2)	27.04 ± 1.4 (23%)a	27 ± 1.4 (23.4%)a
Obese subjects' BMI (kg/m2)	40.6 ± 15.2 (20.7%)a	35.1 ± 6.3 (10.6%)a

[a]Between parentheses, percentages of subjects

Table 2. Demographics of the sample (n=690)

Variable	Frequency (N=690)	Percent
Gender		
Male	87	12.6
Female	603	87.4
Nationality		
Kuwaiti	557	80.7
Non-Kuwaiti	133	19.3
Year in College		
Freshmen	78	11.3
Sophomore	146	21.1
Junior	153	22.2
Senior	313	45.4
Area of Residency		
Urban	446	64.6
Semi-urban	244	35.4
College of Major		
Practical colleges	375	54.3
Theoretical colleges	180	26.1
Not-specialized	135	19.6
Social-Status		
Single	593	86
Married	92	13.3
Divorced	5	0.7
Having Children		
Yes	44	6.4
No	646	93.6
Income		
Low	3	0.4
Medium	565	81.9
High	122	17.7

daily consumption of milk and about 82% of them specified drinking one to two glasses of milk per day. However, more males (10%) than females (3.4%) reported intake of at least four cups of milk/yogurt per day. Students consumed rice, pasta, bread, and potatoes regularly, as 67% of the students reported intake of one to two portions daily. Less than half of the sample (44%) reported daily intake of fruits and vegetables and about one-quarter reported eating two to three portions daily. The percentage of fruit and vegetable consumption for both males and females was too close, with a slightly higher proportion for male students (45% vs. 44%, respectively). In a similar vein, 13% of male students reported daily intake of meat compared to 10% of females. More than one-third of the students (33% vs. 35% respectively) reported fish intake once every 15 days and eggs one to two times per week. However, the percentage of males (45%) consuming fish one to two times per week was higher than females (35%). Cheese was consumed in similar proportions by males (22%) and females (21.5%). About one-quarter of the students (23%) reported intake of ready-to-eat meat (such as mortadella and sausages) once every 15 days. Approximate proportion (22%, 25%, respectively) reported intake of cakes/sweets and pizzas at least one to two times weekly. More than one-third of the sample (40%, 38%, 35%, respectively) consumed legumes, fried potatoes, and fast food at least one to two times per week. Consumption of ready-to-eat meat was more frequent among males (30%) than females (15%), whereas sweets consumption was more frequent among females (25%) than males (18%). Males (39%) consumed fast food more frequently than females (35%), although the percentages are close to each other.

Table 3: Percentage distribution of subjects' food frequency

Variable	**Yes**					**No**		
1- Do you drink milk/milk and coffee/ cappuccino or do you eat yogurt every day?	**64.2**					**35.8**		
	1-2/day	**3-4/day**	**>4/day**	**1-2 /week**	**3-4 /week**	**>4 /week**	**1/10-15days**	**Never**
	82	13.7	4.3	43.7	28.3	5.3	10.9	11.7
2-Do you eat pasta/rice/bread/ potatoes every day?	81.4					18.6		
	1-2/day	**3-4/day**	**>4/day**	**1-2 /week**	**3-4 /week**	**>4 /week**	**1/10-15days**	**Never**
	67.3	25.4	7.3	31.1	50.8	12.1	5.3	0.8
3- Do you eat fruit and vegetable every day?	43.8					56.2		
	1-2/day	**3-4/day**	**>4/day**	**1-2 /week**	**3-4 /week**	**>4 /week**	**1/10-15days**	**Never**
	70.6	23.8	5.6	43	35	10.7	6.6	4.7

Times of food intake						
Variable	**1-2**	**3-4**	**1/day**	**2/day**	**1/10-15days**	**Never**
4- How often do you eat meat in 1 week?	**29**	**31.9**	**22**	**4.8**	**6.2**	**6.1**
5- How often do you eat fish in 1 week?	**36.4**	**4.4**	**0.7**	**0.6**	**32.8**	**25.1**
6- How often do you eat eggs in 1 week?	**34.5**	**23.9**	**10.4**	**1.6**	**19.4**	**10**
7- How often do you eat cheese in 1 week?	**21.6**	**37**	**24.7**	**6.1**	**6.7**	**3.9**
8- How often do you eat mortadella and sausages (ready to eat meat) in week?	**17.1**	**5.1**	**2.3**	**1.3**	**22.6**	**51.6**
9- How often do you eat legumes in 1 week?	**38.9**	**19**	**6**	**1.2**	**21.2**	**13.8**
10- How often do you eat sweets and cakes in 1 week?	**24.2**	**29.8**	**26.6**	**10.9**	**6.5**	**2**
11- How often do you eat fried potatoes in 1 week?	**37.3**	**25.7**	**9.3**	**2.6**	**19.2**	**6**
12- How often do you eat in a fast food in 1 week?	**35.3**	**17.4**	**7**	**1.7**	**27.7**	**10.9**
13- How often do you eat pizza in 1 week?	**25.5**	**4.6**	**1.3**	**0.6**	**53.1**	**17.9**

Eating Habits

The total score for this section (39) was divided into tertiles, where the lowest one referred to "inadequate eating habits", the medium one referred to "partially satisfactory eating habits" and the highest one referred to "satisfactory eating habits". The mean score for this section was 23.3 ± 4.9, without any statistical significant difference between males and females (p = 0.277) (Figure 2). The scores of eating habits section did not differ significantly between seniors, juniors, sophomores, and freshmen (p = 0.328), as well as between students' with high, medium and low household income (p = 0.781). Approximately, 2.6% of the students showed "inadequate eating habits", 71.4% had "partially satisfactory eating habits", while more than one-quarter of the sample (26%) showed "satisfactory eating habits". The most health adverse eating habit noted was eating calorie-dense breakfast. Approximately, 41% of the sample reported having fat-dense breakfast, which mainly consisted of pastries, 27.7% reported consuming breakfast rich in whole grains, while only 7.5% reported eating fruits for breakfast. Overall, students' eating habits were poor in fruit and vegetable intake. Only 8.1% and 13.8% of the subjects reported eating at least two portions of fruits and vegetables respectively, on a daily basis. About 16.8% of the sample reported consuming high amounts of soft drinks.

Data on eating habits revealed that the worst eating habit is eating calorie-dense, high fat breakfast, which makes up 41% of the sample. In addition, data showed that most students' should increase their intake of fruits and vegetables, and decrease their intake of soft drinks and calorie-dense snacks. Furthermore, findings suggest that eating three meals a day should be encouraged since only 22% of the sample consume three meals daily. Frequent eating has been considered one of the common dietary approaches for managing body weight (49), based on the concept that eating frequent meals has led to increased satiety, and decreased hunger and food intake (50, 51, 52). Our findings are inconsistent with the official food-based dietary guidelines for Kuwait (53). One good dietary habit that was practiced by the majority of students was favoring water over carbonated and energy drinks. Besides, about 64% of students reported having a daily breakfast regularly or usually. Our findings are consistent with the findings of a Lebanese study, which showed that 53% of students ate their breakfast daily (54). Nonetheless, there was a gender variance in terms of daily breakfast consumption. Generally, males were found to have breakfast more frequently than females. Available evidence suggests that breakfast consumption plays a role in reducing intake of dietary fat as well as minimizing impulsive snacking (54, 55). No gender differences were found between males and females regarding milk/yogurt consumption (at breakfast). Daily milk/yogurt was consumed by approximately 64% of the students. Out of those who consumed milk/yogurt daily, about half of the students consumed one to two glasses of milk per day. Findings suggest that excess weight gain can be avoided by consuming calcium-rich dairy products, particularly if intake was in sufficient amounts (three or more servings per day) and accompanied with energy balance (9). Results suggest that males experience better daily fruit consumption, whereas vegetable consumption was of similar proportions in both genders. However, about 6% of the students met the recommendation of consuming at least five servings of fruits and vegetables per day (53). Our data reveal that males' consumption was higher than females with regards to red meat, fast food and soft drinks, which may cause excessive body fat, weight gain and obesity (11, 56). Another study conducted on an African tribe found that food preferences are influenced by gender, with males favoring red meat (18). The high percentage of obese males may be explained by these dietary behaviors. Researchers concluded that the high glycemic index of sweetened beverages increases blood insulin, which may cause insulin resistance and finally obesity (Bachman et al., 2006). It was hypothesized that female students would score better in both eating habits than male students. However, our results showed that males had insignificant higher eating habits scores than females. Our results are consistent with one Korean study which found that women's dietary habits were not healthier than their male counterparts, although they scored higher in the nutritional knowledge test ($p<0.01$) (57).

Nutritional Knowledge

The total score for this section (10) was divided into tertiles, where the lowest one referred to "insufficient nutritional knowledge", the medium one referred

to "good nutritional knowledge" and the highest one referred to "quite good nutritional knowledge". The mean score for this section was 5.7±2 with a statistically significant difference between males (4.8 ± 2.2) and females (5.8 ± 2) (p= 0.000). Approximately 62% had good nutritional knowledge (most female parents), and only17.1% of the parents had insufficient nutritional knowledge (30% of total males vs. 15% of total females). Less than one-quarter of the parents (20.9%) had quite good nutritional knowledge (higher among females). The most common incorrect answers were related to dietary fiber, food protein content and energetic values. The most frequent mistake was to the question "which is the nutrient that contains the most energy?". In response to this question, only 18.8% answered correctly; "Fat". In addition, 41% of the parents answered carbohydrates, 36.2% answered protein and 3.9% answered alcohol.

Impact of Parents' Nutritional Knowledge on Child's Eating Habits

The total score for this section (60) was divided into tertiles, where the lowest one referred to as "inadequate eating habits", the medium one referred to as "partially satisfactory eating habits" and the highest one referred to as "satisfactory eating habits". The mean score for this section was 36.3 ± 7.6, without any statistically significant differences between males and females (p = 0.312). The results showed that 4.5% reflected "inadequate eating habits", 61.4% reflected "partially satisfactory eating habits", while more than one-third of the sample (34.1%) showed "satisfactory eating habits". The eating habit that recorded the lowest score was that parents do not force their children to consume fish at least twice per week.

It was concluded that parents with quite good nutritional knowledge usually force their children to eat breakfast and to have at least 1 cup of milk per day, as compared to parents with insufficient nutritional knowledge. In addition to that, it was revealed that more knowledgeable parents do not offer their kids ready-to-eat meat, artificially flavored foods, and carbonated beverages in comparison with less knowledgeable parents. Results suggest that there is a significant positive "weak" relationship between parents' nutritional knowledge and childrens' eating habits (r=0.229). Therefore, parents who have better nutritional knowledge, their children practice better dietary habits. Our results are in parallel with several studies, which indicated a significant positive association between higher nutritional knowledge of parents and better dietary intake in children (32, 44, 58). Our finding could be explained by the effect of the numerous environmental and individual factors that affect dietary behaviors, such as taste, availability, food cost or security, cultural or religious beliefs and perceptions about food and health (58, 59, 60). It is of high importance that future studies direct their focus into distinguishing the most substantial aspect of nutritional knowledge having the most significant correlation with dietary intake, which will not be only helpful for public health policy decision making, but would extend even to clinical counseling.

Ethical considerations

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

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Competing interests

There are no competing interests to declare of any kind by the researchers.

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Effects of walnut-enriched diet on blood lipids and glucose profiles in hyperlipidemic subjects: a randomized-controlled trial

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Summary. *Background and Objectives:* Walnuts have been shown to reduce serum lipids in hyperlipidemic individuals with a well-controlled feeding trials. Current study have been determined the effects of daily walnut consumption on serum lipids, fasting glucose and insulin levels in hyperlipidemic subjects. *Subjects and Methods:* In this, randomized controlled trial, mild to moderate hyperlipidemic subjects were randomly divided into 2 groups as walnut-enriched (n=20) and control (n=17) groups for 6 weeks. All subjects adhered to a medical nutrition therapy as low-fat and low-cholesterol diet. The walnut-enriched group was supplemented with 40 g/day of walnuts added to their diets. In order to follow nutritional and physical activity status of all subjects, they were visited every 15 days (in total 4 times). Anthropometric measurements of the subjects were taken and were monitored at each visit during the study. Blood samples were measured at the beginning and at the end of the study. *Results:* Our study showed that enriching a well controlled diet with walnuts (40g/day) improves the plasma lipid as well as serum glucose levels after the 6-week. Both groups showed a decrease in serum lipids with adaptation to the AHA (Americen Heart Association) diet, but statistically significant reductions ($p<0.05$) in serum glucose, insulin and HOMA-IR levels were found especially walnut-enriched group showed significant decrease in their total cholesterol and low-density lipoprotein cholesterol (LDL-C) by %5.3 ($p= 0.02$) and %8.8 ($p= 0.0$)respectively. Also LDL:HDL ratio and total cholesterol:HDL cholesterol ratio was significantly decreased in walnut-enriched group ($p<0.05$). Fasting glucose and fasting insulin levels decreased by %15.7 and %15.4 in walnut-enriched group, respectively. Walnut consumption did not show any significant changes in either high-density lipoprotein (HDL) cholesterol and triglyceride (TG) levels. *Conclusions:* This study indicated that, walnut-enriched diet improves serum glucose and serum lipids in hyperlipidemic subjetcs, suggesting a potential reduction in overall cardiac risk.

Key words: Walnut, Hyperlipidemia, Serum Cholesterol, Serum Glucose, Serum Insulin, HOMA-IR

Introduction

There has been an increase in the incidence of cardiovascular disease worldwide since 1900, including in developed and developing countries (1). In both developed and developing countries, the prevalence of cardiovascular disease has emerged with changes in dietary habits, lifestyle and environmental factors. Especially in many developed countries, consumption of energy-dense foods with a physical inactivity are the primary factors which cause an increase in obesity prevelance. The epidemic proportions of obesity leads to a significant increase in the number of cardiovascular diseases (1,2).

Hyperlipidemia is a medical condition characterized by the elevation of any or all of the lipid profile and/or lipoproteins. American Heart Association (AHA) defines hyperlipidaemia as the presence of a high lipid ratio in serum (3). Reducing intake of saturated fats (SFA) and refined carbohydrates, increasing consumption of monounsaturated fats (MUFA) and high fiber foods are among the basic targets in order to improve hyperlipidemia risk factors (4). Consumption of saturated fats cause an increase in LDL-C and tryglycerides, while monounsaturated and polyunsaturated fat (PUFA) consumption decrease serum LDL-C (5). At the same time, it appears that mortality due to coronary diseases has decreased as a result of regular consumption of omega 3 polyunsaturated fatty acids (6). It was observed that not only LDL cholesterol but also other plasma lipoprotein levels were positively affected as a result of balanced diet and consequently decreased CVD risk (7).

Large prospective studies have consistently indicated that, increased nut consumption cause a reduction in CVD risk and mortality risk associated with CVD (8,9). Clinical trials also have shown effects on CVD risk factors such as lipid profiles, vascular inflammation and blood pressure after various interventions that have included nuts, such as a Mediterranean diet (10-12). Nuts are a complex food composed of a number of nutrients and phytochemicals that may lower CVD risk. Many nuts are rich in monounstaurated fatty acids, while walnuts are composed mainly of polyunsaturated fatty acids (47.2g/100g) (13). Not many foods are rich in alpha linolenic acid (ALA), which is a type of omega-3 fatty acid found in plant foods. Walnut in particular have a unique profile; which has a quite high amount of both αlinolenic and linoleic acid, studies indicated that while walnut improves serum lipid levels positively and it also cause a reduction in plasma cholesterol levels and in particular improve CVD risk (14, 15, 24).

Although consumption of MUFA and PUFA appear to have similar lowering effect on total cholesterol and LDL cholesterol, it have meaningless to minimal effect on HDL cholesterol (16). Studies indicated that increased consumption of nuts with a controlled diet, especially in CHD patients tends to favorably decrease LDL cholesterol by %9 - %16 (16-18). Previous studies determined that, diets which are low in saturated fats appear to cause a reduction in LDL-cholesterol levels and overall cardiovascular risk (14,17). Furthermore, some studies supporting that, replacing saturated and trans fatty acids with unsaturated fats, including nuts in the diet, may help to prevent from diabetes and other CVD (18).

Walnuts containing components like fiber, potassium, magnesium, vitamin E and magnesium; all those components synergistically have a potential to decrease blood pressure, serum gluocse and serum lipid levels (20-22). Walnut also contain substantial amounts of L-arginine. L-arginine is the precursor amino acid of the endogenous vasodilator nitric oxide (NO) (23). Walnut consumption increases the levels of L-arginine in the body by 0.9 to 1.4 g/d, which that factor appears to reduce the blood pressure of individuals (23). EFSA-2011 indicated a relationship between the consumption of walnuts and improvement of endothelium-dependent vasodilation. The Panel considers that in order to obtain the claimed effect, at least 30 g of walnuts should be consumed daily; these amounts can be consumed in the context of a balanced diet (25). Aim of the current study was to investigate the effects of daily walnut consumption on serum lipids, fasting glucose and insulin levels in hyperlipidemic subjects.

Subjects and Methods

Subjects

In the current study**,** 37 moderate hyperlipidemic subjects (43,3±6.2 years for control group, 47.1±5.44 years for walnut-enriched group) were randomly divided into 2 groups as walnut-enriched (n=20 [10 women and 10 men]) and control group (n=17 [6 women and 11 men]). They were asked to participate from the local government hospital in Turkish Republic of Northern Cyprus. Patients with triglycerides above 300mg/dl and patients with total cholesterol above 500 mg/dl were excluded from the study. All subjects were required not to be obese, be non-smokers, and non frequent alcohol users, free of dietary restriction/food allergies and not taking medications known to alter plasma lipids. They were also screened for diabetes, renal disorders, thyroid diseases, hepatic diseases, cancer and other major diseases. Patients who have other health problem rather than CHD did not include in the study (Figure 1).

Study Design

During the baseline period, all accepted subjects (control and walnut-enriched group), were first admitted to Famagusta Hospital, Northern Cyprus to have a detailed physical examination. Then, a well controlled, 2 armed randomized controlled study was designed. In total, 6 weeks was used to examine the effects of a walnut-enriched diet compared with the baseline and control diet. All subjects in both groups were informed about American Heart Association (AHA) low-fat and low-cholesterol diet (26) advises by an experienced dietitian. Only difference between the groups were; walnut-enriched group have incorporated 40g of walnut to their diet for 6 weeks while control group only adopt to AHA diet and asked not to consume any nut during study period. During the 6-week diet period, participants were visited once in every 15 days and in order to follow nutritional status of participants during each visit '3-Day consecutive Food Records' were taken (including one day weekend each week). Beside, in every visit, body composition analysis and physical activities of participants were recorded. Subjects were instructed individually on how to complete the food records and how to estimate or measure the food portions at home by an experienced dietitian. At the same day of each visit the packed and pre-weighed packages of walnut (40g) were delivered to participants. Walnut was consumed as snacks (40g) once a day. Nutrient intake of participants was estimated by using the BEBİS-Beslenme Bilgi Sistemi (nutrition information system) computer program. Plasma measurements were obtained at baseline and at the end of the study. The study protocol was clearly explained to each subject, who signed an informed consent. The study protocol was approved by the Ethical Committee of the Eastern Mediterranean University and also approved by the Protocol Registration and Results System. Clinical tiral ID number for the current study is; NCT03680027.

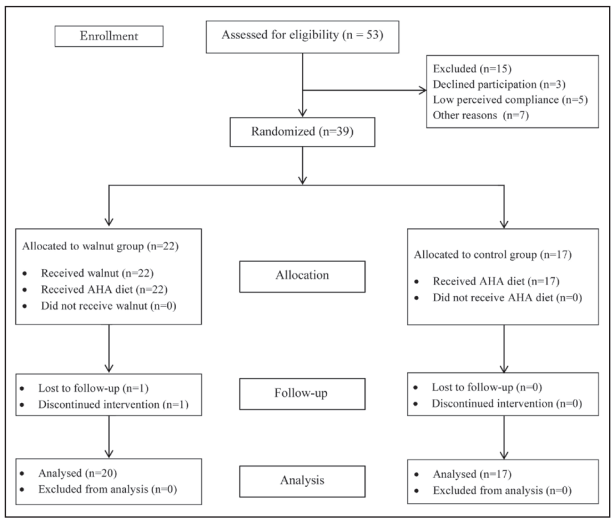

Figure 1. Flowchart of study subjects. In total, 37 subjects were randomized. Three participants droped out since they declined participation (n=3). Five of the participants dropped with a reason of low perceived compliance (n=5) and seven of them were droped out because of other reasons (n=7). A total of 37 subjects were included in statistical evaluation.

Anthropometric parameteres and habitual physical activities

All anthropometric measurements were carried out by dietitian according to the method described by Lohman et al. (27). Body weight and percentage of body fat were measured using a body composition analyser (tanita-BC 420s). Body height, waist and hip circumferences were measured using a standard tape measure. Body mass index (BMI) and waist/hip ratio were calculated (27).

Plasma Measurements

At the beginning and end of the study, blood samples were drawn into vacutainer tubes containing Na2EDTA (1 g/l final concentration) from the antecubital vein after an overnight fast. The tubes were then immediately stored into ice water. Within 2h, plasma was seperated by centrifugation at 2500g for 20 min at 4°C**.** All the measurments were made immediately after the plasma collection. Glucose concentrations were measured by glucose oxidase and peroxidase reactions. Total cholesterol was measured by cholesterol esterase, cholesterol oxidase and peroxidase reactions. Total TAG was measured by glycerol-phosphate-oxidase and peroxidase reactions. Method for direct determination of HDL-cholesterol uses polyethylene glycol ('PEG') based system in which sulfated a-cyclodextrin, dextran sulfate and MgCl2 form water soluble complexes with the non-HDL lipoproteins present in a sample, after which pegylated cholesterol esterase and cholesterol oxidase are introduced. LDL cholesterol concentration were calculated using the Friedewald formula: (total cholesterol)-(HDL cholesterol)-(VLDL cholesterol)=LDL cholesterol. VLDL cholesterol concentrations were estimated as TAG divided by 5, when concentrations are expressed in mg/dl (28).

Statistical analysis

Data were analyzed by statistical analytical systems software (package 20.0). The normality assumption was tested by using One-Sample Kolmogrow–Smirnov test. The mean ± SD were determined, and the differences among baseline, control diet, and walnut-enriched diet were compared by analysis of paired sample t-test. Pearson correlation test was used because of the normal distribution of the data set from the relationships between the parameters.

Nutrient intake (total fat, SFA, MUFA, and PUFA) were also compared with the changes of blood lipid concentrations. Also, Chi Square was used in order to make an assumption and compare the two qualitative (categorical) variables. The level of significance was P<0.05.

Results

In total, 37 hypercholesterolemic subjects completed the trial as detailed in the study protocol. Table 1 shows the changes of some variables of the subject characteristics over the 6-week study period. The mean age for the control group was 43,3±6.2 years, while walnut-enriched diet group mean age was 47.1±5.4 years. In terms of body composition measurment results, there were no significant difference were

Table 1. Initial and final body composition measurements of all subjects.

Anthropometric Measurements	Grup	n	Before			After			B-A
			mean	Sd	p1	mean	s	p1	p2
Body weight (kg)	Control	17	73,59	14,45	0,95	73,70	14,23	0,99	0,69
	WE-D	20	73,32	11,07		73,66	11,20		0,15
Body fat (%)	Control	17	25,89	6,76	0,33	26,56	6,44	0,38	0,14
	WE-D	20	28,20	7,42		28,59	7,23		0,07
Height (cm)	Control	17	169,29	10,17	0,86	169,29	10,17	0,86	-
	WE-D	20	168,70	9,93		168,70	9,93		-
BMI (kg/m2)	Control	17	25,47	3,16	0,84	25,55	3,10	0,75	0,39
	WE-D	20	25,64	2,04		25,84	2,22		0,09
Waist circumference (cm)	Control	17	84,06	14,64	0,89	84,18	14,60	0,86	0,16
	WE-D	20	83,50	8,70		83,50	8,82		
Hip cirfumference (cm)	Control	17	99,35	9,69	0,34	99,35	9,69	0,34	-
	WE-D	20	102,05	7,21		102,05	7,21		-
Waist/hip ratio	Control	17	0,84	0,08	0,32	0,84	0,08	0,32	-
	WE-D	20	0,82	0,06		0,82	0,06		-
Body fat (kg)	Control	17	19,14	6,17	0,51	19,69	5,92	0,53	0,15
	WE-D	20	20,42	5,53		20,91	5,80		0,06
FFM (kg) (fat free mass)	Control	17	54,55	11,57	0,65	54,09	11,14	0,70	0,12
	WE-D	20	52,91	10,58		52,67	10,83		0,13
TBW (kg)	Control	17	38,04	8,01	0,57	37,74	7,79	0,64	0,05
	WE-D	20	36,62	7,14		36,58	7,24		0,66
BMH (kcal)	Control	17	1709,76	340,36	**0,02***	1698,59	343,20	**0,03***	0,14
	WE-D	20	1478,45	249,60		1482,75	244,53		0,46

Abbreviation: BMI, body mass index, WE-D, walnut enriched diet. B-A stands for Before-After. Values are means ± s.d., n = 37. p1=Differences among control and walnut enrcihed-diet group, p2=Differences among pre and post study . For p1; independent t-test and for p2; paired sample t-test was used. The level of significance was P<0.05.

observed in both group throughout the study period (p<0.05). Daily habitual physical activities, energy intake and expenditure of subjects are given in Table 2. The physical activity level (PAL) of both control and walnut-enriched diet subjects was considered equivalent to mild activity (around 1.6).

The nutrient intake of subjects at baseline and the end of diet period are shown in Table 3. As it was expected inclusion of walnut (40 g/day) into the diet resulted in a significant (p<0.05) increase in MUFA. Whereas, unexpectedly the percentage of energy which comes from SFA was significantly (p<0.05) increased in walnut-enriched diet (13.6g) compared to that of the baseline (15.6g). Although AHA diet advises were given to participants walnut-enriched group also increased their dietary cholesterol intake significantly (p<0,05) while control group have decreased their dietary cholesterol intake. In fact, despite an AHA diet was advised to all participants, an increase in saturated fat intake was observed in both groups. As seen in Table 3, saturated fat intake have been increased in control group almost as much as walnut group but there was no statistically significant difference was found in control group. Dietary fiber consumption was increased in walnut-enriched diets compared with baseline however the difference was not determined as significantly different. On the contrary, control group have decreased their fiber consumption throughout the study period.

Compared with baseline, the walnut-enriched diet favorably decreased the concentrations of total serum cholesterol (p<0.05), LDL cholesterol, blood glucose and fasting insulin by 5.3%, 8.8%, 15.7% and 15.4%, respectively, while there were no significant differences found on HDL cholesterol concentrations and triglyceride levels in both groups (Table 4).

Compared to that of the baseline, although there was no statistical significant difference found in Total/HDLcholesterol in walnut-enriched group, it was appeared a decreasing trend for the parameter (%2). In addition, the walnut-enriched diet favorably altered the ratio of LDL/HDL cholesterol (6.2%) compared with the control diet (p<0.05) (Table 4). Therefore, walnut-enriched diet, despite its high fat content, in terms of serum lipid concentrations as well as blood glucose and insulin concentrations was superior to that of the control diet (Table 4).

Table 2: Measurments of BMH, PAL, energy intake and expenditure of subjects

	MEN				WOMEN				TOTAL			
	Control x ± s		**Walnut enriched** x ± s		**Control** x ± s		**Walnut enriched** x ± s		**Control** x ± s		**Walnut enriched** x ± s	
	Before- After		**Before-After**		**Before-After**		**Before-After**		**Before-After**		**Before- After**	
BMR (kcal)	1797, 6± 152	1819,6±146	1761, 2±175	1792,4±171	1388,2±117	1397,3±115	1371,7±121	1385,1±128	1495,8±168	1505,1±141	1489,4±141	1508,7±146
PAL	1,64± 0,2	1,62±0,1	1,51±0,1	1,53±0,1	1,57±0,2	1,57±0,1	1,67± 0,1	1,68±0,2	1,62±0,2	1,60± 0,1	1,59±0,1	1,61± 0,2
Energy expenditure (kcal)	2947,1± 560	2946,8±585	2659,1±530	2741,8±535	2179,2±335	2193,3±345	2289,4±375	2326,1±389	2421,9±447	2408,0± 465	2367,5±452	2427,9±462
Energy intake (kcal)	2166,9±1193	2167,0±121	2085,4±952	2076,7±916	1308,3±116	1341,8±145	1333,5±143	1348,5±128	1898,6±1059	1911,2±1070	1691,6±752	1695,2±724

Abbreviation: PAL, physical activity level. Values are means ± s.d., n = 37. p1=Differences among control and walnut enrcihed-diet group, p2=Differences among pre and post study . For p1-independent t-test and for p2-paired sample t-test was used. The level of significance was P<0.05.

Table 3. Nutrient composition of the two group (control vs walnut-enriched) during the study period.

Nutrients	Group	n	Before			After			B-A
				s	p1		s	p1	p2
Energy (kcal)	Control	17	1897,79	1059,43	0,49	1910,135	1070,20	0,47	0,32
	WE-D	20	1697,59	752,90		1692,98	724,88		0,74
Prot. (g)	Control	17	93,47	47,66	0,25	96,88	47,94	0,34	0,30
	WE-D	20	78,09	32,01		83,24	38,54		0,13
Prot. (%)	Control	17	19,69	3,48	0,20	20,27	2,42	0,36	0,60
	WE-D	20	18,45	2,78		19,68	3,21		0,21
Fat (g)	Control	17	62,35	30,62	0,74	61,43	33,71	0,82	0,93
	WE-D	20	64,68	30,64		69,78	26,60		0,54
Fat (%)	Control	17	29,57	2,94	0,00*	28,93	2,38	0,00*	0,57
	WE-D	20	34,42	3,51		37,01	31,21		0,46
CHO (g)	Control	17	240,69	132,90	0,33	242,49	134,31	0,27	0,49
	WE-D	20	200,43	88,60		183,55	82,93		0,61
CHO (%)	Control	17	50,74	3,68	0,07	50,80	4,25	0,04	0,89
	WE-D	20	47,13	5,05		43,31	4,54		0,69
Fiber (g)	Control	17	31,41	19,89	0,68	29,27	11,10	0,44	0,19
	WE-D	20	33,15	16,02		34,16	17,21		0,71
Vit.E (mg)	Control	17	14,20	11,66	0,68	10,68	4,41	0,51	0,15
	WE-D	20	13,04	4,96		12,00	6,83		0,37
Saturated fat (g)	Control	17	17,29	7,15	0,11	19,76	13,56	0,23	0,25
	WE-D	20	15,66	6,25		17,61	6,64		0,01*
Monounsaturated fat (g)	Control	17	31,62	9,27	0,19	30,63	10,17	0,52	0,11
	WE-D	20	34,93	5,79		36,95	6,69		0,04*
Polyunsaturated fat (g)	Control	17	12,14	5,61	0,00*	11,04	3,64	0,00*	0,04*
	WE-D	20	14,09	10,65		15,22	12,25		0,10
EPA (g)	Control	17	0,34	0,22	0,39	0,37	0,31	0,74	0,37
	Walnut-	20	0,39	0,27		0,41	0,15		0,70
DHA (g)	Control	17	0,13	0,17	0,20	0,11	0,11	0,07	0,00*
	WE-D	20	0,26	0,37		0,30	0,38		0,00*
Cholesterol (mg)	Control	17	279,86	82,64	<0,01*	271,42	188,83	0,29	0,84
	WE-D	20	166,44	91,66		212,68	142,39		0,01*
Omega 3 (g)	Control	17	1,22	0,44	0,00*	1,24	0,71	0,00*	0,85
	WE-D	20	2,53	2,05		4,12	1,84		0,03*
Omega 6 (g)	Control	17	10,65	5,45	0,00*	8,38	3,12	0,00*	0,03*
	WE-D	20	10,21	8,72		13,08	10,56		0,10

Abbreviation: WE-D, walnut enriched diet. p1=Differences among control and walnut enrcihed-diet group, p2=Differences among pre and post study . For p1-independent t-test and for p2-paired sample t-test was used. The level of significance was P<0.05.

Table 4. Serum lipid, glucose and insulin concentrations of all subjects.

Serum biochemical parameters	Grup	n	Before			After			B-A	Differences among pre-post study (%)
				S	p1		S	p1	p2	
Total cholesterol	Control	17	237,00	37,93	0,47	231,76	36,54	0,83	0,00*	% - 3.5
	WE-D	20	247,45	41,12		234,35	35,54		0,02*	% - 5.3
LDL-C	Control	17	168,59	29,18	0,52	156,76	27,59	0,98	0,00*	% - 7.1
	WE-D	20	171,45	36,01		156,50	33,02		0,00*	% - 8.7
HDL-C	Control	17	48,47	7,95	0,12	45,18	7,24	0,03*	0,05	% - 6.2
	WE-D	20	53,80	11,73		52,10	10,34		0,31	% - 3.3
Triglyceride	Control	17	143,82	83,59	0,17	151,59	105,26	0,15	0,51	-
	WE-D	20	110,25	61,38		112,10	53,42		0,73	-
LDL-C: HDL-C.	Control	17	3,48	1,01	0,18	3,47	0,99	0,23	0,05	% - 0.3
	WE-D	20	3,22	1,19		3,00	1,12		0,01*	% -6.8
Total Cholesterol: HDL-C	Control	17	4,89	1,37	0,18	5,13	1,33	0,31	0,06	-
	WE-D	20	4,60	1,46		4,50	1,43		0,39	-
Fasting glucose	Control	17	88,41	11,15	0,69	92,24	7,30	0,43	0,04*	% + 4.5
	WE-D	20	98,65	7,31		83,20	159,51		0,03*	% -15.7
Fasting insulin	Control	17	9,61	3,22	0,89	10,49	3,94	0,22	0,22	% +10
	WE-D	20	9,43	4,54		7,98	3,39		0,04*	% -15.4
HOMA-IR	Control	17	2,09	0,6	0,45	2,4	0,9	0,15	0,22	% + 14.8
	WE-D	20	2,29	0,8		1,63	0,7		0,03*	% - 28
VLDL-C	Control	17	28,76	16,72	0,98	30,32	21,05	0,31	0,50	-
	WE-D	20	22,05	12,28		22,42	10,68		0,73	-

Abbreviation: WE-D, walnut enriched diet. p1=Differences among control and walnut enrcihed-diet group, p2=Differences among pre and post study . For p1-independent t-test and for p2-paired sample t-test was used. The level of significance was P<0.05.

Discussion

In terms of nut family, walnuts have been found to be one of the most vulnerable source of plant based, omega 3 fatty acid by which having a cardioprotective benefit. Current well controlled, randomized study results have shown similar benefit as Sabaté *et al* study (17,50) and that daily consumpiton of 40g of walnut for 6 weeks significantly lowered the serum total cholesterol and LDL concentrations respectively by %5.2 and 8.7%. There is strong epidemiologic and clinical evidence that diets rich in omega-3 (n-3) fatty acids are protective and may reduce cardiovascular and overall mortality (29,30). As firstly shown by Sabaté et al.(17) there is an inverse relation between the daily consumption of walnut and serum cholesterol levels.

Nuts, especially walnuts play a key role due to its unique fatty acid composition with high content of unsaturated fatty acids, specifically polyunsaturated fatty acids (PUFA). Furthermore, the high levels of antioxidants found in walnuts conferred an improvement in antioxidant status as noted by increased enzyme activity and stable oxidation of LDL cholesterol. Some inflammatory markers tends to be improved with walnut consumption compared to other diets (31). It has been shown that walnut consumption can affect clinically relevant endpoints (such as cardiac death or endothelial dysfunction), and that this may be mediated through effects on oxidative stress, inflammation, and altered lipid profiles (31,32).

Most of the studies investigated the effect of nuts as part of a diet compared with nut-free control diets, which were either low in total fat (33,34), high in fat

(35), as part of a Mediterranean diet (36,37), or on a habitual diet (38). Although dietary controls have been variable, the overall results of these clinical trials have consistently shown a cholesterol-lowering effect of regular nut consumption.

Our results showed a significant reduction in LDL-C (–14.95 mg/dL), which represents a reduction of 8.8%. These findings resonate with the results of Wu et al. study (2014), in which a similar pattern was observed (non-HDL-C: –5.8%, TC: –3.9%, apoB: –6.2%, VLDL-C: –13.2%, TG: –5.4%, VLDL-TG: –4.0%) (38). Our results suggest that the increased n-3-PUFA consumption was principally responsible for the cholesterol-lowering effect of walnuts. There is evidence that a high n-3-PUFA intake provides cholesterol-lowering effects through several potential mechanisms (30); however, the exact underlying mechanisms are still not fully understood (40).

Although it was invested considerable time and effort (frequent visits with dietetian; detailed analysis of food records), especially subjects in the walnut-enriched group did not fully comply with the recommended diet. During the study period, it was found that compared to the baseline, walnut-enriched subjects, have been increased saturated fat intake in a statistically significant amount. It is estimated that, an increase in saturated fat intake in the walnut group decreased the power of the hypolipidemic effect that will be obtained from the walnut. In fact, despite an AHA diet was advised to all participants, an increase in saturated fat intake was observed in both groups. As seen in Table 3, saturated fat intake have been increased in control group almost as much as walnut group but there was no statistically significant difference was found in control group. A similar increase in both groups suggests that it did not affect the overall outcome of the study. Many studies investigated that, dietary saturated fat intake has been shown to increase LDL cholesterol, and the replacement of saturated fat with monounsaturated fat has been associated with decreased TC, LDL-C, and HDL-C (41). According to those data, increasing consumption of saturated fat in walnut-enriched group have been one of the most key point which was predicted to affect the result of the current study. Despite a significant decrease of LDL-cholesterol and total cholesterol of walnut-enriched subjects, hypolipidemic effect of walnut expected to be more powerful compared to the control group.

Studies give suggestive evidence that both the dietary fat and the dietary fatty acid composition affects glucose metabolism (42,43). Imamura et al., 2016 (43) indicated that, most consistent favorable effects were seen with PUFA, which was linked to improved glycaemia, insulin resistance, and insulin secretion capacity. In our study, both fasting serum glucose concentrations and fasting serum insulin concentrations of walnut-enriched diet have favourably reduced. Results of the current study concluded that, compared to control group, incorporation of walnut to a controlled diet have reduced fasting serum glucose and fasting serum insulin level respectively by 15.7% and 15.4% ($p<0,05$). In addition to those results, HOMA-IR which is a method used to quantify insulin resistance and beta-cell function (44), HOMA-IR concentrations were significantly decreased in walnut-enriched group compared to the control group. Similar and consistent results were determined in The Nurses Health Study which they have found an inverse association between the consumption of nuts and the risk of type 2 diabetes (45).

The intake of SFA generally increases the risk of CHD by increasing the concentrations of total and LDL cholesterol and by increasing the ratios of total/HDL cholesterol and LDL/HDL cholesterol ratio (46). The results presented in table 3 suggests that, in the current study, in spite of walnuts' high fat content and despite to an increase in saturated fat consumpiton of the walnut-enriched group, incorporation of walnut to a controlled diet seems to have a beneficial effect on total cholesterol and LDL cholesterol which is supported by several studies (12,17,31,32). As it demonstrated in table 3, inclusion of walnut into a control diet caused a reduction in total cholesterol by 5.3% and %8.8 in LDL-cholesterol compared with baseline. Ros et al. (2004), in order to determine the effect of walnut consumption carried out a study with 21 hypercholesterolemic men and women. During study period, subjects were asked to have a controlled Mediterranean diet, and they have arranged subjects diet by providing them walnuts which have accounted 18% (40–65 g/d) of their daily energy needs. Similarly to current study results, Ros et al. (37) found a significant reduction ($P<0.05$) in subjects' total plasma cholesterol as

well as LDL-cholesterol concentrations. However, the amount of walnut consumed was more than that used in the present study.

Some studies observed that changes in the ratios of total/HDL cholesterol and LDL/HDL cholesterol concentrations were better predictors of CHD than the changes in LDL cholesterol alone (48,49). In women, the risk of CHD is increased when the ratios of total/HDL cholesterol and LDL/HDL cholesterol concentrations exceed > 4.5 and >3.0 respectively. For men, the risk of CHD is increased when the ratios of total/HDL cholesterol and LDL/HDL cholesterol concentrations exceed >5 and >3.5 respectively (48). In the current study, compared with the baseline, those subjects who were recruited in walnut-enriched diet favorably altered LDL-C/HDL-C ratio (p=0.01) in cardioprotective direction.

Theoretically, walnut is a fatty food and its regular consumption may be expected to lead to body weight gain. However, as seen in table 1, in both group there were no significant changes in the body weight throughout the study period (p> 0.05). None of the well-controlled metabolic-type feeding studies show signficant changes in body weight compared to the nut and the nut-free control diet (50,51). Our study showed a consistent result and indicated that a walnut-enriched diet did not cause any change in body weight or BMI. This finding is consistent with previous clinical studies (50,51).

Interpretation is limited by the fact that the study relied on self-reported food records completed by the participants. These data may be highly susceptible to recall bias. Furthermore, dietary intake was not monitored daily, but rather recorded for three consecutive days in each 15 days. In addition to that, current study may have another limitation about the amount of walnut that the subjects have consumed. Since some of the studies are focusing on dose-response cholesterol lowering effect and indicating that, an average daily intake of 67 g of nuts (roughly equivalent to 20% of energy) is better as a therapeutic effect for CVD (13, 52).

In conclusion, according to epidemiological data, a 10 percent reduction of LDL cholesterol leads to a 20 percent decrease in the coronary heart disease risk throughout life (52). On the basis of the results of the present study, although the limited number of subjects were included, high-PUFA-rich walnut diet is preferred with a low-fat control diet since it showed a favorable effect on the CHD risk profile. Current results indicated that, walnut enriched diet decreased total and LDL concentrations by %5.2, %8.8 respectively. This means adding of walnut to a controlled diet may have a potential to decrease the risk of CHD by 10-15%.

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An imaging method for the evaluation of early atherosclerosis in inflammatory bowel disease: epicardial adipose tissue

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Summary. The aim of this study was to detect early atherosclerosis in patients with inflammatory bowel disease (IBD) that were strictly selected according to traditional cardiovascular risk factors, and to demonstrate whether evaluating epicardial adipose tissue can serve as an imaging modality through which to detect early atherosclerosis. Forty-three patients with IBD and 29 controls were enrolled in the study. Participants with well-known cardiovascular risk factors were excluded. Carotid intima media thickness (CIMT) and epicardial adipose tissue (EAT) were evaluated by the same physician blinded to the study groups. CIMT and EAT values were significantly higher in the IBD group when compared to the control group ($p<0.01$ and $p<0.001$, respectively). EAT was correlated with CIMT in the IBD group ($r=0.574$, $p=0.001$). CIMT and EAT are functional imaging methods that are used to detect early atherosclerosis in IBD patients without classic cardiovascular risk factors. EAT may be used as an additional diagnostic tool through which to detect early atherosclerosis in clinical practice in IBD patients.

Keywords: Inflammatory Bowel Disease, Atherosclerosis, Imaging Techniques, Chronic Diseases

Introduction

Inflammatory bowel disease (IBD) is characterized by chronic inflammation and a relapsing clinical course. In addition, it is also associated with increasing cardiovascular risk. Although the exact mechanism underlying this relationship is not clearly understood, substantial studies on chronic inflammatory disorders (including rheumatoid arthritis and systemic lupus erythematosus) have suggested that chronic inflammation plays a crucial role in the development of cardiovascular disease induced by atherosclerosis (1,2).

Therefore, non-invasive imaging methods such as carotid intima media thickness (CIMT), flow-mediated dilatation, and carotid femoral pulse wave velocity have been utilized to assess the subclinical atherosclerosis in IBD patients (3-6). In addition, epicardial adipose tissue (EAT), which is correlated with atherosclerotic coronary artery disease (CAD), can be easily evaluated by a basic imaging method called transthoracic echocardiography (7). On the other hand, the lack of a strict similarity between patients and controls according to their cardiovascular disease risk factors revealed conflicting results in patients with IBD when compared with healthy controls who were evaluated by CIMT (5,8-10).

The aim of this study was to evaluate CIMT and EAT in highly selected group of patients with IBD

and healthy controls with respect to cardiovascular risk factors, and also to assess whether measuring EAT can be used as a diagnostic tool through which to gain knowledge of early atherosclerosis in IBD patients.

Material and Methods

Forty-three patients with IBD (10 with Crohn's disease [CD] and 33 with ulcerative colitis [UC]) and 29 healthy age- and gender-matched volunteers were enrolled in the study; the patients' ages ranged from 18–50 years old. Diagnosis was established according to clinical, endoscopic, and histopathological criteria. Disease activity was assessed according to the Crohn's Disease Activity Index (CDAI) for CD and the Disease Activity Index (DAI) for UC (11,12). All patients were under treatment for their respective conditions. The exclusion criteria for both groups included a history of coronary, peripheral artery, or cerebrovascular diseases; inflammatory disorders other than IBD; chronic renal failure; total colectomy for IBD; and cardiovascular risk factors including diabetes mellitus, hypertension, hyperlipidemia, and smoking. Participants using anti-hypertensive drugs and vitamin supplements (including B_{12} and folic acid) were also excluded. The study was conducted in accordance with the tenets of the Declaration of Helsinki. In addition, the study protocol was also approved by the local ethics committee. Written informed consent was obtained from all participants.

On the day of the assessment, the patients' detailed medical histories were obtained, and physical examinations were performed. In addition, current medications, disease duration, involvement of disease, history of surgical intervention, and smoking status were also evaluated. Body weight (kg) and height (m) were measured, and body mass index (BMI) was calculated according to the formula (kg/m^2) for all participants. In addition, blood samples for laboratory evaluation were also obtained after a 12-hour fasting period. Hemoglobin, hematocrit, platelet, white blood cell, and glucose levels, as well as lipid parameters, electrolytes, liver, and renal functions were determined using autoanalyzers. C-reactive protein (CRP) was measured using the nephelometric method.

Measurement of Carotid Intima Media Thickness

Carotid artery intima-media thickness (IMT) measurements were ascertained with a high-frequency (3.0–12.0 MHz) ultrasound scanning probe (Philips L12-3 broadband linear array, Best, Netherlands) while the patients were in the supine position, with their necks extended and chins turned away from the side being examined. The right and left common carotid arteries were imaged proximal to the bulb in multiple longitudinal planes for the clearest resolution of the IMT of the far wall. The mean IMT was obtained by manually tracing the intima-media in the far wall of the artery. Measurements were performed on three end diastolic images and they were subsequently averaged.

Evaluation of Epicardial Adipose Tissue

The EAT of the participants was evaluated by two-dimensional transthoracic echocardiography with a 4 MHz, sector-type transducer probe (Philips HD11 XE Ultrasound System, Best, Netherlands). Images were digitally stored with standard parasternal long- and short-axis views, and they were reviewed by one echocardiologist. The maximum EAT was measured at a point on the free wall of the right ventricle at end-systole, perpendicular to the aortic annulus for the parasternal long-axis view, and perpendicular to both the interventricular septum at the mid-chordal view and the tip of the papillary muscle level for the parasternal short-axis view. Epicardial fat was defined as the relatively echo-free space between the outer wall of the myocardium and the visceral layer of the pericardium. EAT was defined as the average of three cardiac cycles from each echocardiographic view. CIMT and EAT were evaluated by the same physician, who was blinded to the study groups.

Statistical Analysis

Data were evaluated by IBM SPSS version 21 (SPSS inc., Chicago, IL, USA). The normal distribution of the variables was evaluated with the Kolmogorov–Smirnov test, and logarithmic transformations were performed to normalize data with skewed distributions. Student's t-test and the χ^2 test were performed for continuous variables and categorical variables, respectively. The Mann–Whitney U test was also performed for

non-parametric data. Pearson's correlation analysis of the variables was performed. Categorical variables are expressed as numbers and percentages. All continuous variables are expressed as the mean ± standard deviation. Finally, p<0.05 was considered significant.

Results

Baseline Features of the Study Population

Among the patients in the IBD group, 33 with UC and 10 with CD participated in the study. In addition, the control group included 29 participants. The patients' mean age was 31.3±7.3 years in the IBD group, while it was 31.3±6.9 years in the control group. There were 26 (60.5%) men and 17 (39.5%) women in the IBD group, and 12 (41.4%) men and 17 (58.6%) women in the control group. The mean BMI of the study groups (IBD versus control) was 23.7±3.9 kg/m^2 and 24.3±3.3 kg/m^2, respectively. Furthermore, the patients in the IBD and control groups did not exhibit signs of cardiovascular diseases, including hyperlipidemia, hypertension, diabetes mellitus, smoking, or a family history of cardiovascular disease, according to the exclusion criteria. No significant differences were observed between the IBD group and the control group with respect to age, gender, and BMI (Table 1).

Clinical Characteristics and Biochemical Evaluation of IBD Patients

The clinical characteristics of the IBD patients are shown in Table 2. In the IBD patients, the disease duration was 16.8±9.7 months. Among patients with UC, 7 had proctitis, 21 had left-sided colitis, and 5 had extensive colitis, whereas among patients with CD, 8 cases had ileocolonic involvement and 2 cases had colonic involvement. The inflammatory type was predominant in CD patients (8 of 10, 80%), and a previous enterocutanous fistula was determined in one patient with CD. The CD patients' mean CDAI score and the mean DAI score among UC patients was 90±51 and 3.9±2.2, respectively. The erythrocyte sedimentation rate (ESR) and CRP values were significantly higher in IBD patients than in the control group (Table 1). At the time of the study, there were 18 (41.9%) patients in remission and no patients with

Table 1. Characteristics and Values of CIMT and EAT of Study Population

Parameters	IBD (n=43)	Control (n=29)	*p*-value
Age (year)	31.4±7.4	31.3±6.9	*NS*
Gender (M:F)	26:17	12:17	*NS*
BMI (kg/m^2)	23.7±3.9	24.3±3.3	*NS*
EAT (cm)	0.448±0.208	0.238±0.119	<0.001
CIMT (mm)	0.541±0.150	0.413±0.182	<0.01
ESR (mm/h)	22.7±21.3	6.8±5.9	<0.001
CRP (mg/dl)	23.9±45.9	4.6±2.8	<0.001

IBD: Inflammatory bowel disease, BMI: Body mass index, EAT: Epicardial adipose tissue, CIMT: Carotid intima media thickness, ESR: Erythrocyte sedimentation rate, CRP:C-reactive protein, M: Male, F: Female.

Table 2. Clinical Features of IBD Patients

	IBD (n=43)	UC (n=33)	CD (n=10)
Disease duration (months)	16.8±9.7	16.2±8.6	18.8±13.2
Disease Activity n (%)			
Remission	18	10	8
Mild	21	19	2
Moderate	4	4	-
Severe	-	-	-
Extent of UC			
Proctitis		7	-
Left sided colitis		21	-
Extensive colitis		5	-
DAI		3.9±2.2	
Extent of CD			
Ileal			-
Ileocolonic			8
Colonic			2
Behavior of CD			
Non-stricture non-penetrating			8
Stricture			1
Penetrating			1
CDAI			90±51
Current Treatment			
5-ASA	43	33	10
Steroid	3	2	1
Azathiopurine	7	3	4
Anti-TNFα	4	3	1

IBD: Inflammatory bowel disease, UC: Ulcerative colitis, CD: Crohn disease

IBD had previous surgery. IBD patients were taking 5-ASA (100%), steroids (7%), azathioprine (16.3%), and anti-tumor necrosis factor (TNF)-α (9.4%).

Imaging Methods: CIMT and EAT

CIMT and EAT were significantly higher in the IBD group when compared to the control group ($p<0.01$ and $p<0.001$, respectively; Table 1, Figure 1). EAT was correlated with CIMT in the IBD group ($r=0.574$, $p<0.001$, Figure 2). On the other hand, there were no significant correlations between imaging modalities and disease duration, involvement, and activity. In addition, when we organized the IBD group according to remission type, we did not reveal any significant differences with respect to the CIMT and EAT values (CIMT [active versus remission]: 0.544±0.153 mm versus 0.537±0.151 mm; and EAT [active versus remission]: 0.460±0.222 cm versus 0.430±0.191 cm). When we stratified the different groups of patients with IBD, we did not observe any significant difference between UC and CD in terms of CIMT and EAT ($p>0.05$, for both). However, CIMT and EAT were significantly higher in patients with UC and CD when compared to controls (Figure 1). In addition, we allocated participants according to their BMI (≥25 kg/m^2). In all, 23 of 43 cases in the IBD group and 11 of 29 cases in the control group had a BMI ≥25 kg/m^2. We did not find a significant difference in the distribution of obese participants in the study groups. In addition, we did not observe a significant difference between a BMI ≥25 kg/m^2 and a BMI <25 kg/m^2 in terms of the CIMT and EAT values in IBD patients.

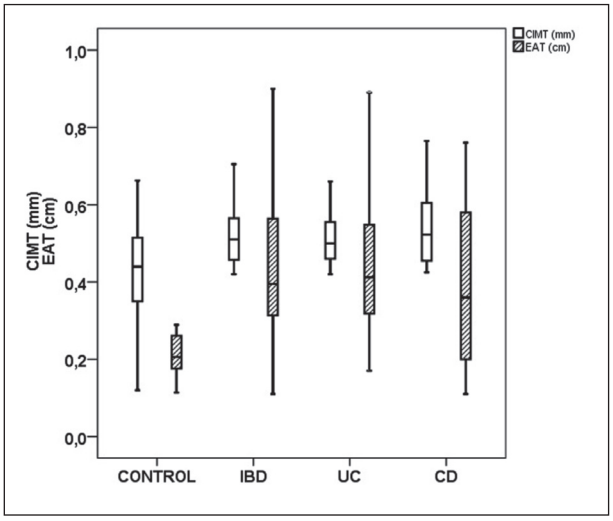

Figure 1. Carotid Intima Media Thickness (CIMT) and Epicardial Adipose Tissue (EAT) values of Study Groups; IBD vs Control (CIMT and EAT $p<0.01$, $p<0.001$ respectively), UC vs Control (CIMT and EAT $p<0.01$, $p<0.001$ respectively), CD vs Control (CIMT and EAT $p<0.05$, $p<0.05$ respectively), IBD: Inflammatory bowel disease, UC: Ulcerative colitis, CD: Crohn disease 220x185mm (300 x 300 DPI)

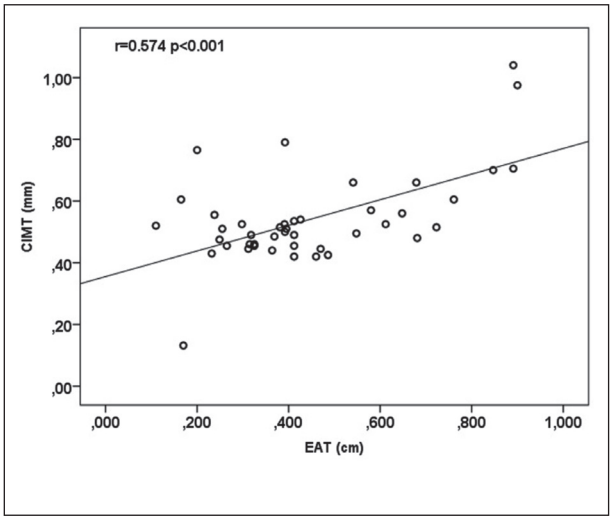

Figure 2. Correlation between carotid intima media thickness and epicardial adipose tissue thickness in IBD patients (n=43), CIMT: Carotid Intima Media Thickness, EAT: Epicardial Adipose Tissue 220x166mm (300 x 300 DPI)

Discussion

In this study, we demonstrated that IBD patients without well-known cardiovascular risk factors had significantly higher CIMT and EAT values when compared to the control group. Furthermore, EAT was positively correlated with CIMT in the IBD group. To the best of our knowledge, this study is the first to suggest that the EAT measurement could be used to evaluate early atherosclerosis in IBD.

Growing evidence has indicated that several chronic inflammatory and immunological disorders are associated with increased cardiovascular morbidity and mortality due to atherosclerosis (13). Furthermore, substantial increasing consequences suggested that chronic inflammatory disorders such as rheumatoid arthritis, Hashimoto's thyroiditis, psoriasis, and systemic vasculitis have some capacity to cause future

cardiovascular events in an independent manner, even without the presence of traditional cardiovascular risk factors (14-17). Nevertheless, the clinical course of IBD is also based upon chronic and relapsing inflammation. Bernstein et al reported that the risk of ischemic heart diseases increased in all IBD patients, regardless of diagnosis and the sex of the patients (18). However, studies that investigate the relationship between IBD and cardiovascular disease present conflicting results due to the undetermined and strict cardiovascular risk factors observed between the IBD and control groups (8-10). Therefore, the IBD and control groups in our study were selected to compose strictly stated groups without cardiovascular risk factors. Conversely, even though the study groups did not exhibit cardiovascular risk factors, the levels of inflammatory markers (including ESR and CRP) in our IBD patients were significantly higher when compared with the control group. This finding pointed to the idea that chronic inflammation alone may cause inflammatory disorders in our study groups, despite the use of anti-inflammatory medications.

Geroulakos et al suggested that CIMT is a non-invasive marker that reflects early vascular structural changes due to atherosclerosis, and it also predicts the presence of CAD with a specificity of 77%, a sensitivity of 43%, and a positive predictive value of 83% (19,20). Furthermore, the current epidemiological data concluded that the normal range of CIMT could change in accordance with age. When analyzing age, the normal CIMT ranges for those <30 years, 31–40 years, and 41–50 years are 0.44–0.57 mm, 0.42–0.50 mm, and 0.44–0.57 mm, respectively. However, a CIMT ≥1 mm at any age is related to significant cardiovascular risk (21,22). The mean age of our study groups, including the IBD patients and controls, was 31.3±7.4 years and 31.3±6.9 years, respectively. Our patients had higher CIMT values according to their age range. In addition, the CIMT value in our IBD patients was significantly elevated when compared to that of the control group (IBD versus control: 0.541±0.150 versus 0.413±0.182 mm, respectively).

EAT is associated with conventional cardiovascular risk factors and cardiovascular events due to subclinical atherosclerosis. Ahn et al reported that EAT is higher in patients with CAD, as compared to patients without CAD. In addition, EAT ≥0.3 cm was an independent factor for CAD on multiple logistic analysis (odds ratio=3.357; 95% CI: 2.177–5.175; p<0.001) (7). On the other hand, EAT may exert detrimental effects by releasing inflammatory mediators (23). Overall, it is suggested that EAT has an effect on coronary vascular abnormalities via atherosclerosis (24-27). Therefore, we thought that EAT could be used as a non-invasive marker to detect early atherosclerosis in IBD patients. Interestingly, we observed that EAT in the IBD group had significantly higher values than in the control group (IBD versus control: 0.448±0.208 versus 0.238±0.119 cm, respectively; p<0.001). Furthermore, EAT was also significantly correlated with CIMT. In fact, Ahn et al also pointed out that EAT was not only thicker in patients with metabolic syndrome, but it also increased linearly with respect to metabolic syndrome components (7). Despite the fact that we did not evaluate insulin resistance, our study groups had homogenous features irrespective of the presence of metabolic syndrome components, including hypertension, hypertriglyceridemia, and diabetes mellitus. On the other hand, we had participants with a BMI ≥25 kg/m^2. Therefore, we evaluated BMI to determine whether a BMI ≥25 kg/m^2 had an effect on CIMT and EAT values; we found out that a BMI ≥25 kg/m^2 had no effect on CIMT or EAT.

We did not observe any relationships between the imaging modalities and disease-related factors including disease duration, activity, and involvement. However, these findings could be a consequence of our highly selected IBD patients, who did not have well-known cardiovascular risk factors. In addition, the presence of inflammatory burden in IBD patients was the most powerful factor observed across a number of imaging modalities, despite the absence of cardiovascular risk factors, as was mentioned previously. Furthermore, CRP – which is a predictor of cardiovascular events (28) – was significantly higher in IBD patients than in the control group. Therefore, CRP may also actively play a crucial role in the development of atherosclerotic cardiovascular diseases via inflammation. Overall, long-term patient follow-up to illuminate the relationship between the aforementioned IBD-related factors, as well as imaging methods to detect early atherosclerosis, might constitute a prudential approach.

In conclusion, this study suggests that patients with IBD without well-known cardiovascular risk factors exhibit an increased risk of early atherosclerosis. In addition, both CIMT and EAT serve as functional imaging methods that can detect atherosclerosis in patients with IBD. EAT may be used as an additional diagnostic tool through which to extrapolate early atherosclerosis in clinical practice.

Authors' contributions

Nevzat Gozel, Orhan Kursat Poyrazoglu and Bahadir Sarli designed the studies and wrote the protocols. Yasemin Dogan, Abdullah Eyvaz and Banu Demet Ozel were followed patients in the study and were collected data. Agah Bahadir Ozturk and Omer Bilgehan Poyrazoglu undertook the literature searches and statistical analyses. Nevzat Gozel wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

Declaration of interest

The authors report no conflict of interest and have not received any payment for the preparation of this manuscript. The authors alone are responsible for the content and writing of the paper.

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The effect of vitamin B1 on heavy menstrual bleeding

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Summary. Profuse menstrual bleeding is a main reason for a poor quality of life and iron deficiency anemia in women of reproductive age. Therefore, it is necessary to take measures to reduce the volume of such bleeding. Therefore, this study was conducted to determine the effect of vitamin B1 on the volume of menstrual bleeding. This is a double-blind clinical trial. The study was performed on 98 single students of paramedicine faculty (aged 18-26 years) who suffered from menstrual bleeding volume during 2016-17. The samples were selected provided their bleeding lasted for longer than 7 days and in case more than 14 pads were used by them. Then, 100 mg of vitamin B1 and placebo were administered to the intervention and control groups for 3 months during three consecutive menstrual cycles, respectively. The samples were evaluated for bleeding 1 month before and after intervention (without taking medication or placebo). In this study, Higham's chart was used to measure bleeding. Data analysis was done using Mann-Whitney test, Wilcoxon rank sum test, and repeated measures test. 83 subjects completed the study. In the intervention group, mean duration of bleeding was decreased from 8.7±1.5 to 5.7±1.21 and from 9.15±1.08 to 8.01±0.78 in intervention and control groups, respectively. The number of pads used in the intervention group decreased from 18.5±3.2 to 11.8±4.2 and from 19.5±2.6 to 18.69±2.23 in control and intervention groups, respectively (P <0.001). The results of this study showed that vitamin B1 is a useful supplement for reducing menstrual bleeding.

Keywords: Heavy menstrual bleeding; Vitamin B1; Thiamine; Girls; Iran

Introduction

The onset of physiological menstrual bleeding that begins on average from 12 years of age and lasts up to menopause is an indicator of health during reproductive age of women (1). In abnormal uterine bleeding, the menstrual period may be longer than 7 days with a blood volume of over 80 milliliters, sometimes during intervals shorter than 21 days, and with blood clots of different sizes. Although menorrhagia does not increase the mortality rate of women, physical, mental, and social consequences of these bleedings frequently occur, which are the main causes of deteriorated work performance and education efficiency, a poor quality of life as well as iron deficiency anemia in women of reproductive age, increasing the expense of medical services during infection (2).

In the study of Jitesh and colleagues (2015), 50% of patients under 40 years of age and approximately 30% of women experienced menorrhagia, and 20% of patients with menorrhagia were anemic (3). In the study of Shahghaybi, 13.9% of subjects had hypermenorrhea (4). There is no obvious reason for menorrhagia in over 50% of cases; however, if a certain abnormality such as genital tract infection, excessive secretion of prolactin hormone, thyroid gland disorders, etc. is diagnosed, the underlying condition must be treated. Nevertheless, there is no definite cause for abnormal bleeding in most cases, and the patient is subject to conventional medical treatment (5). Tranexamic acid is the first effective, well-tolerated, and non-hormone drug used in the treatment of idiopathic menorrhagia (6). Nonsteroidal anti-inflammatory drugs (NSAID) reduce the production of prostaglandin, decreasing

general prostaglandin levels by inhibiting the cyclooxygenase enzyme and increasing the contraction of uterine arteries (7). NSAIDs such as mefenamic acid are preferred over other drugs since they should be administered during menstrual period, especially in cases where contraception is not needed (8). The application of hormonal IUD in women who do not intend to become pregnant slowly releases progestin, decreasing the volume of menstrual bleeding and the number of bleeding days through the strong anti-proliferative effect of levonorgestrel on endometrium (9). Endometrial ablation, during which the endometrium is damaged, is performed by a hysteroscope or another other tool. Hysteroscopy is used to remove fibroids or polyps, and laser beam or cauterization is often performed in cases where ulceration and chronic cervical infection cause abnormal hemorrhage. Dilation and curettage (D &C) has diagnostic and therapeutic value for primary functional hemorrhagic abnormalities, and the patient's problem is completely resolved and bleeding ceased using curettage and ablation of endometrium (10), although the lack of evidence-based therapies and unnecessary surgeries are a major concern in these cases (11).

There are several therapies for controlling menorrhagia with different efficacies and complications, which are often effective for patients. Evaluation of the efficacy of various treatments for menorrhagia is difficult due to problems in measuring the volume of bleeding in patients, and on the other hand, the attitude and understanding of patients from menorrhagia plays a key role in the decision-making process to select different treatments for menorrhagia (12). Treatment using medicinal herbs and vitamins is a type of treatment with much lower side effects than traditional therapies (13). Thiamine (vitamin B1) is a vital vitamin for protein metabolism and growth (14) that plays a role in the formation of hemoglobin, which is a protein carrying oxygen in red blood cells (15). Oxygen transfer is highly important for bodybuilders' performance. The higher the intensity and the time of training, the more important the role of oxygen transfers. According to investigations, thiamine is a vitamin supplement that should be added while repeating and increasing the duration and intensity of exercises (16). Although little information is available regarding the effects of vitamin B1 on vascular system, studies have shown that high doses of thiamine improve endothelial activity of the arteries in diabetic patients (17, 18). The aim of this study was to evaluate the effect of vitamin B1 on the volume of menstrual bleeding, which should be used as a treatment to reduce menstrual bleeding as well as a dietary supplement for women's health in case of efficacy.

Subjects and Methods

After being registered with Ethics Code of IR.IAU.ARAK.REC.1395.2 from Ethics Committee and clinical trial center with registration number IRCT2017070710451N2, this double-blind clinical trial was conducted on 98 female students aged 18-26 years who lived in dormitories of Boroujerd Islamic Azad University in 2016-17. Girls with regular menstrual periods lasting 26-30 days, menstrual bleeding time of over 7 days, taking more than 14 pads with no history of kidney disease and stone, abdominal or pelvic surgery, mental and psychological disease nor liver, kidney, thromboembolic, and coagulation disorders, oral contraceptive pills or other steroid hormones were enrolled. The subjects were monitored for bleeding for 1 month and they were asked to record a checklist of their menstrual profile during the menstrual cycle, and the volume of bleeding and the number of pads were recorded according to Higham's chart. Subsequently, each research unit consistent with inclusion criteria in terms of bleeding duration, the length of menstrual cycle, and the number of pads with no underlying disease was selected as the sample and signed the written consent form. Then, the subjects were randomly assigned to intervention and control groups (sample size was 98 with regard to $\alpha \leq 0.05$ and 95% strength according to studies). Medications included 100 mg vitamin B1 and the placebo that was prepared as a compound drug with lactose and dry starch formulation in a pharmacy. To prepare the placebo, the powders were poured into a capsule after weighing and mixing steps. The medications (vitamin B1 and placebo) were submitted to subjects monthly for 3 months or 3 menstrual periods in 30 packs separated from each other as A and B packets. Subjects were also given checklists after

receiving the drugs. Vitamin B1 pill was delivered to the intervention group and placebo was given to the control group in one dose per day during the second, third, and fourth menstrual cycles of the study. Then, one month after the end of the intervention, the samples were examined for bleeding without receiving the drug and placebo.

In addition, the subjects were notified that they should refrain from using any other drug as much as possible. In case they received a drug, they were asked to explain the name of the drug or any treatment other than the selected treatment as well as its amount.

After these 3 treatment steps with medication and placebo, subjects who did not consume the drug regularly or failed to continue their treatment for any reason were considered as drop-outs of our study. According to research objectives, content validity was used to determine scientific credibility. Data were collected by a questionnaire containing 28 questions concerning demographic features as well as midwifery and menstrual characteristics of the research units. The second part of the observation sheet (checklist) involved the information extracted from the bleeding record sheet using Higham's chart. Content validation was used to check for the validity and reliability of the tool. We first used ANOVA for repeated measures to analyze the response variables (outcome), which were quantitatively measured. Due to the fact that Mocheli test rejected the sphericity assumption of variance-covariance matrix, modified Greenhouse Gray's test was used to observe the TIME*Group interaction results. If the response variable was measured as qualitative grade response or did not have a normal distribution, Friedman test was used to measure dependent sizes, and Cochran test was used for dependent sizes when the response variable was in qualitative-nominal form.

Results

15 out of 98 cases (9 from control and 6 from intervention group) were excluded from the study for a variety of reasons. Statistical analysis based on per protocol analysis (figure 1) was performed on 43 subjects in the intervention group and 40 subjects in the control group (a total of 83 subjects). The results

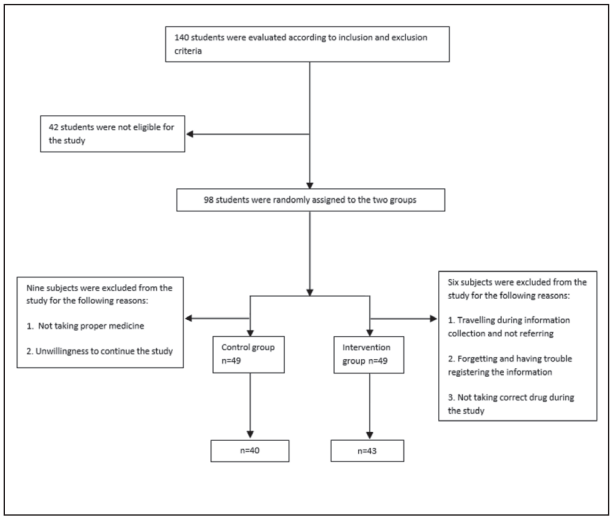

Figure 1. Flowchart of research units evaluated in the study

showed that mean age in the intervention and control groups was 20.3±1.6 and 20.1±1.07 years, respectively. There were no significant differences between the two groups regarding demographic factors (Table 1). The average number of pads used in the intervention group decreased from 17.9 in the second month of study to 11.8 in the fifth month. Also, ANOVA for repeated measures using modified Greenhouse Gray's test showed that the interpersonal effects of intervention were significant in reducing the number of used pads (P<0.001). There was no significant difference in the number of pads used by the control group relative to the previous month (P>0.05), and the number of pads used by the control group decreased to 18.69 in the fifth month from 19.04 in the second month of study (P<0.05) (Table 2), (Figure 2).

Table 1: Demographic and menstrual characteristics of participants in the study

p	Control group (n=40)	Intervention group (n=43)	Variable
	Mean ± SD		
0.549	20.1±1.07	20.3±1.6	Age (years)
0.741	12.14±1	12.3±1	Onset age of menstruation (years)
0.467	14.98±1	15.17±1	Onset age of menstrual pain (years)
0.885	28.2±1.8	27.7±1.7	Duration of menstrual cycle (days)
0.645	9.1±1.07	8.7±1.4	Duration of menstruation (days)

Table 2: Comparison of the mean number of pads used in both intervention and control groups

Group	Duration of intervention (month)	Mean	SD	95% Confidence Interval		P-Value	P-Value
				Lower Bound	Upper Bound	(Within subjects impact test)	(Between groups impact test)
Control	First	19.5	2.6	18.71	20.31	<0.001	<0.001
	Second	19.04	2.80	18.17	19.91		
	Third	18.98	2.33	18.08	19.88		
	Fourth	18.97	2.25	18.04	19.90		
	Fifth	18.69	2.23	17.65	19.74		
Average total		14.58	2.60	13.82	15.35		
intervention	First	18.5	3.2	17.55	19.55		
	Second	17.92	4.22	17.08	18.76		
	Third	15.51	3.99	14.64	16.38		
	Fourth	13.05	3.98	12.15	13.95		
	Fifth	11.87	4.20	10.86	12.87		
Average total		14.58	3.70	13.82	15.35		

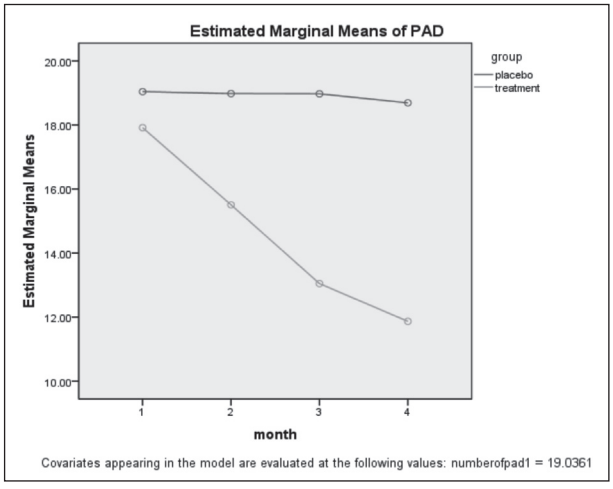

Figure 2. Average number of pads in intervention and control groups

The mean bleeding time was 8.74±1.5 and 9.15±1.08 in the intervention and control groups, respectively, as shown in the Figure. According to the results of Mann-Whitney test, there was no significant difference between the two groups before the intervention (P=0.121). Mean duration of bleeding during the second month of study in the intervention group was 7.98±1.68, which decreased to 5.77±1.21 days during the fifth month. The result of ANOVA test for repeated measures using modified Greenhouse Gray's test showed that the interpersonal effects of intervention were significant in reducing the number of bleeding days (P<0.001).

However, in the control group, the duration of bleeding in second month of study was 8.70±0.76, which reached 8±0.78 in the fifth month. According to paired t-test, there was no significant difference between the second and fifth months in the control group, which was not significant within all months after bleeding (P<0.05) (Table 3), (Figure 3).

Discussion

In this study, administration of vitamin B1 significantly reduced the duration of menstrual bleeding and the number of pads used. Severe menstrual bleeding, which occurs physiologically in women of reproductive age, is one of the most common causes of iron deficiency anemia in these women (3).

Although only 10% of women discharge more than 80 mL of menstrual blood and 60% of them become anemic (i.e. hemoglobin concentration <12) (19), 15% of referrals to gynecologic clinics are due to severe menstrual bleeding. Approximately 50-75% of hysterectomy operations performed in the age range of 25-49 years are due to menorrhagia, although no pathologic finding is observed in 50% of hysterectomy cases (20). Recent studies indicate the involvement of fibrinolysis and imbalance of prostaglandins in abnormal menstrual bleedings (21). Pacal et al. found that a high dose of vitamin B1 could potentially reduce the adverse effects of hyper-

Table 3: Comparison of bleeding time between intervention and control groups

Group	Duration of intervention (month)	Mean	SD	95% Confidence Interval		(within subjects impact test)	(Between groups impact test)
				Lower Bound	Upper Bound		
Control	First	9.15	1.08	8.74	9.56	<0.001	<0.001
	Second	8.70	0.76	8.28	9.12		
	Third	8.38	0.90	7.98	8.77		
	Fourth	8.08	0.73	7.76	8.39		
	Fifth	8.00	0.78	7.68	8.32		
Average total		8.46	0.82	8.14	8.77		
Intervention	First	8.74	1.50	8.35	9.14		
	Second	7.98	1.68	7.58	8.38		
	Third	7.02	1.52	6.64	7.41		
	Fourth	6.07	1.18	5.77	6.37		
	Fifth	5.77	1.21	5.46	6.08		
Average total		7.11	1.42	6.81	7.41		

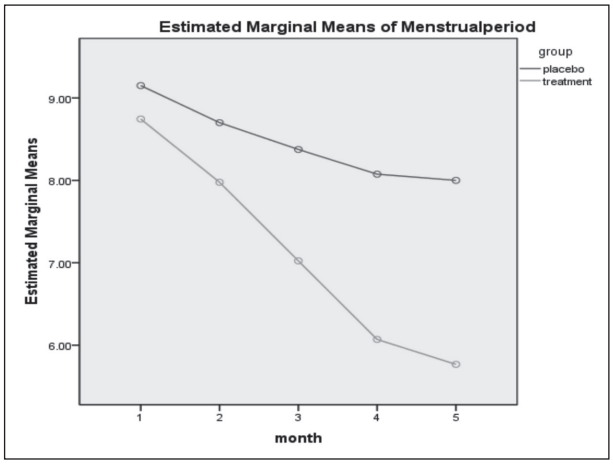

Figure 3. Mean number of bleeding days in intervention and control groups

glycemia on vascular cells in diabetic patients with its positive effects through improving vascular endothelial function (22). Tornali and colleagues demonstrated that vitamin B1 supplementation can prevent vascular problems and dyslipidemia among diabetics (18). Gokhal concluded that all common treatments for menstruation are based on suppression, but vitamin B1 is not a suppressor but a treatment and a cure for the disease without side effects (23).

Jafari and colleagues in a study showed that the use of vitamin B1 could significantly reduce the mean duration of bleeding and spotting in women using IUD after intervention relative to after it. On the other hand, the level of satisfaction with IUD use was increased, so that the IUD withdrawal rate in the intervention group had a significant difference with the control group (P<0.001) (24).

This study is consistent with the results of the present study in terms of vitamin B1 effect on reduction of bleeding and the number of pads used. Our knowledge about the effect of vitamin B1 on menstrual bleeding is limited because there has been no study to be compared with ours in this respect. There is also no comparative study in this regard except for the investigation by the author on the effect of this vitamin on bleeding due to the insertion of IUD. The mechanism of vitamin B1 action in reducing bleeding is not known and requires further studies. It is recommended to study the effect of vitamin B1 on menstrual bleeding during luteal phase. The results of this study suggested that vitamin B1 use is a safe and inexpensive way to reduce the duration of bleeding and the number of pads used and that the research units were satisfied with drug use.

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The impact of astaxanthin on adverse effects of hyperglycemia induced by STZ in retinal tissue of rat

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Summary. Astaxanthin (ATX) is a powerful natural antioxidant belongs to xanthophylls and the aim of this study is to investigate its protective roles on adverse effects of hyperglycemia in retinal tissue. Sixty rats were randomly divided into Controls, and hyperglycemic groups. ATX (20 mg/kg) was administrated over 47 days. After 47 days the final blood glucose concentration and body weight also the expression of vascular endothelial growth factor (VEGF), tumor necrosis factor α (TNF-α) proteins, antioxidant capacity and vessels dimension in ganglionic cell layer (RGC) layer in retinal tissue were measured as well as immunohistochemistry and histopathological assessments. Hyperglycemia-induced decrement in Catalase (CAT) (0.096 ± 0.026) and Glutathione (GSH) (133.80 ± 65.10) activity in retinal tissue but increase Superoxide dismutase (SOD) (15.52 ± 1.36 mU/mg) and Malondialdehyde (MDA) (2.64 ± 0.12) content. Administration of ATX increased the antioxidant capacity in the treated group ($p<0.05$). An increment in the expression of VEGF and TNF-α and vasodilation were shown in the hyperglycemic groups ($p<0.01$). Immune and histopathological assessments indicated that the ATX treatment could repair vasodilation in RGC layer vessels and also reduce the TNF-α content in retinal tissue ($p<0.05$). ATX could repair vasodilation and inflammation presumably because of removal of oxidizing and inflammatory agents in retinal tissue but during 47- day treatment it could not significantly decrease expression of VEGF in retinal tissue of hyperglycemic group.

Keywords: Astaxanthin; Oxidative stress; Hyperglycemia; Retinal tissue; RGC layer; VEGF

Introduction

Diabetes is a main public health problem. Several factors such as genetic susceptibility, environmental factor, poor control of blood sugar, lifestyles and comorbidities have an impact on the prognosis of diabetes complication (1, 2). Hyperglycemic retinopathy is one of the prevalent complications of diabetes (3). During diabetes, hyperglycemia increases free radicals through different metabolism pathways (4). Oxidative stress is the lack of balance between overproduction and eliminating ROS in the body. During diabetes, the retinal cells and their capillaries experience oxidative stress (4). Research has shown that advanced glycation end products increase vascular endothelial growth factor (VEGF) and inflammatory factors such as tumor necrosis factor α (TNF-α) (5, 6). Inflammation plays an important role in the pathology of hyperglycemic retinopathy (6). Overexpression of VEGF and TNFαinduces the adverse effect on tight junction proteins in vascular endothelial cells in the retina which they cause blood-retinal barrier break down. This event increased vascular permeability and edema in retinal tissue in the hyperglycemic condition (7).

Any organism has enzymatic and non-enzymatic defense mechanisms that remove harmful free radicals.

The enzymatic defense mechanisms include superoxide dismutase (SOD) and catalase(CAT) (8). The non-enzymatic defense mechanisms consist of glutathione, acid ascorbic, vitamin E and etc. Some studies confirm that antioxidant capacity decreases in diabetes and some other demonstrate increment of antioxidant agents in saliva and serum along with the oxidants enhancement in diabetes. Also, it was found that during hyperglycemia vitamin E, C and Beta-carotene have been decreased (9).

Some of the carotenoids are used as a nutritional supplement to prevent oxidative lesions in the retinal tissue. Carotenoids are the phytochemical substance which has hydrocarbon chain with carbon-carbon double bonds, therefore, they can scavenge free radicals and inhibit lipid peroxidation by the unique structure (10). In human one of the significant role of carotenoids are in the macula. Lutein and Zexanthin are the macular pigment carotenoids which exist in the eye in different amounts (11). Research results have shown that during the past two decades the antioxidant action of macular carotenoids, as a filter, is removing optical radiation damage in the retina (11).

Astaxanthin (AXT) is a carotenoid from xanthophylls category that shows pharmaceutical potential (12, 13) and also some of the investigators attribute to it therapeutic role in neurodegeneration (14). The unique feature of AXT is the existence of oxygen with a double bond in the ionic ring at the end of the hydrocarbon chain. This property and the carbon-carbon double bonds contribute to the powerful antioxidant AXT (12).

However, the interaction of this potent antioxidant with other cellular and molecular targets and their exact mechanisms are not clear in the hyperglycemic condition in retinal tissue. The aim of this study is to investigate the effect of AXT on antioxidant capacity, vascular variations and expression of VEGF and TNF ά in retinal tissue in an uncontrolled hyperglycemia condition. In

Methods

Ethics statement

The experimental methods of this study were approved by bioethics committee of the animal house in Baqiyatallah University of Medical Science and have followed the NIH guidelines for use and care of animals(Approval code: IR.bmsu.Rec.1396.620).

Animals

In this study, 48 male Wistar rats weighing 200-225 were used. The rats were kept in standard cages with free access to food and water in temperature of 22 ± 2˚C, humidity of 40% - 60%, and 12 hours light/dark cycle condition.

Diabetes induction

Diabetes was induced by a single tail vein injection of Streptozotocin (Sigma UK) (45 mg/kg) dissolved in 0.1 M citrate buffer. Blood glucose was measured in the first day before streptozotocin (STZ) injection and days 5th and 47th after injection of streptozotocin by Accu-Chek Blood Glucose Meter. Animals with a blood glucose of more than 350 mg/dl were selected as hyperglycemic animals.

Retina Sampling

The animals were euthanized by a lethal dose of Ketamine and Diazepam. The eyeballs were removed and washed with cold PBS (phosphate buffer saline, PH 7.4). The retinas were detached from the retinal pigmented epithelium cell layer and submerged in liquid nitrogen to freeze then stored at -80˚ C for protein extraction or enzyme assays. For the histopathological and immunohistochemical studies, the whole eye caps were fixed in formalin or paraformaldehyde.

Protocols and Groups of Experiment

Animals in the control group were randomly divided into two groups of (n=12) control and AXT treated rats. Treated- control rats group was fed with AXT (1-800-921-8482 manufactured for Viva Labs Inc, made in the USA), 20 mg/ kg orally once a day by gavages during six weeks. Hyperglycemic animals were randomly divided into two groups (n=12) hyperglycemic and treated-hyperglycemic rats. The treated-hyperglycemic rats group were treated with AXT, 20 mg/kg orally once a day by gavages over six weeks. After six weeks the final blood glucose concentration and body weight were checked.

Enzyme Assays and Protein measurement

Isolated retinas were homogenized and sonicated on ice-cold in PBS solution (pH=7.4) incubated at 4˚C for 30 minutes then centrifuged (15,000 RPM

at 4°C for 30 minutes). The supernatants were used for protein (Bradford method) (15) and enzyme assays. All measures were based on the spectrophotometric assay. The method of Tietz was used to determine the GSH content of retinal tissues (16). Briefly interaction of cell lysate with Na2HPO4 and 5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB) and the absorbance of DTNB was monitored at 412 nm for 5 minutes. The SOD activity of the retina tissues was measured according to Winterbourne method (17). In this method, potassium phosphate buffer was added to the EDTA solution containing sodium cyanide, NBT and homogenized sample. In presence of the riboflavin, the reaction initiates. The absorbance of samples was measured at 560 nm. For Catalase activity assay the Abei method was used (18). The principle of this method was based on measuring the decrease in absorbance of the test sample by the induced decomposition of H2O2 in the presence of the analytic enzyme at 240 nm wavelength.

MDA content of the retinal tissues was measured by the method of Satoh (19). The homogenized sample was mixed with TCA (Trichloroacetic acid) and centrifuged. MDA content in the supernatant was determined by the interaction of thiobarbituric acid (TBA) and n- Butanol after centrifuging the light absorption of the upper supernatant was monitored at 532 nm. A standard curve of MDA was produced and sensitivity of measurement was determined to be between 1 and 100 μM.

Histological assessment

At the end of the experiment, animals were euthanized by a lethal dose of Ketamine and Diazepam. Eyes were removed and fixed in formalin (10%). After fixation and tissue processing, paraffin- embedded sectioning (each 50 μm intervals) was processed routinely for Hematoxylin and Eosin (H&E) staining (20). The histological changes were observed by a light microscope (Nikon, Japan) connected to the digital camera (CMEX, Holland).

Immunohistochemical (IHC) method

The retina was fixed in paraformaldehyde. Then it was sustained in a sucrose solution and rinsed with acetone at optimal cutting temperature for one night. It was stored at -80º temperature for cutting. Each slide had 10 μm diameters. The slides were defrosted and blocked in a goat serum for one hour. The retinal vasculature and adherent leukocytes were imaged by DPI or VEGF antibody (Santa Cruze) which was diluted in a goat serum 3% incubated overnight in 4°C. Finally, the secondary antibody conjugated to FITC was added. After dehydrating and contrasting, the images were observed under fluorescent microscope. The vascular dimension and area of vessels were measured by Olysia software.

Protein extraction and western blot analysis

Isolated retinas were homogenized and sonicated in a lysis buffer [0.5 M Tris-HCl (pH 7.4), 150 mM NaCl, 0.5% deoxycholic acid, 1% Triton X100, and protease inhibitors(1 tablet/50 ml Tris Buffer (pH 7.2),0.1% SDS] incubated at 4°C for 30 minutes and centrifuged with 14,000 rpm at 4°C for 20 minutes. Protein concentration was determined by the Bradford assay, each sample (50 μg of protein) were mixed 1:1 with sample buffer (60 mM Tris, 10% glycerol, 2% sodium dodecyl sulfate, 5% 2-beta-mercaptoethanol, 0.01% bromophenol blue, pH 6.8) and boiled for 5 minutes. The proteins were separated by electrophoresis in a 12% polyacrylamide gel (for 1.5 hours at 90 V) and were transferred into nitrocellulose membranes via wet blotting protocol (overnight, 15 vol). After washing the nonspecific bindings were blocked by Western blocking solution (free fat milk in Tris Buffer Solution (TBS)) for 5hour at 4°C. The blots were then incubated with the primary rabbit polyclonal IgG VEGF antibodies (1:1000; sc-152), diluted in2.5% milk in TBS overnight at 4°C,or with Rabbit polyclonal beta Actin βactin antibody (1:2000; Abcam Inc., ab8227)or with Rabbit polyclonal Anti-TNF alpha (1:2000 ab66579 Abcam). After washing, the membranes were incubated with horseradish peroxidase-conjugated secondary antibody (1:5000; Abcam Inc., ab8227) for one hour and were visualized by TMB solution (Sigma T0565). The relative expression of proteins was quantified by densitometry scanning of blots with ImageJ software.

Statistical Analysis

In the current study, all values were expressed as mean ± SEM. The analyses of data between groups

were performed using one way ANOVA followed by Tukey post hoc test analysis and in some cases, the nonparametric tests of Kruskal Wallis and Mann-Whitney were applied. In all states, P<0.05 was considered to be statistically significant.

Results

The results indicated that STZ injection (50 mg/kg) induced hyperglycemia in rats and after 6 weeks the blood glucose level reached 509.8 ± 16.18 mg/dl in the Hyperglycemic group. In the Hyperglycemic-treated group that received daily AXT (20mg/kg) over 6 weeks the blood glucose reached 568 ± 15 mg/dl. This result showed that AXT could not prevent the elevation in blood glucose (Figure 1). There was no significant difference in blood glucose levels between the control group (126.4 ± 2 mg/dl) and treated-control group (124.16 ± 2.5 mg/dl) that received AXT. Changes in body weight during the six week study period were compared with the initial weight. There was an increment of approximately 40% in the control group from (198.83 ± 2.2 g) to (272.8 ± 3 g). In the Hyperglycemic group weights did not change from the beginning (221.45 ± 3.7 gr) to the end of study period (225.85 ± 5.35 gr) but in the Hyperglycemic-treated group slightly increase occurred (Figure 2).

The Glutathione contents in the rat›s retina in four groups illustrated the minimum amount of GSH contents in the Hyperglycemic group (29.76 ± 4.91 pMol/mg) which was significantly lower than the control group (212.89 ± 72.75). The amount of GSH increased prominently in the Hyperglycemic treated group (133.8 ± 65.10 pMol/ mg) however, this amount was lower than the control group (± SEM 72.75 pMol/ mg) (Table 1).

The superoxide dismutase (SOD) activities in the retina of four groups showed more significant activities in the Hyperglycemic group (15.52 ± 1.36 mU/

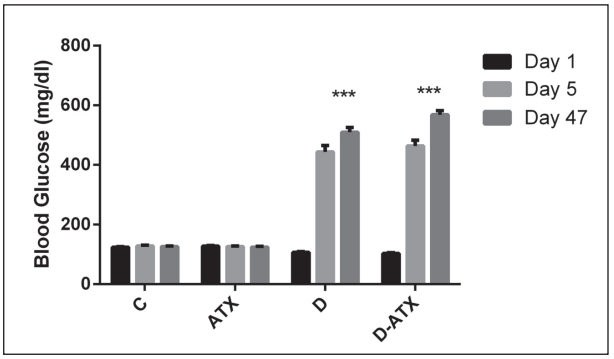

Figure1. Changes in blood glucose
No changes were observed during days 1,5 and 47 in control group and ATX treated group, STZ increased blood glucose up to 506 (mg/dl) in hyperglycemic or D group. ATX treatment could not prevent the glucose elevation (D-ATX). The significant increment was observed 5 days after ingection of STZ. *** represents P<0.0001.

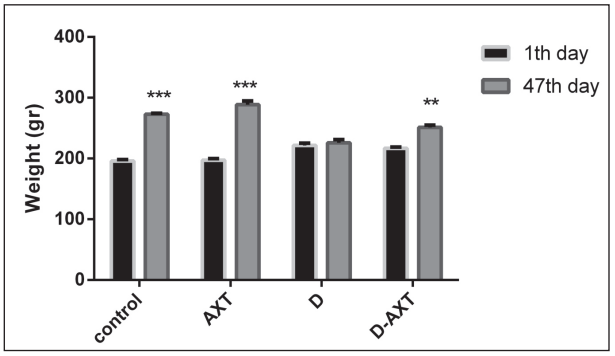

Figure2. Changes in weight
The significant increase were observed in control and ATX treated groups.no increment was seen in hyperglycemic(D) rats during 47 days but ATX treatment could induce weight gain in them (D-ATX). *** P<0.0001, **P<0.001.

Table 1. The amount of CAT, GSH, SOD, and MDA in Different Groups

	CAT (mu/mg)	GSH (pMol/mg)	SOD (mu/mg)	MDA (nMol/mg)
Control	0.06 ± 0.008	212.89 ± 72.75	2.62 ± 0.37	2.38 ± 0.105
ATX	0.2 ± 0.059**	130.65 ± 22.9	11.47 ± 3.21*	2.37 ± 0.106
D	0.036 ± 0.006*	29.76 ± 4.91*	15.52 ± 1.36*	2.86 ± 0.15*
D-ATX	0.096 ± 0.026#	133.80 ± 65.10#	19.17 ± 1.56*	2.64 ± 0.12

The Catalase(CAT), Superoxide dismutase(SOD) activity and Glutathione (GSH), Malondialdehyde (MDA) content in control, control- treated (ATX), ,Hyperglycemic (D) and Hyperglycemic treated (D-ATX) groups.**p<0.01,*p<0.05 compare with control,# p<0.05 compare between D and D-ATX.

mg) compared with the control group (2.62 ± 0.377 mU/mg). However, the activity of SOD in the Hyperglycemic rats that were treated with AXT (19.17 ± 1.562 mU/mg) was higher than the Hyperglycemic group (Table 1).

The measurement of catalase enzyme activity showed that diabetes decreased the activity of catalase in the Hyperglycemic group (0.036 ± 0.006 mU/ mg) compared with control group (0.06 ± 0.008 mU/ mg). Treatment with AXT significantly increased catalase activity in the hyperglycemic-treated group (0.096 ± 0.026 mU/mg). Interestingly, treatment with AXT in control-treated group significantly increased the catalase enzyme activity (0.2 ± 0.059mU/ mg) (Table1).

The Malonaldehyde (MDA) contents in the retinal tissue of rats in the four groups showed that the MDA contents were higher in the Hyperglycemic group (2.86 ± 0.15 nMol/mg) compared with the control group (2.38 ± 0.105 nMol/mg protein) ($P< 0.05$). In spite of the reduction of MDA contents in Hyperglycemic treated group (2.86 ± 0.124 nMol/mg) it was not significant compared with the Hyperglycemic group.

The Histopathological H&E staining showed six layers of retinal tissue respectively: the photoreceptor layer, outer nuclear, outer plexiform, inner nuclear, inner plexiform and ganglionic cell layer in four groups of experiment. Increased permeability and edema were visible in the ganglionic cell layer (RGC) of the retina in the Hyperglycemic group (Figure 3a-f). One case of vitreous humor hemorrhage in the hyperglycemic group is shown in Figure 3f.

The Immunohistochemistry (IHC) assessment showed some dilated vessels in RGC layer of the retinal tissue in the both hyperglycemic and treated Hyperglycemic groups. However, vasodilatations were severe in the hyperglycemic group than the treated hyperglycemic group (Figure 4). Quantitated measuring of vessels area in RGC layer indicated that there were significant increases in the cross-section of the vessels of hyperglycemic rats (4807 ± 731 μm^2) in comparison with control rats (1973.6 ± 200 μm^2) and treatment with Astaxanthin could repair this impairment (2480±437 μm^2) (Figure 5).

The results of Western Blot analysis for the VEGF and TNF-α proteins showed no expression in the con-

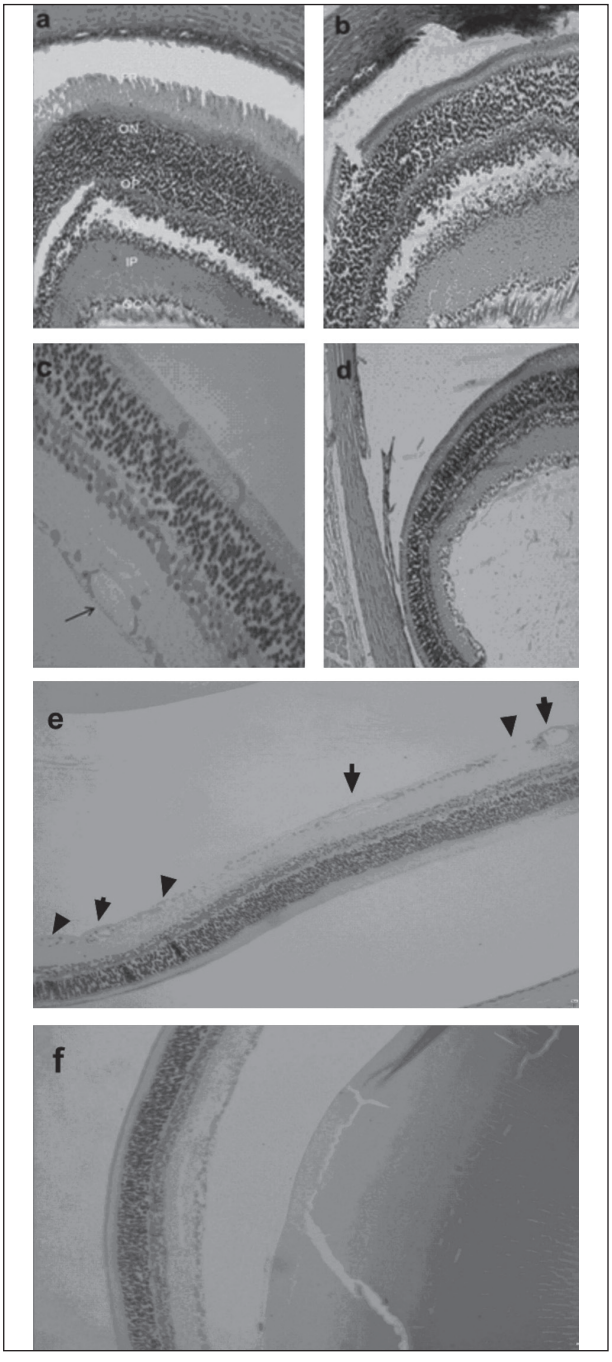

Figure3. Pathological signs
H&E staining revealed the six layers of retina in all groups, a) no specific pathologic sign were observed in control (a) and ATX treated (b) groups. In hyperglycemic group the arrows indicated the inflammation and vasodilation in ganglionic cell layer (c) (e). (f) represented the significant hemorrhage in vitreous of diabetic group. No obvious pathologic signs were observed in hyperglycemic treated with astaxanthin cases (d). Retinal layers: Fotoreceptor cells (FC), outer nuclear (ON), outer plexiform (OP), inner nuclear (IN), inner plexiform (IP) Magnification: a-d 400X and e-f 100X.

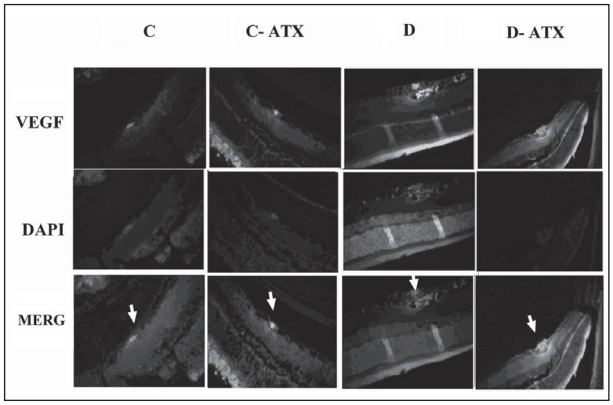

Figure 4. RGC vessels vasodilation
Immunofluorescence staining indicated severe dilation in vessels in RGC layer of hyper glycemic rats (D) (arrows) in upper trace, DAPI staining represent RBC locations in middle trace, merge of tow staining in lower trace. In all images the magnification = 100X.

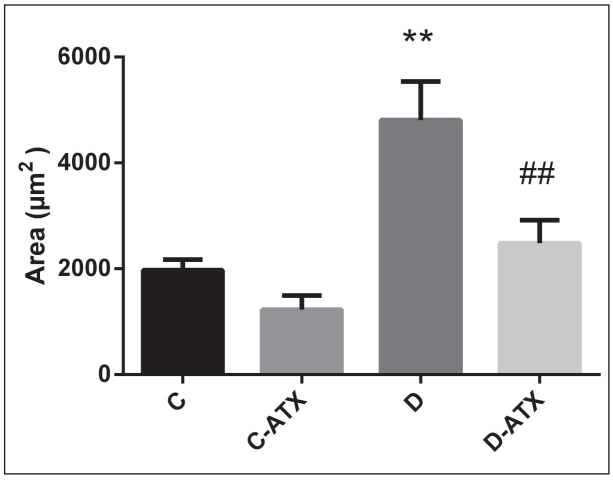

Figure 5. Area measurement of vessels
Counting the RGC vessels area represented increment in their dimension in hyperglycemic(D) rats compare with control and control treated with Astaxanthin (ATX) groups, **p<0.001, treatment with ATX in hyperglycemic rats showed significant reduction of vessels dimension, ## P<0.01.

trol and Astaxanthin treated animals but elevation in the expression of these two proteins in retinal tissue of hyperglycemic groups were prominent. Although Astaxanthin treatment could prevent the elevation of TNF-α in hyperglycemic condition but it could not significantly prevent the expression of VEGF protein in retinal tissue (Figure 6 a, b, c).

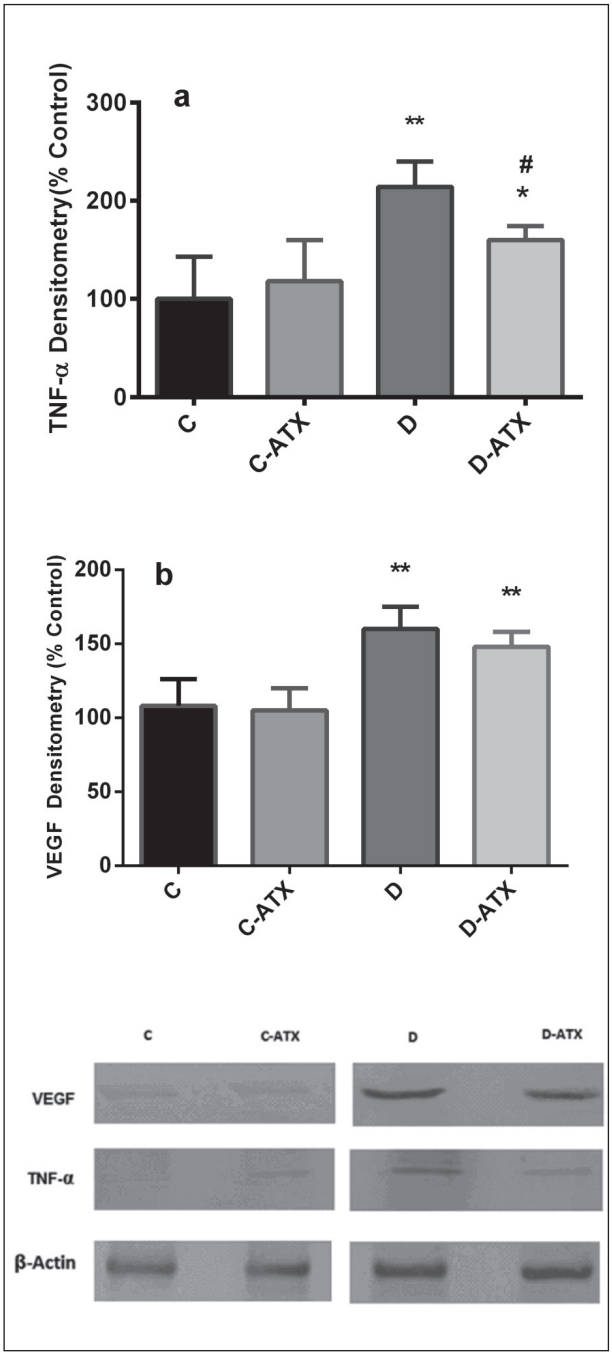

Figure 6. Expression of VEGF and TNF α
(a) Densitometry analysis of VEGF expression indicated significant increase in hyperglycemic group (D) (** p<0.01). The decrease in hyperglycemic treated (D-ATX) group in comparison with hyperglycemic was not significant. (b) The TNF-α as an inflammation marker were increased in hyperglycemic group (** p<0.01 * p<0.05) There was significant decrease with ATX treatment (D-ATX) compare with hyperglycemic group (D) (# p<0.05). The images of western blot membranes were demonstrated in below.

Discussion

In the current study treatment of hyperglycemic rats with Astaxanthin could not prevent the elevation of blood glucose but it could prevent weight loss in diabetic animals. In consistency with other studies, there is no evidence for Astaxanthin as a decreasing blood glucose element. This attributes to the correction of metabolic disorder which is induced by STZ or it aims to the adverse effect of Astaxanthin on insulin resistance which has been mentioned in other studies (21).

According to our results, in hyperglycemic conditions, there were increase in SOD and decrease in CAT and GSH activity or amounts. Previous studies demonstrated that the activity of complex III in mitochondria decreases in hyperglycemic condition. In this condition, the action of electrons transportation in the electron transport chain in mitochondria are reversed and superoxide anions will be produced (22). Overexpression of superoxide dismutase (SOD) in the mitochondria of retinal cells in hyperglycemic condition prevents the harmful effects of superoxide production (23). In diabetes glutathione content decrease and increase in expression of SOD by mitochondria has little effect on the reduction of GSH (23). In normal condition the activities of these three antioxidants are essential in decreasing the harmful effects of free radicals in hyperglycemic conditions.

Xanthophyll carotenoids, AXT and lutein increased the levels of antioxidant enzymes in the ocular tissues of STZ induced hyperglycemic rats (23). Some studies confirm that potency of AXT to remove superoxide radicals is higher than other antioxidants such as vitamin E (24). Related to AXT molecular structure it can eliminate free radicals with three mechanisms which include oxidation, hydrogen abstraction, and electron transfer. However, AXT does not exists in the retinal tissue but crosses easily through the blood-retinal barrier and protects the ganglionic cells from oxidative stress (25). This study demonstrated elevation of malondialdehyde level in hyperglycemic groups. In vitreous fluid, the ROS elevation is concurrent with severity of diabetes (26). It is probable that the increase of ROS causes peroxidation lipid, protein, and carbohydrate in two hyperglycemic groups. Also, NADPH oxidase induces Xanthine oxidase which in turn reduces GSH level and finally antioxidant defense mechanisms are disturbed (27).

At the hyperglycemic condition, ROS reacts to the double bond of free fatty acids and accordingly MDA is produced (19) it attacks to biological substances then advanced lipid peroxidation end products appear in the cells. All these events led to the creation of pseudo hypoxia in retinal tissue. Hypoxia is the strong operating of VEGF expression (28). It is an important factor to vasodilatation and increases permeability in the vasculature network within the inner retina (29). In addition, the ROS stimulates inflammatory response (30). It is demonstrated that TNF-α has a role in hyperglycemic vascular outflow and in the complication of hyperglycemic retinopathy and the AXT inhibits pre inflammatory cytokine via inhibition NF-βB expression (31). Also, it has been shown the suppressive effect of AXT in ocular hypertension (32). Other studies demonstrated that TNF-α and VEGF increased in two weeks after the beginning of diabetes and the inhibition of TNF-α decreased blood retinal breakdown in retinal tissue (33). Our study demonstrated the same increase of VEGF and TNF-α after six weeks of the beginning of Hyperglycemia. Although treatment with AXT could prevent the expression of TNF- α in the hyperglycemic group but the decrement of VEGF was not significant in this group. As the study of Hashimato H. et al in 2016 in spite of positive correlation between total hydroperoxide and VEGF in aqueous humor in diabetic patients but AXT treatment did not induce significant changes in VEGF level in aqueous humor (33).

Conclusion

In this study we revealed that, during hyperglycemia (47 days) the antioxidant capacity in retinal tissue decreased and vasodilation and inflammation in RGC layer were induced. The VEGF expression was also increased in this tissue. Treatment with the Astaxanthin during experience some deal could prevent antioxidant deficiency and helped to repair of vasodilation. This may be due to its first antioxidant and anti-inflammatory effects and not directly through reduction in VEGF expression. The unaffected expres-

sion of VEGF with Astaxanthin treatment may be related to insufficiency of treatment time or to the stage of pathologic condition and also related to a specific sampling site.

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Functional food knowledge and use in individuals with type 2 diabetes and the relevant factors

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Summary. This study aimed to determine the knowledge and use of functional foods in individuals with type 2 diabetes who applied to Dokuz Eylul University Hospital's Endocrinology Polyclinic, were aged 18 years or older, and diagnosed with type 2 diabetes no earlier than six months from before the study began. The study also focused on the associated factors. This is a cross-sectional study. In an infinite population, the smallest sample size to be achieved was 196 with 50% frequency and 7% error margin. The study was conducted with individuals who visited Dokuz Eylul University Hospital's Endocrinology Polyclinic between May and July of 2016. The dependent variable is the knowledge and use of functional foods. The independent variables are the socio-demographic and economic characteristics, health status variables, and information sources for functional foods. The study data were collected using a face-to-face questionnaire as well as chi-square and logistic regression analyses. The mean age of the participants was 57.9±11.5 years (21-77), and of them, 58.7% were female (n=115). The frequency of knowing at least one functional food that is effective for balancing the blood sugar was 95.9% (n=188), and the frequency of using this knowledge was 83.7% (n=164). In this study group, the current use of functional food was not affected by sociodemographic variables, diabetes history in family, health perception, compliance with treatment, presence of a chronic disease other than diabetes, receiving nutritional counseling, following a diabetic diet, receiving functional food counseling and having friends and/or acquaintances as information source. The participants who did exercise regularly (OR=3.370, 95%CI=1.201-9.458, p=0.021), provided information from health professionals (OR=3.921, 95%CI=1.106-13.894, p=0.034), and provided information from the internet (OR=4.152, 95%CI=1.176-14.661, p=0.029) had a significantly higher use of functional food currently. Diabetic individuals should be supported to become more informed about functional food that has a growing popularity, and they should also be taught not to consume it without consulting a physician or dietitian. The study suggests that further studies should be conducted to assess functional food, its effects on health, and individuals' knowledge and frequency of using it, and to make interventions in relation to these subjects.

Key words: diabetes mellitus, functional food, type 2 diabetes

Introduction

According to the World Health Organization (WHO), diabetes mellitus (DM) is a metabolic disorder of multiple etiology, characterized by chronic hyperglycemia with disturbances of carbonhydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both (1). DM is becoming more common in Turkey and worldwide. It is reported by WHO to be the most significant health problem that occurs in relation to a chronic disease. DM is included in epidemic diseases group (2).

Many individuals fall victim to ill-advised health recommendations and nutrition advice that leaves us-

ers lacking in necessary nutritional elements which can increase the number of health problems they may face (3). Society has become more conscious of healthy diets, which leads to an overall shift towards attentiveness in food and lifestyle choices. In the first quarter of the twentieth century, individuals started to use natural sources to treat DM which lead to the development of new medicinal molecules. Different plants and foods are used throughout the world for traditional methods of treating DM. Some traditional treatments are embraced by scientific authorities including the WHO, which supports studies in this field (4). The increase in the prevalence of chronic diseases and desire of leading a quality life directs individuals to functional foods (3).

Functional foods are defined as the foods or food elements that meet basic nutritional needs. They provide additional benefits for human physiology and metabolic functions and help reduce the occurrence of disease (5). Today, there is a considerable amount of misinformation and misuse regarding functional foods. It is critical to provide correct information about functional foods for improving personal and social health. To prevent misuse of functional foods, it is mandatory to determine the knowledge level, attitudes, and behaviors of individuals and eradicate any errors (6). Functional foods may bring harm to individuals with DM if they do not follow the daily dosing instructions and/or switch their treatment plan without professional permission.

The aim of this study was to determine the knowledge, usage and related factors of functional foods on patients diagnosed with type 2 diabetes who applied to Dokuz Eylul University Endocrinology Polyclinic.

Methods

Subjects and setting

The population of this cross-sectional study was 196 individuals. 196 individuals who applied to Dokuz Eylul University Hospital Endocrine Polyclinic between May and July of 2016 were included in the study.

Ethics committee approval was obtained on 05.05.2016 and 2016/12-23 numbered decision of Dokuz Eylul University Non-Invasive Clinical Research Ethics Committee.

Conducting data

A total of 196 individuals who older than 18 years and applied to Dokuz Eylul University Hospital Endocrine Polyclinic between May and July of 2016 were included in the study. Informed consent form was at the beginning of the questionnaire and data were collected by researcher with face-to-face interview.

Variables of the study

The independent variables were sociodemographic and economic characteristics (age, gender, education level, marital status, working status, income-expense perception, the longest living place) health status features (presence of a chronic disease other than diabetes, diabetes history in family, blood sugar control status, compliance with treatment, use of insulin status, receiving nutritional counseling, exercising regularly, following a diabetic diet, use herbal products) functional food counseling and information source of functional food. Dependent variables were knowledge of functional foods and use of functional foods.

Knowledge of functional foods: Participants were given lists of functional foods used to balance blood sugar level. Participants were asked the following question for each food in the list: "Did you know that this food is effective for balancing blood sugar level?" Those who stated that they knew at least one of the functional foods were accepted as "they know."

Use of functional foods: The list of functional foods that are used to balance blood sugar level was given to the participants. The questions, "Do you use this food to balance your blood sugar level?" and "Did you know that these foods are effective for balancing blood sugar level?" were asked for the functional foods. Those who state that they currently use these foods were accepted as "they use."

Statistical Analysis

Chi-Square and Logistic Regression Analysis were used in SPSS 15.0. Logistic regression analysis variables were performed for three groups, which are sociodemographic variables, health status variables, and functional food variables.

Results

Of the participants, 34.2% were 65 years old or older, and the mean age was 57.9±11.5 years old. Of them, 58.7% were female. Of the participants, 36.8% received a primary education or lower, 81.1% have lived in a provincial center for the majority of their lives, and 71.9% were married. Additionally, 37.8% were retired and 36.7% were housewives (Table1). The participants' economic status data indicates that the incomes and expenses of 48.0% are equal. Incomes of 39.8% of participants were lower than their expenses while 12.0% earned more than their expenses.

The participants' health status data is presented in Table 2.

The number of those who knew at least one of the functional foods in the list that are effective in balancing the blood sugar level was 188 (95.9%). The most popular foods recognized were cinnamon (89.8%), olive leaves (58.5%) and dietary fiber, respectively. 164 participants (83.7%) stated that they currently use at least one of the functional foods to balance their blood sugar. 139 participants (70.9%) mentioned that they used a functional food "each day" currently or in the past. In addition, 168 participants (85.7%) expressed that they did not consider the amount consumed when using a functional food. The functional foods used were presented in Table 3.

Of the participants, 89.3% stated that they had not ever received counseling regarding functional foods. The three most common information sources for functional foods are acquaintances or friends (71.2%), television (41.4%), health professionals (32.4%), or the internet (32.4%) displayed in Table 4.

Causal analyses could not be performed on the variable of knowing a functional food because of the high number of individuals knowing at least one functional food effective in balancing blood sugar level.

Age group (p= 0.212), gender (p=0.276), educational level (p=0.200), marital status (p=0.194), the longest living place (p=0.051), income-expense perception (p=0.197), and working status (p=0.409) did not significantly affect functional food use.

The presence of a chronic disease other than diabetes, diabetes history in the family, blood sugar control status, thinking about to compliance with treatment, using insulin, receiving nutritional counseling from a dietitian, and following a diabetic diet did not significantly affect functional food use (p>0.05) (Table 5). Those who exercise regularly as a way to regulate blood sugar level used functional foods more frequently when compared to those who did not exercise. This difference was significant (p=0.008) (Table 5).

Receiving counseling on functional foods and receiving information from books, advertisements, ac-

Table 1. Socioeconomic characteristics of diabetic individuals

Characteristics (n=196)	n	%
Educational level		
Illiterate	7	3.6
Literate	5	2.6
Primary school	60	30.6
Secondary School	18	9.2
High school	56	28.6
University and over	50	25.5
The longest living place		
Province	159	81.1
District	29	14.8
Village	8	4.1
Working status		
Retired	74	37.8
Housewife	72	36.7
Officer	25	12.8
Self-employed	16	8.2
Worker	5	2.6
Employer	2	1.0
Unemployed	1	0.5
Not working	1	0.5

Table 2. Health status characteristics of individuals

Characteristics (n=196)*	n	%
Presence of a chronic disease other than diabetes (Yes)	177	90.3
Diabetes history in family (Yes)	133	67.9
Blood sugar control status(Yes)	57	29.1
Compliance with treatment (Yes)	146	74.5
Use of insulin status (Yes)	69	35.2
Receiving nutrition counseling from dietitian (Yes)	148	75.5
Exercising regularly to regulate blood sugar (Yes)	125	63.8
Following a diabetic diet to regulate blood sugar (Yes)	153	78.1
Use herbal products to regulate blood sugar (Yes)	77	39.3

*More than one option checked

Table 3. Distribution of functional foods currently used by participants

Functional foods (n=196)*	n	%
Cinnamon	105	53.6
Dietary fiber	100	51.0
Walnut	51	26.0
Almond	48	24.5
Apple	45	23.0
Nigella sativa	39	19.9
Pumpkin	11	5.6
Purslane	11	5.6
Olive leaves	11	5.6
Broccoli	10	5.1
Pomegranate	10	5.1
Coffee	10	5.1
Blueberries	9	4.6
Oats	9	4.6
Black mulberry	7	3.6
Persimmon leaf	7	3.6
Turmeric	7	3.6
Green tea	6	3.1
Allium porrum	5	2.6
Dead nettle	5	2.5
Quince	4	2.0
Garlic	3	1.5
Cumin	3	1.5
Onion	2	1.0
Teucrium polium	2	1.0
Sweet basil	1	0.5
Soy beans	1	0.5
Fennel	0	0.0
Fenugreek	0	0.0
Algea	0	0.0
Ginseng	0	0.0

* More than one option checked

quaintances-friends, food producers and newspapers or radio did not significantly affect functional food use (p>0.05). Functional foods were significantly used more frequently by those whose information sources were internet (p=0.004), health professionals (p=0.004) and health or diet-related journals and books (p=0.017).

Logistic regression analyses were performed by separating the variables into three groups. First group

Table 4. Counseling on functional food and distribution of information resources

Characteristics	n	%
Received counseling (Yes) (n=196)	21	10.7
Information sources of functional foods which known (n=188)*		
Acquaintances or friends	134	71.2
Television	78	41.4
Health professionals	61	32.4
Internet	61	32.4
Health or diet-related journals and books	23	12.2
Newspaper	13	6.9
Books	11	5.8
Food producers	5	2.6
Advertisement	3	1.5
Radio	2	1.0
Herbalist	2	1.0

* More than one option checked

Table 5. Functional food use according to health status

Characteristics (n=196)	Using functional foods				p*
	Yes		No		
	n	%	n	%	
Presence of a chronic disease other than diabetes					
No	17	89.5	2	10.5	0.744**
Yes	147	83.1	30	16.9	
Diabetes history in family					
Yes	114	85.7	19	14.3	0.261
No	50	79.4	13	20.6	
Thinking about the ability to control the blood sugar level					
Yes	49	86.0	8	14.0	0.578
No	115	82.7	24	17.3	
Thinking about the compliance with treatment					
Yes	122	83.6	24	16.4	0.942
No	42	84.0	8	16.0	
Using insulin					
Yes	58	84.1	11	15.9	0.915
No	106	83.5	21	16.5	
Receiving nutritional counseling from a dietitian					
Yes	124	83.8	24	16.2	0.942
No	40	83.3	8	16.7	
Exercise regularly to regulate blood sugar					
Yes	66	93.0	5	7.0	0.008
No	98	78.4	27	21.6	
Following a diabetic diet to regulate blood sugar					
Yes	40	93.0	3	7.0	0.060
No	124	81.0	29	19.0	

* Chi-Square Analysis; **Fisher's p

variables were the sociodemographic variables (Table 6). Age group, gender, educational level, marital status, the longest living place and income-expense perception did not significantly affect functional food use (Table 6).

The logistic regression analysis performed by the health status variables indicates that health perception, presence of a chronic disease other than diabetes, diabetes history in the family, compliance with treatment, receiving nutritional counseling, and following a diabetic diet did not significantly affect functional food use. Those who performed regular exercises used the functional foods three or four times more frequently (OR=3.370, 95%CI=1.201-9.458 p=0.021) (Table 7).

Receiving functional food counseling and friends or acquaintances as an information source did not significantly affect the use of functional foods. Functional foods were used 4.2 times higher by those whose information source is the internet (OR=4.152, 95%CI=1.176-14.661 p=0.027) and 3.9 times higher by those whose information source is health professionals (OR=3.921, 95%CI=1.106-13.894 p=0.034) (Table 8).

Discussion

Participants stating that they knew at least one of the functional foods were accepted as "they know." The number of those who knew at least one of the functional foods in the list was 188, or 95.9% of patients. This rate indicates that participants' awareness about balancing blood sugar levels using certain foods is high. Of the participants, 86.2% knew cinnamon is effective for balancing blood sugar levels, while 56.1% mentioned olive leaves, 55.6% mentioned dietary fiber, 52.0% mentioned black cumin, and more than 30% mentioned almond, walnut, and cranberry.

It is believed that cinnamon balances blood sugar levels by boosting insulin resistance. In a study 109 individuals with type 2 DM were administered one gram

Table 6. Logistic regression analysis data: the effect of sociodemographic variables on functional food use

Characteristics (Reference group)	Using functional foods		
	p	OR	95% CI
Age group (65 years old or older)	0.428	1.409	0.603-3.295
Gender (Male)	0.090	2.116	0.889-5.040
Education (Lower than high school)	0.395	1.469	0.606-3.562
Marital Status (Single)	0.173	0.545	0.228-1.305
The longest living place(District- village)	0.107	2.123	0.851-5.300
Status of income and expense (Income lower than the expense)	0.351	1.482	0.649-3.383

*Age group (reference group; 65 years or older), gender (reference group; male), education (reference group; lower than high school), marital status (reference group; single), the longest living place (reference group; district- village), status of income and expense (reference group; income lower than the expense.)

Table 7. Logistic regression analysis data: Health status effects on functional food use

Characteristics (Reference group)	Using functional foods		
	p	OR	95% CI
Health perception (Poor)	0.490	0.726	0.292-1.803
Presence of a chronic disease other than diabetes (Not Present)	0.483	0.562	0.112-2.818
Diabetes history in family (Not Present)	0.166	1.791	0.785-4.086
Compliance with treatment (Yes)	0.986	1.008	0.397-2.560
Receiving nutritional counseling (No)	0.219	3.732	0.457-30.504
Exercising regularly (No)	0.021	3.370	1.201-9.458
Following a diabetic diet (No)	0.146	2.600	0.718-9.416

*Health perception (reference group: poor), presence of a chronic disease other than diabetes (reference group: not present), diabetic family history (reference group: not present), compliance with treatment (reference group; yes), receiving nutritional counseling (reference group; no), exercising regularly (reference group=no), following a diabetic diet (reference group=no.)

Table 8. Logistic regression analysis data: variables related to the effects of functional food information counseling on the use of functional foods.

Characteristics (Reference group)	Using functional foods		
	p	OR	95% CI
Receiving functional food counseling (No)	0.274	3.226	0.395-26.340
Internet as information source (No)	0.027	4.152	1.176-14.661
Health professionals as information source (Yes)	0.034	3.921	1.106-13.894
Friends or acquaintances as information source (No)	0.283	1.598	0.679-3.763

*Receiving functional food counseling (reference group: no), internet as information source (reference group: no), health professionals as information source (reference group: yes), friends-acquaintances as information source (reference group: no)

of cinnamon daily for 90 days, and their HbA1c values decreased significantly (14). In a recent study conducted on 60 volunteers with type 2 DM, participants were administered 1, 3, and 6 grams of cinnamon right after a meal for the first 40 days. In the last 20 days, they were administered a placebo. Blood sugar levels of the participants who were administered cinnamon decreased by 18-29% in the first 40 days. At the end of the last 20 days, no significant decrease was present in blood sugar levels (15). Thirty individuals with type 2 DM were administered 1, 3, and 6 grams of cinnamon daily for 40 days, and a daily intake of 1-3 grams of cinnamon significantly decreased blood glucose levels (4).

Of the participants, 58.5% stated that they know olive leaves are effective for balancing blood sugar levels. A study conducted by Gonzalez et al. to examine the glycemic responses to cooked rice indicates that olive leaf extract significantly decreases blood sugar levels, and oleuropein in oil leaves boosts the rate of glucose intake to the cells. Diabetic rabbits were administered 20 mg/kg oleuropein for 16 weeks and their glucose levels were found to decrease after the eighth week (16).

Dietary fiber, which was mentioned by 57.9% of participants, delays glucose absorption in diabetic individuals and reduces insulin levels. Thus, diabetic individuals are recommended to consume 30-50 grams of dietary fiber (17). In a randomized controlled study, individuals with type 2 DM consumed two types of diets with the same macro and micro food elements for six weeks. One of these diets was recommended by American Diabetes Association (ADA). The other diet contained high amounts of fiber. Pre and postprandial glucose levels, insulin concentrations, total cholesterol level and TG and LDL cholesterol levels significantly decreased after dietary fiber was consumed when comparing the two diets. Consumption of dietary fiber by individuals with type 2 DM upon the recommendation of ADA ensured that glycemic control was maintained, hyperinsulinemia rate decreased, and blood lipid concentrations were reduced (18).

In the current study, 54.2% of the participants stated that they knew black cumin is effective for balancing blood sugar levels. Thymoquinone, dithymoquinone, thymohydroquinone, and thymol components increase insulin secretion in beta cells. Thus, black cumin increases insulin levels. A study conducted with volunteers indicated that consuming 1 gram of black cumin twice daily for two weeks reduces blood glucose levels (4). Another study suggests that black cumin seeds traditionally used to treat type 2 DM in North Africa and the Middle East display insulin-related effects. Ethanol extract of black cumin seeds displays antihyperglycemic effects on beta, skeletal, muscle, and fat cells (19). In a study where diabetic rabbits were orally administered black cumin seeds for two months, high blood glucose levels were found to decrease (4). In a six-week study conducted to compare the effects of 2g/kg black cumin to those of metformin (300mg/kg/day), used to treat DM, black cumin was found to improve glucose tolerance as effectively as metformin. Using black cumin as a supplement for individuals with DM reduces the glucose absorption rate and glucose tolerance. In addition, metformin and black cumin helps weight loss without causing any toxic effects (20).

More than 30.0% of the participants stated that they knew almond, walnut, and blueberries are effective for balancing blood sugar. A relevant study was conducted on individuals with type 2 DM in China, where 20% of the diet calorie of the experimental group had almond derivatives (60 grams/day). At the end of the two-week study, adding almond to a healthy diet reduced lipoidosis, insulin resistance, and the cholesterol of participants with type 2 DM, while also increasing glycemic control (21). A similar study was conducted to examine the metabolic effects of polyunsaturated fatty acids during walnut consumption. Fifty overweight individuals with type 2 DM who did not use insulin consumed a low-fat diet (2000 kcal, 30% fat) for one year. The intervention group consumed approximately 30 grams of walnut derivatives in their diet. The preprandial insulin levels of those who consumed walnut sources significantly decreased in the first three months (22). A study conducted on nurses showed that the prevelance of type 2 DM among women who consumed a fatty seed (almond, walnut, peanut, hazelnut, etc.) for five times or more in a week was 27% lower than the rate for those who did not consume fatty seeds at all (23). Almond and walnut consumption ensures glycemic control for those with type 2 DM and reduces the risk of cardiovascular

disease (24). In a study conducted on individuals with type 2 DM, daily consumption of 200 ml blueberry juice for three months reduced fasting blood glucose, HbA1c and blood lipid levels (25).

Of the participants, 89.3% stated that they had not received counseling regarding functional foods before. The three most common information sources for functional foods were acquaintances or friends (71.2%), television (41.4%), health professionals (32.4%), or internet sources (32.4%). In a study conducted to determine individuals' attitudes towards functional foods, it was found that even scholars with the highest academic degrees did not have sufficient knowledge of functional foods. The most common reference source for functional foods used by participants was "acquaintances and friends," which is similar to the our study. Health professionals, (doctor/dietitian), advertisements, and company personnel, respectively, follow acquaintances and friends (6).

The current study analyzes whether participants consumed functional foods. Findings indicate that 53.6% used cinnamon, 51.0% consumed dietary fiber, 26.0% ate walnut, 24.5% consumed almond, 23.0% ate apples, and 19.9% used black cumin to balance their blood sugar levels. A study conducted in İzmir on academic staff awareness, acceptance, and attitudes towards functional foods indicated that the three most common functional foods participants consumed were mineral water, cereals that contained whole grain, and wholegrain dietary biscuits. Foods that contain plenty of grains were regarded as dietary fiber and the second most common food in our study (26). Dietary fiber, walnut, almond, and apple, which were all commonly consumed by participants, are among the foods recommended by the dietitians within the medicinal dietary treatments for diabetic individuals. The most commonly consumed functional foods can be attributed to counseling advice, with 75.5% of the participants receiving DM-related counseling from a dietitian before and 78.1% following a diabetic diet to balance blood sugar levels.

Age group, gender, educational level, marital status, the longest living place, and income-expense status were not found to have a significant effect on functional food consumption. Two studies conducted in Turkey indicate that educated young individuals are more inclined to consume functional foods (26). Studies conducted in USA suggest that functional foods are consumed more frequently by the middle-aged, highly-educated, female population (27). European women who are older than middle-aged and from high socioeconomic status use functional foods the most (28). The consumption of functional foods is more frequent among individuals with high educational and socioeconomic status. This can be attributed to the increase of health consciousness in popular culture. In addition, perceiving functional foods to be more expensive than other food products may prevent individuals with low socioeconomic status from buying and consuming these foods (6).

The presence of another chronic disease was not related to the consumption of functional foods. When a person with a chronic disease other than DM uses functional foods to balance their blood sugar level, care should be taken to make sure that the functional food(s) will not negatively affect the progression of the chronic disease. Functional foods are consumed more frequently by diabetic individuals that did not have cardiovascular disease than those who did. The reason why individuals that had both DM and cardiovascular diseases consumed less functional foods to balance their blood sugar levels may be attributed to the assumption that the functional food to be consumed may negatively affect the cardiovascular disease or interact with a medicine.

The effects of DM-related characteristics on the use of functional foods indicate that DM history in the family, thinking about the ability to control the blood sugar level, thinking about the compliance with treatment, using insulin, receiving nutritional counseling from a dietitian, and following a diabetic diet did not significantly affect the use of functional foods. Those who exercised regularly to regulate blood sugar levels used functional foods more frequently when compared to those who did not exercise, and the difference was significant. A study conducted to determine consumers' attitudes towards functional foods indicates that individuals who regularly exercise have more positive attitudes towards functional foods compared to those who do not exercise (6). This trend may be attributed to the assumption that individuals who exercised regularly seek non-medicinal solutions to lower their blood sugar level.

Receiving counseling on functional foods and acquaintances or friends being a source of information on functional foods did not significantly affect the use of functional foods. The internet and health professionals as information resources increase the rate of using functional foods. This may be attributed to general trust in health professionals and the internet.

Limitations

The greatest limitation of the current study is that it was conducted with the individuals who applied to the relevant polyclinic for DM and received healthcare. Educational and awareness levels of the individuals who live in the area around the endocrine polyclinic where the study was conducted were high, and the study was conducted with the individuals who already received healthcare, which prevent the generalization of the outcomes. In addition, data related to the balancing of blood sugar levels, exercising regularly, and following a diabetic diet were spontaneously collected in the first and only interviews with each person. This may be regarded as another limitation. Finally, only a few studies on diabetic individuals' awareness and consumption of functional foods have been conducted, which makes it challenging to compare results.

Strengths

Our study is the first study that examines diabetic individuals' awareness and consumption of functional foods in Turkey. All data were collected by the researcher. Findings from this study may guide diabetic individuals to pursue a better understanding of the specific features of functional foods. In addition, this study may show which subjects diabetic individuals need to educate about functional foods.

Conclusion

Diabetic individuals should be informed about functional foods that are becoming more popular and educated on the necessity of consulting a doctor or dietitian before consuming them. In addition, when diabetic individuals use functional foods, they may be harmed if they don't attention amount of usage. This should be the responsibility of doctors, dietitians, and all health professionals who meet with diabetic individuals.

The development of functional food science research and proper media coverage of consequent findings could lead to potential progress in the treatment of DM. Raising awareness of the society and increasing the number of studies measuring the frequency of knowledge levels and consumption frequencies are important. Although scientific studies indicate that foods affect our quality of life, it should be remembered that no food can create miracles and foods cannot be used as medicines for the treatment of diseases.

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Fatty acid compositions of the seeds of different *Sanguisorba minor* genotypes

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Summary. The seed oils of twenty *Sanguisorba minor* (Leguminosae) genotypes were investigated for their oil contents and fatty acid compositions. The oil contents of the seeds were found to be between 8.85% and 15.66%. The fatty acid compositions of these twenty different genotypes were determined by the GC of the methyl esters of their fatty acids. The oilseeds of *Sanguisorba minor* genotypes contain palmitic acid as the major component of their fatty acids, among the saturated acids, with small amounts of steraric acid. The major unsaturated fatty acids found in the oilseeds of the genotypes were oleic, linoleic and linolenic acids. In this study, the total saturated fatty acids of *Sanguisorba minor* genotypes were between 6.40% and 15.84% while the total unsaturated fatty acids were between 84.16% and 93.60%.

Key words*:* Oil content, unsaturated fatty acid, saturated fatty acid, *Sanguisorba minor*

Introduction

Sanguisorba minor is a perennial plant with pointed leaves and redis-green flowers (1). The plant is grown in Eurpope, North Africa, Canarias Islands, Southeastern Asia, New Zealand and England and it is widely used in animal nutrition (2). It has also been used in folk medicine for centuries (3,4). Despite low oil content, the plant has several clinical advantages, thus used as an alternative oil crop. Plants exhibit quite much diversity in macro-micro elements (5) and chemical composition (especially in fatty acids) (6). Genetic diversity is the most significant factor in breeding studies (7). Selection of proper parents is also a significant factor in improving efficiency of breeding programs and performing promising hybridizations. Therefore, all characteristics of local genotypes should be identified in detail.

The present study was conducted to characterize 20 different salad burnet (*Sanguisorba minor*) genotypes with regard to fatty acid compositions.

Material and Methods

Plant samples

In this study, a total of 20 salad burnet (*Sanguisorba minor*) genotypes from Kirsehir, Kahramanmaras, Sivas, Kayseri and Yozgat provinces in Turkey were used as the seed material (Table 1). The seeds were sown and propagated under controlled conditions of Kayseri province of Central Anatolian (39°48'N, 38°73'E). Resultant plants were subjected to fatty acid analyses.

Table 1. Codes and abbreviations of *Sanguisorba minor* genotypes

No	Code	No	Code	No	Code	No	Code
1	ÇD2	**6**	ÇD12	**11**	ÇD26	**16**	ÇD38
2	ÇD5	**7**	ÇD16	**12**	ÇD28	**17**	ÇD40
3	ÇD9	**8**	ÇD17	**13**	ÇD32	**18**	ÇD45
4	ÇD10	**9**	ÇD19	**14**	ÇD33	**19**	ÇD47
5	ÇD11	**10**	ÇD24	**15**	ÇD34	**20**	ÇD57

Oil extraction and preparation of fatty acid methyl esters (FAME)

Impurities were removed from the seeds and the cleaned seeds were ground using mill into powder. The seed material of *Sanguisorba minor* genotypes was homogenized in 10 mL of hexane / isopropanol (3:2) at 10.000 rpm for 30 sec and centrifuged at 5000 rpm for 10 min (8). The upper part was removed and placed in a test tube by filtration.

Capillary GLC

The fatty acids in lipid extracts were converted to methyl esters in methanol with 2% sulfuric acid (v/v) (9). The fatty acid methyl esters were extracted with n-hexane. Then the methyl esters were separated and quantified by gas chromatography and flame ionization detection (Schmiadzu GC, 17 Ver.3) coupled with a glass GC 10 software computing recorder. Chromatography was performed with a capillary column [GC-MS instrument (USA) of Agilent brand 7890A / 5970 C and SGE Analytical BPX90 100 m x 0.25 mm x 0.25 μm column (Australia) were used] using nitrogen as carrier gas (flow rate 1 mL/min) the temperatures of the column, detector and injector valve were 120-220, 240-250 °C, respectively. Identification of the individual method was performed by frequent comparison with authentic standard mixtures analyzed under the same conditions.

Results and Discussion

In this study, oil content and fatty acid compositions of the seeds of 20 different *Sanguisorba minor* genotypes were determined and the results were given in Table 2. Oil content of *Sanguisorba minor* genotypes was detected between 8.85% and 15.66%. The ÇD33 genotype had the highest oil content, while the lowest percentage was found in ÇD19 genotype. The main components in the seed oils of *Sanguisorba minor* genotypes are palmitic, oleic, linoleic and linolenic acids. Palmitic acid, a saturated fatty acid, was ranged from 4.55-10.40%, which was lower than those given by Viano et al. (2) as 29.1%. Stearic acid was detected in only 3 genotypes (ÇD2, ÇD19 and ÇD28), ranging from 2.28% to 7.90%. These results were in agree-

Table 2. Fatty acid composition of studied samples (%)

Fatty acid	ÇD2	ÇD5	ÇD9	ÇD10	ÇD11	ÇD12	ÇD16	ÇD17	ÇD19	ÇD24
Oil content	10.83	12.27	11.14	11.38	11.10	13.18	12.94	10.49	8.85	9.77
16:0	4.55	7.98	7.41	7.56	6.92	7.44	6.40	7.80	7.94	10.40
18:0	2.28	-	-	-	-	-	-	-	7.90	-
18:1	34.34	24.78	30.84	23.09	19.57	23.40	25.98	29.54	24.88	-
18:2	31.14	36.76	33.92	38.81	36.62	40.46	36.92	32.16	30.51	49.84
18:3	27.69	30.48	27.83	30.54	36.89	28.70	30.70	30.50	28.77	39.76
∑TSFA	6.83	7.98	7.41	7.56	6.92	7.44	6.40	7.80	15.84	10.40
∑TUSFA	93.17	92.02	92.59	92.44	93.08	92.56	93.60	92.20	84.16	89.60
	ÇD26	ÇD28	ÇD32	ÇD33	ÇD34	ÇD38	ÇD40	ÇD45	ÇD47	ÇD57
Oil content	12.44	13.43	11.33	15.66	13.98	13.11	10.23	10.81	10.49	10.80
16:0	7.55	6.42	7.67	6.52	7.59	7.54	7.37	6.57	8.33	7.47
18:0	-	3.52	-	-	-	-	-	-	-	-
18:1	25.58	26.22	33.02	22.51	28.57	21.28	28.07	30.53	30.33	22.31
18:2	36.12	32.80	33.25	39.62	34.76	37.84	35.70	33.74	32.17	35.53
18:3	30.75	31.04	26.06	31.35	29.08	33.34	28.86	29.16	29.17	34.69
∑TSFA	7.55	9.94	7.67	6.52	7.59	7.54	7.37	6.57	8.33	7.47
∑TUSFA	92.45	90.06	92.33	93.48	92.41	92.46	92.63	93.43	91.67	92.53

C16:0 Palmitic acid; C18:0 Stearic acid; C18:1 Oleic acid; C18:2 Linoleic acid; C18:3 Linolenic acid; TSFA: Total saturated fatty acid; TUSFA: Total unsaturated fatty acid

ment with Viano et al. (2) who reported that stearic acid was detected from *Sanguisorba minor* ssp. *muricata* as 6.90%.

Oleic acid was detected between 19.57% and 34.34% in all genotypes except for ÇD24 genotype. From the data presented it could be seen that the highest oleic acid was found in ÇD2 genotype, while the lowest percentage was found in ÇD11 genotype. The values we obtained about oleic acid were found to be higher than those obtained by some research (2,10). It is thought that this is due to the different genotypes used in the research. Linoleic acid was the major unsaturated component in the oil of these seeds, followed by linolenic and oleic acids. Linoleic acid was found to be the highest in the ÇD24 genotype (49.84%) and the lowest in the ÇD19 genotype (30.51%). These results were in disagreement with Viano et al. (2) who reported that linoleic acid was detected from *Sanguisorba minor* ssp. *muricata* as 22.6%. On the other hand, Petersen et al. (10) reported that linoleic acid was found to be 2.6% in DM of *Sanguisorba minor*. The highest linolenic acid was obtained in ÇD24 genotype with 39.76%, while the lowest linolenic acid was obtained in ÇD32 genotype with 26.06%. The values we obtained for linolenic acid were higher than the values of some scientists (2,10).

Total unsaturated fatty acid (TUSFA) of studied *Sanguisorba minor* genotypes was between 84.16% and 93.60% (Table 2). It could be seen in Table 2 that the highest TUSFA was found in ÇD16 genotype, while the lowest percentage was found in ÇD19 genotype. Total saturated fatty acid (TSFA) of studied *Sanguisorba minor* genotypes was between 6.40% and 15.84%. ÇD19 genotype has highest level of TSFA; also in the *ÇD24* genotype (10.40%) and *ÇD28* genotype (9.94%). The lowest percentages of TSFA were found in ÇD16 genotype. The values we acquired related to TSFA and TUSFA were disagree with Viano et al. (2) and Petersen et al. (10).

Conclusion

The oil contents of the studied legumes belonging to the *Sanguisorba minor* genotypes showed quantitative differences but the oilseeds showed uniform fatty acid composition. The results revealed that the oilseeds of the *Sanguisorba minor* genotypes studied with a substantial amount of very long chain fatty acids might have attracted attention because of their value for industrial, nutritional and renewable resources.

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Association between subclinical hypothyroidism and coronary artery disease

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Summary. Coronary artery disease (CAD) is a common health problem with high morbidity and mortality. In recent years overt hypothyroidism is shown as an independent risk factor of CAD. Thyroid hormones have more effects on the cardiovascular system, and both hypothyroidism and hyperthyroidism have harmful effects on the cardiovascular systemç Subclinical hypothyroidism (SCH) is a common clinical situation with 4-20% frequency. SCH is associated with endothelial dysfunction, coronary atherosclerosis, and cerebrovascular disease. SYNTAX score is the angiographic scoring system and is widely used to evaluate the severity and complexity of CAD. The aim of this study to evaluate the association between SCH and SYNTAX score. This study is a retrospective cohort of participants who underwent coronary angiography and resulted in coronary artery bypass graft surgery. Participants divided into two group according to their SYNTAX score as high SYNTAX score (SYNTAX ≥ 23) and low SYNTAX score (SYNTAX<23). There is no statistically significant difference between two groups regarding age, male, height, weight, smoking, hypertension, diabetes mellitus, hyperlipidemia, peripheral artery disease, total cholesterol, HDL-C, LDL-C, TG, free thyroxine (fT4), free triiodothyronine (fT3), thyroid stimulating hormone (TSH), and uric acid. Serum LDL, total cholesterol levels are significantly higher in the SCH group than non-SCH group (respectively; p=0.029, p=0.024). There is a positive correlation between SCH and age, SCH prevalence increase with older age (p=0.017). Patients were divided into two group according to their TSH levels as SCH (fT3 and fT4 normal, TSH≥4). We used the SYNTAX score to evaluate the severity of CAD severity. However, there is no significant difference. Further studies are needed to evaluate the association between SCH and CAD.

Keywords: Coronary artery disease, subclinical hypothyroidism, ageing

Introduction

Coronary artery disease (CAD) is a common health problem with high morbidity and mortality risk. The elimination of cardiovascular risk factors such as hypertension (HT), diabetes mellitus (DM), hyperlipidemia (HL), smoking and obesity is the important part of cardiovascular disease prevention. In recent years overt hypothyroidism (OH) is shown as an independent risk factor of CAD. Thyroid hormones have more effects on the cardiovascular system, and both hypothyroidism and hyperthyroidism have harmful effects on the heart and vascular system (1). OH is defined as elevated thyroid stimulating hormone (TSH) levels with abnormally T4 levels. Subclinical hypothyroidism (SCH) is a common clinical situation with 4-20% frequency. The most common reason of SCH is autoimmune thyroiditis, and only a small group of patients have symptoms (2,3). SCH is associated with endothelial dysfunction, coronary atherosclerosis, cer-

ebrovascular disease, and defined as elevated (TSH) and normal serum free thyroxine (fT4), free triiodothyronine (fT3) levels (4). This association is thought to be due to several mechanisms such as increased low-density lipoprotein-cholesterol (LDL-C), HT, impaired coagulation mechanism and high C-reactive protein levels (5). Many studies showed a positive correlation with thyroid hormone and serum total cholesterol (TC), LDL-C, non-high-density lipoprotein cholesterol (non-HDL-C), triglycerides (TG) and negative correlation with serum HDL-C (6). Besides that carotid intima-media thickness is found significantly higher in SCH and OH group in previous studies as an indicator of atherosclerosis (7).

There are small clinical studies about thyroid replacement treatment on dyslipidemia and HT. However, there is no clinical data with this therapy and effects on cardiovascular mortality and morbidity (8-10). Even so, recent guidelines suggest thyroid hormone replacement therapy in SCH with special conditions such as pregnancy, infertility, symptomatic patients, high risk of progression to OH. Some of them suggest treating SCH in most patients with a serum TSH level below 10 IU/L (11).

SYNTAX score is used to evaluate CAD complexity and grade. Also for deciding coronary artery bypass surgery (CABG) or percutaneous coronary intervention in patients with serious CAD predicting long-term mortality. It includes anatomical complexity of all coronary arteries and indicates the severity of CAD (12).

On the basis of the possible association between SCH and the presence of atherosclerosis, the aim of this study was to the evaluıate association between more complexity of coronary artery disease and TSH levels.

Materials and Method

Participants

Our study is a retrospective and conducted with cohort of participants who undergo coronary angiography (CA) and resulted with coronary artery bypass graft surgery at Near East Medical Faculty Hospital from September 2015- September 2018. We included patients with the indication of CABG for severe CAD and were decided with the heart team. Patients with previous CAD history, thyroid disorder or operation history from thyroid gland and taking thyroid replacement therapy or antithyroid therapy, valve disease and undergo valve surgery at the same time with CABG and admit with acute coronary syndrome (ST-elevation myocardial infarction or non ST elevation myocardial infarction), have liver function abnormalities (serum alanine transaminase >2 times the upper normal limits) or renal insufficiency (serum creatinine >2.0 mg/dL) were excluded from study.

A detailed medical history of each patient was compiled, included the history of DM, HT, HL, peripheral artery disease, smoking, alcohol use, family history of CAD and treatment history. The patients' height and fasting weight were measured, body mass index (BMI) (weight/height in kg/m2) was calculated. Glucose, lipids profile (TC, TG, HDL-C, LDL-C), creatinine levels, white blood cell (WBC), C-reactive protein (CRP), TSH, free T3 and free T4 were assayed in blood samples after 12h overnight fasting. We defined SCH as elevated serum TSH level (TSH≥4 IU/L) with normal levels of fT3 and fT4. Patients were divided into two group according to their TSH levels as SCH (fT3 and fT4 normal, TSH≥3) or not. Blood pressure (BP) was measured using a mercury sphygmomanometer in a sitting position, after 10 minutes of rest.

The study was approved by the local Ethical Committee, and all patients provided written informed consent.

Coronary angiography

We performed CA using the Judkins technique through the femoral or radial artery. Each coronary artery was visualised in at least 2 different plane images. According to baseline CA, the SYNTAX score was calculated for all patients by two experienced interventional cardiologists unaware of the patients' clinical or laboratory results. SYNTAX score was determined for all coronary lesions with > 50% diameter stenosis in a vessel > 1.5 mm based on SYNTAX score calculator 2.1 (www.SYNTAXscore.com). Patients were divided into two groups as high SYNTAX score (≥ 23) or low SYNTAX score (<23).

Statistical analysis

Statistical analysis was performed using the SPSS (version 20.0, SPSS Inc., Chicago, Illinois) software package. Continuous variables were expressed as the mean ± standard deviation (mean± SD), and categorical variables were expressed as a percentage (%). The Kolmogorov-Smirnov test was used to evaluate the distribution of variables. Student's t-test was used to evaluate continuous variables showing normal distribution, and Mann-Whitney U-test was used to evaluate variables that did not show normal distribution. A p-value < 0.05 was considered statistically significant.

Results

Baseline characteristics

Five of participant excluded because of the missing data, 124 of them were included in the analysis. Participants divided into two group according to their SYNTAX score as high SYNTAX score (SYNTAX ≥23) and low SYNTAX score (SYNTAX<23).

There is no statistically significant difference between two groups regarding age, male, height, weight, BMI, smoking, HT, DM, HL, PAH, TC, HDL-C, LDL-C, TG, fT3, fT4, TSH, and uric acid (Table 1).

Baseline characteristics of the randomised patients in SCH and non SCH group are listed in Table 2. 6.8% of them comprising SCH (n=8), 86.8% (n=112) of patients were male, 13.2% (n=17) of them female between 45 and 90 years. Serum LDL, TC levels are significantly higher in the SCH group than non-SCH group (respectively; p=0.029, p=0.024) (Table 2). There is a positive correlation between SCH and age, SCH prevalence increase with older age (p=0.017). There is no statistically significant difference between SCH and SYNTAX score (p=0.724).

Table 1. Comparison of basal demographics features in SYNTAX groups

Variable	SYNTAX score ≥23 (n=56)	SYNTAX score <23 (n=73)	p-value
Age (year)	65.8	66.3	0.780
Male (n)	51	61	0.211
Height (cm)	169.2	169.7	0.769
Weight (kg)	81.6	84.6	0.296
Smoking (n)	20	24	0.736
Hypertension (n)	40	56	0.495
Hyperlipidemia (n)	17	19	0.587
Diabetes mellitus (n)	23	26	0.527
PAH (n)	6	7	0.833
TC (mg/dl)	194.3	195.5	0.893
LDL-C (mg/dl)	115.0	123.3	0.297
HDL-C (mg/dl)	37.3	40.1	0.114
TG (mg/dl)	213.2	161.2	0.234
FT3 (pg/ml)	2.5	2.6	0.442
FT4 (pg/ml)	1.1	1.0	0.423
TSH (mIU/ml)	1.6	1.7	0.696
Uric acid (mg/dl)	6.3	6.2	0.838

BMI, body-mass index; PAH, peripheral artery disease; TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TG, triglyceride; FT3, free triiodothyronine; FT4, free thyroxine; TSH, thyroid stimulating hormon

Table 2. Comparison of basal demographics features between SCH and non-SCH groups.

Variable	SCH (n=8)	Non-SCH (n=116)	p value
Age (years)	8	116	0,017
Male (n)	5	103	0,066
Height (cm)	168,8	169,5	0,858
Weight (kg)	89,4	83,3	0,340
Smoking (n)	3	40	1,0
Hypertension (n)	6	87	1,0
Hyperlipidemia (n)	1	35	0,436
Diabetes mellitus (n)	3	45	1,0
PAH (n)	1	11	0,568
TC (mg/dl)	230,8	189,9	0,024
LDL-C (mg/dl)	151,2	115,3	0,029
HDL-C (mg/dl)	41,8	37,9	0,373
TG (mg/dl)	189	185	0,967
FT3 (pg/ml)	2,54	2,60	0,722
FT4 (pg/ml)	1,07	1,06	0,974
SYNTAX score (≥23)(%)	21,8	23,1	0,263
Uric acid (mg/dl)	6,07	6,30	0,775

BMI, body-mass index; PAH, peripheral artery disease; TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TG, triglyceride; FT3, free triiodothyronine; FT4, free thyroxine

Discussion

We used SYNTAX score to evaluate the severity of CAD and aim to show a correlation between SCH and CAD severity, however, there is no significant association between serum TSH level and SYNTAX score. Also, SCH and non SCH prevalence are similar between high and low SYNTAX score group. SYNTAX score indicates the anatomical structure and quality of coronary vessels, besides that complexity of the CAD. It is found associated with cardiovascular morbidity and mortality (13-21). In previous studies SCH is found an independent risk factor for CAD in groups without previous CAD. In our study participants have already multivessel CAD and they underwent CABG. Therefore SCH is not found associated with the CAD complexity and severity.

Hypothyroidism is a significant health problem due to its high prevalence and associated risks of atherosclerosis, endothelial dysfunction and weight gain. It's well known that there is a strong association between CAD and OH. Dyslipidemia is considered for the main mechanism of increased atherosclerosis risk in OH (22). In the same manner, in our study serum TC and LDL levels are found significantly higher in the SCH group than the non-SCH group. Also, patients with SCH are older than the non-SCH group. The other mechanisms which are thought to be responsible are obesity, arterial stiffness, hypercoagulability and endothelial dysfunction (22-25). Desai et al. (26) demonstrated the association with OH and cardiovascular comorbidities and complications, all-cause mortality in hospital after percutaneous coronary intervention.

Although previous studies showed a correlation between SCH and coronary atherosclerosis some of them did not confirm it (27-29). It is reported that SCH increases the risk of atherosclerosis and cardiovascular system disease with a similar mechanism as OH (30). SCH is defined as mild (TSH<10 IU/L) or severe (TSH ≥10 IU/L). In previous studies, SCH and cardiovascular disease link were shown especially in a group with TSH levels ≥ 10 μIU/ml (31). In our study mean TSH levels in the SCH group was lower than 10 IU/L, we exclude the severe SCH because of the perioperative and postoperative risks of CABG. The insignificant results of our study may be due to study inclısion criteria. Also, Lee et al. (32). showed that repeat PCI risk is higher in SCH than euthyroid patients for in-stent restenosis, but there is no difference for de-novo lesions.

TSH level increases with ageing, and there is no consensus about the harmful effects of SCH. It is thought to be a compensatory mechanism and may have beneficial or neutral effects in the elderly population (33). It may have resulted in an underestimation of SCH in the elderly population. Rodondi et al. (34) showed a positive correlation between cardiovascular mortality/morbidity and SCH. On the other hand, other studies with elderly population did not demonstrate association between cardiovascular risk and SCH (35).

There is no consensus about the TSH criteria for the SCH definition. Previous studies defined SCH as TSH>4 IU/L although some of them accept TSH>5 IU/L, this may result in a misclassification and underestimation of SCH. We defined SCH as TSH>4 IU/L however, SCH prevalence is 6.8%, and there is no association with severity of CAD.

Conclusion

The coexistince of OH and CAD is known for many years. In the light of this findings we aim to evaluate the association between the SCH and CAD severity, however, there is no significant association. Further studies are needed to evaluate the association between SCH and SYNTAX score.

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Nutritional value of *Lycium ruthenicum Murr*. and its relieving resistance to exercise-induced fatigue

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Summary. *Lycium ruthenicum Murr.* has high medicinal value. This paper mainly studied the nutritional value of *Lycium ruthenicum Murr.* and its alleviating resistance to exercise-induced fatigue. Firstly, the nutritional composition and value of *Lycium ruthenicum Murr.* were introduced briefly. Then the alleviating resistance of Lycium ruthenicum Murr. to exercise-induced fatigue was analyzed by swimming experiment in mice. The mice were divided into low, medium and high *Lycium ruthenicum Murr.* groups. and a blank group which took distilled water. Swimming experiment was conducted after 30 days of continuous feeding, and then the changes of blood sugar, lactic acid and glycogen in mice before, after exercise, after exercise and 30 minutes after exercise were compared. The results showed that 30 minutes after exercise, the group with high dose of *Lycium ruthenicum Murr.* had higher content of blood sugar, hepatic glycogen and muscle glycogen and lower content of lactic acid. The experimental results demonstrate that *Lycium ruthenicum Murr.* can increase blood sugar, reduce lactic acid content and increase glycogen reserve in mice, which has good alleviating resistance to exercise-induced fatigue.

Key word: *Lycium ruthenicum Murr.*, nutritional value, exercise-induced fatigue, anti-fatigue

Introduction

Lycium ruthenicum Murr. is a perennial shrub which belongs to Solanaceae and Lycium (1), and its fruit is atropurpureus. It usually distributes in areas with high degree of salinization, such as the northwest area of China (2). It has strong resistance to high temperature, drought (3) and cold, showing extremely strong adaptivity (4), and it can prevent wind and fix sand, showing a high ecological value as a kind of shrub for controlling salinization and desertification. In addition, *Lycium ruthenicum Murr.* has functions of nourishing the liver and eyes, invigorating the kidney and benefiting the essence, and its nutritional value is much higher than that of Chinese wolfberry. It is a kind of medicinal and edible substance (5). Its chemical constituents and pharmacological effects have been extensively studied. Wang et al. (6) studied the anthocyanin content of *Lycium ruthenicum Murr.* from different areas and found that the total composition of anthocyanin was the same, but the concentration of anthocyanin was significantly different, indicating that the regional environment would have a significant impact on anthocyanin. Duan et al. (7) studied the effects of *Lycium ruthenicum Murr.* on radiation damage of peripheral blood system in mice. Taking *Lycium ruthenicum Murr.* as a variable, mice were divided into different groups. Then the DNA, Caspase-3, Caspase-6 and P53 of mice were compared. The results showed that *Lycium ruthenicum Murr.* could significantly increase hemoglobin and DNA of mice and reduce the serum content of Caspase-3, Caspase-6 and P53, which showed that it could protect radiation damage. Peng et al. (8) analyzed the effect of lycium barbarum polysaccharide and found that LRGP3 could reduce cell inflammation through inhibiting TLR4/NF-ϰB signal. Gong et al. (9) studied the immune regulation function of *Lycium ruthenicum Murr.* by injecting low, medium and high doses of *Lycium ruthenicum Murr.* into

mice and found that *Lycium ruthenicum Murr.* could promote the recovery of spleen and thymus indicators, enhance the phagocytic function of macrophages, and thus play an immunoregulatory role. In this study, the alleviating resistance of *Lycium ruthenicum Murr.* to exercise-induced fatigue was analyzed by swimming experiment in mice. The alleviating effect of *Lycium ruthenicum Murr.* on fatigue in mice was proved by comparing the blood sugar and lactic acid of different mice, which provides some theoretical bases for the further application of *Lycium ruthenicum Murr.* in alleviating fatigue.

Nutritional value of Lycium ruthenicum Murr

Nutritional components

Lycium ruthenicum Murr. contains abundant anthocyanin (10). It is the natural wild plant with the highest anthocyanin content. In *Lycium ruthenicum Murr.,* the content of polysaccharide is between 10% and 17%, and the content of flavone is about 2.71%; therefore it can reduce blood lipid and antioxidant (11). The content of protein is about 11%. The content of fat is about 5%-6%. *Lycium ruthenicum Murr.* contains abundant unsaturated fatty acids and rich minerals including macroelements such as sodium, magnesium and iron and microelements such as manganese, zinc and chromium; therefore it has anti-cancer efficacy.

Nutritional value

(1) Bacteriostasis and anti-inflammation

The ethanol and water extracts of *Lycium ruthenicum Murr.* have inhibitory effects on *Escherichia coli*, *Penicillium niger* and *Aspergillus niger*, and anthocyanin plays the main function. Anthocyanins can significantly inhibit the growth of *Escherichia coli* (12) and promote the abnormal growth of bacterial cells, leading to disintegration of bacteria.

(2) Prevention and treatment of diabetes mellitus

The main epitope of diabetes mellitus is persistent hyperglycemia. Polysaccharides in *Lycium ruthenicum Murr.* can alleviate the symptoms of diabetes mellitus to a certain extent and reduce blood sugar concentration. Flavone in *Lycium ruthenicum Murr.* also play a very good role in regulating blood lipids.

(3) Antioxidation

Aging is caused by free radicals produced by cell metabolism. Polyphenols, polysaccharides, anthocyanins and flavones in *Lycium ruthenicum Murr.* have scavenging effects on free radicals (13); therefore it can relieve aging, resist oxidation, and potentially prevent Alzheimer's disease (14).

(4) Improving immune function

Polysaccharides in *Lycium ruthenicum Murr.* can activate macrophages to promote its immune function.

(5) Anticancer

Anthocyanin can inhibit the growth of cancer cells and induce apoptosis of cancer cells. It can inhibit cancer by regulating the activity of enzymes.

(6) Fatigue resistance

Exercise-induced fatigue refers to a phenomenon that the body can not maintain the established intensity of exercise (15). After a long period of high-intensity exercise, the metabolism of the body will produce a large number of unstable free radicals, destroy human cells and tissues, consume a large amount of glycogen, and disturb blood sugar level; as a result, the body is in a serious hypoxic condition, lactic acid accumulates, muscle tissue is acidic, and body proteins are metabolized in large quantities, leading to the decrease of vitality of the body and exercise-induced fatigue. Exercise-induced fatigue will not only make athletes perform poorly, but also increase the risk of sports injury (16). Anthocyanins and flavones in *Lycium ruthenicum Murr.* can scavenge free radicals. Polysaccharides can increase glycogen reserves, supplement amino acids, stabilize blood sugar levels, and alleviate exercise-induced fatigue. In this study, the fatigue resistance of *Lycium ruthenicum Murr.* was studied in mice.

Relieving resistance of Lycium ruthenicum Murr. to exercise-induced fatigue

Experimental materials

Ninety-six specific pathogen free (SPF) mice with weight between 20 g and 22 g were purchased from the Animal Laboratory Center of Jilin University (license number: SCXK (Ji) 2013-0001). 600 g of *Lycium ruthenicum Murr.* which was purchased from Huirentang in Lanzhou, China was immersed in distilled water for

30 min and decocted twice with slow fire after adding ten times of distilled water, 0.5 h each time. The water decoction obtained was combined and condensed to 500 ml, i.e., concentration of 1.2 g/ml. Moreover liver glycogen, muscle glycogen and lactic acid reagent kits (Nanjing Jiancheng Bioengineering Institute, China), fully automatic biochemical analyzer (Beckmancuolter Company, USA) and centrifuge (Shanghai Precision Instrument Co., Ltd., China) and electronic balance, water box and swimming box from laboratory were also used. The temperature of the laboratory was between 20 °C and 25 °C.

Experimental methods

The ninety-six mice were randomly divided into four groups: low *Lycium ruthenicum Murr.* group (3 mg/g), middle *Lycium ruthenicum Murr.* group (6 mg/g), high *Lycium ruthenicum Murr.* group (12 mg/g) and blank group (0.2 mL/g distilled water). Each group was divided into a pre-exercise group, a post-exercise group and a post-exercise 30 minutes group with 8 mice in each group. The mice were fed for 30 days by means of gavage, once a day, and 20 minutes of swimming training was given every other day. One hour after the last time of feeding, the mice were placed in a swimming box with a diameter of 1 m and a water depth of 30 cm to swim for 40 minutes. Samples were taken immediately in the pre-exercise group, immediately after swimming in the post-exercise group and 30 minutes after swimming in the post-exercise 30 minutes group.

Observation indicators

(1) Blood sugar and lactic acid: 1.5 ml of orbital blood was taken and transferred to a 2 mL EP tube, and the serum was precipitated one night at 4 °C. Then the blood sugar and lactic acid were measured by the fully automatic biochemical analyzer.

(2) Liver glycogen and muscle glycogen: Mice were killed, and the liver and quadriceps femoris muscle were taken. The liver and quadriceps femoris muscle were washed and dried, and 1 g of tissue was cut into pieces and put into a tube which was loaded with 1.5 ml of 30 % KOH. After 15 min of boiling water bath, it was cooled and diluted to 100 ml. Then anthranone color developing agent was added, and colorimetric assay was performed using a 721 spectrophotometer at the wavelength of 620 nm. The content of hepatic glycogen and muscle glycogen was calculated.

Statistical Analysis

The collected data were analyzed by SPSS17.0 software. When $P < 0.05$, it means that there was a statistically significant difference.

Experimental results

Effect of Lycium ruthenicum Murr. on blood sugar in mice

Figure 1 is a comparison of blood sugar values among groups. It can be seen from Figure 1 that before the experiment, there was no significant difference in the blood sugar of mice. After exercise, the blood sugar of each group decreased, and then recovered slightly 30 minutes after exercise, but there were significant differences among the groups. Firstly, after exercise, the blood sugar of the blank group was 6.34 ± 2.07 mml/L, which was significantly lower than that before exercise. Compared with the blank group, the blood sugar of the low *Lycium ruthenicum Murr.* group was $P > 0.05$, but there were significant differences between the middle *Lycium ruthenicum Murr.* group and the high *Lycium ruthenicum Murr.* group ($P < 0.05$). There were also significant differences between the high *Lycium ruthenicum Murr.* group and the middle *Lycium ruthenicum Murr.* group. From the data of 30

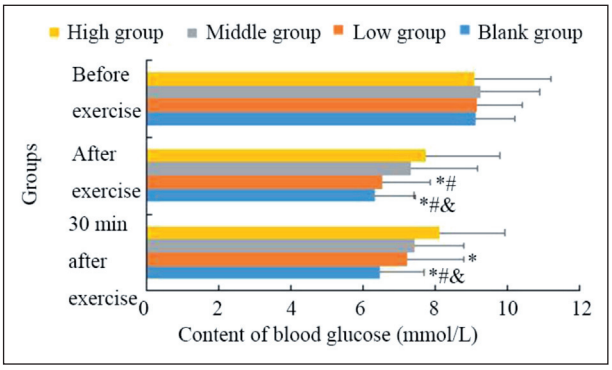

Figure 1. Effects of *Lycium ruthenicum Murr.* on blood sugar in mice
* indicated $P < 0.05$, compared with the blank group
# indicated $P < 0.05$, compared with the low *Lycium ruthenicum Murr.* group
& indicated $P < 0.05$, compared with the middle *Lycium ruthenicum Murr.* group

minutes after exercise, there was significant difference between the *Lycium ruthenicum Murr.* group and the blank group in blood sugar values. The blood sugar rebound value of the *Lycium ruthenicum Murr.* groups was higher than that of the blank group. The blood sugar value of the high *Lycium ruthenicum Murr.* group was the highest, which was 8.12 ±1.21 mmol/L. Compared with the other two groups, P < 0.05, indicating that the intake of *Lycium ruthenicum Murr.* could promote the recovery of blood sugar after fatigue in mice.

Effect of Lycium ruthenicum Murr. on blood lactic acid in mice

The blood lactic acid content of mice before exercise was about 4 mmol/L, which increased significantly after exercise. The blood lactic acid value of the blank group reached 11.36 ± 2.07 mmol/L, which was significantly higher than that of the mice which took *Lycium ruthenicum Murr.*; the higher the content of *Lycium ruthenicum Murr.*, the lower the value of the blood lactic acid. The blood lactic acid value of the high *Lycium ruthenicum Murr.* group was the lowest, 8.27 ±1.33 mmol/L, which was significantly different with the low and middle *Lycium ruthenicum Murr.* groups. 30 minutes after exercise, the blood lactic acid content of the *Lycium ruthenicum Murr.* groups was also significantly lower than that of the blank group, and the blood lactic acid content of the high *Lycium ruthenicum Murr.* group was 7.13 ± 1.21 mmol/L, which was significantly different with that of the other three groups (P < 0.05). It indicated that the supplementation of *Lycium ruthenicum Murr.* could significantly reduce the blood lactic acid content, thus alleviating fatigue.

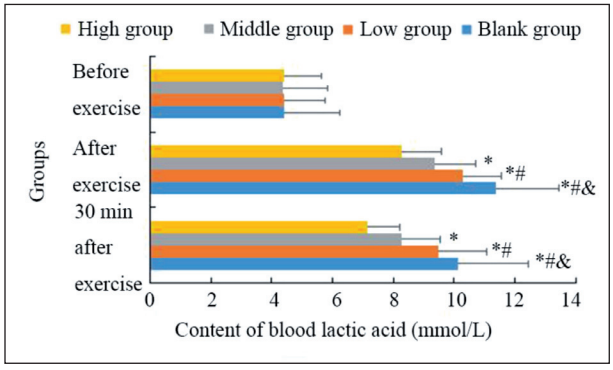

Figure 2. Effects of *Lycium ruthenicum Murr.* on blood lactic acid in mice
* indicates P < 0.05, compared with the blank group,
# indicates P < 0.05, compared with the low *Lycium ruthenicum Murr.* group
& indicates P < 0.05, compared with the middle *Lycium ruthenicum Murr.* group

Effects of Lycium ruthenicum Murr. on hepatic glycogen and muscle glycogen in mice

It was found from Table 1 that the content of liver glycogen and muscle glycogen in mice decreased due to exercise-induced fatigue, but gradually recovered 30 minutes after exercise. Firstly, in the comparison before exercise, the content of two glycogens in mice which took *Lycium ruthenicum Murr.* were higher than that in the blank group; the content of hepatic glycogen in the blank group before exercise were 4.42 ± 0.51 mmol/L, while that in the high *Lycium ruthenicum Murr.* group was 6.48 ± 0.41 mmol/L. After exercise, the content of hepatic glycogen in the blank group was 4.12 ± 0.27 mmol/L, while that in the *Lycium ruthenicum Murr.* groups were 3.95 ± 0.31, 4.22 ± 0.29 and 4.89 ± 0.32 mmol/L, respectively, which were significantly higher than that in the blank group after exercise (P < 0.05).

Table 1. Effects of *Lycium ruthenicum Murr.* on hepatic and muscular glycogen in mice (mmol/L)

		Blank group	Low *Lycium ruthenicum Murr.* group	Middle *Lycium ruthenicum Murr.* group	High *Lycium ruthenicum Murr.* group
Glycogen	Before exercise	4.42± 0.51	4.81±0.44	5.42±0.48*	6.48±0.41*#
	After exercise	4.12± 0.27	3.95±0.31	4.22±0.29*#	4.89±0.32*#&
	30 minutes after exercise	4.27± 0.12	4.01±0.13	4.54±0.11*	5.31±0.14*#&
Muscle glycogen	Before exercise	1.32± 0.08	1.41±0.07	1.83±0.09*#	2.34± 0.11*#&
	After exercise	1.24± 0.34	1.28±0.36	1.41±0.32*#	1.81±0.38*#&
	30 minutes after exercise	1.31± 0.21	1.35±0.23	1.68±0.22*#	2.21±0.21*#&

* indicates P < 0.05 compared with the blank group; # indicates P < 0.05 compared with the low *Lycium ruthenicum Murr.* group; & indicates P < 0.05 compared with the middle *Lycium ruthenicum Murr.* group

Similar results were found in the values 30 min after exercise, and the comparison of results of muscle glycogen was similar to that of liver glycogen. These findings suggested that the intake of *Lycium ruthenicum Murr.* could increase the content of liver glycogen and muscle glycogen in mice, thus achieving the function of anti-fatigue.

Discussion

Resistance of Lycium ruthenicum Murr. to exercise-induced fatigue

Lycium ruthenicum Murr. increased glycogen energy in mice *in vivo*, thus alleviating fatigue after swimming for 30 minutes. According to the mechanism of exercise-induced fatigue, *Lycium ruthenicum Murr.* increased the energy material reserve in mice, reduced the accumulation of blood lactic acid, and promoted the rise of blood sugar, so that the body can continue to exercise after vigorous exercise. It was found from the results that the more the intake of *Lycium ruthenicum Murr.*, the more obvious the alleviating resistance to exercise-induced fatigue. Taking 30 minutes after exercise as an example, the blood sugar content of the blank group was 6.47 ± 1.83 mmol/L), while that of the high *Lycium ruthenicum Murr.* group was 8.12 ± 1.21 mmol/L, which was significantly higher than that of the blank group, indicating that *Lycium ruthenicum Murr.* promoted the recovery of blood sugar in mice, thus helping mice recover from exercise-induced fatigue more quickly; the blood lactate content of the blank group was 10.12 ± 1.83 mmol/L 30 minutes after exercise, and that of the high *Lycium ruthenicum Murr.* group was 7.13 ± 1.21 mmol/L. *Lycium ruthenicum Murr.* can effectively reduce the production of blood lactic acid. Lactic acid accumulation may be one of the causes of exercise-induced fatigue, and the inhibition of *Lycium ruthenicum Murr.* on lactic acid alleviated exercise-induced fatigue in mice. The glycogen reserve in *Lycium ruthenicum Murr.* groups was always significantly higher than that in the blank group, indicating that *Lycium ruthenicum Murr.* could increase the glycogen content in mice to alleviate exercise-induced fatigue.

According to the experimental results, *Lycium ruthenicum Murr.* can increase glycogen reserve, promote blood sugar recovery and reduce lactic acid accumulation, thus achieving the alleviation of fatigue. Exercise-induced fatigue is similar to exercise-induced fatigue of mice. It can be inferred that *Lycium ruthenicum Murr.* as an anti-fatigue food has certain feasibility and it can improve the energy content of human body, stabilize blood sugar, reduce lactic acid, regulate metabolic substance, and increase glycogen reserve, so as to relieve fatigue of body.

Economic value analysis of Lycium ruthenicum Murr.

Lycium ruthenicum Murr. can be eaten raw, cooked or medicated. It has significant effect in reducing blood lipid and blood sugar. It can develop hypoglycemic products suitable for diabetic patients and obese people. It can be used for developing health food as it can regulate immune function and resist fatigue (17). Anthocyanin in *Lycium ruthenicum Murr.* can be used as natural colorant (18). It can also reduce the morbidity and mortality of cardiovascular and cerebrovascular diseases (19), protect the liver, regulate cholesterol, and inhibit lipid accumulation (20). In addition, *Lycium ruthenicum Murr.* as fodder has a high feeding value for animals such as camels and goats and plays a role in in health care for livestock.

The role of *Lycium ruthenicum Murr.* in food and medicine suggests its great economic value. But at present, the yield of wild *Lycium ruthenicum Murr.* is low, the price is high, and the market penetration rate is not high. With the continuous excavation of value of *Lycium ruthenicum Murr.*, people's demand for *Lycium ruthenicum Murr.* has begun to increase. We can reasonably develop *Lycium ruthenicum Murr.* production areas, coordinate planting and processing, and develop ecological industries, which can not only protect the ecological functions of the western region, but also achieve sustainable economic development.

The alleviation of exercise-induced fatigue has attracted more and more attention. This study has proved that *Lycium ruthenicum Murr.* can alleviate exercise-induced fatigue in mice and achieved some results. In the future work, the application of *Lycium ruthenicum Murr.* as food and medicine will be further studied, and moreover the alleviation resistance of *Lycium ruthenicum Murr.* to human exercise-induced fatigue will be analyzed.

Conclusion

Lycium ruthenicum Murr., a kind of precious Chinese medicinal material, has functions such as resisting aging and fatigue, reducing blood lipid and lowering blood sugar. In this study, the alleviating resistance of *Lycium ruthenicum Murr.* to exercise-induced fatigue in mice was analyzed. It was found that *Lycium ruthenicum Murr.* could effectively promote the recovery of blood sugar, reduce the production of lactic acid, and increase the glycogen reserve in mice, so as to alleviate the fatigue of mice caused by intense exercise.

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Effect of *Lepidium meyenii Walp.* on the immune function of boxers

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Summary. *Objective:* To explore the effect of *Lepidium meyenii Walp.* on immune function of boxers. *Methods:* Thirty athletes who received boxing training in Pukyong National University were randomly divided into three groups and then divided into three intervention stages to ensure that each group underwent different doses of *Lepidium meyenii Walp*. The levels of red blood cells, white blood cells and immunoglobulin (Ig) in the blood of the athletes were detected before and after each intervention stage. *Results:* After taking different doses of *Lepidium meyenii Walp.*, there was no significant change in the level of red blood cells ($P > 0.05$); the level of white blood cells in the low and high dose groups increased significantly ($P < 0.05$; $P < 0.01$); the levels of IgA, IgM and IgG in the high and low dose groups increased significantly ($P < 0.05$), but the difference of increase amplitude was not large. *Conclusion: Lepidium meyenii Walp.* can improve the immune function by raising the level of white blood cells and Ig in athletes.

Key words: *Lepidium meyenii Walp.*, immune function, white blood cells, immunoglobulin

1. Introduction

Boxing is a sport of unarmed fighting with gloves. Its victory condition is to get more points or knock down opponents while avoiding attack as much as possible in the course of attack and defense. Boxers need to fight with their bare hands on a square platform. This process is a mental, physical and technical confrontation. In order to gain advantages in the process of confrontation, boxers need a lot of training, including pace and various punching skills. Therefore, for boxers, a large amount of daily exercise is essential. In order to maintain the healthy development of the body, besides a balanced diet, it is essential to improve their anti-fatigue ability and recovery ability (1). Especially when the boxing match enters the see-saw fight, the endurance and resilience of the athletes become the key to victory or defeat. *Lepidium meyenii Walp.* has anti-fatigue and endocrine regulation functions, so that it can assist boxers in daily exercise (2). In addition, when boxers exercise or compete, they will inevitably have wounds, and skin, especially, will have varying degrees of damage. Once the wounds are improperly handled, bacteria or viruses will invade the body, causing varying degrees of illness, as well as internal injuries. Diseases caused by bacterial viruses will greatly affect the daily performance of athletes, so how to improve the immune function of boxers has become an important part of the sports field (3). In addition to the above functions, *Lepidium meyenii Walp.* can also improve the immunity of the human body. Therefore, *Lepidium meyenii Walp.*-related products have been applied in the field of sports. Li et al. (4) detected the molecular structure of *Lepidium meyenii Walp.* using fiber chromatography and found that it had reductive ability through antioxidant experiments and that it could promote the proliferation and phagocytosis of immune cells. Lee et al. (5) fed Andean trout with

different doses of *Lepidium meyenii Walp.*. The results showed that the trout groups which were fed with *Lepidium meyenii Walp.* not only grew fast, but also had a high survival rate; *Lepidium meyenii Walp.* increased the number of white blood cells in trout, so as to improve the survival rate of trout and larvae. Through virtual screening of estrogen receptors and verification of osteoblast pharmacological activity, Hao et al. (6) revealed that *Lepidium meyenii Walp.*'s effective anti-osteoporosis component, N-benzyl-palmitamide, could promote osteoblast proliferation, differentiation and mineralization and promote bone formation by enhancing the expression of osteogenesis-related genes. This paper briefly introduced the origin, shape and nutrient composition of *Lepidium meyenii Walp.*, and then took 30 athletes who were trained for boxing in Pukyong National University as an example.

2. *Lepidium meyenii Walp.*

Lepidium meyenii Walp. (7), a herb growing at high altitudes in the Andes, is divided into annual and biennial varieties, but there are no significant differences in functional pharmacodynamics and cell chromosomes. *Lepidium meyenii Walp.* is regarded as food locally, but also as a natural herb. *Lepidium meyenii Walp.* belongs to Lepidium (8) in the perspective of botanical classification. It is like white radish in overall appearance. The main edible and medicinal parts of *Lepidium meyenii Walp.* are the tuberous rhizomes buried in the soil. The leaves exposed to the ground during growth are basically linear drill-shaped. The edges of the leaves are serrated and can grow up to 23 cm. They droop to the ground in the process of growth. The underground tuber parts are usually 10-14 cm long and the maximum diameter can be about 3-5 cm. The natural rhizome has two colors, yellow and purple, and the longest root fibrous is up to 15 cm at the bottom. *Lepidium meyenii Walp.* with purple rhizome contains much iodine; the darker the color, the more the content. It tastes sweet and bitter. In the growth process of *Lepidium meyenii Walp.*, the tuberous rhizomes can be obtained only 7 to 9 months after sowing, and after flowering for about one month, the fruit can be obtained. *Lepidium meyenii Walp.*'s growth conditions are very strict. In addition to planting in high altitude areas, it is necessary to ensure that the temperature difference between day and night exceeds 30°C, and there must be sufficient water in the growing area (9). Because of the harsh growth conditions, the cultivation of *Lepidium meyenii Walp.* is difficult to expand and the yield is difficult to increase. Another key reason is the predatory absorption of soil fertility. After harvest, it is necessary to make the cultivated land rest for more than seven years. Therefore, the yield of *Lepidium meyenii Walp.* can not be improved in any case.

Lepidium meyenii Walp. is rich in nutrients. 100 g of dried *Lepidium meyenii Walp.* contains 10.2 g of protein, 2.2 g of fat, 8.5 g of dietary fiber and 59 g of carbohydrate. Compared with common tuber crops such as sweet potato and radish, it has higher protein and dietary fiber (10). Alkaloids that regulate human endocrine system can also be extracted from *Lepidium meyenii Walp.*, which is the secondary metabolite of *Lepidium meyenii Walp.*

As *Lepidium meyenii Walp.* is rich in nutrients and secondary metabolites such as alkaloids, it also has a considerable degree of health care functions besides being a food (11), including improving fertility, regulating endocrine, improving anti-fatigue ability and antioxidant capacity, and strengthening immune function. Moreover, *Lepidium meyenii Walp.*, as the main food of local people for many years, is not found to be toxic to human beings. Various toxicity studies have proved that *Lepidium meyenii Walp.* is a non-toxic crop. Although *Lepidium meyenii Walp.* has been proved to be a non-toxic crop, due to its fertility and endocrine regulation function, excessive consumption may cause physical damage, so it is necessary to regulate the dosage.

3. Example analysis

3.1 Research subjects

As shown in Table 1, 30 athletes who received boxing training in Pukyong National University were selected. All of them have been trained for more than three years. Their average age was about 20 years old, their average height was about 175 cm, and their average weight was about 70 kg. They had been informed. They have no family history and have not been injured

Table 1. Basic information of research subjects

Various indicators	Group 1	Group 2	Group 3
Number/n	10	10	10
Average age/year	20.1±0.1	20.2±0.1	20.0±0.2
Boxing training time/year	3.5±0.2	3.6±0.1	3.4±0.3
Height/cm	175.1±2.3	175.2±1.2	175.3±0.3
Weight/kg	70.2±0.3	71.1±0.2	70.1±0.1
Family history	None	None	None
Being injured in recent 3 months?	None	None	None

in the past three months. Among them, no family history was required (12) to prevent some genetic-related immune diseases from affecting the experiment, and no injury in the last three months was required to prevent the immune system from working after injury, resulting in immune indicators beyond the normal range. Thirty athletes were randomly divided into three groups and numbered **1**, **2** and **3**.

3.2 Main instruments and reagents

Main instruments included automatic biochemical analyzer, automatic blood cell analyzer, high-speed centrifuge, refrigerator, disposable transfusion tube, aseptic test tube, and pipette.

Main reagents included sterile water, saline, anticoagulant, biochemical analyzer kit reagent, and blood cell analyzer kit reagent.

The preparation of *Lepidium meyenii Walp.* tonic: *Lepidium meyenii Walp.* dried tablets produced from Lijiang, Yunnan province, were processed into fine powder and made into capsules in a size of 250 mg each capsule. Moreover, the capsules which was made of edible starch and had the same specification were used as placebo. There was no difference in appearance between the two tonics. All the above preparation works were carried out in sterile environment.

3.3 Experimental process

As shown in Table 2, the experiment was divided into three stages of tonic intervention to ensure that each group of athletes were given three different doses of tonic intervention. Four *Lepidium meyenii Walp.* capsules were given to the high dose group, two *Lepidium meyenii Walp.* capsules was given to the low dose group, and the placebo group was given four starch capsules. During the whole experiment, the athletes were not told what kind of capsules they were taking, and the prescribed dosage was not allowed to talked between groups.

Table 2. The experimental scheme of supplement intervention stage

	The first stage	The second stage	The third stage
Group 1	High dose	Placebo	Low dose
Group 2	Low dose	High dose	Placebo
Group 3	Placebo	Low dose	High dose

Firstly, each stage lasted for three weeks, during which each group took daily supplements as shown in Table 2, and there was an interval of 2 weeks between every two stages to eliminate the effects of supplements on the body. During the whole experimental process (including three intervention stages and two elution stages), all three groups of boxers had the same daily training and eating. Daily training included: **1** jogging for 2 km in the morning and having breakfast after 30 minutes' rest; **2** doing warm-up activities for 15 minutes, including rope skipping and sandbags beating, after resting for one hour; **3** group antagonistic boxing training after warming-up; **4** resting until lunch time after group boxing training; **5** free activity time for 2 h after lunch; **6** warming up for 15 minutes after the free exercise and doing boxing exercises and confrontation

training; **7** the time after dinner and before 22:00 was free time, and the time after 22:00 was sleep time.

3.4 Index test

At the beginning and end of each stage, the indicators were detected, including blood physiological indicators (red blood cell and white blood cell count) and specific immune detection indicators (immunoglobulin (Ig) A, IgM and IgG) (13).

The procedure of each test is the same. First, the athletes kept fasting for 12 hours the night before, and then 10 mL of venous blood was collected with a disposable transfusion tube in the morning at the fasting state, 5 mL for the detection of blood physiological indicators and 5 mL for the detection of specific immune indicators.

Detection of blood physiological indicators: 15 μL of venous blood was transferred into a sampling bottle with a pipette. The red blood cell and white blood cell counts were detected by putting the sampling bottle into the automatic blood cell analyzer. The blood sample of each person was detected three times, and the average value was taken.

Specific immunoassay indicators: Samples containing venous blood were centrifuged with a high-speed centrifuge at a centrifugal speed of 3500 r/s, for 15 minutes, to obtain the separated serum; the separated serum was loaded into a sterile test tube and stored in refrigerator at -20°C; through automatic biochemical analyzer and associated reagents, the content of IgM, IgG and IgA in the serum was detected using immune turbidimetry (14). The detection of each subject repeated three times, and the average value was taken as the final result.

3.5 Statistical analysis

The collected data were input into EXCEL and analyzed by SPSS software (15). The calculation results are expressed as $\bar{x} \pm s$ and processed by t test. Difference was thought having statistical significance if the value of P was smaller than 0.05.

3.6 Experimental results

As shown in Table 3, the number of red blood cells in the normal human blood was 3.5~*5.5*x10^{12}/L, the number of white blood cells was 4.0~*10.10*x10^{9}/L, and the red blood count and white blood count of the boxers before and after taking different doses of *Lepidium meyenii Walp.* were within the normal range. Different doses of *Lepidium meyenii Walp.* had no effect on the red blood count in the athletes' blood. Although there was slight change before and after taking *Lepidium meyenii Walp.*, there was no significant difference, and the value of P was larger than 0.05. There was no significant change in the white blood count in athletes' blood before and after taking placebo, and the value of P was higher than 0.05. The white blood count in the blood increased significantly before and after taking low dose of *Lepidium meyenii Walp.*, and the value of P was smaller than 0.05. The white blood count in the blood before and after taking high dose of *Lepidium meyenii Walp.* significantly increased, and the value of P was smaller than 0.01. As shown in Figure 1, the white blood cells in the blood of athletes after taking *Lepidium meyenii Walp.* increased with the dosage, but did not exceed the normal range.

As shown in Table 4, the serum IgA content in the normal subjects ranged from 0.76 to 3.90 mg/mL, the IgM content ranged from 0.40 to 3.45 mg/mL,

Table 3. Changes of red blood cells and white blood cells before and after taking different doses of *Lepidium meyenii Walp*

		High dose	**Low dose**	**Placebo**
Erythrocyte count (10^{12}/L)	Before taking	4.95±0.28	4.93±0.31	4.95±0.25
	After taking	4.93±0.29	4.92±0.29	4.94±0.26
Leukocyte count (10^{9}/L)	Before taking	5.33±0.75	5.32±0.74	5.34±0.78
	After taking	5.67±0.66**	5.53±0.67*	5.38±0.76

*Note: * indicated that there was a significant difference compared to before taking, P < 0.05; ** indicated that there was a significant difference compared to before taking, P < 0.01.*

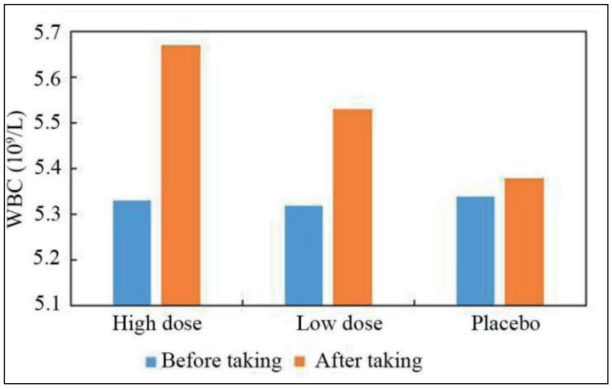

Figure 1. Changes of number of white blood cells before and after taking different doses of *Lepidium meyenii Walp*

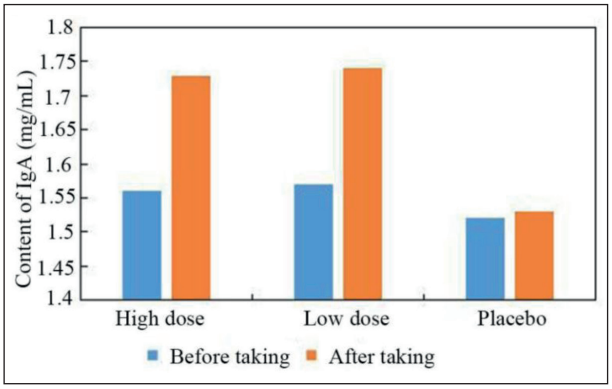

Figure 2. Changes of serum IgA level before and after taking different doses of *Lepidium meyenii Walp*

and the IgG content ranged from 6.00 to 16.00 mg/mL; the serum IgG content of athletes in three groups before and after taking different doses of *Lepidium meyenii Walp.* was within the normal range; the serum IgG content was the highest, followed by the content of IgA and IgM. The three kinds of Ig increased significantly after taking low and high doses of *Lepidium meyenii Walp.*, and the value of P was smaller than 0.05; the three kinds of Ig had no significant changes after taking placebo, and the value of P was larger than 0.05.

The change of the serum IgA content before and after taking different doses of *Lepidium meyenii Walp.* is shown in Figure 2. The specific values are shown in Table 4. It was found from Figure 2 that the serum IgA content of athletes after taking *Lepidium meyenii Walp.* did not change significantly with the increase of dosage, and the final value does not exceed the normal range of IgA.

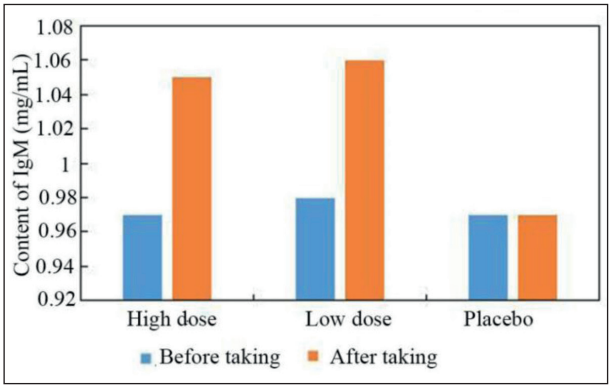

Figure 3. Changes of serum IgM level before and after taking different doses of *Lepidium meyenii Walp*

The change of serum IgM content before and after taking different doses of *Lepidium meyenii Walp.* is shown in Figure 3. The specific values are shown in Table 4. It was found from Figure 3 that the serum

Table 4. Changes of serum Ig levels before and after taking different doses of *Lepidium meyenii Walp*

		High dose	**Low dose**	**Placebo**
IgA (mg/mL)	Before taking	1.56±0.53	1.57±0.56	1.52±0.51
	After taking	1.730.58*	1.74±0.59*	1.53±0.52
IgM (mg/mL)	Before taking	0.97±0.38	0.98±0.38	0.97±0.46
	After taking	1.05±0.41*	1.06±0.43*	0.97±0.39
IgG (mg/mL)	Before taking	9.25±1.65	9.26±1.59	9.23±1.98
	After taking	10.06±2.09*	10.02±1.55*	9.25±1.91

** indicated that there was a significant difference compared to before taking, P < 0.05.*

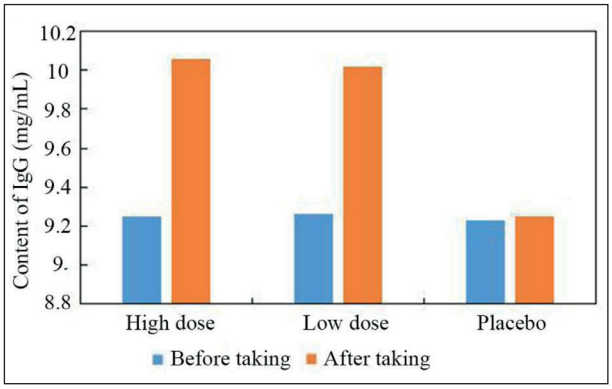

Figure 4. Changes of serum IgG level before and after taking different doses of *Lepidium meyenii Walp.*

IgM content after taking *Lepidium meyenii Walp.* did not change significantly with the increase of dosage, and the final value did not exceed the normal range of IgM.

The change of serum IgG content before and after taking different doses of *Lepidium meyenii Walp.* is shown in Figure 4. The specific value is shown in Table 4. It was found from Figure 4 that the increase of serum IgG content of athletes after taking *Lepidium meyenii Walp.* did not change significantly with the increase of dosage, and the final value did not exceed the normal range of IgG.

4. Discussion and analysis

Boxers need to consume a lot of energy in competition or training, at which time the oxygen demand is greatly increased. As hemoglobin in red blood cells can combine with oxygen and carbon dioxide to achieve intracellular and extracellular material exchange and promote energy conversion, the content of red blood cells in the body can affect the endurance of athletes: more red blood cells can transport more oxygen, promote cell respiration, and produce more energy, and more oxygen can also inhibit cellular anaerobic breathing, reduce the production of lactic acid in muscle, and improve exercise endurance. The results of red blood cell test of boxers showed that the change was not obvious after the athletes took different doses of *Lepidium meyenii Walp.*, and moreover its content was within the normal range. Therefore, it was speculated that the fluctuation of blood cell content was caused by training and normal metabolism, and *Lepidium meyenii Walp.* had little effect on blood cells.

The number of white blood cells will reduce after boxers experience a large number of high-intensity exercises, and sometimes leukopenia may occur. The former can recover to the normal level after conditioning, while the latter may develop into a long-term low immunity, which will not only affect the daily life of boxers, but also force them to end their boxing career earlier. After all, boxing is a fierce antagonistic sport, and injury is common in competition. White blood cells is a part of non-specific immunity in human immune system. It is differentiated from hematopoietic stem cells in bone marrow. It is generally divided into granulocyte, monocyte and lymphocyte by morphological function. The results of this study showed that the number of white blood cells increased after taking different doses of *Lepidium meyenii Walp.*, and the larger the dosage, the greater the increase, but not beyond the normal range. The reason is that *Lepidium meyenii Walp.* can promote the production of testosterone, and testosterone can promote the proliferation and differentiation of hematopoietic stem cells. In addition, secondary metabolites in *Lepidium meyenii Walp.* can also promote the proliferation and differentiation of white blood cells, neutralize the hypochlorous acid in white blood cells, and prolong the life of white blood cells.

Ig in serum is a component of specific immunity in human immune system. Its principle is to combine with pathogens *in vivo* to label pathogens and activate other immune cells. Ig is classified into five types: A, M, G, E and D. The content of IgE is too low, and the function of IgD is not clear. Therefore, only three Igs, i.e., IgA, IgM and IgG, were in this study. The results showed that the content of IgG was the highest, followed by the content of IgA and IgM. Three kinds of Ig increased after the athletes took different doses of *Lepidium meyenii Walp.*, but the dose had no significant influence on the increased content of Ig.

In conclusion, *Lepidium meyenii Walp.* can improve the content of non-specific immune white cells and specific immune globulin, thereby enhancing the immune function of athletes.

5. Conclusion

This paper briefly introduced the origin, shape and nutrient composition of *Lepidium meyenii Walp.* and then took 30 athletes who received boxing training in Pukyong National University as an example. The athletes were randomly divided into three groups and then divided into three stages of supplement intervention to ensure that each group receive different doses of *Lepidium meyenii Walp.* intervention. Finally, the content of red blood cells, white blood cells and Ig in athletes' blood were detected before and after each intervention stage. The results are as follows. *Lepidium meyenii Walp.* had no effect on the content of red blood cells in the blood of boxers, but it can effectively improve the level of white blood cells; the larger the dosage of *Lepidium meyenii Walp.*, the better the effect. *Lepidium meyenii Walp.* could effectively raise the level of IgA, IgM and IgG in athletes' serum, but the effect had no significant relationship with the dosage of *Lepidium meyenii Walp*. In conclusion, *Lepidium meyenii Walp.* can effectively improve the immune function of boxers by improving the level of immune cells and immune substances.

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Effects of Mediterranean diet and weight loss on blood-lipid profile in overweight adults with hypercholesterolemia

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Summary. Blood cholesterol has been positively associated with increased cardiovascular risk as a modifiable risk factors together with the lifestyle and diet. Furthermore, an improvement of the blood-lipid profile seems to be able to produce a decrease in cardiovascular events. Cholesterol plasma levels are related to the body mass index (BMI) and are affected by diet. The aim of this study was to evaluate the effectiveness of a Mediterranean diet (MD) weight-loss programme to improve blood cholesterol profiles in overweight adults subjected to real-world outpatient diet. Forty-nine hypercholesteraemic, overweight adults of both sexes were subjected to a dietary weight-loss intervention. Patients were prescribed a slightly hypocaloric MD for 16 weeks, followed by an 8-week follow-up period with a normocaloric diet. Data showed significant weight loss and cholesterol blood profile improvement both under the hypocaloric diet and during the follow-up period. In particular, the decrease in both Total and LDL-cholesterol was greater than their critical differences indicating the clinical relevance of blood lipid improvement induced by MD.

Keywords: Cholesterol; Critical Difference; Mediterranean Adequacy Index; Nutritional Counselling; Triglycerides; Lifestyle.

Introduction

The blood-lipid profile has been positively associated with an increase in cardiovascular risk (CVR) by a large number of epidemiological studies. In particular, the reduction of total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) plasma levels induces a decrease in the incidence of cardiovascular events (1,2). By contrast, high-density lipoprotein cholesterol (HDL-C) is inversely related to CVR (3). An increase in TC -particularly in the LDL-C concentration - is a marker of atherogenic CVR, whereas a decrease in HDL-C concentration is correlated with various risk factors, including the development of metabolic syndrome (i.e., MetS) (4,5). Starting from this assumption, the TC/HDL-C ratio (well-known as "*Castelli atherogenic index*") seems to be a strong predictor of coronary heart disease and has got a high discriminatory capacity. For primary prevention, the TC/HDL-C ratio risk-thresholds have been set at 5 for males and 4.5 for females, with desirable targets of < 4.5 and < 4, respectively (5-7). European guidelines (2) focus on the reduction of LDL-C and TC blood concentrations as the main target in primary cardiovascular disease prevention. Furthermore, it has been suggested that pharmacological interventions (i.e., Statins) should be administered when the LDL-C concentration is greater than 190 mg/dL to achieve a desirable outcome of 155 mg/dL in patients with low CVR. Additionally, in patients with moderate CVR, the goals are to achieve a treatment threshold of 155 mg/dL with a desirable level of less than 120 mg/dL. In subjects with low-to-moderate CVR, recommendations include maintaining a healthy TC blood concentration less than 200

mg/dL, with borderline values considered between 200 mg/dL and 240 mg/dL.

In each person, cholesterol plasma levels are due to a balance between absorption after food-intake and endogenous synthesis (8,9). Therefore, genetic tendencies are influenced and modulated by metabolic factors, such as BMI (10) and the amount of visceral or liver fat (11). Furthermore, diet composition affects various plasma cholesterol concentrations and ratios (12,13). In particular, on one hand a reduction in the dietary intake of saturated fat acids (SFAs) is strictly related to both improvement of the blood-lipid profile (14,15) and lowering of CVR (16-18). On the other hand, epidemiological and clinical evidence is consistent with finding reporting a reduction of CVR depending on nutrients used to replace SFAs (15,19). Specifically, it has been demonstrated that the replacement of SFAs with unsaturated fatty acids, either monounsaturated fatty acids (MUFAs) or polyunsaturated fatty acids (PUFAs), can reduce CVR (17,20-22). Conversely, SFA-replacement with high-glycaemic-index (GI) refined carbohydrates actually increases CVR (23,24). Nevertheless, studies exploring the effects of nutritional interventions on lipid profiles never compared TC and/or LDL-C reduction with their critical differences (CDs) (12,14, 17, 25-28) CD, also known as the reference change value, is a parameter used to assess laboratory results (29,30). The CD is defined as the smallest difference between sequential laboratory results which is likely to indicate a true change for a given analyte in the patient. Particularly, the difference between two consecutive analyses is significant and clinically relevant if the difference between the two measurements is higher than the CD (30, 31).

Many studies provide direct evidence that a Mediterranean diet (MD), characterized by a high percentage of unsaturated fatty acids, especially MUFAs, and a high proportion of low-GI whole grains, is inversely associated with CVR(32-35) as well as MetS development (36, 37). However, in the Mediterranean region in recent years, a Westernization of dietary habits occurred with a corresponding shift away from the traditional Mediterranean nutritional (38, 39, 40).

Thus, the aim of this study was to assess the efficacy of a weight-loss programme based on the characteristics of an MD under real-world outpatient conditions to improve the blood-lipid profile in overweight patients with moderate to high hypercholesterolemia.

Materials and Methods

Participants

Fifty-five overweight adults with hypercholesterolemia, including 31 males and 24 females aged between 31 and 65 years (49 ± 7.4 years SD), were consecutively recruited from a larger sample of overweight adults subjected to a dietary weight-loss intervention. All participants had a sedentary job and did not engage in sufficient physical activity according to the recommendations of the World Health Organization (i.e., less than 150 minutes of moderate-intensity activity each week). The Ethics Committee for clinical trials of the University of Pisa (CEAVNO) approved the study protocol (nr. #271/2014). All participants provided written informed consent and data were processed anonymously by assigning an identification number to each patient. Six subjects (3 males and 3 females) withdrew before the end of the protocol, leaving 21 females and 28 males to be included in the study. Patients who dropped out of the study underwent pharmacological therapy. Table I shows the baseline characteristics of the participants who completed the protocol.

The inclusion criteria comprised 25 < BMI <30, TC ≥ 250 mg/dL (to convert mg/dL into mmol/L, multiply by 0.0259), and TC/HDL-C >5. Exclusion criteria included triglycerides ≥ 220 mg/dL (to convert mg/dL into mmol/L, multiply by 0.011), currently taking lipid-lowering medications, a personal history of cardiovascular disease, cancer, hypertension (ie., blood pressure ≥ 140/90 mm Hg), and diabetes, renal or liver disease.

Experimental Protocol

A nutritional assessment and medical (i.e., baseline blood profile), anthropometric, and psychological examinations were performed for each patient. Height was measured without shoes to the nearest centimetre at baseline using a wall-mounted stadiometer. Body weight was measured without clothes to the nearest 0.5 kg. During the first visit, the Eating Habits Structured Interview (EHSI) was administered, too. The EHSI is

Table 1. Characteristics of Male and Female groups at baseline. For normally distributed data, the Mean and Standard Deviation are reported; the Median, 1st and 3rd quartiles are shown in non-Gaussian distributions. Significance of the baseline differences between the sexes was calculated by * ANOVA Mixed design and Post Hoc test, ° Mann-Whitney U and + Unpaired Student t-test.

		Females	Males	P between sexes
Normality	**Subjects N°**	21	28	
Yes	Age (years) +	49.5 ± 7.4	48.7 ± 7.5	NS
Yes	Height (cm) +	162.9 ± 5.5	176.5 ± 5.2	< 0.0001
Yes	Weight (kg) +	72.7 ± 5.8	86.2 ± 7.1	< 0.0001
Yes	BMI (kg/m^2) *	27.4 ± 1.5	27.7 ± 1.3	NS
No	MAI °	2.5 (2.1-4.0)	2.6 (2.4-3.1)	NS
Yes	TC (mg/dl) *	278 ± 17.5	279 ± 17.9	NS
Yes	LDL-C (mg/dl) *	192 ± 15.5	194 ± 16.2	NS
No	HDL-C (mg/dl) °	48 (44-50)	42 (40-45)	< 0.001
No	TG (mg/dl) °	213 (199-215)	214 (205-216)	NS
Yes	TC/HDL-C *	5.9 ± 0.61	6.6 ± 0.63	< 0.001

an interview on dietary habits and lifestyles covering 5 sheets (i.e., master data and medical history, lifestyle, physical activity, body perception, and eating habits) with 53 items (41). The eating habits sheet included a report of the diet composition pertaining the previous week. Dietary composition data were processed in order to evaluate the adherence to an MD model using the Mediterranean adequacy index (MAI) (42), as described below.

At baseline and after fasting for 12 hours, venous blood samples were drawn without stasis into evacuated glass tubes. TC and triglyceride (TG) concentrations were measured enzymatically, HDL-C was measured through precipitation (43) and LDL-C was measured using Friedewald's formula. Biological Variability (CVb), Analytical Variability (CVa) and Critical Difference (CD) for TC, LDL-C, HDL-C and TG are reported in Table II, according to the Tuscany quality assessment program for clinical biochemistry. (44, 45).

Table 2. Biological Variability (CVb), Analytical Variability (CVa) and Critical Difference (CD) for Total Cholesterol (TC), LDL-Cholesterol (LDL-C), HDL-Cholesterol (HDL-C) and Triglycerides (TG) according to the Tuscany quality assessment program for clinical chemistry (Barsotti, 1995; La Gioia, 2013).

Analytes	CVb (%)	CVa (%)	CD (%)
TC	6.1	2	17.8
LDL-C	7.6	5	25.2
HDL-C	7.4	5	24.7
TG	8.1	3.5	24.4

Immediately after the first medical assessment, each participant received a personalized dietary weight-loss programme. The hypocaloric eating plan was carried out for 16 weeks, and then it was followed by 8 weeks during which the hypocaloric diet was replaced with a normocaloric one. Nutritional counselling sessions were performed every two weeks in order to evaluate both diet adherence and progression in the alimentary education programme. Weight data were processed at baseline, 8 weeks, 16 weeks and 24 weeks. In the fourth week (during the weight-loss programme) and twentieth week (during the follow-up period), each subject compiled a weekly dietary report, and data were processed to evaluate the MAI. Blood tests control was performed at 8 weeks, 16 weeks and 24 weeks. At the end of the protocol, the participants who did not meet the threshold values for the relative class of risk, underwent pharmacologic care, according to the ESC/EAS guidelines for the management of dyslipidaemia (2).

For each analyte, the differences between the end and the beginning of the protocol were calculated and reported as a percentage of the initial value. This percentage was compared to the relative CD and the number of subjects reporting a reduction in blood levels higher than the CD was reported.

Mediterranean Adequacy Index (MAI)

According to the Fidanza's formula, MAI was computed by dividing the sum of the percentages of

dietary energy (from food groups typical of a reference MD) by the sum of the percentages of dietary energy of food groups, that are not characteristic of the referent MD (42).

$$MAI = \frac{\%.energy\ .from.Mediterranean.foods}{\%.energy\ .from\ .non.Me\ diterranean.\ foods}$$

Foods typical of an MD were cereals and their derivatives, legumes, potatoes, vegetables, fresh and dry fruit, fish, wine, and extra-virgin olive oil. In the group of non-MD foods were milk and dairy products (including cheese), meat, eggs, animal fats and margarines, sweet beverages, cakes, pies, and cookies. The choice of the two food groups was based on the healthy MD proposed by Alberti and colleagues following the results of a dietary survey carried out in a sub-sample of Nicoteran (Southern Italy) families in 1960 (42, 46). For each subject, MAI was computed by calculating the weekly energy intake for each food group and relating it to the total energy intake, followed by an application of the Fidanza's formula.

Clinical Psychological Investigation

All subjects underwent a clinical psychological assessment, during which the Pisa Survey for Eating Disorders (PSED) was completed. The PSED is a self-administrated questionnaire for the determination of eating habits and self image (47) Additionally, all subjects underwent continuous clinical psychophysiological registration (PPR) of specific parameters strictly connected with the Autonomous Nervous System (ANS) arousal, in order to evaluate the balance between sympathetic and parasympathetic balance as previously described (48). The multichannel SATEM ("Modulab® 800") was used to carry out the PPR connected to a computer via an infrared cable, and data were detected and processed by the PANDA Works programme® software (SATEM). The purpose of the evaluation was to identify subjects with potentially full-blown eating disorders described above as a particular pattern of the ANS activity (49).

Diet Protocol

The resting energy expenditure was estimated using the Mifflin's formula(50) and adjusted for physical activity levels. Hypocaloric diet was based on total daily energy expenditure (TDEE) levels, removing approximately 25% of kilocalories (26.9% of the TDEE ± 0.8% SD) to achieve a 10% weight loss in a 16-week period for each participant. Eating plans for each subject were based on the MD model, by maintaining a protein intake of approximately 0.8 g/kg (0.83 ± 0.05 SD), as recommended by the Italian guidelines of the National Institute for Research on Food and Nutrition (INRAN) (51).

The daily macronutrient distribution was 52.3% (± 1.9 SD) carbohydrates, 18.7% (± 1.1 SD) proteins, and 29% (± 1.5 SD) fats with approximately 75% UFAs. Approximately 75-80% of the fats in the diet were of vegetable origin, and an MUFA: PUFA: SFA ratio of approximately 2:1:1 was maintained. The daily vegetable fibre intake was 29.5 g (± 2.05 SD). Nutritional characteristics of the foods used in the eating plans derived from the database of food composition published by the Italian Research Institute for Food and Nutrition (52).

Daily caloric contribution was distributed in five daily meals: breakfast (approximately 20% of the total daily caloric intake), morning snack (approximately 5% of the total daily caloric intake), lunch (approximately 35% of the total daily caloric intake), afternoon snack (approximately 10% of the total daily caloric intake), and dinner (approximately 30% of the total daily caloric intake). The daily diet included two servings of mixed vegetables (i.e., approximately 400 g), three servings of fruit (i.e., approximately 450 g), and one serving of semi-skimmed milk or yogurt (i.e., 125 g for serving). There were additional limitations on beef (i.e., once every ten days), pork (i.e., once per week), cheese (i.e., no more than once per week) and eggs (i.e., twice per week). These proteins were replaced with fish, poultry, rabbit and legumes. Complex carbohydrates were provided by pasta, spelt, brown rice, mixed wholegrain and whole-meal bread, while limiting the use of potatoes, rice and white bread. The GI of each meal was always maintained at less than 55%, as well as the glycaemic load (GL) was always less than 50. GI and GL were calculated as indicated by Wolever et al (53). Butter usage was replaced with 20-35 g/day of extra-virgin olive oil, depending on energy intake (i.e, approximately 15% of the total daily caloric intake).

Moreover, a diet integration of 10–15 g/day of nuts (i.e., approximately 6% of the total daily caloric intake) was provided. A daily consumption of 100 ml red wine was also granted. The same directions were applied in the follow-up diet without caloric restriction.

Statistical Analysis

Correlations at baseline between MAI and values of BMI, TC, LDL-C, HDL-C and TG were performed by non-parametric Spearman rs. Significance of correlation coefficients were tested by Fisher's formula.

The normal distribution of the samples and the homoscedasticity of their variances were evaluated with Shapiro-Wilk and Levene's tests, respectively. In normally distributed data (i.e., BMI, TC, LDL-C and TC/HDL-C), statistical evaluation was performed using a between-within Analysis of Variance in a within-between mixed design (sex - time (0, 8, 16 and 24 weeks)). A post hoc multiple comparison analysis of between data was performed, by the Tukey-Kramer HSD Test. For within data, paired Student t-tests with Bonferroni's correction were used. The effect size for a mixed between-within ANOVA was calculated by using the partial eta squared ($P\eta 2$). In non-parametric data (i.e., MAI, HDL-C and TG), within effects were analysed using Friedman's ANOVA. A post hoc multiple comparison analysis was performed by the Wilcoxon tests (Bonferroni corrected). Differences in gender were evaluated using multiple Mann-Whitney U tests (Bonferroni corrected). The effect size for non-parametric tests was calculated through an application of the non-parametric *r* to post hoc analysis.

To assess variations in the effects over time, we calculated differences between the eighth week and baseline ($\Delta 1$), the sixteenth and eighth weeks ($\Delta 2$), and the twenty-fourth and sixteenth weeks ($\Delta 3$) for all variables that changed during the protocol. For each analyte (i.e., TC, LDL-C, HDL-C and TG), the blood-levels variation (Δ_{Total}) between the end of the protocol (at the twenty-fourth week) and baseline was compared with related CDs. The correlation between the baseline values of TC, LDL-C, and TG and the corresponding Δ_{Total} was evaluated by Pearson ϱ in normally distributed data, and by Spearman rs in non-Gaussian data distributions. Significance of correlation coefficients were tested by Fisher's formula.

Results

Primary analyses

The overall adherence to the protocol was quite good, with a drop-out rate of 10.9% (6 subject out of 55). Adherence to the MD in the week before the start of the dietary programme was low (i.e., MAI Median = 2.61, 1st quartile = 2.32; 3rd quartile 3.48), but a highly significant reverse correlation between MAI and both TC and LDL-C concentrations at baseline was detected (rs = -0.57, $F_{(1,47)} = 22.86$, $P < 0.0001$ and rs = -0.52, $F_{(1,47)} = 17.33$, $P < 0.001$, respectively). No significant correlation between MAI and BMI, HDL-C or TG was found, too.

All the subjects showed a significant change of their eating habits, increasing adhesion to the Mediterranean diet. Indeed, statistical analysis showed a significant MAI increment over time (Friedman $\chi^2 = 74.5$, df = 2, $P < 0.0001$). The MAIs calculated during the weight-loss programme (at the fourth week) were significantly higher than those at baseline (Median = 5.11, 1st quartile = 5.0; 3rd quartile = 5.26; Wilcoxon $T_{(49/49)} = 0$, $p < 0.0001$, r = 0.87). These values were similar to the MAIs calculated in the twentieth week, during the follow-up period (Median = 5.15, 1st quartile = 5.04; 3rd quartile = 5.30; Wilcoxon $T_{(49/49)}=0$, $p < 0.0001$, r = 0.87), and no differences were found between the 4th and 20th weeks (Wilcoxon $T_{(49/49)} = 526$, $p > 0.25$, r = 0.05). We did not find any difference in eating habits between males and females ($p > 0.1$).

Weight loss

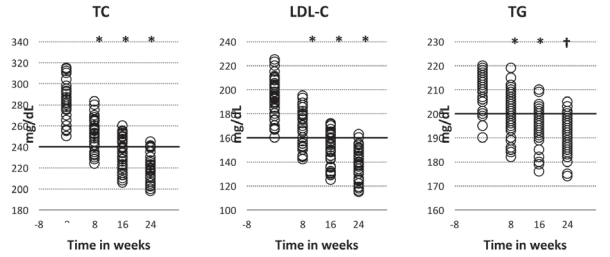
During the dietary weight-loss intervention (baseline-sixteenth week), all the subjects showed a constant reduction in BMI. No sex-related differences were detected in BMI reduction (ANOVA, between subjects $p > 0.5$; $F_{(1,47)} = 0.01$, $P\eta^2 = 0.000$), whereas a high statistical significance was found in diet-treatment (ANOVA, within subjects $p < 0.0001$; $F_{(3,141)} = 1468$; $P\eta^2 = 0.97$). Post hoc analysis showed an awesome decrease in BMI after 8 and 16 weeks of the hypocaloric diet (Bonferroni's paired Student t-test, $p < 0.0001$). However, no differences were observed between the sixteenth and twentieth week after the normocaloric diet period (Bonferroni's paired Student t-test, $p > 0.1$). The mean difference between baseline values and those collected at the sixteenth week was 2.67 ± 0.34 SD, approximately

10% of the baseline BMI (9.6% ± 1.27% SD). Weight loss was gradual, and no differences between D_1 (1.4 ± 0.26) and D_2 (1.3 ± 0.27) were found (Wilcoxon $T_{(49/49)}$ = 310.5, p > 0.5, r = 0.09). No correlation between baseline BMI and the TC, LDL-C, HDL-C, and TG concentrations were found.

Total Cholesterol (TC) and LDL-Cholesterol (LDL-C)

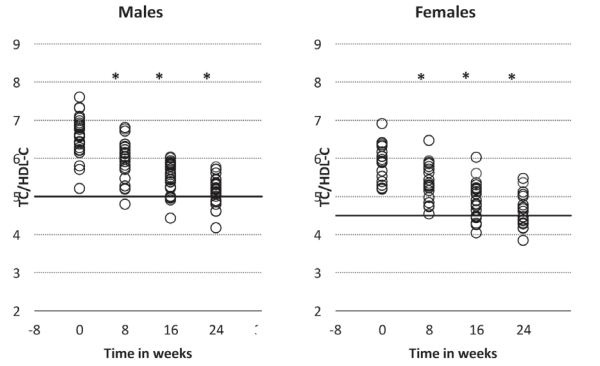
TC and LDL-C showed similar patterns of variation. No differences or interactions in sex were found in blood concentrations. Conversely, all the subjects manifested a consistent and significant reduction of both analytes concentration during the study protocol (Figure 1). Post hoc analysis showed significant differences among all the measurements as well as between the end of the weight-loss programme (sixteenth week) and the end of the normocaloric diet follow-up (twenty-fourth week) (Figure 1). Nevertheless, the decrease in TC and LDL-C slowed down over time. In fact, there were significant differences among Δ_1, Δ_2 and Δ_3, and post hoc analysis detected that $\Delta_3 < \Delta_2 < \Delta_1$ (Figure 2). The decrease during the follow-up period (Δ_3) was approximately 20% of the D_{Total} for both TC and LDL-C (19.2% ± 3.5% SD and 20.2% ± 3.9% SD, respectively). Furthermore, both TC and LDL-C showed a strong correlation between baseline values and D_{Total} (ϱ = -0.75, $F_{(1,47)}$ = 55.77, p < 0.0001 and ϱ = -0.71, $F_{(1,47)}$ = 43,9.77, p < 0.0001, respectively). At the end of the protocol, 94% of the subjects (n = 52) showed a reduction of TC greater than the CD. Similarly, 86% of the subjects (n = 49) showed a reduction of LDL-C higher than the CD (Table III).

Although 92% of the subjects (26 males (93%) and 19 Females (90%)) showed a TC concentration less than 240 mg/dat at the end of the protocol, only



		baseline	Weight loss 8th week	Weight loss 16th week	Weight loss 24th week	Follow up	Within Subjects (Time)
TC (mg/dl)	Males	280 ±18.0 SD	252 ±15.9 SD	234 ±15.0 SD	223 ±14.0 SD		**P <0.0001 ANOVA** $F_{(3,141)}$ = 3049.34 $Pη^2$ = 0.98
	Females	278±18.5 SD	253±16.1 SD	234±14.5 SD	223±12.8 SD		
	Between Subjects (Sex) ANOVA		**P >0.5**; $F_{(1,47)}$ = 0.002, $Pη^2$ = 0.001				**Interaction (Sex x Time) P >0.5** $F_{(3,141)}$ = 0.69 $Pη^2$ = 0.01
LDL-C (mg/dl)	Males	194±16 SD	164±14 SD	141±13 SD	133±12 SD		**P <0.0001 ANOVA** $F_{(3,141)}$ = 1468.06 $Pη^2$ = 0.97
	Females	194±16 SD	165±14 SD	142±14 SD	135±13 SD		
	Between Subjects (Sex) ANOVA		**P >0.5**; $F_{(1,47)}$ = 0.27, $Pη^2$ = 0.006				**Interaction (Sex x Time) P >0.5** $F_{(3,141)}$ = 0.269 $Pη^2$ = 0.006
TG (mg/dl)	Males	213(208-216)	201(197-205)	196(193-199)	195(191-197)		**P <0.0001 Friedman** c^2 = 120 df = 3
	Females	213(210-215)	203(195-207)	195(191-199)	192(185-196)		
	Between Subjects (Sex) Mann W U		**P>0.25**	**P>0.5**	**P>0.25**	**P>0.1**	

Figure 1. Changes in Total Cholesterol (TC), Low-Density Lipoprotein-Cholesterol (LDL-C) and Triglycerides (TG) during the treatment of males and females. Within post hoc comparison significances: * P<0.0001 and † P<0.001. Paired Student t-test with Bonferroni's correction in normally distributed data and Wilcoxon Matched Pairs Test with Bonferroni's correction in non-parametric analyses. In the table, the mean ± standard deviation (SD) is reported for each time point, and the results of the split-plot (between-within mixed design) ANOVA for TC and LDL-C are reported. For TG, the median and 1st and 3rd Quartiles are shown, and non–parametric statistical analyses are reported (Friedman for within data and Mann Whitney U with Bonferroni's correction for multiple between-data comparisons).



	baseline	Weight loss 8th week	Weight loss 16th week	Follow up 24th week	ANOVA Within Subjects (Time)
Males	6.58±0.51 SD	5.98±0.48 SD	5.47±0.38 SD	5.20±0.38 SD	**P <0.0001** $F_{(3,141)}$ = 741.3 $Pη^2$ = 0.94
Females	5.86±0.46 SD	5.35±0.49 SD	4.89±0.48 SD	4.66±0.43 SD	
ANOVA Between Subjects (Sex)		**P <0.0001**; $F_{(1,47)}$ = 23.02, $Pη^2$ = 0.33			**Interaction (Sex x Time) P <0.01** $F_{(3,141)}$ = 4.50 $Pη^2$ = 0.09
Post Hoc between (Sex) Tukey-Kramer	P <0.001	P <0.01	P <0.01	P <0.01	

Figure 2. Changes in Total Cholesterol (TC), Low-Density Lipoprotein-Cholesterol (LDL-C) and Triglycerides (TG) during the treatment of males and females. Within post hoc comparison significances: * P<0.0001 and † P<0.001. Paired Student t-test with Bonferroni's correction in normally distributed data and Wilcoxon Matched Pairs Test with Bonferroni's correction in non-parametric analyses. In the table, the mean ± standard deviation (SD) is reported for each time point, and the results of the split-plot (between-within mixed design) ANOVA for TC and LDL-C are reported. For TG, the median and 1st and 3rd Quartiles are shown, and non–parametric statistical analyses are reported (Friedman for within data and Mann Whitney U with Bonferroni's correction for multiple between-data comparisons).

Table 3. Mean and Standard Deviation of the total variation (Δ_{Total}) of each analysis at the end of protocol (24th week) compared with the Critical Difference (CD) according to the Tuscany quality assessment program for clinical chemistry (22). N Δ_{Total} > CD Numbers of subjects showing a reduction higher than the Critical Difference.

		Δ_{Total} (mg/dl)	Δ_{Total} (%)	CD (%)	N Δ_{Total}>CD	N Subjects
TC (mg/dl)	M	-63.7±8.7 SD	22.7±2.3 SD	17.8	27	28
	F	-62.6±9.6 SD	22.3±2.4 SD		19	21
LDL-C (mg/dl)	M	-60.5±9.2 SD	30.9±3.5 SD	25.2	25	28
	F	-60.3±9.0 SD	31.3±3.2 SD		17	21
TG (mg/dl)	M	-17.7±11.2 SD	8.2±5.2 SD	24.4	0	28
	F	-12.6±8.7 SD	6.2±4.2 SD		0	21

4% of them (2 males (7%)) reached a TC concentration less than 200 mg/dl. By contrast, all the subjects showed an LDL-C concentration lower than 190 mg/dl, and 86% of participants (20 females (95%) and 22 males (79%)) reached a concentration less than 155 mg/dl. Furthermore, in 3 males (11%) and 2 females (10%), LDL-C decreased under 120 mg/dl.

Triglycerides (TGs)

TG plasma concentrations showed a pattern of variation similar to that of TC and LDL-C with no significant differences between sexes and a statistically significant decrease of the analyte concentration during treatment. Post hoc analysis showed significant variations between the eighth week and baseline, between the sixteenth and eighth weeks, and between the twenty-fourth and sixteenth weeks (Figure 1). Similarly to the decrease in TC and LDL-C, TG decrease slowed down over time, particularly $\Delta_3 < \Delta_2 < \Delta_1$ (Figure 2). Moreover, TG showed a correlation between baseline values and D_{Total} (ϱ = -0.65, $F_{(1,47)}$ = 34.4, p < 0.0001). The decrease during the non-energy-restricted diet follow-up period was less than that of TC and LDL-C (10.7% ± 7.5% SD). Despite the significant TG blood-concentration decrease during treatment, no subjects showed a reduction of TG greater than the CD at the end of the protocol (Table III).

HDL-Cholesterol (HDL-C)

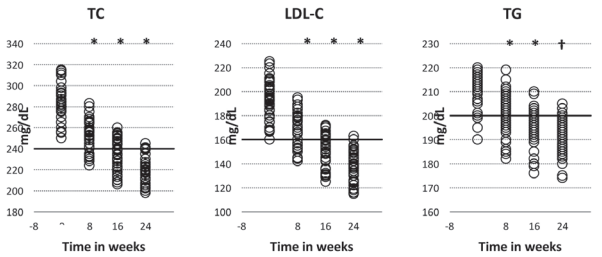
Significant differences between sexes in HDL-C blood concentrations were found, with females showing higher values than males in every instance (p < 0.005, Mann-Whitney U). In contrast, significant effects of diet were not found in males or females (females, Friedman: c^2 = 1.49, df = 3, p > 0.5; males, Friedman: c^2 = 5.99, df = 3, p > 0.1).

TC/HDL-C

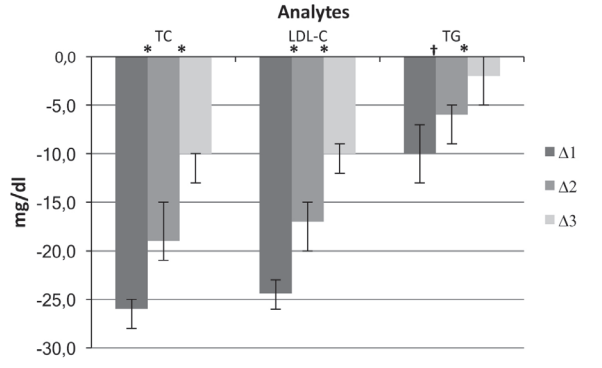
A significant difference between sexes was found in the TC/HDL-C ratio, with males manifesting consistently higher values. Both males and females showed a highly significant reduction of the TC/HDL-C ratio over the study period, and post hoc analysis indicated significant differences during all treatment periods (Figure 3). In addition, a significant interaction between sex and diet effects was found (Figure 3). In fact, the reduction in the TC/HDL-C ratio in males (DTotal = -1.38 ± 0.26 SD) was significantly higher than in females (DTotal = -1.22 ± 0.21 SD; Student t-test, p < 0.05) at the end of the protocol. At the end of the treatment, 42.9% of females (n. = 9) reached a TC/HDL-C ratio less than 4.5, and 21.4% of males (n. = 8) had a TC/HDL-C ratio less than 5.

Discussion

To the best of our knowledge, the results of this study show for the first time a reduction in TC and LDL-C blood concentrations achieved by a lifestyle intervention greater than the CD. Previous studies exploring the effects of weight loss, nutritional interventions, and/or both on lipid profiles has never compared TC and/or LDL-C reduction with CD (12,14, 17, 25-28). We found a clinically relevant reduction in TC and LDL-C without a decrease in HDL-C induced by both MD and weight-loss. These findings are supported by the significant decrease in Castelli's index for each participant. The decrease in TG - although statistically significant - never reached a rate of decline that was higher than the CD. Differences found between males and females in HDL-C concentration and in TC/HDL-C ratio are consistent with previously pub-



	baseline	Weight loss			Follow up	Within Subjects (Time)
		8th week	16th week	24th week		
TC (mg/dl)	Males	280±18.0 SD	252±15.9 SD	234±15.0 SD	223±14.0 SD	**P <0.0001** ANOVA $F_{(3,141)} = 3049.34$ $Pη^2 = 0.98$
	Females	278±18.5 SD	253±16.1 SD	234±14.5 SD	223±12.8 SD	
	Between Subjects (Sex) ANOVA	**P >0.5**; $F_{(1,47)} = 0.002$, $Pη^2 = 0.001$				**Interaction (Sex x Time) P >0.5** $F_{(3,141)} = 0.69$ $Pη^2 = 0.01$
LDL-C (mg/dl)	Males	194±16 SD	164±14 SD	141±13 SD	133±12 SD	**P <0.0001** ANOVA $F_{(3,141)} = 1468.06$ $Pη^2 = 0.97$
	Females	194±16 SD	165±14 SD	142±14 SD	135±13 SD	
	Between Subjects (Sex) ANOVA	**P >0.5**; $F_{(1,47)} = 0.27$, $Pη^2 = 0.006$				**Interaction (Sex x Time) P >0.5** $F_{(3,141)} = 0.269$ $Pη^2 = 0.006$
TG (mg/dl)	Males	213(208-216)	201(197-205)	196(193-199)	195(191-197)	**P <0.0001 Friedman** $\chi^2 = 120$ df = 3
	Females	213(210-215)	203(195-207)	195(191-199)	192(185-196)	
	Between Subjects (Sex) Mann W U	**P>0.25**	**P>0.5**	**P>0.25**	**P>0.1**	



	Weight loss			Follow up	ANOVA
	baseline	8th week	16th week	24th week	Within Subjects (Time)
Males	6.58±0.51 SD	5.98±0.48 SD	5.47±0.38 SD	5.20±0.38 SD	**P <0.0001** $F_{(3,141)} = 741.3$ $Pη^2 = 0.94$
Females	5.86±0.46 SD	5.35±0.49 SD	4.89±0.48 SD	4.66±0.43 SD	
ANOVA Between Subjects (Sex)	**P <0.0001**; $F_{(1,47)} = 23.02$, $Pη^2 = 0.33$				**Interaction (Sex x Time) P <0.01** $F_{(3,141)} = 4.50$ $Pη^2 = 0.09$
Post Hoc between (Sex) Tukey-Kramer	P <0.001	P <0.01	P <0.01	P <0.01	

Figure 3: Changes in Castelli's index (TC/HDL-C) during the treatment of both males and females. * indicates the within-data post hoc comparison significances (P< 0.0001 paired Student t-tests with Bonferroni's correction). The solid lines represent the risk-threshold: 5 for males and 4.5 for females. In the table, the mean ± standard deviation (SD) is reported at each time-point, including the results of the split-plot (between-within mixed design) ANOVA.

lished data (53, 54).

The changes that occurred during the hypocaloric diet period are clearly due to both the weight loss and the nutritional profile and TC, LDL-C and TC/HDL-C also decreased during the follow-up period without any caloric restriction, demonstrating a direct effect of the MD nutritional pattern in improving cholesterol profile. The magnitude of the changes during the normocaloric period was lower than in the first periods of the weight-loss programme, and this is partially due to the direct effect of negative energetic balance during the hypocaloric diet (56). However, we found a progressive decrease in the efficacy of the programme in improving the lipid-profile that could partially explain the lower rate of decline of TC, LDL-C and TC/HDL-C at the end of the protocol. The magnitude of changes in TG during the follow-up period of the normocaloric diet was less than that in TC and LDL-C. This could be partially explained by the progressive slowing of the TG decrease but it would seem to indicate that the decrease in TG was more closely related to weight loss than to nutritional pattern. Moreover, we found a statistically significant reverse-correlation between MAI scores and both TC and LDL-C concentrations before the start of the program, confirming data previously reported by Platania et al (57). These findings seems to indicate a positive correlation between the adherence to MD and an healthier blood lipid profile.

Furthermore, our findings showed a key role of the MD on the control of the blood-lipid profile, particularly in lowering atherogenic TC and LDL-C without decreasing the healthy HDL-C fraction. Despite traditional recommendations suggesting that low-cholesterol and low-fat diets may improve dyslipidaemia and metabolic disorders, the typical fatty acid profile and carbohydrate sources rich in fibres, folates and phytosterols (i.e., fruits, vegetables, and whole grains) that characterize the MD have been demonstrated to be more effective than interventions based on the limitation of individual food categories alone (39). These features of the MD are likely partially responsible for the protective effect of this diet against CV disease and follow the European guidelines for CV prevention(2).

Our data further showed a low baseline adherence to the Mediterranean dietary pattern, confirming previous indicating a clear tendency towards Westernization of eating habits (38-40). Nevertheless, temporal variations in the MAI that were induced by the dietary programme showed the possibility for improvement in dietary habits, accompanied by a positive progres-

sion of clinical parameters in the blood-lipid profile. Moreover, MD seems to have a good rate of compliance with drop-out rate of 10.9%.

Our study was a single-arm study, and the lack of a control group represents its principal limitation. However, our aim was to test the efficacy of a MD weight-loss programme to improve the blood-lipid profile under real-world outpatient conditions. Furthermore, the comparison of the CD demonstrates the clinical relevance of our findings and their applicability for outpatients.

The overall results of our study suggest that the Mediterranean Diet should be treated as a valuable supportive tool in controlling the serum blood-lipid profile, particularly if the MD is associated with a negative energetic balance. Indeed, the MD may be considered a healthy and sustainable lifestyle that plays a key role in both weight control and the primary prevention against CV disease.

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The relationship between healthcare professionals' mindful eating, eating attitudes, and body mass index

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Summary. *Purpose:* This observational research study was conducted to determine the relationship between healthcare professionals' eating attitudes, mindful eating, and body composition. *Methods:* Participants were 535 healthcare professionals, 325 (60%) working at Çorum (Turkey) Elitpark Hospital and 210 (40%) at Çorum Private Hospital. The participants filled a questionnaire with questions on demographic characteristics, body mass index (BMI), nutritional habits, Eating Attitudes Test, and Mindful Eating Scale. *Results:* The participants categorized as "other healthcare professionals" (28.6%) had the highest BMI value. The participants with impaired eating attitudes (92.2%) had high BMI values in general. The other healthcare professionals also constituted the occupational group with the highest impaired eating attitude score (66.9%). With regard to the magnitude of the relationship between mindful eating and BMI, obese and overweight people ranked first in terms of disinhibition, emotional eating, and interference. The lowest level of mindful eating was observed in the other healthcare professionals and auxiliary health personnel. Doctors were the occupational group with the highest level of mindful eating. The healthcare professionals with impaired eating attitudes had a statistically significantly higher average score on interference compared to those with normal eating attitude. *Conclusion:* The majority of the healthcare professionals participating in this study had high BMI values. Significant correlations were found among eating attitudes, mindful eating, and BMI. The other healthcare professionals and the auxiliary health personnel had the highest level of impaired eating attitudes and a low level of mindful eating. *Level of Evidence:* No level of evidence, basic science.

Key words: mindful eating; healthcare professionals; obesity; body mass index; eating attitude

Introduction

According to the World Health Organization (1), obesity is a health problem that has doubled over the last 35 years and causes many chronic diseases. Obesity occurs based on life-style changes as well as genetic factors (2).

It has been known for decades that eating behavior relates to body weight and body mass index (BMI), hence profoundly to obesity (3). Eating is a learned behavior (4). Eating behavior can be retaught to individuals, and thus, eating may become more sustainable by using verbal or visual instructions, by considering the process of change in conventional eating habits (5, 6).

A healthy and persistent practice of nutritional treatment can be ensured by having individuals gain mindful eating habits. Mindful eating means to stop, think and then take action whenever you feel hungry; it means being aware of what one eats; that is, it means to be aware of eating, not to eat as a reflex (7).

Although mindfulness has been associated with many health conditions (8), it plays a significant role especially in intrinsic and extrinsic factors such as ensuring portion control (9, 10), preventing emotional eating (8, 10, 11), and being able to stop excessive eating (12, 13) within the scope of mindful eating, as well as in the management of bodyweight.

It has been shown that the decrease in body weight is higher in individuals who are highly mindful and who have self-compassion, and additionally, there is a strong correlation between negative automated thoughts and bodyweight gain (14).

In a study on awareness and bodyweight management, it has been argued that improving mindfulness and self-compassion would be helpful in reducing bodyweight (14). They have been determined that mindfulness affects bodyweight loss independently and that bodyweight loss is positively correlated with mindfulness and self-compassion. They have been also found a strong negative correlation between automated thoughts and bodyweight loss. In the intervention section of the study, they have been offered training sessions on mindfulness and self-compassion, and observed that mindful eating has been improved at the end (14). In a similar study, the effect of mindfulness training on avoidance, impulsivity and bodyweight management was observed. Bodyweight and BMI values were shown to be reduced based on the training when pre and post assessments were compared (15). In another randomized controlled study, bodyweights and mindfulness of participants were compared after mindfulness training. At the end of the training, it was observed that the BMI value decreased and physical activity increased in the intervention group.

A relationship between food consumed by mindful eating — improved through mindful eating training — and bodyweight loss was reported (16). Although eating attitude is the basis of motor, cognitive, social and emotional development, it is regarded as a complex phenomenon that is regulated by environmental factors (17). It is the inclination of people that creates the feelings, thoughts and behaviors about eating and nutrition (18). Eating behavior is considered to vary depending on different emotions such as anxiety, joy, sadness, anger, depression, loneliness or happiness (19).

Eating behaviors are known to be responsible for all obese people being overweight (20). It has been considered that there is a relationship between anxiety levels and eating attitudes in obese people. Studies have shown that obese individuals eat significantly more food than normal-weight individuals when they face anxiety-causing situations (20-22). In a study, eating disorder was found in about 10% of all obese people (23).

Material and Method

Purpose and Significance

The aim of the study was to examine the relationship between healthcare professionals' mindful eating, eating attitudes, and their body mass index. The hypothesis is that there is a positive relationship between healthcare professionals' emotional eating, eating attitudes and BMI; that is, as emotional eating increases, eating attitudes deteriorate and BMI increases. In the study planned with this aim, the results are thought to contribute to the literature as examples for new studies. Moreover, it is thought that the results will contribute to the presentation of the status of healthcare professionals, who have a large share in work life in the fight against obesity, and to their training.

Population and Sample

The sample of this study consisted of all 535 healthcare professionals at Corum Private Hospital and Corum Private Elitpark Hospital in Corum province. They were categorized into groups as follows: doctors, nurses, auxiliary health personnel (dietitians, physiotherapists, biologists, psychologists, pharmacists, anesthesia specialists, and technical staff working in laboratory and imaging services), administrative staff (managers, human resources staff, administrative employees) and other healthcare professionals (cleaning, security, food, technical, cafeteria and porter staff).

Data Collection Instruments

This is an observational research study. A questionnaire consisting of socio-demographic questions identifying the participants and their nutritional habits, the Mindful Eating Scale (MES), and the Eating Attitudes Test (EAT-26)were used to collect data.

Data Collection

The questionnaire was administered after receiving permission to conduct the study from the Bahcesehir University Scientific Research and Publication Ethics Committee dated February 13, 2019 (Docu-

mented No. 2019/02) Participation in the questionnaire was on voluntary basis and with informed consent. This study was conducted in accordance with the principles of the Declaration of Helsinki.

Data Analysis

The BMI values of the healthcare professionals were categorized according to the BMI classification of the World Health Organization, and each BMI value was calculated by dividing the body weight (kilograms) of the individual by the square of his or her height (meters) (24). The SPSS 21.0 for Windows software program was used to analyze the data. First, the data were tested for normality, and it was found that the data were normally distributed. Parametric tests were carried out as a result of the normal distribution of the data. The data obtained from the healthcare professionals were analyzed, and the results were presented in tables. Frequencies, percentages, averages, cross-tables and Chi-square analyses were prepared. The relationships between body mass indices (BMIs), mindful eating levels, and eating attitudes were analyzed according to the occupations of the healthcare professionals. The following parametric tests were carried out: t-tests, ANOVAs and post-hoc tests. The categorical data were analyzed through Chi-square analyses.

Results

The occupational distribution of healthcare professionals participating in this study was as follows: 13.3% of the participants were doctors, 38.7% were nurses, 24.5% were auxiliary health personnel (dietitians, physiotherapists, biologists, psychologists, pharmacists, anesthesia specialists, and technical staff working in laboratory and imaging services), 17% were administrative staff (managers, human resources staff, administrative employees) and 23.2% were other healthcare professionals (cleaning, security, food, technical, cafeteria and porter staff). In addition, the body mass indices (BMIs) of the healthcare professionals were calculated, and it was found that 2.8% were thin, 38.4% were normal, 44.1% were overweight, and 14.4% were obese (Table 1).

Table 1. Demographic information

Variables	n	%
Gender		
Female	323	60.4
Male	212	39.6
Total	535	100.0
Marital Status		
Married	269	50.3
Single	266	49.7
Occupation		
Doctor	71	13.3
Nurse	118	22.1
Auxiliary health personnel	131	24.5
Administrative staff	91	17.0
Other healthcare professionals	124	23.2
BMI		
Thin	15	2.8
Normal	207	38.7
Overweight	236	44.1
Obese	77	14.4
Total	535	100.0

When the distribution of body mass indices of the healthcare professionals was examined according to their occupations, a significant relationship was found between their body mass indices and occupational groups (^{2} = 41.288, p = .000 < .05). The occupational groups were as follows according to their BMI values in descending order: the other healthcare professionals (28.6%), auxiliary health personnel (26%), nurses (24.7%), administrative staff (14.3%) and doctors (6.5%) (Table 2).

There was a statistically significant difference between the eating attitudes and body mass indices (^{2} = 200.395, p = .000 < .05). The groups, who had the worst impaired eating attitudes, were the obese and overweight individuals with a high BMI level (Table 3). That is, there was a significant relationship between weight gain and eating attitudes. When the eating attitude was impaired, the BMI value increased, which affected the body composition.

A statistically significant difference was found when the eating attitudes were assessed according to occupations (^{2} = 18.661, p = .001 < .05). The group with the highest rate of impaired eating was the other healthcare professionals with 66.9%, who were fol-

Table 2. Body mass index distribution by occupation

BMI		Occupation[a]				
		Doctor	Nurse	Auxiliary health personnel	Administrative staff	Other healthcare professionals
Thin	n	3	8	1	2	1
	%	20.0	53.3	6.7	13.3	6.7
Normal	n	24	58	48	23	54
	%	11.6	28.0	23.2	11.1	26.1
Overweight	n	33	44	58	39	62
	%	14.0	18.6	24.6	16.5	26.3
Obese	n	5	19	20	11	22
	%	6.5	24.7	26.0	14.3	28.6
Total	n	71	118	124	91	131
	%	13.3	22.1	23.2	17.0	24.5

[a]Pearson Chi-Square Value = 41.288, p = .000.

Table 3. Comparison of Eating Attitudes by Body Mass Index

		Eating Attitude[a]		
BMI		Normal eating attitude	Impaired eating attitude	Total
Thin	n	15	0	15
	%	100.0	0	100.0
Normal	n	182	25	207
	%	87.9	12.1	100.0
Overweight	n	89	147	236
	%	37.7	62.3	100.0
Obese	n	6	71	77
	%	7.8	92.2	100.0
Total	n	292	243	535
	%	54.6	45.4	100.0

[a]Pearson Chi-Square Value = 200.395, p = .000.

Table 4. Comparison of Eating Attitudes by Occupation

		Eating Attitude[a]		
Occupation		Normal eating attitude	Impaired eating attitude	Total
Doctor	n	37	34	71
	%	52.1	47.9	100.0
Nurse	n	47	77	124
	%	37.9	62.1	100.0
Auxiliary health personnel	n	39	52	91
	%	42.9	57.1	100.0
Administrative staff	n	63	68	131
	%	48.1	51.9	100.0
Other healthcare professionals	n	39	79	118
	%	33.1	66.9	100.0

[a]Pearson Chi-Square Value = 18.661, p = .001.

lowed by nurses with 62.1%, auxiliary health personnel with 51.7%, administrative staff with 51.9%, and doctors with 47.9% (Table 3).

The "disinhibition" dimension of the Mindful Eating Scale had a statistically significant difference in terms of the body mass index (F = 255.18, p = .000 < .05). The averages of disinhibition of the overweight and obese healthcare professionals with a high body mass index were found to be higher than those of the thin and normal healthcare professionals with normal and low body mass indices (Table 5).

The "control of eating" dimension of the Mindful Eating Scale had a statistically significant difference in terms of the body mass index (F = 208.25, p = .000 < .05). The averages of the overweight and obese healthcare professionals with a high body mass index were found to be higher than those of the thin and normal healthcare professionals with normal and low body mass indices.

A statistically significant difference was found when the "emotional eating" dimension of the Mindful Eating Scale was examined with regard to the body mass index (F = 437.53, p = .000 < .05). The averages of emotional eating of the overweight and obese healthcare professionals with a high body mass index were found to be higher than those of the thin and

Table 5. Comparison of Mindful Eating Habits by Body Mass Index

		BMI					
Mindful Eating		**Thin**	**Normal**	**Overweight**	**Obese**	***F***	***p***
Disinhibition	M	2.41	2.86	4.60	5.20	255.18	.000
	SD	.92	.91	.94	.84		
Emotional Eating	M	2.1	2.66	4.38	5.22	208.25	.000
	SD	1.01	.94	.94	.85		
Control of Eating	M	5.01	4.80	2.35	1.75	437.53	.001
	SD	.81	.75	.89	.83		
Concentration	M	4.85	4.83	2.28	1.71	486.31	.000
	SD	1.08	.77	.83	.75		
Eating Discipline	M	5.34	5.07	2.17	1.61	769.69	.000
	SD	1.02	.71	.74	.66		
Mindfulness	M	5.12	5.02	2.22	1.64	668.93	.000
	SD	1.10	.72	.78	.65		
Interference	M	2.16	2.65	4.44	5.23	217.472	.000
	SD	1.01	.94	.94	.85		

normal healthcare professionals with normal and low body mass indices. A relationship was determined between concentration on eating and high BMI values. The averages of concentration on eating were found to be low among the individuals with high BMI values.

When the mindful eating habits of the healthcare professionals were compared according to the occupational groups, "disinhibition" was found to differ statistically significantly depending on the occupational groups ($F = 24.16$, $p = .000 < .05$) (Table 6). Disinhibition was observed to be at the highest level among the other healthcare professionals and auxiliary health personnel, whereas it was at the lowest level in the doctor group.

Table 6. Comparison of Mindful Eating by Occupation

		Occupation						
Mindful Eating		Doctor	Nurse	Auxiliary health personnel	Administrative staff	Other healthcare professionals	*F*	*p*
Disinhibition	M	1.43	2.55	4.67	2.44	4.88	24.16	.000
	SD	.62	.74	.91	.90	.84		
Emotional Eating	M	1.78	2.95	5.10	2.4	5.20	23.66	.000
	SD	.98	.96	.97	.88	.94		
Control of Eating	M	5.13	2.46	2.2	4.45	1.8	28.36	.000
	SD	.81	.92	.96	.89	.92		
Concentration	M	5.1	3.21	2.3	4.49	1.65	26.48	.000
	SD	.93	.87	.96	.86	.79		
Eating Discipline	M	5.23	3.18	2.01	4.24	1.72	25.54	.000
	SD	.88	.90	.94	.89	.93		
Mindfulness	M	5.32	3.33	3.10	3.4	2.2	24.88	.000
	SD	.89	.77	.95	.98	.91		
Interference	M	1.87	3.20	5.4	2.5	5.6	27.61	.000
	SD	.68	.76	.84	.86	.98		

Table 7. Comparison of Mindful Eating Habits by Eating Attitude

Mindful Eating		Eating Attitude: Normal eating attitude	Eating Attitude: Impaired eating attitude	t	p
Disinhibition	M	3.3	5.21	-11.501	.000
	SD	1.19	1.21		
Emotional Eating	M	2.17	4.47	-12.372	.000
	SD	1.22	1.21		
Control of Eating	M	5.24	2.44	13.737	.000
	SD	1.08	1.20		
Concentration	M	4.02	2.30	14.265	.000
	SD	1.43	1.18		
Eating Discipline	M	5.15	2.32	14.903	.000
	SD	1.57	1.21		
Mindfulness	M	4.75	2.35	14.761	.000
	SD	1.53	1.21		
Interference	*M*	2.1	4.52	-12.812	.000
	SD	1.21	1.22		

When the mindful eating habits were assessed according to the eating attitudes, the healthcare professionals with impaired eating attitudes had a high average in terms of the disinhibition dimension, and they had a statistically significantly different score compared to that of the healthcare professionals with normal eating attitudes ($t = -11.501$, $p = .000 < .05$) (Table 7). Similarly, when the emotional eating habits were examined according to the eating attitudes, there was a statistically significant difference between the averages of those with normal eating attitudes and those with impaired eating attitudes ($t = -12.372$, $p = .000 < .05$). The healthcare professionals with impaired eating attitudes were found to have an emotional eating disorder.

The healthcare professionals with low scores on the dimensions of the Mindful Eating Scale, such as control of eating, concentration, eating discipline and mindfulness, were found to have impaired eating attitudes. The levels of mindful eating were found to be higher among the individuals with normal eating attitudes.

When the control of eating, concentration, eating discipline and mindfulness dimensions of the Mindful Eating Scale were examined according to the eating attitudes, these four dimensions also differed statistically significantly between the normal and impaired eating attitudes. The control of eating, concentration, eating discipline and mindfulness were found to be at poor levels among the individuals with impaired eating attitudes.

The healthcare professionals with impaired eating attitudes had a high average in terms of the "interference" dimension, and they were found to be statistically significantly different from those with normal eating attitudes ($t = -12.812$, $p = .000 < .05$). The individuals with impaired eating attitudes were found to eat more due to the influence of external factors and influences.

Discussion

In this study, when the healthcare professionals were examined according to their body compositions, it was found that the obese individuals with the highest BMI value were 14.4% of all healthcare professionals and the overweight individuals with a high BMI value were 41.1%. Similarly, Turner et al. (25) and Campos-Matos et al. (26) found that obese healthcare professionals with the highest BMI were 8% and 16.9% of all participants, and overweight professionals with a high BMI value were 31% and 38.4%, respectively. Healthcare professionals may have to consume fast food and skip meals due to patient examinations, patient care processes, and limited breaks for eating, as per their working conditions. This condition can lead to weight gain and may affect their body compositions.

In this study, when the occupational groups were assessed according to the body compositions, the group of healthcare professionals with the highest level of body composition was found to be the other healthcare professionals, followed by the auxiliary health personnel, nurses, and administrative staff. The group, which had the lowest BMI value, was found to be the doctors. Similarly, in the study of Turner et al. on healthcare professionals, the highest BMI values were found among the auxiliary health personnel and nurses, while the lowest one was among the doctors (25). Similarly, in their study on healthcare professionals, Kyle et al. (27) found that other healthcare professionals had the highest BMI values, followed by nurses and auxiliary health personnel. Due to the intensity of being on call

and patient monitoring, other healthcare professionals and nurses may have insufficient time to eat; they can be sleepless for being on call, and they may have to eat or snack during this process, which can affect weight gain and consequently their body compositions.

In this study, impaired eating attitudes were found to be high among individuals with high BMI levels. Similarly, research shows impaired eating attitudes of individuals with high BMI levels are high (28, 29). Correspondingly, a relationship has been identified between impaired eating attitudes and BMI in studies in the literature, and BMI values of individuals with impaired eating levels have been found to be high (30, 31). Individuals with impaired eating attitudes may tend to eat inadequately, unstably and excessively, which can consequently lead to weight gains.

In this study, the occupational group with the highest level of impaired eating attitude was found to be the other healthcare professionals, followed by the nurses, auxiliary health personnel, administrative staff, and doctors. Similarly, in the study of Ho et al. (32) on healthcare professionals, doctors have been found as an occupational group with the lowest impaired eating attitudes. This finding may be explained by the fact that other healthcare professionals have limited time to eat or have limited access to food because of the operational processes of hospitals such as food service, toilet cleaning, and security, or that they skip their meal with snacks.

In this study, a relationship was found between high BMI values and disinhibition. The mean of disinhibition of the obese and overweight individuals with high BMI value was found to be higher than that of the thin and normal individuals with normal and low BMI values. Similarly, disinhibition has been found to be associated with high BMI values in studies in the literature (33-35).

In this study, a relationship was found between the control of eating and BMI, and the control of eating averages of the overweight and obese healthcare professionals with a high body mass index were found to be higher than those of the thin and normal healthcare professionals with normal and low body mass indices. Likewise, a relationship has been determined between impulsive eating and high BMI values in studies in the literature (15, 36, 37).

In this study, a relationship was found between the emotional eating and high BMI values. The averages of emotional eating of the overweight and obese healthcare professionals with a high body mass index were found to be higher than those of the thin and normal healthcare professionals with normal and low body mass indices. Similarly, a relationship has been determined between emotional eating and high BMI values in studies in the literature (12, 14, 38).

In this study, a relationship was determined between concentration and high BMI values. The averages of concentration on eating were found to be low among the individuals with high BMI values. Similarly, a relationship has been determined between concentration on eating and high BMI values in studies in the literature (5, 11, 39).

In this study, when the mindful eating habits were assessed according to the eating attitudes, the healthcare professionals with impaired eating attitudes had a high average in terms of the disinhibition dimension, and they had a statistically significantly different score compared to that of the healthcare professionals with normal eating attitudes. Similarly, when the emotional eating habits were examined according to the eating attitudes, there was a statistically significant difference between the averages of those with normal eating attitudes and those with impaired eating attitudes. The healthcare professionals with impaired eating attitudes were found to have an emotional eating disorder. The healthcare professionals with low dimension scores of the Mindful Eating Scale, such as control of eating, concentration, eating discipline and mindfulness, were found to have impaired eating attitudes. The levels of mindful eating were found to be higher among the individuals with normal eating attitudes.

In this study, when the control of eating, concentration, eating discipline and mindfulness dimensions were examined according to the eating attitudes, these four sub-factors differed statistically significantly between the normal and impaired eating attitudes. The control of eating, concentration, eating discipline and mindfulness were found to be at poor levels among the individuals with impaired eating attitudes.

In this study, the healthcare professionals with impaired eating attitudes had a high average in terms of the “interference” dimension, and they were found

to be significantly different from those with normal eating attitudes. The individuals with impaired eating attitudes were found to eat more due to the influence of external factors and influences.

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Conflict of Interest

No potential conflict of interest relevant to this article was reported by the author.

Ethical approval

"All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee (Bahcesehir University Scientific Research and Publication Ethics Committee, dated February 13, 2019 and numbered 2019/02) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards."

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Evaluation of eating attitudes of nursing students for type-2 diabetes and cardiovascular diseases

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Summary. Eating disorders/attitudes might lead to disrupted metabolic control. When body releases insulin insufficiently and irregularly, this causes the blood glucose level to rise. Abnormal eating attitudes and behaviors, which develop especially during adolescence, are acknowledged to be the strongest precursor to eating disorders at later ages. The study aimed to evaluate eating attitudes of nursing students with regard to the risk of type 2 diabetes and cardiovascular diseases. This study was designed as a descriptive. The sample contained 356 volunteers. The data were collected using A Questionnaire For The Risk Of Type 2 Diabetes (FINDRISC), the Cardiovascular Disease Risk Factors Knowledge Level Scale (CARRF-KL) and the Eating Attitudes Test (EAT) and various measurements such as waist circumference measurement, blood pressure measurement and body mass indeks. The mean scores on the CARRF-KL Scale suggested a statistically significant difference between the students of different grades. Additionally, the students' scores on the FINDRISC were significantly correlated with their weight, body mass index, waist circumferences, family history of diabetes or heart disease, and level of exercising. The findings suggest that higher weight and body mass indexes and larger waist circumferences lead to a corresponding increase in the scores on the FINDRISC and pose a risk in terms of type 2 diabetes and that higher educational status means a higher knowledge level about cardiovascular diseases.

Keywords: nursing students, type 2 diabetes, cardiovascular diseases, eating behavior.

Introduction

Eating disorders involve excessive eating that leads to obesity, refusing to eat, limiting the amount of eating as a result of being a vegetarian or some psychological reasons, eating non-food items, digesting and discharging food rapidly, or exhibiting uncontrollable night eating behaviors (1).

Eating disorders/attitudes might lead to disrupted metabolic control. When body releases insulin insufficiently and irregularly, this causes the blood glucose level to rise. In such situations, individuals start to get hungry quickly and suffer from thamuria, xerostomia, and excessive dehydration. This long hyperglycemic condition paves the way for long-term complications (neuropathy, retinopathy, hypertension, and cardiovascular diseases) (2).

Abnormal eating attitudes and behaviors, which develop especially during adolescence, are acknowledged to be the strongest precursor to eating disorders at later ages. Adolescence and youth are risky periods in terms of eating behaviors. During these periods, individuals tend to eat in non-family contexts, which change their food choices and might lead to risky eating behaviors. Increased consumption of fast food, which is especially rich in fat and calories, and decreased physical activity, might give rise to an increase in the rates of obesity and eating disorders (3).

Obesity not only poses a great risk to health but also leads to various diseases. The primary obesity-induced health problems with high morbidity and mortality are type 2 diabetes (DM), hypertension (HT), dyslipidemia, and cardiovascular diseases (CVDs) (2). Wrong nutritional attitudes and behaviors displayed during adolescence and youth may lead to obesity and pave the way for the above mentioned health problems.

During their university education, students try to adapt to a new school and environment. This attempt, when coupled with their ideals of having a profession and leading their future, causes many students to start suffering from social, psychological, and health-related problems. Adequate and balanced nutrition plays a key role in the prevention of such health-related problems. However, obesity emerges as a problem among university students as fast-food habits are becoming more and more popular, as their physical activity is getting more and more limited to pave the way for a more sedentary lifestyle, and as malnutrition is becoming increasingly prevalent (4). It is essential that young people of university age, who are at risk of obesity and chronic diseases, should be enabled to develop a healthy lifestyle. Nevertheless, it is also known that these young people rarely follow the advice about healthy nutrition, that they consume vegetables, fruit, and wholegrain food less often than processed and convenience food, and that they take in an insufficient amount of vitamins, minerals, and fiber but excessive amounts of salt and saturated fat. Besides, they usually have a sedentary lifestyle, which puts them at risk of developing not only obesity but also hypertension, diabetes, coronary heart diseases, and certain types of cancer (4).

Within the scope of global fight against chronic diseases, nurses have great responsibilities for the reduction and control of the risk factors associated with chronic diseases (nutrition counselling, physical activity counselling, hypertension treatment, smoking cessation, and weight loss/maintenance) (5). Considering that nurses, who receive health education at university, will provide the public with health training throughout their professional life, set a role model for the public, and raise public awareness about health issues, they should be the first to exhibit proper health behaviors. Therefore, the purpose of this study is to evaluate nursing students' eating attitudes with regard to the risk of type 2 DM and CVDs.

Methods

Study Design

This study was designed as a descriptive one to evaluate nursing students' eating attitudes with regard to the risk of type 2 diabetes and cardiovascular diseases.

Setting and sample

The study was carried out at the School of Health between December 2016 and February 2017 in Turkey. The population of the study comprised of 487 students who studied Nursing at the School of Health during the fall semester of the 2016-2017 academic year. The sample contained 356 students who were not speech, hearing or vision impaired, who did not suffer from any chronic diseases, and who volunteered to take part in the study.

Data collection tools

The data were collected using the Demographic Information Form, which contained questions releated to age, weight, height, waist circumference, place of accommodation, dieting, skipping meals, smoking, exercising, and stress level, the FINDRISC, which is a questionnaire to estimate the risk of type 2 diabetes, the Cardiovascular Disease Risk Factors Knowledge Level (CARRF-KL) Scale, and the Eating Attitudes Test (EAT). After the students filled out the data forms, the researcher measured and recorded their height, weight, waist circumferences, and blood pressure levels. The students' weight measurements were taken using a digital scale, and their waist circumference measurements were taken using a non-elastic tape measure. The students were instructed to take off their shoes for height measurements, which were performed using a measurement stick with 0.1 cm spaces on it. Their blood pressure levels were measured after they had rested at least 15 minutes and while they were in the sitting position. The measurements were performed using a pre-calibrated pneumatic pressure gauge in accordance with the techniques for blood pressure measurement.

The Eating Attitudes Test (EAT-40):, The Eating Attitudes Test Developed by Garner and Garfinkel

is a self-report scale used across the world to identify problematic eating behaviors. Its validity and reliability for the Turkish context were tested by Savaşır and Erol (6). The rating is based on responses that are at pathological extremes. Responses to the items are assigned a score of zero to 3. The rating for items 1, 18, 19, 23, 27, and 39 are as follows: 1 for "sometimes," 2 for "rarely," 3 for "never," and zero for other responses. For the other items in the scale, the responses "always," "very often," and "often" are assigned a score of 3, 2, and 1 respectively, and a score of zero is assigned for other responses. The total score on the scale is the sum of the scores assigned for each item. Higher scores mean a greater disruption in eating attitudes. In the original form of the scale, the cut-off point was 30. Savaşır and Erol found that the Cronbach's alpha coefficient for the scale was 0.7(6).

A Questionnaire For The Risk Of Type 2 Diabetes (FINDRISC): Developed to identify individuals who are at high risk of type 2 diabetes, the FINDRISC is based on an evaluation of the responses to eight questions and can easily be administered on the public level. The distribution of the total scores by the level of risk is as follows: low risk for a score less than 7, slight risk for a score between 7 and 11, moderate risk for a score between 12 and 14, high risk for a score between 15 and 20, and very high risk for a score more than 20(7).

The Cardiovascular Disease Risk Factors Knowledge Level (CARRF-KL) Scale: The scale was developed by Arıkan et al. to identify the knowledge level about risk factors associated with cardiovascular diseases, and its validity and reliability were tested by them (8). Knowledge Level (CARRF-KL) Scale: a validity and reliability study. The items in the scale are in the form of complete statements. The items are introduced to the participants, who are then asked to respond to them in three ways: "Yes," "No," and "I do not know". Each correct answer is assigned a score of 1. Of the 28 items, six (items 11, 12, 16, 17, 24, and 26) are reversely scored. The correct responses to the items are as follows: "Yes" for the first ten items, "No" for items 11 and 12, "Yes" for items 13 to 15, "No" for items 16 and 17, "Yes" for items 18 to 23, "No" for item 24, "Yes" for item 25, "No" for item 26, and "Yes" for items 27 and 28. The maximum possible score on the scale is 28. A higher score means a higher knowledge level. Arıkan et al. reported that the Cronbach's alpha coefficient for the scale was 0.7 (8).

Data analysis

The data were analyzed using SPSS 20.(SPSS Inc., Chicago, IL, USA) The Mann-Whitney U test and Kruskal-Wallis H test were used since the variables did not have normal distribution for the differences between the groups. The level of significance was accepted to be .050.

Ethical Considerations

Consent was obtained from the School of Health. Additionally, ethical approval was granted by the Ethics Committee for Non-Interventional Clinical Research (Ethics committee decision no: 2016/114/10/04). Finally, verbal consent was obtained from the participants, who had already been informed about the study.

Results

More than three quarters of the participants (76.9%) were women, and 36.8% had graduated from a regular high school. As for the family history, 30.9% reported having a family history of type 2 diabetes or heart disease. Approximately two-thirds of the participants (64.0%) reported skipping meals and 50.8% exercises sometimes or played sports. Finally, 73.6% reported that their nutrition was affected by stress, 61.8% reported that they did not diet, and 37.9% were satisfied with their weight (Table 1).

The study identified certain statistics regarding the participants and their scores on the data collection tools: their mean age was 20.63 ± 2.1 years, mean height 166.95 ± 7.84 cm, mean weight 63.08 ± 13.08 kg, mean body mass index 22.55 ± 3.88 kg/m^2, mean waist circumference 78.78 ± 10.62 cm, mean systolic blood pressure 68.81 ± 9.22 mmHg, mean score on the FINDRISC 5.5 ± 3.56, mean score on the EAT 18.39 ± 10.44, and their mean score on the CARRF-KL Scale 19.92 ± 3.10. Approximately two-thirds of the participants (62.0%) were found to be at low risk of type 2 DM.

There was a statistically significant difference between the students of different grades in terms of their

Table 1. Frequency Distribution Table for Demographic Information (n=356)

		n	%
Gender	**Woman**	274	76.9
	Man	82	23.0
Grade	**Freshman**	84	23.6
	Sophomore	100	28.0
	Junior	93	26.1
	Senior	79	22.1
Do you have a family history of Type 2 Diabetes or Heart Disease?	**Yes**	110	30.9
	No	246	69.1
Do you diet?	**Yes**	25	7.0
	No	220	61.8
	Sometimes	111	31.1
Are you satisfied with your weight?	**Yes**	135	37.9
	No	125	35.1
	Partly	96	26.9
Do you exercise?	**Yes**	64	17.9
	No	111	31.1
	Sometimes	181	50.8
Is your nutrition affected by stress?	**Yes**	262	73.6
	No	33	9.2
	Sometimes	61	17.1

mean scores on the CARRF-KL Scale ($p< 0.050$). The freshman students had a significantly lower mean score on the CARRF-KL Scale than the sophomore, junior, and senior students. Also, the students with a family history of heart disease or type 2 DM had a highly statistically significantly higher mean score on the FINDRISC (7.45 ± 3.77) than those without a family history of heart disease or type 2 DM (4.63 ± 3.10) ($p< 0.001$). The students who dieted had a statistically significantly higher mean score on the EAT than those who did not diet or those who sometimes dieted ($p< 0.001$). The students who were satisfied with their weight had statistically significantly lower mean scores on the FINDRISC and EAT than those who were not satisfied with their weight ($p< 0.050$; $p< 0.001$, respectively) (Table 2).

The students who exercised had a statistically significantly lower mean score on the FINDRISC than those who exercised sometimes and those who did not exercise ($p< 0.001$). The students whose nutrition was not affected by stress had a statistically significantly lower mean score on the FINDRISC than those whose nutrition was affected by stress and those whose nutrition was sometimes affected by stress ($p< 0.050$) (Table 2).

The weight, BMIs, and waist circumferences of the students were positively correlated with their scores on the FINDRISC, and the correlation was statistically significant (r= 0.336, r= 0.421, r= 0.468, respectively). In addition, there was a statistically significant correlation between age and the overall scores on the CARRF-KL Scale (r= 0.116). There were not significant correlations among the overall scores on the EAT, FINDRISC, or CARRF-KL Scale ($p> 0.050$).

Discussion

Apart from factors affecting the eating attitude negatively, many factors (such as personal eating choices, eating habits of family, behaviors, beliefs, family, friends) may lead to the development of eating disorders (9). Thus, nurses who will provide service to society and raise awareness of protecting and improving health and become a role model should display healthy behavioral patterns themselves first.

The study found that the freshman students had a significantly lower mean score on the CARRF-KL Scale than the sophomore, junior, and senior students, which suggests that education makes a positive difference in students' knowledge level about cardiovascular diseases and associated risk factors. A similar finding was reported by Yılmaz and Boylu, who found that high school graduates had a lower score on the CARRF-KL Scale than university graduates (10). In another study on nursing students, Badır et al. reported that higher educational status led to a corresponding increase in the scores on the CARRF-KL Scale (5). In other words, the findings of the present study are supported by the literature.

The students with a family history of heart disease or type 2 DM had a highly statistically significantly higher mean score on the FINDRISC than those without a family history of heart disease or type 2 DM ($p< 0.001$). In their study on nurses, Yurtsever et al. found a significant correlation between having close

Table 2. Socio-demographic variables and the overall scores on the EAT, CARRF-KL Scale and FINDRISC (n=356)

		n	Mean	ss	H	p
			Grade		**Kruskal-Wallis H Test**	
The Overall Score on the CARRF-KL Scale	Freshman	84	18.57	3.04	31.50	**0.001**
	Sophomore	100	20.35	2.24		
	Junior	93	19.77	3.36		
	Senior	79	20.99	3.30		
The Overall Score on the EAT			**Do you diet?**		**Kruskal-Wallis H Test**	
	Yes	25	28.88	16.48	32.44	**0.001**
	No	220	15.95	7.94		
	Sometimes	111	20.87	11.12		
The Overall Score on the FINDRISC			**Are you satisfied with your weight?**		**Kruskal-Wallis H Test**	
	Yes	135	4.87	3.04	9.53	**0.009**
	No	125	6.47	4.21		
	Partly	96	5.13	3.05		
The Overall Score on the EAT			**Are you satisfied with your weight?**		**Kruskal-Wallis H Test**	
	Yes	135	16.76	9.32	25.19	**0.001**
	No	125	22.03	11.93		
	Partially	96	15.94	8.48		
The Overall Score on the FINDRISC			**Doing Exercise**		**Kruskal-Wallis H Test**	
	Yes	64	3.95	3.63	25.41	**0.001**
	No	111	6.51	3.66		
	Sometimes	181	5.43	3.28		
The Overall Score on the FINDRISC			**Is your nutrition affected by stress?**		**Kruskal-Wallis H Test**	
	Yes	262	5.80	3.54	8.90	**0.012**
	No	33	4.64	3.46		
	Sometimes	61	4.69	3.57		
The Overall Score on the FINDRISC			**Family history of Type 2 Diabetes or Heart Disease**		**Mann-Whitney U Test**	
	Yes	110	7.45	3.77	-6.99	**0.001**
	No	246	4.63	3.10		

Table 3. Certain variables and the overall scores on the EAT, CARRF-KL Scale and FINDRISC (n=356)

		FINDRISC (Overall Score)	EAT (Overall Score	CARRF-KL Scale (Overall Score)
Age	**r**	0.07	-0.05	**0.11**
	p	.161	0.302	0.029
Height	**r**	-0.06	-0.06	-0.06
	p	0.245	0.206	0.237
Weight	**r**	**0.33**	0.03	-0.05
	p	0	0.460	0.349
BMI	**r**	**0.42**	0.06	-0.03
	p	0	0.196	0.514
Waist Circumference	**r**	**0.46**	0.06	-0.05
	p	0	0.224	0.296

relatives with diabetes and insulin resistance (11). In another study on nurses who worked shifts, Çekinmez et al. found a significant correlation between the family history of diabetes and the risk of developing diabetes (12). They reported that most of the participants who had first degree relatives diagnosed with diabetes and more than half of the participants who were on medication for high blood pressure had high/very high diabetes risk scores. Other studies have reported a positive correlation between the rise of blood pressure levels and diabetes risk scores (13,14). These findings suggest that individuals with a family history of diabetes and hypertension have insulin resistance and are predisposed to DM.

The students who dieted had a statistically significantly higher mean score on the EAT than those who did not diet and those who sometimes dieted. The students who were satisfied with their weight had a statistically significantly lower mean score on the EAT than those who were not satisfied with their weight ($p<0$ .001). Similar findings were reported in a study by Kadıoğlu and Ergün on university students (3). In another study on high school students, Büyük and Duman reported that the students who were not satisfied with their weight and tried to lose weight were at statistically significantly higher risk of having an eating disorder (15). In a study on two groups, one with eating disorders and the other without eating disorders, the former group had a higher score on the EAT, and they were more overweight (16). These findings support those of the present study. Nevertheless, Ünalan et al. studied the eating habits among students from a school of health and found that the students with normal weight were at significantly higher risk of developing an eating disorder (17).

The students who exercised had a statistically significantly lower mean score on the FINDRISC than those who did not exercise and those who exercised sometimes ($p<$ 0.001). Kutlu et al. and Viitasalo reported that individuals who had sedentary lifestyles had higher diabetes risk scores (18,19). Çekinmez et al. found a significant correlation between physical exercise and the risk of diabetes and reported that 95.9% of those who did not do physical exercise at least for 30 minutes a day were at high/very high risk of diabetes (12). Physical activity is a primary risk factor associated with diabetes. The findings of the present study are supported by the literature. The students whose nutrition was affected by stress had a statistically significantly higher mean score on the FINDRISC than those whose nutrition was not affected by stress ($p<$ 0.001).

It is reported in the literature that individuals whose nutrition is affected by stress are more likely to have risky eating attitudes. The primary causes of stress among university students are their ideals of having a profession and leading their future lives, being away from their families, and attempting to adapt themselves to a new environment. Eating attitudes and behaviors are likely to be affected by stressors especially experienced by the young since they experience more stressors and they do not know about the methods for coping with stress yet (20-22).

The students who were not satisfied with their weight had a statistically significantly higher mean score on the FINDRISC than those who were satisfied with their weight and those who were partly satisfied with their weight. Research has shown that the most common method of weight control used by university students is dieting (23).

Higher weight and BMIs and larger waist circumferences led to a corresponding increase in the overall scores on the FINDRISC. Similarly, Çekinmez et al. reported that higher BMIs and larger waist circumferences meant a higher risk of diabetes (12). Yurtsever et al. studied nurses of different age groups and reported that 53.5% of the nurses who were under 45 years old were overweight and obese and 76.8% of those who were above 45 years old were overweight and obese. They also found that 39.9% of the nurses had high scores on the FINDRISC. This finding among nurses is worrying considering that they are professional health care members (11).

As the students' age increased, so did the overall scores on the CARRF-KL Scale. Nevertheless, Yilmaz and Boylu studied individuals with desk jobs and reported that age was not a factor in the scores on the CARRF-KL Scale (10). This could be attributed to the idea that as students get older, their educational status gets higher and thus they have better awareness.

In the present study, it was determined that there was no statistically significant difference between

the total scores of YTT, Findrisk and KARRIF-BD (p>0.05). Positive nutritional attitudes and behaviors are important for nursing students. These positive attitudes and behaviors are important for the future public health. In a study, it was reported that university students consumed more frequently foods like sweets, cake, chips and fast food, but fruits and vegetables less (24,25). Negative behaviors especially in such eating attitudes may lead to obesity in the first place and also diabetes and cardiovascular diseases in the future. This result of in the present study suggests that the increase students' knowledge concerning nutrition and health reflects on their nutritional behaviors and eating attitudes positively.

Conclusion

Nurses play an important role in protecting andpromoting an individual's health and promoting public health. It can be recommended to train nurse candidates who will contribute awareness of the society and become a role model before starting their professionespecially on precautions to take concerning methods of coping with stress, healthy nutrition and protection from the risk factors of DM and cardiovascular diseases in order to protect their health.

Limitations

The results obtained from the study can be generalized to only the nursing students studying at the faculty where the study was conducted. It is recommended to conduct the study in more than one centers.

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Original article

Type 2 Diabetes Mellitus (Type 2 DM) incidence and associated factors: a cross sectional study in Istanbul, Turkey

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Summary. *Background:* Incidence of Type 2 Diabetes Mellitus (Type 2 DM) is considerably high in Turkey and has been associated with risk factors as obesity, nutrition, socioeconomic status and education. Lifestyle-intervention programs may be of special significance in impaired glucose tolerance and development of DM. *Objective:* This research was planned as a observational cross-sectional study, in order to determine the major factors associated with the incidence of DM. *Methods:* The study was carried out in Beykoz Public Hospital Tepeustu, Istanbul. The patient population comprised of 18970 patients with Type 2 DM. Incidence of diabetes were evaluated according to gender, age, education, income level with descriptive statistical analyses. *Results:* DM incidence was highest in females of age group 61-75 years (31,29%) followed by men within the same age group (16,35%). Also the incidence was highest in the individuals with primary school education (37,97%) followed by individuals with no formal education (32,43%). DM increased as income decreased with highest percentages (36,56%) recorded among patients having personal income less than 1000 TL per month. *Conclusion:* As the study indicates poverty and lack of education are major contributing factors; effective policies aimed at decreasing poverty and increasing education level should be implemented in the lower strata of the society.

Keywords: diabetes mellitus, socioeconomic status, gender, education, age

Introduction

Type 2 Diabetes Mellitus (Type 2 DM) is one of the major chronic disease that has been increasing progressively over the last two decades worldwide as well as in Turkey (1). Presently, 13,7% of the Turkish population is affected by DM (1,3% Type 1 and 12,4 % Type 2), exhibiting a 90% rise in the last 12 years (2).

The association of many chronic diseases and their risk factors with lifestyle, nutrition and health awareness has been well documented (3,4). Evidences from prospective observational studies and clinial trial performed over the past couple of decades emphasize on low glycemic index carbohydrates and vegetarian style dietary pattern for prevention against obesity, type 2 diabetes mellitus and cardiovascular diseases (5). The rise in the incidence of type 2 diabetes is closely linked to the increase in obesity. Approximately 90% of type 2 diabetes is attributable to excess weight especially abdominal obesity which leads to impaired glucose intolerance and metabolic syndrome (6). According to the reports of Turkish Diabetes, Hypertension, Obesity and Endocrinology Diseases Prevalence Studies 2016, TURDEP II (2), incidence of obesity (BMI ≥30) for Turkish adult males and females was found to be 23,9% and 35,0% respectively.

Apart from factors such as obesity, gender, physical inactivity, smoking and low birth weight; socioeconomic status (7,8) and educational level (9) have also been described as risk factors implicated in the development of Type 2 DM Considering the fact that obesity prevalance is also considerably high in Turkey (10,11) lifestyle-intervention programs for increasing awareness may be of special significance in individu-

als with obesity related impaired glucose tolerance and those exhibiting high risk for the development of DM.

This research was planned as an observational cross-sectional study, in order to determine the major factors associated with the incidence of Type 2 DM namely gender, age, education and income level in a population diagnosed with the disease. Obesity has been shown to have a direct relation with Type 2 DM in several studies (6,12). So obesity factor was left out of the parameters considered in this study.

Methods

The study was carried out in Beykoz Public Hospital Tepeustu, one of the two public hospital in the Beykoz county, located on the Anatolian part of Istanbul with a population of about 251.087 (13), having a mixed socio-economic status. All patients visiting the Diabetes Polyclinic of Internal Medicine Department of the Hospital and diagnosed with Type 2 DM as per standardized diagnostic criteria (14) during 2011-2017 years were included in the study (n=18970). Information regarding demographic properties were recorded (Table 1). Level of education (Table 2) and income level (Table 3) were recorded in the hospital database based on respondent's self report. Incidence of Type 2 DM based on gender, age group, level of education and income was evaluated from the database.

Education was categorized into five groups. The first group consisted of individuals who stated having no formal education in school, followed by subjects having 4 years of primary school education, 4 years of secondary school, 4 years of high school education and university graduates.

Based on TEKSIF (Turkish textile, weaving, garment, leather industries labour union) declaration and national wage level classfication (15,16), mimimum monthly wage for an individual to be able to cross the poverty level is approximately 1000 TL. Therefore in this study, personal monthlyincome was classfied into four categories; upto 1000 TL/month (poverty level), 1001-2000 TL/month (lower middle category), 2001-3000 TL/month (upper middle category) and 3001-4000TL/month and above (upper category).

Ethical Issues

The data belonging to the patients have been included in this study with their permission and their identities were kept confidential. This study was approved by the Ethical Committee of Istanbul Aydin University.

Statistical Analyses

SPSS (Statistical Package for the Social Sciences) package program (version 18) was utilized for statistical analyses of the obtained data comprising demographic characteristics (age, gender, education, and income level) of the patient population. Descriptive statistical analyses namely frequencies (n), and percentages (%) were calculated where applicable.

Results

Total number of subjects included in the study was 18970 out of which 7827 (41,26%) were males. Based on age and gender, Type 2 DM incidence was mostly seen in women in the age group of 61-75 years (31,29%) followed by men within the same age group (16,35%). The incidence of Type 2 DM rose noticeably at 46-60 years in both genders and peaked at 61-75 age group. (Table 1, Figure 1).

When evaluated on the basis of education, incidence of Type 2 DM was highest in the individuals with primary school education (37,97%) followed by individuals having no formal education (32,43%). Incidence of Type 2 DM fell drastically among subjects who were high school (5,78%) and university graduates (1,93%). (Table 2)

In Table 3, incidence of diabetes among the patients have been listed against income categories. According to the data the incidence of Type 2 DM increased as income decreased with highest percentages (36,56%) of the disease recorded among patients belongining to the poverty level category followed by lower middle income category (Table 3).

Table 1. Incidence of Type 2 DM According to Demographic Characteristics

	Incidence of Type 2 DM (n=18970)			
Age Group (years)	**Males**		**Females**	
	n	**%**	**n**	**%**
30-45	328	1,73	562	2,96
46-60	1957	10,32	1789	9,43
61-75	3102	16,35	5936	31,29
76 and above	2440	12,86	2856	15,06
TOTAL	**7827**	**41,26**	**11143**	**58,74***

* Incidence of Type 2 DM in females were significantly higher than males significantly higher as compared to males (p<0,05)

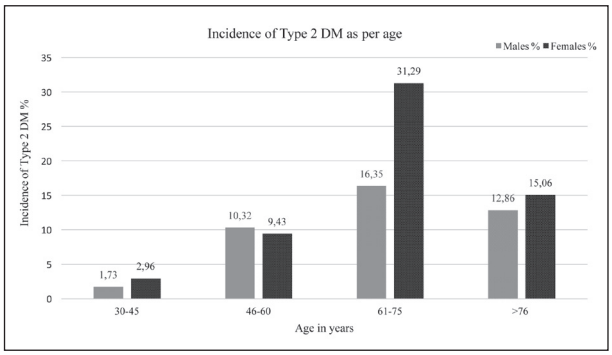

Figure 1. Incidence of Type 2 DM (%) as per age and gender

Table 2. Incidence of Diabetes and Level of Education

	Incidence of Type 2 DM (n=18970)	
Education	**n**	**%**
No formal Education	6152	32,43
Primary School Graduate	7204	37,97
Secondary School Graduate	4153	21,89
High School Graduate	1095	5,78
University Graduate	366	1,93
TOTAL	**18970**	**100**

Table 3. Incidence of Type 2 DM based on Personal Income Level

Income Level	**Income category**	**Incidence of Type 2 DM (n=18970)**	
		n	**%**
0-1000 TL	Poverty level	6936	36,56
1001- 2000 TL	Lower middle	5571	29,37
2001-3000 TL	Upper middle	4520	23,83
3001-4000 TL	Upper	1943	10,24
TOTAL	**TOTAL**	**18970**	**100**

Discussion

The aim of this study was to to determine the major demographic factors associated with the incidence of Type 2 DM in a population and important correlation was identified for factors such as gender, age, economic status and education. (Table 1,2,3, Figure 1).

In 2015, the total number of Turkish population suffering from diabetes mellitus was calculated to be approximately 7 million (9% of total population) of which 85% were diagnosed to have Type 2 DM. Also, difference in diabetes prevelance between males and females or rural and urban life was not found to be significant. (2). However in this study, out of 18970 patients diagnosed with Type 2 DM; incidence of the disease in females was significantly higher than in males (p<0,05). The findings are similar to a study performed in Nigeria investigating gender and age specific incidence and associated risk factors of type 2 diabetes mellitus on 3500 subjects (17). Type 2 DM incidences were higher in females (11,2%) as compared to males (9,6%). Also, the incidence of the disease was highest in the most elderly age group (46-60 years) age group. In a population based study (n=28,788) by Turkish Diabetes, Hypertension, Obesity and Endocrinology Diseases (TURDEP) investigating factors affecting type 2 DM, it was found that education level in men and socio-economic status in women had an effect on impaired glucose tolerance (IGT). Diabetes incidence was also found to be higher in women (p<0,0001) (18).

In this study the elderly groups comprised of individuals above 60 years of age and highest incidence of diabetes occurred in the elderly groups especially between 61-75 years of age (16,35% for males and 31,29% for females) (Figure1). According to TUIK (Turkish Statistical Institute) 2012 figures presented in International Diabetes Leadership Forum, 11% (approximately 8,4 million) of the Turkish population were 65 years of age and above and the Incidence of diabetes in this group was 34,8% (2,4 million) (19) rendering this group to be at a risk and increased predisposition to type 2 diabetes. Moreover, the demographic characteristics of the elderly population in Turkey and the World indicate that majority of older persons are women, poor, lonely and have lower educational levels (20). Older people have been suggested to be vulnerable to malnutrition

for many reasons including physiological and functional changes that occur with age, lack of financial support and inadequate access to food as well as their inability to carry out their day to day activities including preparation of food and intake (21).

In this study, incidence of Type 2 DM decreased with the rise in educational level (Table 2). Of the patients diagnosed with type 2 diabetes, 70% had a education level of primary school or lesser. Literacy and health literacy level has been found to be considerably low in elderly Turkish population in other studies performed (22). Other studies also suggest that age and sex adjusted diabetes incidences are highest in a population with lower socio-economic status or in poorer countries as compared to wealthier countries (23-25). Type 2 DM can be managed well by proper diet, physical activity and lifestyle management, a need for education is essential for health awareness and self management of the disease.

In this study, prevelance of Type 2 DM also fell as the income level increased, with 75% of incidences in patients belonging to the two lowest income level categories having a monthly income of less than 2000 TL. In Turkey (Table 3). Minimum wages in Turkey in 2018 remain approximately at 1600 TL, whereas elderly pension may fall below 1000TL/month due to variable reasons as incomplete payment of premium and so on (15,16). These results are in accordance with several other studies relating diabetes and other chronic diseases with socio-economic status of a population (25-28).

Limitations of the Study

In this study, obesity (or BMI) was not included among the parameters to be considered because there is adequate literature relating BMI/Obesity with Type 2 DM. Apart from that, it would also be of interest to study how the studied parameters (age, income, level of education) correlate among themselves. On the other hand, income and level of education may be expected be strongly correlated to each other. Nevertheless, the study may be repeated on another group including these parameters and others such as ethnic group, marital status, rural or urban life, other chronic diseases as hypertension, cardiovascular diseases and so on.

Conclusion

Effective diabetes care requires a collaboration and active communication between the patient and healthcare professional. This cooperation should include the provision of training to enable the patient to fully understand his illness, effects of the illness on health, providing appropriate and timely information. In addition, diabetes involves a proper balance between medical treatment, nutrition, exercise, blood glucose monitoring and most of all a behavioral change on the aprt of the patient. The consequences of diabetes treatment are to a large extent dependent on whether the patient continues to have a healthy lifestyle and whether he maintains the motivation to adapt to diabetes treatment.

Considering the increasing prevelance of obesity related chronic diseases including diabetes, in Turkey, the following health reforms may be suggested. Health care policies aimed at promoting health awareness, health care reforms introducing health coverage for all, and finally as the study indicates poverty and lack of education are major contributing factors; effective policies aimed at decreasing poverty and increasing education level should be implemented in the lower strata of the society.

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Conflict of Interest

None stated by the authors.

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Effect of different music tempos on aerobic performance and recovery

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Summary. The aim of this study was to determine effect of different music tempos on aerobic performance and recovery period. Thirty-five healthy male students (age=22.63±2.94, body mass=71.40±10.71, height=178.24±9.95) who were studying at Faculty of Sport Sciences voluntarily participated in this study. Participants carried out Bruce treadmill test with 72 hour intervals under three conditions: slow tempo music (100 bpm), fast tempo music (140 bpm) and no music. Anova was applied for statistical analysis. Fast tempo music (18.28±3.01 minute) was determined to improve running time by 4.63% and 3.10% compared to no music (17.47±2.83 min) and slow tempo music (17.73±3.09 min), respectively ($p<0.01$). Slow and fast tempo music did not have any effect on heart rate (HR) and rate of perceived exertion (RPE) during and after exercise ($p>0.05$). Lactate accumulation during 15 min recovery was found lower with slow tempo music compared to fast tempo and no music conditions ($p<0.05$). As a result, it can be concluded that fast tempo music improved running time and performance by creating ergogenic effect while slow tempo music led to fast decreasein heart rate and lactate accumulation during 15 min recovery.

Keywords: Fast music, Heart rate, Lactate, Running time, Slow music

Introduction

Music, an important part of human life, is known to be important for physical and psychological healing (1,2), accelerate and decelerate brain wave, coordinate movements with muscle tension and create anxiolytic effect (3). In addition, the effect of music on movement is defined as miscellaneous as psycho-emotional supporting coordination of repeated cognitive, affective, motor movement patterns during exercise (4). Music has been used to relax people for a long time. Music is a reliable, effective and non-pharmacological painkiller and tranquilizer. It has been reported that music decreases the need for ataractic drug (5), therapy with music decreases anxiety, increase life quality and is effective to decrease pain (6-8). Many ideas have been suggested to explain the strength which makes athletes to participate long duration physical activities and continue this for a long time (9). The latest one is music which is thought to be effective in athletic performance. Accordingly, the number of studies investigating ergogenic effects of combination of music and physical activity has increased (10). Because music has been used in different areas and successful results have been obtained, its effects on sport practitioners have been investigated and some studies highlighted its positive effects on athletes in terms of physiological and psychological aspects (11,12). While many studies have investigated effects of music pre and during exercise (13, 14) there is not enough number of studies investigating effect of music on recovery following a strenuous exercise. Some of these studies suggested sedative music at least 15 min following an exercise session to stimulate recovery (15, 16). Thus, the aim of this study was to determine effect of different tempos music on aerobic performance and recovery.

Materials and Methods

Participants

Thirty-five healthy male students (age=22.63±2.94, body mass=71.40±10.71, height=178.24±9.95) who were studying at Faculty of Sport Sciences voluntarily participated in this study. Participants who has a chronic disease or regularly uses medicine were excluded from the study.

Measurements

Fifty fast and slow English songs were determined by the researcher before the measurements. Then, the loudness of the music was set at 82 decibels (17) and beats were set at 100 bpm (beat per minute) for slow music tempo and 140 bpm for fast music tempo (18). Songs were sent to participants via e-mail and they were requested to choose 5 songs for both fast and slow tempos they liked most and send back them to researcher. Each participant involved in measurements 3 times with 72-hour intervals under three condition: fast tempo music, slow tempo music and no music. Blood pressure and heart rate were monitored pre and post-test and during recovery with Omron 10 and Polar Team, respectively. Blood lactate measurement was performed with Lactate Scout + lactate analyzer. When participants arrived at the laboratory, they were requested to sit for 15 min to determine resting values of heart rate and blood pressure. Participants listened to the music they chose during the test and recovery for 15 min with earphone. Heart rate (HR) was recorded before, after and at 5th, 10th and 15th min during the test. Borg Scale was used to determine rate of perceived exertion (RPE) at 5th, 10th and 15th min during the test and following the test. Participants state their RPE using a chart (between 6-20) (19). Running performance was tested using a treadmill (RAM 770-M, CAMIN, Italy) with Bruce protocol. Bruce treadmill protocol is a test starting with 2,7 km/h speed and 10% grade and continue with increasing speed and grade every 3 min (20). When participants finished the test, they were seated on a chair for recovery with listening to selected music for 15 min. Meanwhile, peak HR, blood pressure and lactate accumulation were measured. HR, systolic and diastolic blood pressures were recorded 5th, 10th and 15th min of recovery. Blood lactate was measured at the end of the recovery (15th min). When tests were implemented under the third condition they did not listen to or were exposed to any music during the test and were seated for 15 min during recovery in a quiet environment.

Statistical Analysis

Data were analyzed with SPSS-23. Normality of the data was determined using Shapiro Wilk Test. As data was normally distributed, Repeated Measures Anova was used. When any difference was found, Bonferroni Test was applied to see which group caused the difference. The significant level was set at 0.05 and 0.01.

Results

This study aimed to determine the effect of music with different tempos on aerobic performance and recovery duration in healthy active males. Performance variables under no music condition were compared with those during fast tempo music and slow tempo music in terms of aerobic running performance, HR and RPE during the test and HR, blood pressure and blood lactate accumulation during the recovery.

Effect of different music tempos on running performance is presented in Figure 1. It was observed that fast music tempo (18.28±3.01min) improved running duration compared to slow music tempo (17.73±3.09 min) and no music (17.47±2.83min) at percentages of 3.10% and 4.63%, respectively ($p<0.01$). No significant difference was found between slow tempo music and no music ($p>0.05$).

Figure 2 presents effects of different music tempos on HR and RPE during the test. It was observed that HR and RPE at 5, 10 and 15th min of test and

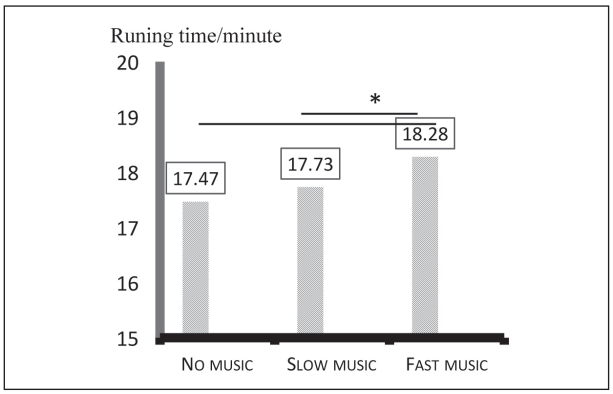

Figure 1. Running performance values under three conditions. Significant differences shown with asterisk (*$p<0.01$).

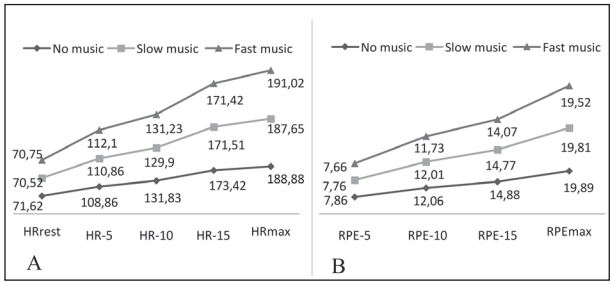

Figure 2. Heart rate (HR) (A) and rate of perceived exersion (RPE) (B) values pre, post and 5th, 10th and 15th min of exercise under 3 different conditions.

post-exercise were not affected by different music tempos (p>0.05).

Resting values of HR, blood pressure, post-test maximal HR, blood pressure, lactate accumulation and HR, blood pressure and lactate accumulation under three condition can be found in Table 1.

Table 2 presents HR, blood pressure and percentage of decrease in lactate accumulation of the participants during 15 min recovery.

Discussion

This study was implemented to determine effect of different music tempos on aerobic performance and recovery time. Fast tempo music increased running performance 3.10% and 4.63% compared to no music and slow tempo music, respectively. However, HR and RPE during and at the end of the test were not affected by different music tempos. In a similar study with Bruce protocol by Karageorghis et al. (2009), while running time was 17.84 min with motivational music, running times were 16.84 and 15.56 for non-motivational music and no music, respectively. They also

Table 1. Physiological variables of the participants pre and post-test and during recovery.

	No music (1)	Slow music(2)	Fast music (3)	Sig. Diff.
	Mean±Sd	Mean±Sd	Mean±Sd	
RestHR	71.62±11.48	70.52±9.95	70.75±8.88	0.143
RestSBP	118.15±9.23	123.89±8.5	120.45±8.23	0.346
RestDBP	71.33±6.32	70.66±7.41	72.45± 6.83	0.928
PeakDBP	79.63±6.65	81.10±6,83	81.02±5.77	0.364
PeakSBP	160.16±15.18	158.30±12.23	161.24±11.89	0.547
PeakHR	188.88±9.19	187.65±9.38	191.02±10.21	0.935
PeakB[LAC]	13.31±2.81	12.64±2.68	12.97±2.45	0.169
RecB[LAC]15min	9.94±2.06	8.82±1.95	9.56±0.98	0.017* 2<1,3
Rec HR-5min	105.94±10.31	105.00±13.25	103.03±11.78	0.626
RecHR-10min	105.00±11.06	100.78±12.80	102.55±10.16	0.093
Rec HR-15min	102.68±9.73	98.42±10.06	101.43±10.11	0.082
RecSBP5min	126.00±10.60	126.78±12.80	125.44±9.56	0.788
RecSBP10min	122.72±8.03	122.66±7.32	121.28±4.56	0.748
RecSBP15min	121.05±10.63	122.15±9.94	120.47±5.43	0.825
RecDBP5min	69.63±4.05	71.10±3.87	69.94±3.04	0.276
RecDBP10min	71.16±7.11	71.55±4.75	70.77±3.63	0.900
RecDBP15min	71.47±5.41	73.21±7.56	69.78±2.76	0.429

*P<0.05.Sd:Standard deviation. RestHR: Heart rate during resting. PeakB[LAC]: Peak blood lactate after maksimal exercise. RecB[LAC]15min: Blood lactate in minute 15 during recovery. RecHR-5min: Heart rate in minute 5 during recovery. RecHR-10min: Heart rate in minute 10 during recovery. RecHR-15min: Heart rate in minute 15 during recovery. RecSBP5min: Systolic blood pressure in minute 5 during recovery. RecSBP10min: Systolic blood pressure in minute 10 during recovery. RecSBP15min: Systolic blood pressure in minute 15 during recovery. RecDBP5min: Diastolic blood pressure in minute 5 during recovery. RecDBP-10min: Diastolic blood pressure in minute 10 during recovery. RecDBP15min: Diastolic blood pressure in minute 15 during recovery. Diff. : Diffrence

reported that motivational music improved running performance by 15% compared to no music condition and music had no effect on RPE. It was concluded by the same researchers that music improved endurance performance during exercise (21). Likewise, Eliakim et al. (2012) also stated that music has positive effect on aerobic components but also highlighted that it did not affect RPE (22). Tenenbaum et al. (2004) implemented treadmill test on participants at 90%of their VO2max under 4 different music condition (no music, rock, sedative and dance) and stated no significant effect on RPE and HR (23). Birnbaum et al. (2009) stated that music (fast, slow and no music) had no effect on systolic-diastolic blood pressure, HR and RPE during 15 min steady state running exercise on a treadmill (24). Most of the studies stated no significant effect of music on HR during exercise (25-27). Likewise, we determined that music did not affect HR responses during the exercise. It was reported that female football players performed a treadmill exercise with increasing speed and grade and running time, HR_{max} and RPE and HR during the exercise were not affected by self-selected fast music (28). Terry et al. (2012) applied a study on 11 elite triathlon athletes where self-selected music improved exhaustion time by 18.1%, 19.7% with motivational music and neutral music, respectively compared to no music. Moreover, emotional state and mood were found more positive with motivational music compared to other two conditions and music decreased oxygen consumption by 1-7% and improved running economy (29). Another study reported that music increased running time and fast tempo music was found more effective than slow tempo music (12). Our findings also indicate that fast tempo music improved running performance compared to slow tempo music. Ghaderi et al. (2009) reported that fast tempo music increased running time by 41% compared to slow tempo music but aforementioned music tempos did not enhance running performance compared to no music condition (30). In contrast to the studies in the literature, some researchers stated that music did not increase running distance and time and concluded that music does not affect sub-maximal performance of individuals (28, 31, 33). Different results suggested by different studies may stem from the type of music, fitness level of the participants, music tempo lacking motivational stimulus.

Table 2. Rate of decrease between peak values after exhaustion exercise and recovery values after 15 min recovery.

	PeakB[LAC]	**RecB[LAC]15min**	**%Δ15**
	Mean±Sd	**Mean±Sd**	
No music	13.31±2.81	9.94±2.06	33.90
Slow music	12.64±2.68	8.82±1.95	43.31
Fast music	12.97±2.45	9.56±0.98	35.66
	PeakHR	**RecHR15min**	**%Δ15**
No music	188.88±9.19	102.68±9.73	83.95
Slow music	187.65±9.38	98.42±10.06	90.66
Fast music	191.02±10.21	101.43±10.11	88.32
	PeakSBP	**RecSBP15min**	**%Δ15**
No music	160.16±15.18	121.05±10.63	32.30
Slow music	158.30±12.23	122.15±9.94	29.59
Fast music	161.24±11.89	120.47±5.43	33.84
	PeakDBP	**PeakDBP15min**	**%Δ15**
No music	79.63±6.65	71.47±5.41	11.41
Slow music	81.10±6.83	73.21±7.56	10.77
Fast music	81.02±5.77	69.78±2.76	16.10

%Δ15: Percentage of decrease 15 min after exercise

There was no significant difference in recovery HR and systolic and diastolic blood pressure at the 5th, 10th and 15th min of recovery between slow, fast tempo music and no music ($p>0.05$). however, slow tempo music enhanced removal of the lactate during recovery compared to fast tempo music and no music ($p<0.05$). While percentage of lactate removal was 43.31% with slow tempo music, it was 33.90% with no music condition and 25.66% with fast tempo music at the 15th min of recovery. HR decreased by 90.66% with slow tempo music while it decreased by 83.95% with no music condition and 88.32% with fast music tempo. Eliakim et al. (2012) stated no effect of music on HR and lactate removal during recovery following an exhaustion exercise but highlighted a faster lactate removal with music (28.1%) compared to no music condition (22.8%). Moreover, lactate removal increases with music for 12-15 min (15). Tan et al. (2014) stated that listening to sedative music for 15 min after an exercise did not enhance recovery in HR compared to no music (34). Karageorghis et al. (2018) suggested that slow tempo and sedative music enhance recovery following an exhaustive exercise in terms of hemodynamic (blood pressure and HR) parameters in spite of a clear explanation (35). Knight and Richard

(2001) indicated that sedative music hampersincrease in subjective anxiety, systolic blood pressure and HR (36). According to literature, music decreases HR by increasing vagal activity and decreasing sympathetic activity (37) while sedative music is thought to lead to fast recovery because it indirectly affects autonomous system by activating hormones that decreases stress by affecting pituitary part of the brain (16). Some studies highlighted that music solely cannot enhance recovery in healthy subjects although it enhance recovery in HR and systolic blood pressure after aerobic exercise (38). In contrast to Gomes et al. (2018), Savitha et al. (2010) stated that slow tempo music during recovery decreased systolic and diastolic blood pressure and HR faster and concluded that sedative music is a very useful tool to enhance recovery after exercise (39). In our study, slow tempo music did not affect recovery in systolic and diastolic blood pressure and HR but it significantly enhanced rate of lactate removal.

Conclusion

Currently many studies have been conducted in sport sciences to increase performance and enhance recovery during/after exercise. The importance of music increases in exercise to create an ergogenic effect on athletes in terms of psychologically, physiologically and physically. In this study, it was observed that fast tempo music increased running time which is a sign of endurance compared to slow tempo music and no music. Slow tempo music was observed to lead blood lactate and HR to turn normal values faster. As a result, it can be concluded that fast music can be used to motivate, activate athletes to increase endurance and provide an ergogenic aid while slow tempo music can be advised to enhance recovery after exercise.

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Quality evaluation and pollen profile of honey samples from different locations

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Summary. In this study, it is aimed to determine some physicochemical properties and pollen types of honey samples collected from different regions. Pollen profile, moisture content, ash, electrical conductivity, frutose/glucose, HMF and proline were the parameters analysed in each honey sample. According to the results obtained, all honey samples obtained from different geographic origins were found to be consistent, in terms of the parameters analyzed, with the values given in the Turkish Food Codex Honey Communiqué. It was found that only one honey sample did not comply with the values given in the codex in terms of F/G ratio. According to the results of the melissopalinological analysis, three honey samples were found to be unifloral (two chestnut and one tragacanth) and the others were found to be polyfloral honey. The results obtained indicate that physicochemical properties of the honey samples produced at different points in Turkey differ greatly in accordance with the diversity of the flora of the region.

Keywords: Honey, melissopalynology, proline, HMF, electrical conductivity

Introduction

Beekeeping is one of the most widely carried out agricultural activities today due to the importance of the products obtained. Honey, pollen, propolis, royal jelly, bee bread, bee venom and apilarnil are among the products produced as a result of this activity. These products are used mainly by people through health-protective functional properties they own. Therefore, different researches have been conducted on the physicochemical properties and biological activities of different bee products (1-6). Honey is the natural sweet substance produced by honey bees from the nectar of plants or from secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honey comb to ripen and mature (7). Honey has an important place in traditional medicine for centuries. The chemical composition of honey is complex, containing approximately 181 substances including proteins, moisture, sugars, minerals, enzymes, 5-hydroxymethylfurfural (HMF), vitamins, flavonoids, phenolic acids and volatile compounds (8). Individual groups of honey (blends, blossom, and honeydew) vary significantly in aroma, colour and taste. There are also differences in the chemical composition which are reflected in many physicochemical properties, such as in the content of ash, electrical conductivity, the spectrum of sugars, the activity of enzymes and pollen types (9).

Chemical and physical properties of different types of honey have been reported by many scientists (10, 11). Criteria such as moisture, water-insoluble content, free acidity, proline, electrical conductivity, HMF, diastase number, fructose/glucose, fructose+glucose, and the amount of naphthalene are used to determine the quality of honey (12). These characteristics of honey vary according to their plant and geographical origin, but are also influenced by certain external conditions such as season, processing, packaging and storage (13). Therefore, the present work was conducted to characterize the quality of honey samples from Turkey (n = 12) in terms of plant sources and physicochemical properties.

Material and Methods

Collection of honey samples

Honey samples of honey bee (*Apis mellifera* L.) were obtained from apiaries at 12 different province of Turkey in 2018 for determination of quality parameters and pollen types. All samples were stored at room temperature until analysis.

Pollen analysis

The pollen analysis of honey samples were was based on the method described by Louveaux et al (1970) (14). Pollen types were classified into three categories: dominant pollen (≥ 45%, D), secondary pollen (16–44%, S), important minor pollen (> 3–15%, M) and trace polen (3%<). When one pollen type represented > 45% of the total number of pollen grains, the sample was classified as a monofloral honey. Although this classification is a generalization, for example, for the classification of chestnut honey as a monofloral honey it must contain 70% to 90% of *Castanea* pollen (15).

Physicochemical analysis

Moisture, sugar, proline and hydroxymethylfurfural (HMF) analyzes were performed according to the IHC (2009) (16). The electrical conductivity measurement of honey samples was carried out by using the method developed by Sancho et al. (1991) (17).

Results and Discussion

In this study, pollen analysis of honey samples collected from 12 different provinces were carried out and plant taxa which were the source of these honeys were identified (Table 1). As a result of microscopic analysis of honey samples, pollen belonging to different families were identified at different rates. Most of these taxa belong to Fabaceae family. This indicates that bees prefer plants of this family in the regions where honeys are obtained and use them as nectar sources. Honey samples obtained from Bursa, Zonguldak and Tunceli provinces are grouped as unifloral honey because they contain dominant pollen grains, and these honeys are defined as *Castanea sativa* honey for honeys from Bursa and Zonguldak and *Astragalus* spp. honey for Tunceli honey. As a result of melissopalinological analyzes, honey samples obtained from 9 other regions were grouped as polyfloral honey. Fabaceae, Asteraceae, *Castanea sativa*, Cistaceae, *Cistus* spp., Fabacae, *Onbrychis vicifolia*, *Trifolium repens* and *Trifolium* spp. were among the taxa represented by secondary pollen in different honey samples. The pollens of *Brassica* spp., Apiaceae, *Astragalus* spp., Boraginaceae, Brassicaceae, Caryophyllaceae, *Castanea sativa*, *Centaurea* spp., *Cornus* spp, *Dianthus* spp., *Echium* spp., *Eryngium* spp., Fabaceae, *Hedysarum* spp., Lamiaceae, *Medicago* spp., *Melilotus alba*, *Melilotus* spp., *Minuartia* spp, *Nepeta* spp., *Rosa canina*, *Rosa* spp., Rosacaee, *Salix* spp., *Teucrium* spp., *Trifolium* spp. and *Verbascum* spp. taxa were represented in the honey samples in minor rate. In addition, pollens of 56 taxa were detected in trace amounts in honey samples. Similar to our study, melissopalinological examinations were conducted using honey samples from different locations of Turkey (18-21). Mercan (2007) (22) in their study, the pollen types of honey samples produced in the Aegean Region and around have determined. Pollen from Chenepodiaceae, *Trifolium* spp., *Trigonella* spp., Cyperaceae, *Zea mays* and *Anthemis* spp. were the most common ones. Dalgıç (1994) (23) examined 50 honey samples collected from different provinces of the Aegean Region between 1991 and 1993 in terms of biochemical and palynological. It was determined that the most common taxa of pollen in honey samples of this region were Fabaceae, Lamiaceae, Apiaceae, Brassicaceae families and *Helianthus annuus* from Asteraceae family, *Cistus* spp. from Cistaceae family and *Castanea sativa* from Fagaceae family. Bağcı and Tunç (2006) (24), as a result of pollen analysis of 21 honey samples collected from Konya region, Apiaceae, Rosaceae, Fabaceae (*Astragalus* spp., *Trifolium* spp., *Lotus* spp., *Onobrychis* spp.), Asteraceae (*Carduus* spp., *Centaurea* spp., *Achillea* spp., *Tragopogon* spp.), Brassicaceae (*Brassica* spp.), Lamiaceae (*Mentha* spp., *Salvia* spp.), Plantaginaceae (*Plantago* spp.) and Scrophulariaceae (*Linaria* spp.) determined that the important honey plants. Kaplan (1993) (25) deteced the pollen of Fabaceae, Brassicaceae, Rubiaceae, Euphorbiaceae family, *Salix* from Salixaceae, *Ranunculus* spp. from Ranunculaceae and *Centaurea triumfetti* from Asteraceae as the dominant in 24 honey samples collected from Konya region. Similar to this study, we identified pollen from different plant species in addition to the pollens belonging to

Onobrychis spp., *Astragalus* spp., *Salix* spp., Fabaceae and *Trifolium* spp. taxa in honey samples from Konya region. Göçmen and Gökçeoğlu (1992) (26) stated that the plants that contain the most nectar in the Bursa region are *Castanea sativa* from Fagaceae, *Helianthus annuus* from Asteraceae, *Daucus carota* from Apiaceae, *Rosa* spp. from Rosaceae, *Trifolium* spp. from Fabaceae and *Tilia argentea* from Tiliaceae. Similarly, in this study, we found that *Castanea sativa* pollen was dominant in the honey sample of Bursa origin and pollen belonging to *Cistus* spp., Fabaeae and *Rosa* spp. taxa were detected in trace rate. In a different research, Apiaceae, *Eryngium* spp. and *Scandix* spp. taxa pollen were found in minor and trace amounts in 18 of 29 honey samples belonging to Erzincan region (27). It can be stated that the similarities and differences between our study and other studies stem from the floristic diversity of the regions where honey samples were obtained. The fact that bees visit different plant species rather than a single one as the source of nectar while creating honey indicates that this affects the taste, aroma, color and physicochemical properties of the honey. In addition to this, the type and proportion of secondary, minor and even trace amount plant pollens explain that each honey has different physicochemical

Table 1. The pollen spectrum of honey samples

Location	Dominant	Secondary	Minor	Trace
İstanbul	-	Fabaceae Asteraceae	*Castanea sativa*	*Achillea millefolium* Apiacae *Anchusa* spp. *Brassica* spp. *Calluna* spp. *Centaurea* spp. *Cerinthe* spp. Dipsaceae Lamicaeae *Geranium* spp. *Malva* spp. *Onobrychis vicifolia* *Silene* spp. *Trifolium repens* *Trifolium* spp.
Sakarya	-	*Trifolium* spp.	Boraginaceae Brassicaceae *Castanea sativa* Lamiaceae *Melilotus* spp. Rosacaee	Apicaeae *Cistus* spp. *Eryngium* spp. Liliaceae *Mentha* spp. *Ornithogalum* Pinaceae *Ranunculus* spp. *Rosa* spp. Rubiacae *Salix* spp. *Tanacetum* spp. *Teucrium* spp. *Trifolium* spp.
Konya	-	*Onbrychis vicifolia*	*Verbascum* spp. *Hedysarum* spp.	*Astragalus* spp. *Centaurea* spp. Chenepodiaceae *Heliansthu*s spp. *Lotus* spp. *Medicago* spp. *Salix* spp. *Trifolium* spp.

Continue on the next page

Table 1. The pollen spectrum of honey samples

Düzce	-	*Castanea sativa* *Trifolium repens*	*Brassica* spp. *Cornus* spp. Fabaceae *Melilotus alba* *Rosa canina*	*Acer* spp. Caryophylaaceae Lamiaceae *Pinus* spp. Rosacaeae *Salix alba* Scrophulariaceae
Bursa	*Catanea sativa*	-	-	*Cistus* spp. Fabacaee *Rosa* spp.
Trabzon	-	*Castanea sativa* *Cistus* spp. Fabaceae	Lamicaeae Rosaceae	Apicaeae Brassicaceae *Campanula* spp. *Hedysarum* spp. *Plantago* spp. Poaceae *Rubus* spp. *Rumex* spp. Scrophulariaceae
Zonguldak	*Catanea sativa*			Fabaceae Lamicaeae
Çanakkale	-	Fabacae	*Castanea sativa*	Apicaeae *Cistus* spp. *Olea* spp. *Papaver* spp. *Quercus* spp.
Balıkesir	-	Fabaceae	*Castanea sativa* *Echium* spp. *Trifolium* spp.	Apiaceae Asteraceae Boraginaceae *Cistus* spp. *Helianthus annuus* *Olea* spp. *Pinus* spp. Rosaceae *Stachys* spp. *Verbascum* spp.
Erzincan	-	*Astragalus* spp. Fabaceae *Onobrychis* spp.	*Centaurea* spp. *Dianthus* spp. *Eryngium* spp. Lamicaeae *Minuartia* spp. *Nepeta* spp. *Trifolium* spp. *Teucrium* spp. *Medicogo* spp.	Apiaceae Betula spp. Caryophyllace Liliaceace *Pinus* spp. *Quercus* spp. Rosacae Rosaceae *Taraxacum* spp.
Tunceli	*Astragalus* spp.		Caryophyllaceae Rosaeae *Salix* spp.	Apiacaee *Centaurea* spp. Liliaceae *Rumex* spp.
Şırnak	-	Fabacea Cistaceae	Apiaceae *Astragalus* spp. Lamiaceae *Rosa* spp.	Apiaceae Asteraceae Boraginaceae Caryophyllacace Cucurbiatace

properties. These differences might stem from the synergistic energy created by all these pollens that contribute to the formation of honey while contributing to honey.
The moisture content of the honey which is harvested after complete maturation is expected to be low. Water content of honey varies depending on harvest period, climatic factors and the degree of maturity reached in hive (28). As the moisture increases in honey, which is an important foodstuff, its quality decreases and the risk of fermentation increases. Therefore, ascertaining the moisture content of honey is one of the parameters used in determining the quality of honey. When the honey samples used in our study were examined, it was found that the moisture content in general varied between 15.3% and 18.9% (Table 2). Among the samples examined, honey samples with the lowest and highest moisture content were from Erzincan and Trabzon, respectively. The main sugars contained in honey are fructose and glucose, and in addition to these monosaccharides, it also contains disaccharides such as sucrose, maltose, isomaltose, lactose, and some oligosaccharides (29). The total amount/proportion of glucose and fructose in the content of honey is one of the parameters commonly used to detect adulteration in honey. In this study, the sugar ratios of honey samples were determined between 0.9 and 1.5, except one honey sample obtained from one region (Çanakkale) the sugar ratios of the samples were found to be compatible with the standard value (0.9-1.4) given in the Turkish Food Codex Honey Communiqué (2012) (12).

HMF amount is another important parameter for the evaluation of freshness and quality of honey, and generally not present in fresh honey, its content increases during conditioning and storage (30). In the Turkish Food Codex Honey Communiqué (2012) (12), it is stated that the HMF content of honey should be maximum 40 mg/kg in the blossom honey, and our results are consistent with this value. HMF contents of honey samples used in our study were found to be between 1.26-26.28 mg/kg and the highest rate was found in the honey sample obtained from Balıkesir region. Similarly, in their study conducted to determine the biochemical properties of highland honey and sunflower honey, Şahinler and Gül (2004) (31) determined the average HMF amounts of highland honey and sunflower honey as 5.73±0.18, 2.17±0.10 mg/kg, respectively and reported that all the samples were in accordance with the criteria specified in the Turkish Food Codex Honey Communiqué (2012) (12). Likewise, the amount of HMF was found in the range of 0.19-41.16 mg/kg in 49 different honey samples which were sold commercially in Southern Spain and were not heat treated, and it was stated that high amount of HMF resulted from the climatic conditions of Southern Spain (32).

In addition, proline values of honey samples were calculated in our study. According to this, proline values of honey samples were determined as 654 mg/kg (İstanbul), 712 mg/kg (Sakarya), 556 mg/kg (Konya), 949 mg/kg (Düzce), 740 mg/kg (Bursa), 652 mg/kg (Trabzon), 1055

Table 2. Quality characteristics of honey samples

	Location	Moisture (%)	HMF (mg/kg)	Proline (mg/kg)	F/G	Electrical conductivity (mS/cm)
1	İstanbul	18.3	6.44	654	0.9	0.49
2	Sakarya	17.6	3.52	712	1.1	0.47
3	Konya	16.5	11.34	556	1.3	0.26
4	Düzce	16.2	10.16	949	1.1	0.32
5	Bursa	16.0	4.80	740	1.3	0.92
6	Trabzon	18.9	8.86	652	1.0	0.11
7	Zonguldak	18.1	7.38	1055	1.2	0.98
8	Çanakkale	16.7	14.16	596	1.5	0.74
9	Balıkesir	17.3	26.28	728	1.3	0.70
10	Erzincan	15.3	4.99	628	1.0	0.25
11	Tunceli	16.2	18.24	856	1.4	0.56
12	ırnak	16.4	1.26	846	1.0	0.74

mg/kg (Zonguldak), 596 mg/kg (Çankkale), 728 mg/kg (Balıkesir), 628 mg/kg (Erzincan), 856 mg/kg (Tunceli) and 846 mg/kg (Şırnak). The lowest proline value was determined to be 556 mg/kg in Konya honey and the highest value was determined to be 1055 mg/kg in chestnut honey (Zonguldak). Proline, which is regarded as one of the quality criteria of honey, is an amino acid that comes from honey bees and mixes with honey during the processing of nectar and shows the maturity of honey. Therefore, it is a parameter that reflects the botanical origin of honey (33, 21). The free amino acid concentration in honey is 100 mg/100g in average and 50-85% of the total amino acid amount is composed by proline (34). The results show that the proline contents of honey samples are significantly different depending on the regions. In addition to this, the electrical conductivity values of honey samples used in our study were determined between 0.11-0.98 mS/cm (Table 2). Electrical conductivity is an important parameter used to determine the source of the honey. In our study, the electrical conductivity value of honey samples was found to be consistent with the values of Turkish Food Codex Honey Communiqué (2012) (12) and it was determined between 0.92-0.98 mS/cm for chestnut honey and 0.11-0.74 mS/cm for other honey samples. Many studies investigating the quality characteristics of the honey produced in different regions of Turkey and the world have been conducted in parallel to our study (19, 35, 36) . Similarly, in order to determine the quality of blossom honey samples, which are released to the market for consumption, it was determined that the average moisture content was 17.56%, the average fructose/glucose ratio was 1.22 and the average electrical conductivity value was 0.46 mS/cm in 50 honey samples (37). Muli et al. (2007) (38) examined the quality characteristics of 72 honey samples and the findings are as follows: the average moisture16.00%, HMF 3.70-389.36 mg/kg; proline 20.83-300.6 mg/kg. Ölmez (2009) (39) analyzed the moisture, glucose, fructose and HMF values of 8 different honey samples harvested in the same conditions from different regions of Turkey in 2006 and 2007 and the total of glucose and fructose was detected as 51.31-68.30%, HMF value as 1.34-31.28 mg/kg, moisture content as 17.1-20.0%. According to the results of our study, no honey sample has the same characteristics with another one, and therefore, it can be inferred that especially geographic differences affect the physicochemical properties of honey significantly. However, anthropogenic factors are also considered to have a significant effect on the physicochemical properties of honey.

Conclusion

It was observed that honey samples produced in different regions of Turkey, whose floral diversity was quite high, did not share the same quality. This can be explained by the differences in regions from which honey samples are collected and accordingly the diversity of plant species that make up the content of honey. Therefore, when the fact that the plant source has a significant effect on the physicochemical properties of honey is considered, making a plant source map of honey with regard to regions is very important in terms of revealing the differences of honey.

Conflict of interest

No potential conflict of interest relevant to this article was reported by the authors.

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Development of prebiotic galactooligosaccharide enriched buttermilkand evaluation of its storage stability

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Summary. Prebiotic Galacto-oligosacchrides (GOS) is reportedly present in human milk and elicits bacterial growth i.e. beneficial bacteria in human intestines. The aim of the present study was to produce GOS through trans-galactosylation and development of GOS based buttermilk. Trans-galactosylation process was carried out in pasteurized milk (100 °C) to produce prebiotic GOS. Yogurt was produced from GOS containing milk and then after the churning of yogurt buttermilk was produced from trans-glycosylated milk.Results showed that, enzyme concentration between 400-600µL/5ml can produce GOS in 30min and 1hr samples. Chemical analysis and sensory evaluation of plain/control and GOS containing buttermilk showed no remarkable difference. While shelf life study showed that there was no significant difference between the overall quality of buttermilk in glass and pouch packaging. Buttermilk in pouch packaging maintained its stability for 6 days without adding any kind of preservative.

Key words: Buttermilk, galacto-oligosaccharides, prebiotic, transgalactosylation

Introduction

Since 20th century greater emphasis is placed on diet and nutrition due to growing concern regarding the diet related diseases prevalence on global scale (1). The concept of "functional foods" is recently introduced, extensively investigated shortly after, and moved forward to somehow fully grown field. Given the widely accepted fact that functional foods are associated with health benefits (2) so they are prepared with specific, either alone or in combination, with more than one specific bioactive component, usually of plant origin. The advancement made in microbial enzyme technology has further modified functional food sector. Specific microbial enzymes can be used to modify, synthesize or convert foods constituents to more beneficial compounds within foodsthat can attain health benefits or at least decrease the risk of certain diseases (3). With this trend, trans-galactosidase enzyme is reportedly used that can convertmilk lactose to galactose-oligosaccharides (GOS), a well-known and valuable prebiotic (4). This reaction may be beneficial for increasing the milk tolerance for people with lactose intolerance andgrowth of gut microbiota (5).

Galacto-oligosacchrides is not digestible and can be fermented in the small intestine by microbiota (6). Growing body of evidences have shown that GOS is associated with reduction in cholesterol (7), diarrhea, lower intestinal inflammation and increases mineral absorption (8), improve immunity (9), and enhance the growth of beneficial microbiota (6), to name a few. In view of the importance of this dietary fibre, technologically and economically feasible processing methods and product development is necessary. This manuscript reports reliable, easy and cost effective methods for preparation of buttermilk with prebiotic which has comparatively low lactose content.

Materials and methods

D-Glucose (99%) (Merck, Germany), D-Galactose (99%) (Merck, Germany), Lactose (99%) (Mer-

ck, Germany), NaHPO4 (96%) (Merck, Germany), 1-Butanol (99%) (Merck, Germany), Ethanol (99%) (Merck, Germany), 2-Propanol, (99%) (Merck, Germany), H2SO4 (<96%) (Merck, Germany), NaOH (99%) (Merck, Germany), Thymol (90%) (Eyer®), oNPG (99) % (Sigma-aldrich, Germany) and LB broth (MP Biochem, France) were purchased.

Bacterial cell growth and enzyme production

β-galactosidase enzyme was isolated from *Escherichia coli (E.coli)*, which was initially from University of Natural Resources and Life Sciences Vienna Department of Food Science and Technology Division of Food Biotechnology (Vienna, Austria). This strain has gene of *Lactobacillus reuteri* L103 which has ability to produce more active enzymes than taken from normal non recombinant *E.coli*.

Bacterial strains and culture conditions

Escherichia coli containing β-galactosidase gene from *Lactobacillus reuteri*L103 was cultured as the source of the β-galactosidase enzyme. *E.coli* cells were grown on Luria-Bertani broth (LB) containing the appropriate antibiotics (100 μg/mL ampicillin) required for maintaining the plasmids. Bacterial culture was grown at 37 ˚C in incubator for 14-16 hours in 5 mL LB medium. Safety measures such asfiltration of lab through UV light, use of ampicillin and gloves were used to avoid contamination.

After the bacterial growth, 0.8 mlof the grown culture (culture A) was transferred into 40 mLLB medium and was grown again at 37 ˚C for 16 hrs. Then,culture A (2mL) was transferred into 2000 mLLB medium and was grown again at 37 ˚C until optical density of this media was between 0.3-0.6, then isopropyl-β-D-thiogalactoside (IPTG) was added (final conc. in media 0.1 mM), after adding IPTG, bacteria was grown at 25 ˚C for 16 hours. These three steps were carried out again and again until the requirednumbers of cells were collected.

Collection and cell breakdown

Cells (90 ml) were collected by centrifugation (Micro 200R, Japan) (5000rpm, 15min, 4˚ C) when lag phase of microbial growth was reached. The cells obtained were washed twice in 50mM sodium phosphate buffer pH 6.5 and suspended in same buffer and stored at 4˚ C. Supernatant was drained while cellswere breakdown with sonicator (Ultrasonic homogenizer) for 2 min at 13000 rpm and cell debris were removed by ultra-centrifugation (Centrifuge 5810R, Japan) (12000rpm, 20min, 4˚ C) to obtain the unpolished cell extract/ crude enzyme (35 mL).

Enzyme assays

β-Galactosidase (β-gal) activity was determined using O-nitrophenyl-β-D-galactopyranoside (ONPG) as the substrates (Nguyen et al. 2006). 20 μL crude enzyme sampleswere added to 480 μL of 22 mM ONPG in buffer (50 mM sodium phosphate buffer, pH 6.5). After 10 minutes, reaction was stopped by adding 750 μL Na_2CO_3 (0.4 M). The release of O-nitrophenol was assessed by measuring the absorbance at 420 nm.

Transgalactosylation experiment on buttermilk

Buttermilk15 mLwas taken in 3 separated falcon tubes. Reaction was started by adding enzyme β-galactosidase 50μL, 200 μL and 400 μL. Reaction were carried out for 4 hrs at 37˚ C and agitation (230 rpm) was applied by putting in shaker. Samples were withdrawn after 0hr, 30 mins, 1hr, 1.5hr, 2hr, 3.5hr, and 4hrs. The enzyme was deactivated by dipping in boiling water for 3 minutes.Similarly, from each time point 400 μL samples were drawn each time dipped in boiling water. The samples were then mixed with 180 μL, centrifuged again. After centrifugation, enzyme was settled at the bottom of falcon tubes while supernatant was kept in hot oven at 70º C for about one hour. After the oven treatment, 20 uL supernatant was diluted with180uLdistilled water and was used for TLC analysis.

Thin Layer Chromatography (TLC)

Almost 1.0 μL from each prepared solution, as mentioned above, along with standards (glucose, galactose, lactose) was taken and applied on silica gel plate, dried the plates for 20 minutes and placed in running buffer used in TLC was prepared by water, n-propanol, ethanol, and n-butanol in the ratio of 2:3:3:2 respectively. Silica gel plates were put in the chromatographic tank till the buffer reached the 2/3rd of the plate at the upper end. TLC was run for 45 minutes. After that, the plates were taken out of the chromatographic tank

and dried in the hot air oven at 100°C. Staining buffer (0.6 g thymol+5ml H_2SO_4(95%) + 95ml ethanol) was sprayed on the dried plates. Sugar stains were visible after 110 °C heating in hot air oven which shows the formation of glucose,galactose and GOS.

Analysis of buttermilk

Buttermilk was analyzed for the following parameters like fat, protein, Lactose, Ash and pH was determined according to the methods of AOAC (10).

Standards and buffers used for TLC

The standards used for thin layer chromatography were of following concentration; 20g/lGalactose, 20g/l Lactose, 20g/l Glucose. 0.02g of each standard samples were taken anddissolved in 1ml of distill H_2O. TLC staining buffer composition was: 0.6 g thymol+5ml H_2SO_4(95%) + 95ml ethanol.

Quantification of Galacto-oligosaccharides

Quantification of Galacto-oligosaccharides was done by measuring lactose, D-galactose and D-glucose through Megazyme kits (Megazyme International, Ireland) and detailed as:

D-glucose Assay conditions

About 3.0 ml of GOPOD reagent was added to 0.1 ml of buttermilk solution and incubated at 40°C to 50°C for 20 minutes. Absorbance was read against reagent blank at 510 nm wavelength in order to get values of ΔAsampleand ΔAD-glucose standard.While for the lactose and D-galactose, absorbance was carried out at 340 nm, and water was taken as blank.Total galacto-oligosaccharides produced were calculated by following formula:

GOS (g/L) =A – B+C+D

Where;A= Initial lactose concentration (g/L); B= Lactose concentration after transgalactosylation (g/L); C=Galactose concentration after transgalactosylation (g/L); D=Glucose concentration after transgalactosylation (g/L)

Preparation of buttermilk

The raw milk was inoculted with 2.5% of mixed culture of *Lactobacillus balgaricus* and *Streptococcus thermophillus* and filled into clean glass containers (250 ml volume) and incubated at 45° C for 3-4 hours. After the curd formation it was cooled to 4 °C (11).
Yogurt was then taken in a butter churner and was agitated vigorously until the whey was separated from the fat. Butter/ fat was removed and remaining liquid (which was buttermilk) was collected for further analysis.

Production of prebiotics GOS

Pasturization of 10 litre of raw milk was done at 85°C for 15 seconds. The β-galactosidase enzyme (35 ml) was added in milk at that temperature optimized at initial trials. Then 100 µL of this milk was used to check the enzyme activity and immediately 400 µL of ONPG (chromogenic substrate o-nitrophenyl-β-D-galactopyranoside) was used for enzyme deactivation at 95 °C for 5 minutes and then cooled to 45 °C temperature.

Chemical Analysis

Once the buttermilk containing prebiotic galacto-oligosaccharides was produced, it was analyzed for the quantity of protein, fat and SNF, pH, glucose, galactose and lactose (Table 4).

Organoleptic acceptability

Prebiotic galacto-oligosaccharide containing buttermilk was presented for sensory evaluation by a semi-trained panel of 10 judges (12). For this purpose judges were contacted through personnel contacts, emails and fliers and consent forms were signed. The judges were given a performa, they marked it according to their individual acceptability. Evaluation was carried out by the panelists using 15-cm unconstructed line for parameters of color, flavor, taste, consistency and overall acceptability on a sensory evaluation.

Shelf life study of buttermilk containing GOS

Pouch and glass packaging of prebiotic GOS buttermilk was done to check the shelf life and stability of the new product. Glass bottles were used for glass packaging. Bottles were sterilized before packaging. Shelf life of the new products was checked by sensory evaluation which was carried out for 6 days. A panel of 10 judges (each day) participated in the evaluation and marked the given performa according to their acceptability of the product. The results of collected data

were analyzed through paired sample T test and two way ANOVA analyses by using SPSS version 16.0.

Results

Chemical analysis of Trans-galactosylated buttermilk

Results showed that enzyme concentration between 400-600μL/5mL can produce GOS in 30min and 1hr samples. By increasing time all lactose present in milk was converted into galactose and glucose by breaking all β-linkages present in lactose. Results showed no significant difference (p>0.05) in the composition of plain and prebiotic buttermilk, suggesting safety of the prebiotic for human consumption (Table 1).

Sensory evaluation of plain and prebiotic buttermilk

Sensory attributes of plain and GOS containing butter milk is presented in Table 2 and Figure 1. According to the results, significant difference was found (P≤0.05) in flavor, taste and consistency of plain and prebiotic GOS containing buttermilk. However, overall acceptability remained similar (P=0.08) for both type of milk. Moreover, GOS buttermilk was slightly sweet as compared to plain buttermilk, but sensory panelist scored preferences was found to be not significantly different (P = 0.335).

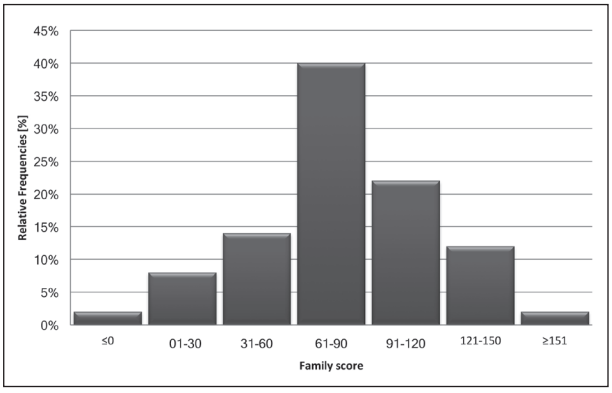

Figure 1. Average Score of Sensory Attributes of plain and prebiotic buttermilk

Shelf Life Study

Buttermilk containing prebiotic galacto-oligosaccharides was pouch packed and glass packed by sterilizing glass bottles and pouring buttermilk in it. Buttermilk was stored at refrigeration temperature for 6 days. Sensory evaluation was carried out for 6 consecutive days to check the shelf life of prebiotic GOS containing buttermilk in both types of packaging.

Table 1. Chemical analysis of buttermilk

Analysis	Plain Buttermilk	GOS containing Buttermilk	Independent samples t- statistic	P Value
Protein	3.233±0.015	3.133±0.100	1.061	0.184
Fat	0.6±0.057	0.533±0.057	1.000	0.561
Ash	0.633±0.073	0.666±0.016	-.267	1.000
Lactose	3.906±0.057	2.763±0.057	3.583	0.073
pH	4.533±0.048	4.433±0.176	2.121	1.000

Note: All differences are statistically non-significant (p>0.05)

Table 2. Sensory analysis of prebiotic buttermilk with plain buttermilk

Parameters	Plain Buttermilk	Prebiotic Buttermilk	Independent samples t statistic	P-value
Colour	8.6±0.665	10.3±0.866	-1.169	0.185
Flavour	8.3±0.568	9.2±1.111	-0.826	0.334
Taste	8.7±0.264	9.8±0.896	-1.281	0.484
Consistency	7.6±1.106	8.5±0.472	-0.988	0.591
Overall Acceptability	7.2±1.457	10.4±0.568	-2.476	0.932

Note: All differences are statistically non-significant (p>0.05)

Table 3. Shelf life of sensory attributes of prebiotic buttermilk in different packages

OAA	Consistency	Taste	Flavour	Colour*	Parameters		
7.22±0.55	7.61±0.32	8.73±0.33	8.39±0.31	8.69±0.45	Day 1	Glass	Packaging
10.46±0.20	8.50±0.42	9.82±0.11	8.39±0.31	10.31±0.50		Pouch	
9.66±0.42	8.53±0.45	8.76±0.53	10.6±0.05	8.6±0.44	Day 2	Glass	Packaging
10.88±0.05	9.34±0.23	9.86±0.09	11.15±0.89	8.97±0.18		Pouch	
7.68±0.1	9.40±0.33	9.11±0.03	8.19±0.19	8.5±0.45	Day 3	Glass	Packaging
9.39±0.33	10.76±0.15	10.06±0.05	9.67±0.43	10.02±0.02		Pouch	
7.68±0.1	9.40±0.33	9.11±0.03	8.67±0.44	8.53±0.45	Day 4	Glass	Packaging
9.39±0.33	10.76±0.15	10.06±0.05	9.53±0.24	9.17±0.08		Pouch	
8.95±0.10	9.62±0.44	9.52±0.43	9.92±0.08	9.68±0.32	Day 5	Glass	Packaging
10.33±0.1	9.24±0.17	9.92±0.08	10.37±0.46	10.3±0.50		Pouch	
7.68±0.1	9.40±0.33	9.11±0.03	8.67±0.44	8.53±0.45	Day 6	Glass	Packaging
9.39±0.33	10.76±0.15	10.06±0.05	9.53±0.24	9.17±0.08		Pouch	

Note: *$p<0.05$

Discussion

The ancient and effective role of prebiotics in our diet is not clear. Continual researches to clear our concepts of the health welfares of prebiotics through latest in-vitro and in-vivo studies would assist from the time complexity delivered by archaeology. A prebiotic was first known as a 'food ingredient that is not digestible and which constructively affects the host by selectively motivating the growth or activity of one or a restricted number of bacteria in the colon, and in this way improves the host health (13). As the archaeological evidence discloses, prebiotics have long been part of the human food and in amounts in some areas and time that far surpass those currently used by modern populations (14).

The production of galacto-oligosaccharide from lactose, by utilizing β-galactosidase enzyme, has been widely examined over the last 50 years because of the functional assets of GOS as prebiotics. The importance in GOS synthesis has increased since its addition in Japanese legislation concerning foods for stated health use (15).

Buttermilk, whey or lassi is a popular drink especially in Punjab regions of Pakistan and India. Buttermilk and lassi has many health benefits. Lassi is a mixture of yogurt, water and many water soluble vitamins. Lassi can be salty or sweet and has more or less the consistency of a smoothie. It provides calcium, vitamin B12, zinc and proteins. Vitamin B12 is very important for converting blood glucose into energy (16). While buttermilk is the liquid remaining after the removal of the butter fat (17), the nutritional value of buttermilk is similar to that of skim milk. It is an excellent source of potassium, vitamin B12, calcium and riboflavin.it is also a good source of phosphorus and contains zinc, magnesium, niacin, thiamin, folic acid and vitamin B6. Low in fat, buttermilk is rich in lactic acid and nitrogen (18).

Buttermilk is also recommended to the individuals suffering from lactose intolerance (19). Lactose intolerance is caused by insufficient production of the lactose enzyme, which is necessary for the breakdown and absorption of lactose, the carbohydrate found only in dairy products. Undigested lactose leads to bloating, nausea, abdominal pain and excessive gas. When dairy products are created by a fermentation process, they contain significantly less lactose. This is especially true for yogurts, aged cheese (e.g cheddar, swiss, Colby) and buttermilk (20).

Use of GOS as food ingredient have been found beneficial because GOS has wide options of usage in food products. Constancy of GOS towards salivary break down and oral microbiota makes GOS less cariogenic carbohydrate alternative in confectionary and chewing gums. GOS have low glycemic index and low calorie index because they are not digested by gastric juice and pancreatic enzymes while passing through

the small intestine. Its glycemic index is almost 50% less than that of sucrose, which makes GOS preferable for diabetic patients and a low calorie food. They are very much soluble and have good moisture holding ability. Their properties are similar to sucrose in such a way that they can improve the mouth feel, texture of food with pleasant taste (21).

In the present study, milk was obtained from UVAS Ravi campus and was transgalactosylated. Yogurt was produced from this milk by adding culture and after the churning of butter from yogurt, butter milk was collected and stored at refrigeration temp for further analysis. 5ml of milk samples were taken for enzyme analysis 0ul, 100ul, 200ul, 300ul and 800ul respectively samples was collected at 30min and 1hr and so on. Enzyme was denatured by putting in hot water and samples were analyzed on TLC with lactose and Yakut Oligomate as standard.

Result showed that. transgalactosylation occurred at 30 mins and at 1 hour. Clear bands could be seen with glucose, galactose and lactose standards. It showed that enzyme can produce GOS (galacto oligosaccharides) in 30min and 1hr samples. By increasing time all lactose present in milk was converted into galactose and glucose by breaking all linkages present in milk lactose. In this study, GOS were produced in milk by transgalactosylation process at optimized conditions.

Buttermilk was prepared with good organoleptic attributes. Organoleptic attributes of the buttermilk containing prebiotic GOS were checked and it was concluded that there was no significant difference between plain buttermilk and prebiotic galacto-oligosaccharide enriched buttermilk. Parameters like color, flavor, taste, consistency and overall acceptance were checked. Chemical analysis were done for plain and GOS containing buttermilk. Chemical analysis showed no significant difference between the composition of both buttermilk, which shows that galacto-oligosaccharides don't change the composition of buttermilk.

Mean values for chemical analysis of buttermilk were: protein (3.133±0.1) while for fat, ash and lactose values were (0.533±0.06), (0.666±0.03) and (3.906±1.1) respectively.

In a study conducted in USA on Chemical Composition, Probiotic Survivability and Shelf Life of Symbiotic Buttermilk, buttermilk was made using a commercial mesophilic starter CHN22 and the probiotics. The control buttermilk was made using CHN22 and the symbiotic buttermilk were evaluated for chemical composition, probiotics survivability, mold, yeast and coliform counts. Changes in pH, titratable acidity and proteolysis were also checked during storage at 4°C for 12 weeks. The chemical composition of the control and symbiotic buttermilk were: protein 3.29±0.05 and 3.30±0.02%; fat 3.28±0.04 and 3.26±0.06%; carbohydrate 4.55±0.05 and 5.16±0.06%; total solids 11.81±0.05 and 12.42±0.03%; ash 0.69±0.03 and 0.70±0.01%, respectively. There were significant differences in pH and titratable acidity between the control and symbiotic buttermilk ($p<0.05$). There was no major difference in proteolysis between the two samples. Results indicated that the symbiotic buttermilk might be considered as a functional food (23).

In another study in Spain on physicochemical properties of buttermilk with protein, fat, ash and lactose (24.82±0.02), (30.11±0.07), (5.22±0.05) and (39.35±0.03) respectively (these values shows the values of total solids), there was an overall difference in the chemical composition of buttermilk, which might be due to differences in the original quality of milk and processing conditions. In this study all visible fat was removed from milk and after the churning of buttermilk fat was reduced to 0.5 % which makes it ideal drink for the patients of diabetes and cardiovascular disease and for those who are on weight reduction program (22).

Results of paired t-test of the present study showed that there was no significant difference in flavor, taste and consistency of plain and prebiotic galactooligosaccharide containing buttermilk as $P>0.05$ but there was a significant difference in overall acceptability of galactooligosaccharide containing buttermilk as it was slightly sweet $P=0.08$. Flavor of plain buttermilk was intense than GOS containing buttermilk but it was not significantly different P value = 0.335 ns.

Buttermilk containing prebiotic galacto-oligosaccharides was pouch packed in University of Veterinary and Animal Sciences, Ravi Campus and glass packed by sterilizing glass bottles and pouring buttermilk in it. Buttermilk was stored at refrigeration temperature for 6 days. Sensory evaluation was carried out for 6 consecutive days to check the shelf life of prebiotic

galactooligosaccharide containing buttermilk in both types of packaging. Results showed that there was significant difference in colour of both packaging P<0.05. Results showed that there was no significant difference in taste in both packaging P>0.05.

Consistency of buttermilk was also checked in both packaging for 6 days. Results showed that there was no significant difference between the consistency of buttermilk in both packaging as p>0.01. Although, solids began to accumulate at the bottom of the bottles in glass packaging and required to shake the bottle every time before consumption.

Results of overall acceptability of buttermilk in both packaging showed that there was no significant difference between the overall quality of buttermilk in both packaging (P>0.05).Buttermilk in pouch and glass packaging maintained its stability for 6 days without adding any preservative at refrigeration temperature.

Conclusion and suggestions

The main aim of this project was to produce galacto-oligosaccharides enriched buttermilk to make it a value added product of high nutritional value and check its shelf life and stability. Buttermilk is an industrial waste which is discarded after the production of butter and cream. Buttermilk provides us protein, minerals, vitamins and is low in fat but with additional functional ingredient GOS will increase its worth. From the present study it can be concluded that the GOS enriched buttermilk can be produced. GOS stability under various pH and temperature conditions increases the options of its unification with other food products. Transgalactosylated buttermilk comparison with plain buttermilk did not show any significant difference in flavor, taste, consistency and overall acceptability of prebiotic buttermilk but there was difference in the colour of both (i.e plain and prebiotic) buttermilks due to the different packaging. Two types of packaging i.e Glass and Pouch packaging was done to check the shelf life and stability of the new product. Results showed that buttermilk in pouch packaging maintained its stability for about 6 days without adding any preservative. Also the functional product prepared was of good bifidogenic effect according to the results. It is recommended that GOS enriched buttermilk should be introduced in the market after proper packaging and safety evaluations. The stability of GOS enriched buttermilk should be checked for more than 6 days and effect of other types of packaging e.g tetra pack should be checked on prebiotic GOS enriched buttermilk. Efforts should be made to create awareness among the general population about the role of prebiotics in human health.

Declaration of interest

Authors declare that there is no conflict of interest.

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Results of an adult weight-management program and reflections as the influence of weight on quality of life in patients with obesity

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Summary. *Background and aim:* Recent studies focus on obesity treatment programs that aim at holistically improving metabolic, physical, psychological and social health. This study was conducted to elucidate the results of a hospital-based adult weight-management program in terms of health status and its reflections as the influence of weight on quality of life (QoL) in patients with obesity. *Methods:* This cross-sectional analytical study was conducted with adult (aged>18 y) patients with obesity (Body Mass Index≥30.0 kg/m^2) (n=73) in Outpatient Clinic for Obesity of Çiğli Regional Education Hospital in İzmir/Turkey. Socio-demographic data were collected through face-to-face interviews. Anthropometric measurements (body weight, height, waist&hip circumferences) were taken. Biochemical findings (fasting blood glucose, HbA1c, insulin, blood lipids) were obtained from patient files. Individualized weight loss program was planned by the dietitian. The influence of weight on QoL was determined with Obesity and Weight-Loss Quality-of-Life (OWLQOL) scale. *Results:* Mean age was 42.3±10.3 years; 87.7% were women. Comorbidities were hypertension (23.3%), hyperlipidaemia (19.2%), diabetes mellitus (12.3%). Significant decreases from baseline to post-treatment were found in weight loss (9.55±3.21%), BMI, waist and hip circumferences, waist-to-hip ratio, waist-to-height ratio and body fat percentage. Post-treatment levels of fasting blood glucose, insulin, total cholesterol, HDL and LDL-cholesterol were significantly lower compared to baseline. The OWLQOL score significantly decreased showing less negative influence of weight on QoL. *Conclusions:* This conventional hospital-based weight management program provided reduction in body weight at desired levels, improved metabolic indicators for comorbidities and resulted in improvement in QoL.

Key words: Obesity; Quality of Life; Weight-Related Quality of Life; Weight Loss; Weight Management.

Introduction

Studies in Turkey demonstrate that obesity is a significant health problem in epidemic levels as in the world. Especially 51-64 years-of-age (in both genders) has been reported to be the most prevalent group recently (1, 2). Turkish population-based studies (2-4) have shown that obesity prevalence in Turkish adults is getting higher (males: from 22% to 40%, females: from 38% to 50%). The relationship between health status and increased body weight is well documented (5). The "Milano Declaration" reported by 24 European countries including Turkey, suggested the collaboration of European countries for obesity prevention, diagnosis, treatment and developing national strategies for a plan of action to struggle with obesity. Besides many governmental steps, Turkey has founded Obesity Research Association, which has

shown that 70% of Turkish people had body mass index (BMI) higher than normal (6). The main striking situation was that most of the individuals did not want to see themselves as obese and were trying to get rid of excess weight. Dissatisfaction from obesity resulted from health issues while people with obesity had mobility limitations, exposed to humiliation, neglect and exclusion by the society. Many of them avoided public transportation vehicles and preferred to imprison themselves at home. Obesity brings occupational problems, marital issues which are threatening the quality of life (QoL) (6). As previously highlighted, the QoL is disrupted due to obesity (7, 8). Psychological problems such as poor body image, binge eating behaviour, depression and social dysfunction accompany with obesity (9). While the aesthetic discomfort of weight has been one of the main reasons for applying to the obesity clinic of hospitals (10), how patients with obesity value their weight is important to the QoL impairment (11). Studies have shown a significant decline in QoL in every high BMI degrees (12, 13). Nevertheless, body weight management programs provide an improvement in metabolic profile, symptoms of health-related issues and comorbidities like diabetes, cardiovascular diseases, asthma, sleep apnoea, osteoarthritis besides the QoL (8). Weight management programs for obesity treatment have been reported to improve the health status and QoL of the patients (14-16). To investigate the effect of a conventional hospital-based weight loss program in an outpatient clinic and its reflections on patients' health status and influence of weight on QoL was aimed in this study.

Materials and methods

Subjects

This cross-sectional analytical study was conducted with adult (aged>18 y) patients with obesity (Body Mass Index≥30.0 kg/m^2) (n=73) who applied the outpatient clinic (Setting: Outpatient Clinic for Obesity of Cigli Regional Education Hospital in İzmir/Turkey) for the first time and who followed the program uninterruptedly for at least 3 months. The sample size was calculated according to Gündüzoğlu et al's study (17) (based on BMI finding (33.92±4.16 kg/m^2); alpha=0.05, effect size=0.409, statistical power=0.90) as (at least) 65 patients. This study was planned for the weight management program applied within 4 months at the hospital, and population was composed of patients who applied to the outpatient clinic within this 4 month-term; totally 119 patients applied, so all patients participated were chosen among these patients who met the inclusion criteria based on census method. The study was completed with 73 patients who met the inclusion criteria and who were regularly checked at the controls. For ensuring regular check, a patient that has not come for longer than a month after the last control was excluded from the study. When those patients applied to the outpatient clinic again, they were not included in the study however, they were treated for obesity.

Inclusion criteria: Being an adult (aged>18 y), with a Body Mass Index≥30.0 kg/m^2, applying the outpatient clinic for obesity for the first time, attending controls regularly and uninterruptedly for at least 3 months, not having a mental disease, being voluntary.

Exclusion criteria: Previous application to and/or monitorization in the same outpatient clinic for obesity, aged <18 y, BMI ≤29.99 kg/m^2, inability to communicate.

Data Collection and Procedures

Data of study were collected with a face-to-face interview at baseline and the end of the study (post-treatment). Anthropometric measurements were taken by the dietitian of the clinic. An individual weight loss program was planned based on patient's baseline anthropometric measurements and dietary intake history by reducing the energy intake by 500-1000 kcal/day to provide 0.5-1 kg/week weight loss by the dietitian (18). This program was delivered by a registered dietitian with over 10 years of experience working with patients with obesity. Patients were advised to do regular exercises like brisk walking for a total of 150 minutes per week or 30 minutes per day (19).

Anthropometric measurements: Body weight, height, waist and hip circumferences were taken by the dieti-

tian with proper methods; BMI, waist-to-height and waist-to-hip ratios were calculated (20). Bioelectric impedance method for body fat percentage was used (TANİTA, MC 480, Japan). Patients were classified as I. degree obesity, II. degree obesity and morbid obesity based on BMI and abdominal obesity or at risk of obesity based on waist circumference and waist-to-hip and waist-to-height ratios (20).

Biochemical findings: Biochemical findings (fasting blood glucose, HbA1c, insulin, blood lipids) were obtained from patient files.

Data Collection Tools

Questionnaire form: Socio-demographic data, physical activity and nutritional habits were collected with a structured questionnaire form. The information about health status and diseases of the patients were based on physician-diagnosed patient files.

Obesity and Weight Loss Quality of Life (OWLQOL): This scale which was developed by Patrick et al (21) determines the effect of overweight-related negative conditions on QoL in patients with obesity. Turkish validity and reliability study was conducted by Gündüzoğlu et al (17). It is a 7-Likert type scale including 17 items; responses are indicated on a seven-point scale that ranges from 0 ("not at all") to 6 ("a very great deal"): 0-not at all; 1-hardly; 2-somewhat; 3-moderately; 4-a good deal; 5-a great deal; 6-a very great deal. The scale includes occasion statements showing the condition that patient lives and its impact on QoL. Some of the statements are "Because of my weight, I try to hide my shape", "I feel guilty when I eat because of my weight", "Because of my weight, I try to avoid having my photograph taken", "My weight prevents me from doing what I want to do". The scale is one factor and has no subfields. All items are summed to determine the single score. The raw scores obtained are calculated using the formula and converted to 0-100 standardized pin (17, 21).

$$\text{Score} = \frac{\text{Total score of all items} - \text{The lowest possible score}}{\text{Possible raw score distribution score}} \times 100$$

As the total score from the scale approaches 0, adverse events that patients experience have a less negative impact on QoL, while adverse events that patients experience affect the QoL more adversely when the total score from the scale approaches 100 (17). Cronbach alpha level was found to be 0.928 in this study.

Outcomes

The main outcomes are body weight loss, change in BMI and waist circumference, change in biochemical findings, change in the weight-related QoL measuring the OWLQOL.

Ethical issues

An ethical approval was taken from Non-Interventional Clinical Trials Ethics Committee of Izmir Kâtip Çelebi University Faculty of Medicine (date: 25.02.2016, number: 32) and an institutional approval was obtained from İzmir Provincial Health Directorate. Written informed consent from the patients was provided, in accordance with the Declaration of Helsinki.

Statistical analysis

The sample size was calculated with PASS 11 software. Data were analysed with the statistical package program (SPSS 22.0) by a biostatistician. Findings were summarized with descriptive statistics. Chi-square analysis for comparing qualitative data was used. Shapiro-Wilk test was performed for testing homogeneity. Dependent Two Samples t tests (Paired Samples t-test and Wilcoxon test) were performed for comparison of two groups. $p<0.05$ was set as statistically significant.

Results

The mean age of the patients was 42.3±10.3 years; 87.7% were female. Patients mostly graduated from primary school (43.8%) and were housewives (61.6%), insured worker (13.7%) and retired (11.0%). More than half (57.5%) did not smoke. Most of the patients applied to the outpatient obesity clinic due to weight loss desire (89.0%). Diagnosed chronic conditions were hypertension (23.3%), hyperlipidaemia (19.2%) and diabetes (12.3%). More than half of the patients had insulin resistance (52.1%) (Table 1).

Table 1. Demographic and descriptive characteristics of the patients (n=73)

Demographic and Descriptive Characteristics	n	%
Gender		
Male	9	12.3
Female	64	87.7
Education status		
Not literate	1	1.4
Literate	1	1.4
Primary school	32	43.8
Secondary school	8	11.0
High school	18	24.7
University and higher	13	17.8
Occupation		
Officer	3	4.1
Insured worker	10	13.7
Self-employment	2	2.7
Retired	8	11.0
Student	2	2.7
Unemployed	3	4.1
Housewife	45	61.6
Marital status		
Married	62	84.9
Single	7	9.6
Divorced	2	2.7
Widow	2	2.7
Smoking		
Smokes	15	20.5
Not smokes	42	57.5
Gave up smoking	16	21.9
Alcoholic Drink Consumption		
Drinks	10	13.7
Not drinks	62	84.9
Gave up drinking	1	1.4
Cause of Application to Outpatient Clinic		
Obesity	65	89.0
Diabetes Mellitus	3	4.1
Fatigue-sleepiness	1	1.4
Hypertension	1	1.4
Sweet craving	1	1.4
Frequent hunger	1	1.4
Hypercholesterolemia	1	1.4
Diagnosed conditions (n=54)		
Cardiovascular disease	7	9.6
Hypertension	17	23.3
Hyperlipidaemia	14	19.2
Eye problems	7	9.6
Diabetes Mellitus	9	12.3

As a result of the weight loss treatment (lasting 111.7±13.3 days), exercising habits changed. The ratio of regularly exercising patients increased to 82.2% from 23.3% (baseline) ($p<0.001$) being the "brisk walking" as the favourite exercise (from 19.2% to 75.4%) and staying same in the duration (Table 2).

Baseline and post-treatment anthropometric measurements of patients showed significant changes (Table 3). Body weight (kg) (95.8±17.5 to 86.7±16.7), BMI (kg/m^2) (37.2±5.5 to 33.6±5.3), waist circumference (cm) (108.4±13.6 to 99.2±11.7), waist to hip ratio (0.67±0.79 to 0.62±0.07) and body fat percentage (%) (42.2±4.7 to 38.0±5.8) significantly decreased compared to baseline levels ($p<0.001$ for each variable). Weight loss rate was found to be 9.55±3.21% (2.77% – 17.03%) (Table 3). Of the patients, 97.3% (n=71) had >5% of weight loss.

Post-treatment biochemical findings demonstrated that fasting blood glucose, insulin, total cholesterol, HDL-Cholesterol, LDL-Cholesterol levels significantly decreased compared to the baseline (Table 4). Mean±SD (Median) OWLQOL scale score was 60.2±22.0 (61.000) at baseline; it was found to be 42.5±20.9 (42.000) after the study (Table 5) ($p<0.001$).

Table 2. Exercise behaviours of patients before and after the study (n=73)

Exercise Behaviours	Baseline		Post-treatment	
	n	%	n	%
Regular exercising	17	23.3	60	82.2
	**X^2=39.000, p<0.001			
Exercise type*				
Brisk walking	14	19.2	55	75.4
Pilates	3	4.1	17	6.6
Exercise severity				
Mild	5	29.4	20	33.3
Moderate	12	70.6	40	66.7
Exercise frequency				
Everyday	8	47.1	19	31.7
Every other day	4	23.5	26	43.3
2 times a week	5	29.4	15	25.0
Exercise duration (minute/week)	50.0±23.1		46.8±15.3	
	45.0 (20.0-90.0)		45.0 (15.0-100.0)	
	*** Z=-0.431, p=0.667			

*More than one type of exercise was marked by a patient.
**McNemar test was performed.
***Wilcoxon test was performed.

Table 3. Anthropometric measurements of patients before and after the study (n=73)

Anthropometric Measurements	Baseline	Post-treatment	Statistical Analysis
Body weight (kg)	95.8±17.5 91.0 (72.1-153.3)	86.7±16.7 80.8 (61.6-140.6)	Z*= -7.425, **p<0.001**
Weight loss rate (%)	9.55±3.21%, 9.39 (2.77% – 17.03%)		
BMI (kg/m²)	37.2±5.5 35.8 (30.0-58.9)	33.6±5.3 32.3 (25.4-55.2)	Z= -7.424, **p<0.001**
Waist circumference (cm)	108.4±13.6 106.0 (81.0-145.0)	99.2±11.7 96.0 (79.0-129.0)	Z= -7.205, **p<0.001**
Hip circumference (cm)	124.0±10.8 123.0 (108.0-168.0)	116.3±10.1 113.0 (101.0-157.0)	Z= -7.330, **p<0.001**
Waist to hip ratio-FEMALE	0.85±0.06 0.86 (0.71-1.01)	0.84±0.05 0.83 (0.74-0.96)	0.87±0.07 (Baseline) 0.85±0.06 (Post-treatment) t**=3.554, **p=0.001**
Waist to hip ratio-MALE	0.96±0.05 0.99 (0.86-1.04)	0.91±0.06 0.93 (0.77-1.00)	
Waist to height ratio	0.67±0.79 0.66 (0.52-0.85)	0.62±0.07 0.61 (0.47-0.81)	t=12.607, **p<0.001**
Body fat percentage (%)	42.2±4.7 42.4 (25.8-55.4)	38.0±5.8 39.0 (15.2-52.6)	Z= -7.183, **p<0.001**

*Wilcoxon test was performed.
**Paired Samples t-test was performed.

Table 4. Biochemical findings of patients before and after study (n=73**)**

Biochemical Measurements	Baseline	Post-treatment	Statistical Analysis
Fasting blood glucose (mg/dL)	95.8±17.5 91.0 (72.1-153.3)	86.7±16.7 80.8 (61.6-140.6)	Z*= -7.425, **p<0.001**
HgA1c	6.0±0.07 6.0 (6.0-6.1)	5.8±0.4 5.8 (5.5-6.1)	Z= -1.000, p=0.317
Insulin	15.0±8.6 14.3 (4.4-48.6)	12.0±6.4 10.6 (1.2-37.4)	Z= -2.852, **p=0.004**
TSH	1.2±0.2 1.2 (1.0-1.4)	2.2±0.1 2.2 (2.1-2.3)	Z= -1.124, p=0.261
Total cholesterol (mg/dL)	213.9±38.7 211.0 (132.0-308.0)	196.0±32.5 191.0 (122.0-277.0)	Z= -5.016, **p<0.001**
Triglycerides (mg/dL)	139.5±69.8 122.0 (48.0-319)	132.2±64.4 111.0 (42.0-318.0)	Z= -1.079, p=0.280
HDL (mg/dL)	46.2±12.7 45.0 (25.0-87.0)	49.6±15.2 47.0 (25.0-109.0)	Z= -3.126, **p=0.002**
LDL (mg/dL)	137.2±31.7 136.0 (74.0-218.0)	121.3±30.5 121.0 (64.0-202.0)	Z= -5.262, **p<0.001**

*Wilcoxon test was performed.

Table 5. Baseline and post-treatment findings of Obesity and Weight Loss Quality of Life (OWLQOL) Scale

OWLQOL Scale	Number of Items	n	Minimum-Maximum Score	Mean± Standard Deviation	Median	Cronbach Alpha	
Baseline	17	73	13.3-100.0	60.2±22.0	61.000	0.928	Z*= -5.307
Post-treatment	17	73	2.9-88.2	42.5±20.9	42.000	0.938	**p<0.001**

*Wilcoxon test was performed.

Discussion

It is not surprising that the wishes and efforts for weight loss of individuals with obesity are intense considering the health status and QoL outcomes of obesity. Although healthcare professionals are interested in health outcomes, patients prioritize QoL-related consequences rather than metabolic ones (11). This hospital-based weight loss intervention study focuses on both clinical outcomes and reflections as the influence of weight on the QoL of patients with obesity. Significant reductions in body weight, BMI, waist and hip circumferences, waist-to-hip ratio, waist-to-height ratio and body fat percentage were succeeded compared to baseline. A behavioural weight loss program was reported to retrieve significant body weight, BMI, waist and hip circumferences, waist-to-hip ratio changes after 6-month intervention (22). Jenkins et al (23) reported small but significant reductions in body weight and waist circumference in all intervened weight loss treatment groups. Latner et al (24) reported that weight loss program was able to reduce waist circumference and cholesterol, triglyceride, HDL, LDL and insulin levels. It is well known that weight loss improves cardiovascular risk factors, helps prevent or hinder the progress of diabetes by stepping with decreases in blood lipid and glucose profile firstly (11, 18, 24). In this study, fasting blood glucose, insulin, total cholesterol, HDL and LDL levels significantly decreased (Table 4). In a study conducted with guided weight loss treatment, amelioration in clinical physiological indicators such as glucose, HDL cholesterol, triglycerides provided greater improvement in Metabolic Syndrome risk with increasing weight loss (25). Improvements in anthropometric measurements and biochemical parameters showing a clinical wellbeing may be the result of weight loss higher than 5%. Latner et al (24) concluded that 4.86±5.05% weight loss changed cardio-metabolic outcomes and waist circumference favourably. An achievement of 9.55±3.21% weight loss in this study accompanied with reductions in blood lipids, glucose tolerance, and a number of anthropometric measurements. Improved cardio-metabolic and anthropometric findings provide evidence for the significance of this conventional hospital-based standard outpatient obesity treatment. These findings are also consistent with previous reports (24, 26) demonstrating significant associations between modest weight loss (higher than 5%) and improved health status. A high ratio (97.5%) with >5% of weight loss in this study may be due to strict controls and interviews with the dietitian (once in every week during the first month, once in two weeks during 2-6th months) (19), the efficient contact through repeated measurements and feedback. Favourable changes in exercising habits (physical activity advice) may have an effect however the baseline duration of the exercising was found not to change, so counselling activities of the dietitian may have a more powerful impact. Also, conducting an individualized, long-term, uninterrupted weight loss program may be one of the key factors for reaching target weight loss in this study. Burmeister et al (27) reported 3.63±2.93% weight loss within 7-week short-term treatment in 57 patients. Latner et al (24) reported that 42.9% of the combined sample, who received 20 sessions of behavioural weight loss treatment over a six-month period, lost >5% of baseline weight (4.68±5.05%) in the post-treatment phase. The proportion of patients who lost >5% was high, however, because there is not a follow-up period in this study, it is not possible to know whether the health-related improvements sustained over 12th or 18th months.

Since weight loss is accepted as the primary determinant of obesity treatment efficacy by the authorities (19, 28, 29), this study revealed a satisfactory improvement in health status after weight loss which also emphasized its influence on the QoL. In a research review of 36 studies conducted by Lasikiewicz et al (30), health-related QoL was found to have the strongest association with weight loss. In this study, the influence of weight on QoL was measured with OWLQOL. The OWLQOL score significantly decreased meaning a less negative influence of weight on QoL. Also, the ratio of patients frequently living negative situations due to their body weight decreased (data of the scale statements not shown). The stigma of obesity causes low self-esteem and body satisfaction, in addition to the hesitation of social life (31). However, patients felt less avoidance to have their photographs taken or bothering about what other people say about their weight or wearing clothes that hide their shape in this study. Weight loss at the desired level (approximately 10%; 9.55±3.21%) improved QoL by decreasing the influence of overweight on QoL. The improvement in QoL is observed when weight loss

is greater than 5%, being more effective if 10% of initial body weight is lost (30). A behavioural weight loss treatment resulted in significant changes in QoL using Impact of Weight on Quality of Life-Lite scale, providing physical function, public distress, work and body image improvements in the core (24). Mensinger et al (22) determined a significant improvement in QoL of patients followed from baseline to 6th month in their weight loss program. It is essential to understand the ways in which obesity impacts the mental and social well-being components of QoL. Studies indicating an increase in QoL within various durations for weight loss reported vitality and self-esteem increase, mental health improvements and distress decrease (16, 22, 32), being specifically reported an association between vitality and OWLQOL in the literature (33). Weight loss permits the individual to "see" physical changes and improvements, improving body esteem (30). This may be called as a snowball effect; starting with dieting and exercising, continuing with weight loss, waist circumference reduction, improvement in the lipid profile and glucose tolerance which brings a total physical and physiological well-being, and resulting in body image satisfaction, self-esteem and less negative social consequences (advanced QoL) that may have a reciprocal potential and assure healthy weight and life maintenance. Obesity is unfortunately often associated with negative social consequences (34). And measuring obesity-related QoL is challenging (34). Therefore measuring the effect of weight on QoL with a short-time-consuming scale provided a strengthened scope of this conventional hospital-based outpatient weight loss program. While the positive health outcomes obtained in this study indicate the success of treatment, this success also manifests itself by reducing the adverse effect of excess weight on QoL. Despite the necessity of caution in generalising the study results due to elimination of the drop outs, this study sheds light on the potential effectiveness of interventions in the real clinical setting.

Conclusions

This study demonstrates that conventional hospital-based adult weight loss treatment was successful in changing healthy lifestyle behaviours like physical activity, in achieving decrements in anthropometric measurements (body weight, BMI, waist circumference, waist-to-hip ratio, waist-to-height ratio) and physiological cardio-metabolic indices (fasting blood glucose, insulin, total cholesterol, HDL, LDL cholesterol). The psychological well-being found in this study has led to understanding the potential of the impact of weight on QoL which should be taken into consideration by the healthcare professionals during weight loss and obesity treatment programs. Future long-term research should continue to test the implementation and sustainability of conventional hospital-based weight loss programs.

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The hydration status and thyroid hormones levels among elite wrestlers

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Summary. *Study Objectives:* Dehydration causes various loss of physical and physiological functions and also thyroid hormones are important for maintaining the normal physiological function of the body and regulate basic metabolism in the human body. The aim of this study was to examine the effect of pre-competition dehydration on elite wrestlers' thyroid hormones levels. Sixty-nine elite wrestlers participated in the study. *Methods:* The retrospective research model was used in the study. In previously obtained blood samples were determined Sodium (Na^+), Blood Urea Nitrogen (BUN), Glucose, Potassium (K), Triiodothyronine (T3), Thyroxine (T4) and Thyroid Stimulating Hormone (TSH) levels. The wrestlers Posm levels were calculated using a mathematical formula and Posm levels >290 who wrestlers as the dehydration group and Posm levels ≤290 who wrestlers as the euhydration group were divided into two groups. The Kolmogorov-Smirnov test was used for the normality test of the data. The independent samples t-test was used in the analysis of the obtained data. Significance was set at 0.05. *Results:* According to the results of this study, it was determined that ~45% of pre-competition wrestlers were exposed to dehydration. When the dehydration and euhydration groups were compared, there was a significant difference in BUN and Na^+ levels as the hydration markers and there was no difference in Glucose and K levels. When the difference between thyroid hormones levels was examined, it was found that there was a statistical difference between T3 levels and there was no difference between T4 and TSH levels. *Conclusion:* T3 levels of elite wrestlers were lower in dehydration group than the euhydration group, whereas T4 and TSH levels were not different between both groups.

Keywords: Hydration status, Triiodothyronine, Thyroxine, Thyroid Stimulating Hormone, Wrestling

Introduction

Exercise is a physical stressor that causes hormonal, metabolic, cardiovascular and immunological changes. The stress can affect the body during heavy exercise is the most prominent elements of stress that threaten homeostatic conditions (1). Preservation of hydration after exercise and exercise-induced dehydration is a common condition that should be minimized in the athletes. The hydration status is determined by Urine Osmolarity (U_{Osm}), Saliva Osmolarity (S_{Osm}) and Plasma Osmolarity (P_{Osm}) in blood, and has a cut-off point for each dehydration measurement method. For U_{Osm} cut-off points are 850 mmol/kg, 50 mmol/kg for S_{Osm}, and 290 mOsm/L for P_{Osm} (2). The reference range of P_{Osm} in blood is 280-290 mOsm/L (3).

Although dehydration has been explained by many studies that cause loss of physical and physiological functions in the athletes (4-8), it has been reported that the prevalence of rapid weight loss is very high (60–90% of competitors) in high school, university and international level wrestlers before the competition (9-11). Therefore, assessing the stress caused by changes in the dehydration-induced metabolism and

the adaptive response of the body to overcome this stress are the main fundamental problems for sports scientists and researchers.

During heavy exercises, the body tries to cope with all of the stresses such as increasing energy and oxygen demand, increasing the use of energy stores (12). In many body tissues, thyroid hormones are involved in maintaining normal physiological functions and regulating basic metabolism. Thyroid hormones affect a number of organs and systems in the neuroendocrine system, growth-development and, most importantly, energy metabolism (13). Moreover, an increase in pituitary-thyroid activity is very important in adaptation to physical exercises. Thyrotropin-releasing hormone is secreted from the hypothalamus, causing the secretion of thyroid stimulating hormone (TSH) from the anterior lobe of the pituitary gland and thus the basic steps in the regulation of thyroid gland functions. In the body, active circulating thyroid hormones are Triiodothyronine (T3) and Thyroxine (T4). T3 and T4 are involved in increasing the metabolic rate in many cells. TSH release is associated with reducing or increasing the level of thyroid hormone (T3 and T4) during circulating in the blood and thus TSH provides protection of basal thyroid hormone levels (14,15). Although there are a few studies investigating an increase in thyroid metabolism through the exercise in the sedentaries (1,16,17). Güllü et al., (2004) have reported that there is no evidence on how to affect changes in the thyroid hormones on the athletes at the risk (outside of the reference ranges) levels (18). Furthermore, it is not clear how dehydration-induced thyroid hormone activation in the weight class athletes. In this context, the adaptive responses of the dehydration-induced thyroid hormones in the organism constitute the hypothesis of the study. The aim of this study was to investigate the effect of dehydration on the thyroid hormone levels of elite wrestlers.

Material and Methods

Participants

The participants were 69 volunteer elite wrestlers (Age: 22.51±2.49 year; Height: 174.54±6.59 cm; Body weight: 78.98±15.87 kg; Body mass index: 25.73±3.77 kg/m2) who had at least 5 years sports experience and did at least one exercise on a daily basis. During the study period, no disease that could affect the blood values of the wrestlers was detected. In this study, official permission for ethical approval of using data was obtained from the Turkey Wrestling Federation with the number of TGF/2171. Additionally, the study was conducted in accordance with the guidelines of the revised Helsinki Declaration.

Experimental Design

The retrospective research model was used in the study. With the help of specialists (nurses), blood samples were taken from the wrestlers during the competition weigh-in time (one day before the competition, between 06:00 and 06:30 pm). In addition, the amount of body weight loss performed by the wrestlers was obtained using a personal information form. The percentage change of body weight loss was determined with the help of formula:

Percentage of body weight loss (**Δ %**) = [(Normal Body Weight - Competition Weight) / Normal Body Weight] × 100.

Sodium (Na^+), Blood Urea Nitrogen (BUN) and Glucose levels were determined from hydration markers in order to determine the P_{Osm} levels in blood samples of the wrestlers and P_{Osm} levels was calculated with the help of a mathematical formula:

$P_{Osm} = (2 \times Na^+) + (BUN / 2.8) + (Glucose / 18)$ (19).

In the literature for the status of euhydration, the P_{Osm} reference range has been reported to be 280-290 mOsm/L. According to P_{Osm} reference range, the group with P_{Osm} level ≤290 was classified as "euhydration" and the group with P_{Osm} >290 was classified as "dehydration" group (20).

Biochemical Analysis

In the blood samples obtained previously, hydration markers (Na^+, BUN, Glucose, and Potassium (K)) were analyzed using a Beckman Coulter AU 2700 Plus biochemical autoanalyzer with Beckman Coulter kits, while hormone analyses (T3, T4, and TSH) were Roche Cobas e601 autoanalyzer with Roche kits.

Statistical analysis

The SPSS 18 was used for the statistical analysis. The normality test of obtained data was tested with

Kolmogorov-Smirnov test. Independent Samples T-test was used for analysis of data showing normal distribution. Significance was set at 0.05.

Results

Information on the research results is given below.

According to the research findings, ~ 45% of the wrestlers have exposed dehydration before the competition. When the amount of body weight lost was calculated, it was determined that dehydrated wrestlers performed weight loss of 4.55% of their body weight.

When the Dehydration and Euhydration groups were compared, there was a statistically significant difference between BUN and Na^+ levels, and there was no difference between Glucose and K levels among hydration markers. When the difference between thyroid hormones was examined, it was found that there was a statistically significant difference between T3 levels, and there was no difference between T4 and TSH levels as thyroid hormones.

Table 1. Descriptive statistics on the classification of wrestlers and percentage of body weight loss

Groups	Frequency	Percentage	Δ% kg
Dehydration	31	44.9	4.55
Euhydration	38	55.1	0.97
Total	69	100.0	2.57

Δ%: change of body weight loss percentage

Discussion

Following the death of three wrestlers in 1997 in the United States of America due to dehydration, National Collegiate Athletic Association (NCAA), attempted to design new rules to ban of unhealthy weight loss practices (21) and decided that new weight classes should be designed by adding +3 kilos (22). Additionally, under Wrestling Weight Certification Program, it is recommended that weekly body weight loss should not exceed 1.5% of body weight (23). For this reason, the United World Wrestling decided in the most recent competition rules for finishing unhealthy weight loss practices to apply weigh-in held the competition morning of the concerned weight category (24). Because many studies have reported that biochemical markers and hormone levels change in the human body due to weight loss (4-6, 8, 25-27).

Table 2. Comparison of wrestlers' hydration markers and thyroid hormones

Variables		Groups	N	Reference Range	Mean±S.D.	p
Hydration Markers	**BUN** (mg/dL)	Dehydration	31	8-20	16.44±3.44	**0.016***
		Euhydration	38		14.31±3.63	
	Glucose (mg/dL)	Dehydration	31	74-106	97.00±15.77	0.951
		Euhydration	38		97.08±12.47	
	Na^+ (mmol/L)	Dehydration	31	136-146	142.45±1.61	**0.001***
		Euhydration	38		137.45±2.74	
	K (mmol/L)	Dehydration	31	3.5-5.1	4.18±0.31	0.951
		Euhydration	38		4.18±0.35	
P_{Osm} (mOsm/L)		Dehydration	31	280-290	296.05±3.14	**0.001***
		Euhydration	38		285.29±5.24	
Thyroid Hormones	**T3** (pg/ml)	Dehydration	31	2-4.4	3.38±0.37	**0.006***
		Euhydration	38		3.67±0.47	
	T4 (ng/dl)	Dehydration	31	0.93-1.7	1.30±0.14	0.224
		Euhydration	38		1.34±0.16	
	TSH (μIU/ml)	Dehydration	31	0.27-4.2	2.23±1.49	0.908
		Euhydration	38		2.19±0.92	

S.D.: Standard Deviation; *p<0.05

According to the primary findings of the study, the percentage of weight loss in the euhydration group before the competition was 0.97% kg. On the other hand, the percentage of weight loss in the dehydration group before the competition was 4.55% kg. Dehydration is a common condition encountered at the end of an exercise. Casa et al. (2000) have reported that 1% of exercise-induced dehydration is well dehydration and it will not cause function losses (28). According to the results of the primary findings of the present study, dehydration group may be exposed to physical and physiological function losses and this situation may cause a decrease in competitors' competition performance.

According to the main findings of the current study, BUN and Na^+ levels of the dehydration group were higher than the euhydration group and the difference between groups was significant. Furthermore, the T3 level of the dehydration group was lower than the euhydration group and the difference between groups was significant. Despite these findings, there was no significant difference between the two groups in terms of glucose, potassium, T4 and TSH levels.

Thyroid hormones are involved in the metabolism of vitamins and minerals, as well as the activity and control of many enzymes in different metabolic processes affecting the response of target tissues to different hormones. Therefore, regulation of general metabolism plays an important role in cell differentiation and growth, thermogenesis and oxygen consumption (29,30). To prevent thyroid disorder, monitoring gland and/or thyroid hormones levels in the blood for effective control of metabolism is necessary. Akander, Rosa, and Moretti (2017) have reported that there were many reports of control of thyroid hormones in the regulation of cardiac function, as an important factor in the control and prevention of diseases of the circulatory system. Furthermore, thyroid function is a new step to the discovery of great champions, since thyroid function is directly related to the athlete's performance, that is, increasingly necessary to control the thyroid gland given that the athlete must achieve greater performances in their competitions (31).

When the literature was investigated, some studies have indicated that the effect of exercise on thyroid hormone levels, whereas some studies have not reported that the effect of exercise on thyroid hormone levels (32-39). These different findings suggest that the level of thyroid hormones may vary depending on factors such as frequency, volume, and content of training protocol, age, and gender. However, there are no studies investigating the relationship between dehydration and thyroid hormones.

In the present study, although there was no significant difference in T4 and TSH levels between dehydration and euhydration groups, there was a significant change in the T3 level. Changes in T3 levels of elite wrestlers as a result of dehydration may result from decreased basal metabolic rate due to loss of body weight. Furthermore, T3 plays an important role in reducing peripheral resistance by altering the Na^+ and K input in smooth muscle cells, leading to a decrease in smooth muscle contractility and vascular tone (40,41). In the current study, a significant difference was found in Na^+ and T3 levels between the dehydration and euhydration groups and this difference caused by dehydration in elite wrestlers would result in a decrease in vascular tone.

In conclusion, dehydration caused a significant change in BUN, Na^+, and T3 levels in elite wrestlers. According to this result, it is suggested to the wrestlers not lose weight before the competition. However, if weight loss is a necessity, weight loss must be carried out gradually according to the NCAA rules. Thus, the markers of hydration may remain within the reference range. This situation can help them to lead a healthy life in the future and maximize their competition performance. Furthermore, further detail studies should be carried out to determine the molecular mechanisms of the effect of dehydration on T3, T4, and TSH levels in elite wrestlers. In addition, determination of the relationship between thyroid hormones and dehydration due to rapid and/or gradual weight loss in other combat sports (Boxing, Judo, Jiu-Jitsu, Muay-thai etc.) will contribute to the sports sciences field.

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Original article

Investigation of the nutritional attitudes and behaviors of the adolescents of the age group of 16-18 who are engaged in sports and the ones who do not

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Summary. The present study was conducted to determine the nutrition exercise attitudes and nutrition exercise behaviors of adolescents the age group of 16-18 who actively perform sport activities and the ones who do not. The study was conducted on a study group comprised of 174 female (44.2%) and 220 male (55.8%) participants; totaling to 394 subjects. In terms of the gender distribution of the participating adolescents, while there is no significant difference between them based on the nutrition exercise behaviors scale, male adolescents displayed significant difference in the sub-factors of healthy nutrition, unhealthy nutrition and meal order in the nutrition exercise behaviors scale compared to the females ($p<0.05$). Furthermore, it was also determined that nutrition exercise attitudes of adolescents who actively performed sport activities were more positive compared to the ones who do not; and this difference was with the healthy nutrition behaviors and meal order; and that adolescents who succeeded to rank in sport competitions had more positive healthy nutrition behavior and meal order behaviors compared to the one who do not ($p<0.05$). Consequently, it can be said that the factor of gender affects the food and exercise attitudes and behaviors of the adolescents of the 16-18 age group; the food and exercise attitudes of the ones who are engaged in sports is more positive compared to the ones who are not, and the children who rank in sports are more attentive to their healthy feeding and the regularity of their meals.

Key words: sport, nutritional behavior, nutritional attitude.

Introduction

The food choices of individuals are affected by many internal and external factors (1), and these factors also continually force people to make decisions concerning their food choices (2). While the factors affecting the food choices have been stated to comprise the taste, convenience, price, and the cultural or religious beliefs (2, 3), their nutritional knowledge can also affect their food choices (4, 5). Athletes know that nutrition affects the performance in a positive way (6), and they obtain such information from their coaches or their sports-related cultural behaviors (7). The concerns regarding their weight (8), performance and aesthetics (9, 10), as well as the effect created by the media (11), pressure the athletes and may change their food attitudes (12, 13).

Nutritional knowledge provides both awareness on nutrition and the practical skills to be used while choosing the healthy foods (4). Thus, the general and sports-related knowledge of athletes affects their food preferences. Although a lot of information is provided concerning the food intake, athletes still do not have adequate and proper food intake (14, 15). The lack of time, difficulty in access to the food, and the lack of the cooking skills and the cooking equipment have

been suggested to be among the reasons for that (16). Although the food intake of athletes is thought to be affected by their nutritional knowledge, there aren't many studies that have taken into consideration the additional factors that might be important in terms of affecting the food attitudes of athletes (17). The previously conducted studies have usually aimed at explaining the processes behind the food attitudes while they mostly focused on the eating preferences (18-20). However, evaluation of the food behaviors of the people engaged in sports is highly necessary in terms of health and performance, since the development of nutritional programs by taking into consideration the training and competition times ensures that the athlete will have the maximum and contributes directly to the recovery after the exercise (21). The food attitude can change depending on the ideals and lifestyles of people, too (22, 23). While people who act with awareness on health attach importance to exercise, nutrition and weight control (24), athletes may attach importance to their food attitudes due to reasons such as performance, competition and being an individual athlete or a member of the team (6). These differences result in the fact that there are nutritional differences between normal individuals and athletes, and between the athletes themselves depending on their level of success.

There are many studies conducted on the food intake of the elite athletes or adult athletes engaged in sports. However, the subject of nutrition in adolescent athletes has not been addressed much. A previous study suggested that non-elite adolescent athletes increased their food intake at a level higher than the normal (25). In addition, it is well known that adolescents prefer consuming certain foods more than young children and adults, and they have different eating habits (26, 27). The people who are not engaged in sports at an elite level may not display a good performance (28) and may be under risk with regards to their health if they cannot ensure their required energy intake. This, in turn, may mean that their nutrition needs to be regulated. Although extensive research has been conducted on human nutrition, the number of studies investigating this subject comparatively between adolescent athletes and normal individuals is low. The purpose of the present study is to investigate the differences between the healthy and unhealthy feeding and food attitudes of the people of the age group of 16-18, who are not engaged in sports, who are actively engaged in sports and who are engaged in sports at an elite level.

Method

Description of Sample and Study Design

The study was conducted on a total number of 394 people between the ages of 16 and 18 (17.5±1.5) who attended the public schools in the province of Uşak, 174 (44.2 %) of whom were female and 220 (55.8 %) male. While 47 % of the participants of the study were engaged in sports, 53 % weren't engaged in sports, and 22.6 % were determined to have ranked. While 46 % of the female children were engaged in sports, the percentage of the male children engaged in sports was found to be 47.7 %. While 11.2 % of the female children were engaged in individual sports and 88.8 % of them in team sports, 26.7 % of the male children were engaged in individual sports and 73.3 % of them in team sports. While the percentage of the female children who had ranked in the branch of sport they were engaged in was found to be 18.4 %, the percentage of the male children who had ranked in the branch of sport they were engaged in was found to be 25.9 %. The data was collected by the four interviewers taking charge in the study by means of the face-to-face interview technique in a classroom setting. The permissions required for the data to be collected from the mentioned schools were obtained from the Ethics Council of Uşak University and the Provincial Directorate of National Education of the Office of the Provincial Governor of Uşak. The adolescents were informed about the study and their verbal consent was obtained before the study was initiated.

Surveys

In the study, the Participant Information Form, the Food and Exercise Attitude Scale (FEAS) and the Food and Exercise Behavior Scale (FEBS) were employed as the data collection tools. The scales have been developed for adolescents of the age group of 12-14. The usability of the same scale in the adolescents who are in the age group of 16-18 has been evaluated by means of the Cronbach's Alpha. The Cronbach's α internal consistency coefficient of the 13-entry Food and Exercise

Attitudes Scale has been determined to be 0.800. The Cronbach's α internal consistency coefficient has been found to be 0.758 for the 'Psychological/Addictive Eating Behavior' sub-dimension, 0.713 for the 'Healthy Nutrition - Exercise Behavior' sub-dimension, 0.723 for the 'Unhealthy Nutrition - Exercise Behavior' sub-dimension, and 0.671 for the 'Order of the Meals' sub-dimension.

Food and Exercise Attitude Scale (FEAS):

This scale, which was developed by Yurt (2008) in order to determine the attitudes of students concerning nutrition, is a five-point Likert-type scale (1: never, 5: always) composed of 13 entries. All entries in the scale include positive expressions and there is no reversely coded question. The total score of the scale can differ between 12 points and 73 points, and a higher point scored in the scale indicates a positive correlation between the food and exercise behaviors (29).

Food and Exercise Behavior Scale (FEBS):

This scale which was developed by Yurt (2008) in order to determine the behaviors of students concerning nutrition is a five-point Likert-type scale (1: doesn't describe me at all, 5: completely describes me) composed of 45 entries and 4 sub-dimensions. The interpretation of the scale is carried out based on the points scored in the sub-dimensions, and the entries numbered 7, 8, 9, 10, 11, 12, 14, 15, 17, 18, 20, 22, 30, 31, 32, 34, 35, 36, 37, 38, 39, 42 and 43 are reversely coded (29). The sub-dimensions of the scale are as follows:

- *Factor 1 (Psychological / Addictive Eating Behavior):* It is composed of 11 entries (7, 8, 10, 20, 22, 34-39). The point that can be scored in this sub-dimension is between 11 and 55. A higher point scored is an indication of a higher level of psychological eating behavior.
- *Factor 2 (Healthy Nutrition - Exercise Behavior):* It is composed of 14 entries (13, 16, 19, 23-28, 33, 40, 41, 44, 45). The point that can be scored in this sub-dimension is between 14 and 70. A higher point scored is an indication of a higher level of healthy nutrition-exercise behavior.
- *Factor 3 (Healthy Nutrition - Exercise Behavior):* It is composed of 14 entries (9, 11, 12, 14, 15, 17, 18, 21, 29, 30-32, 42, 43). The point that can be scored in this sub-dimension is between 14 and 70. A higher point scored is an indication of a higher level of unhealthy nutrition-exercise behavior.
- *Factor 4 (Order of the Meals):* It is composed of 6 entries (1, 2, 3, 4, 5 and 6). The point that can be scored in this sub-dimension is between 6 and 30. A higher point scored is an indication of a better order of the meals.

The scales have been developed for adolescents of the age group of 12-14. The usability of the same scale in the adolescents who are in the age group of 16-18 has been evaluated by means of the Cronbach's Alpha. The Cronbach's α internal consistency coefficient of the 13-entry Food and Exercise Attitudes Scale has been determined to be 0.800. The Cronbach's α internal consistency coefficient has been found to be 0.758 for the 'Psychological/Addictive Eating Behavior' sub-dimension, 0.713 for the 'Healthy Nutrition - Exercise Behavior' sub-dimension, 0.723 for the 'Unhealthy Nutrition - Exercise Behavior' sub-dimension, and 0.671 for the 'Order of the Meals' sub-dimension.

Statistical Method

In the present study, the SPSS-22.0 statistical software package was used for the analysis of the data. The percentage standard deviation and frequency were used for the description of the data concerning the personal characteristics of the participants of the study. Comparison of the average scores of the scales (FEAS and FEBS) was carried out by using the independent samples t-test. The statistical significance level was taken as $p<0.05$.

Results

The general average of the answers which children participating in the study gave to the 13 questions by which they stated their opinions about the food and exercise attitude scale was found to be x=44.27±8.43. Accordingly, the food and exercise attitude of the adolescents participating in the study is at a medium level. When the food and exercise behavior scale of the adolescents participating in the study was examined, their points at the Psychological/Addictive Eating Behavior sub-dimension (33.94±8.68) and in the Unhealthy Food-Exercise Behavior sub-dimension (40.94±8.25) were found to be at a medium level. On the other

hand, their points at the Unhealthy Food-Exercise Behavior sub-dimension (48.29±9.30) and in the Order of the Meals sub-dimension (22.59±4.49) were found to be at a high level (Table 1).

When the results of the study were examined, it was found out that the male adolescents differed statistically significantly compared to the female adolescents in the factors of 'healthy nutrition', 'unhealthy nutrition' and 'order of the meals' ($p<0.05$). Compared to the female children, the 'healthy nutrition behaviors', the 'unhealthy nutrition behaviors' and the 'behaviors related to the order of the meals' of the male children were found to be at a higher level compared to those of the female children (Table 2).

When the food and exercise attitudes and behaviors of the participants of the study were examined depending on the variable of being / not being engaged in sports, it was determined that the level of the children engaged in sports was statistically significantly higher than that of the children not engaged in sports in the food and exercise attitude scale, the food and exercise behavior scale and the factor of the order of the meals ($p<0.05$; Table 3).

When the food and exercise attitudes and behaviors of the adolescents participating in the study were examined depending on their status in terms of having or not having ranked in sports, statistically significant differences were determined in the factors of food and exercise attitude, the healthy food and exercise behavior and the order of the meals ($p<0.05$; Table 4).

Table 1. Average Points scored in the sub-dimensions of FEAS and FEBS

	Min	Max	Mean ± SD
Food and Exercise Attitude Scale	22	64	44.27±8.43 3.40±1.19
Psychological (addictive) Eating Behavior	13	71	33.94 ± 8.68 3.09±1.42
Healthy Nutrition-Exercise Behavior	21	111	48.29 ± 9.30 3.45±1.38
Unhealthy Nutrition-Exercise Behavior	25	67	40.94 ± 8.25 2.93±1.26
Order of the Meals	8	30	22.59 ± 4.49 3.77±1.21

Table 2. Comparison of the Food and Exercise Attitudes and Behaviors depending on the variable of Gender

	Gender	N	Mean ± SD	p
Food and Exercise Attitude Scale	Girls	173	3.35±0.61	0.127
	Boys	220	3.45±0.68	
Psychological (addictive) Eating Behavior	Girls	173	3.11±0.80	0.582
	Boys	220	3.07±0.78	
Healthy Nutrition-Exercise Behavior	Girls	173	3.34±0.61	0.002*
	Boys	220	**3.54**±0.69	
Unhealthy Nutrition-Exercise Behavior	Girls	172	2.85±0.56	0.023*
	Boys	220	**2.98**±0.61	
Order of the Meals	Girls	174	3.65±0.73	0.007*
	Boys	220	**3.86**±0.76	

* P<0.05

Table 3. Comparison of the Food and Exercise Attitudes and Behaviors depending on the variable of Athletic Activeness

	Sports Status	N	Mean ± SD	p
Food and Exercise Attitude Scale	Yes	185	**3.51**±0.58	0.002*
	No	208	3.31±0.69	
Psychological (addictive) Eating Behavior	Yes	185	3.09±0.87	0.965
	No	208	3.08±0.71	
Healthy Nutrition-Exercise Behavior	Yes	185	**3.65**±0.65	0.000*
	No	208	3.27±0.63	
Unhealthy Nutrition-Exercise Behavior	Yes	185	2.89±0.60	0.215
	No	207	2.96±0.58	
Order of the Meals	Yes	185	**4.00**±0.69	0.000*
	No	209	3.56±0.74	

* P<0.05

Table 4. Comparison of the Food and Exercise Attitudes and Behaviors depending on the variable of Having Ranked in Sports

	Sports Rating	N	Mean ± SD	p
Food and Exercise Attitude Scale	available	89	**3.58**±0.64	0.004*
	unavailable	304	3.35±0.64	
Psychological (addictive) Eating Behavior	available	89	3.04±0.77	0.526
	unavailable	304	3.10±0.79	
Healthy Nutrition-Exercise Behavior	available	89	**3.78**±0.74	0.000*
	unavailable	304	3.35±0.61	
Unhealthy Nutrition-Exercise Behavior	available	89	2.94±0.60	0.768
	unavailable	303	2.92±0.59	
Order of the Meals	available	89	**4.03**±0.68	0.000*
	unavailable	305	3.69±0.75	

* P<0.05

Discussion

The age interval of 16-18 is a process of transition from adolescence to adulthood and a period when independence increases. This period when adolescents increase their independence is also an important developmental phase in terms of the formation of the behavioral patterns concerning health (30). In the present study, the Food and Exercise Attitudes, the Psychological/Addictive Eating Behaviors and the Unhealthy Food and Exercise Behaviors points of adolescents were found to be at a medium level on average, while their average points for the Healthy Food and Exercise Behaviors and the Order of the Meals were found to be at a high level (Table 1). The healthy nutritional behaviors, the unhealthy nutritional behaviors and the order of the meals behaviors of the male participants were found to be better than those of the female participants (Table 2). Although the results suggest a high level of the existence of the ones who feed in a healthy way and are attentive to the order of their meals, the existence of the unhealthy food and exercise behaviors among the general population should never be overlooked. The previous studies suggest that the healthy food attitude has a very little effect on the food preferences. It has been stated that the peer influence is highly effective on the food attitudes of adolescents (31-33). Even if healthy nutritional suggestions are tried to be provided and their attitudes and knowledge on nutrition are improved, the individuals at the adolescence period do not follow those suggestions much. Especially among girls, nutrition mostly means only the weight control, and it does not involve any concern about health (34, 35). It has been indicated that girls do not consume an adequate amount of nutritional elements compared to males although the males and females engaged in sports have an adequate intake of calories and have the habit of eating regular meals (34), which is of importance in terms of supporting the present study.

The Unhealthy Food-Exercise Behaviors and the Order of the Meals of the adolescents engaged in sports have been found to be statistically significantly higher compared to the adolescents not engaged in sports (Table 3). Cavadini *et al*. (2000) stated that adolescent athletes had healthier nutritional habits compared to their peers not engaged in sports, and suggested that the programs including the knowledge of sports and nutrition needed to be implemented (36). As a matter of fact, the food intake depends on the nutritional behaviors (37) and the travel- and health-related nutritional attitudes of the people actively engaged in sports may affect their food intake (38). Previous studies conducted on female athletes indicate their energy intake to be less than the recommended amount (39, 40) and the male athletes engaged in weight-class sports may have to undergo calorie restriction (41). These circumstances can create problems in food behaviors of both the female and male athletes (42). However, in team sports, where calorie restriction is not much required, the energy and nutritional needs can be met to a great extent (43). In the present study, the majority of the participants engaged in sports were engaged in team sports. This means they didn't have to undergo calories restriction, which can explain the fact that their levels of healthy food behaviors and the order of meals were found to be high.

In the present study, the adolescents who had ranked at sports were found to have more positive healthy food behaviors and order of the meals behaviors compared to the ones who hadn't ranked (Table 4). In the selection of foods, the purpose for which individuals engage in sports is of great importance (12, 13). Successful elite athletes have more knowledge on nutrition, which may result from the importance they attach to performance and healthy nutrition (44). The purpose for engaging in sports and the goals constitute the difference between the elite athletes and the amateur athletes, and those factors can change the order of importance in the food consumption (45). An athlete with a goal of success does not have the freedom of eating whatever he/she wishes, which pushes him/her to a different and more controlled type of nutrition (12). And this can be effective in athletes having a healthier and more regular nutrition (17).

Consequently, it can be said that the factor of gender affects the food and exercise attitudes and behaviors of adolescents in the age group of 16-18, the food and exercise attitudes of the ones engaged in sports are more positive compared to the ones who are not engaged in sports, and the children who have ranked in sports are more attentive to their healthy nutrition and to the order of their meals. The attitudes of the ath-

letes towards the preference of foods at various stages of seasons should also be investigated. Considering the highly competitive world of sports and the demands the athletes are faced with, it is highly possible that performance plays an important role in the food preferences.

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Nucleotide inclusion in pet food: effect of heat treatment and storage

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Summary. Nucleotide supplementation in pet foods aims to reinforce the immune system and promote intestinal function. Data on alteration of nucleotides during pet food processing are lacking, however. With this study we compared the recovery percentage of nucleotides in dry and canned food after processing and controlled environment storage. Selected dry and canned feed were supplemented with 0.4g/100 g dry matter basis of Prosol petMOD™ (free nucleotide concentrate) before exposure to high temperature. In detail, the highest temperature applied to dry pet food was 110° C and around 125° C to canned food. The recovery percentage for dry food was 75% at the end of processing and 74% after storage for 12 months versus 43% and 41%, respectively, for canned food.. These results indicate that dietary supplementation with nucleotides to pet food may benefit animal health; however, a high loss of these semi-essential nutritional components was observed, particularly in the canned pet food.

Key words: dry pet food, canned pet food, recovery, nucleotides, thermic treatment, storage

Introduction

Supplementation with nucleotides to baby food and parenteral preparations, in particular, has increased following research demonstrating that including nucleotides in these products can reinforce the immune system and improve gut function (1). Nucleotides are now recognized as "semi-essential" nutrients: endogenous production satisfies requirements in normal health conditions, while supplementation is beneficial for the growth and development of young animals and for tissue repair (1). Nucleotides are phosphoric nucleoside esters formed of three components: a weakly basic nitrogenous compound, a pentose sugar, and one or more phosphate groups. They constitute the basic units of the nucleic acids DNA and RNA (2-3). The most important are adenosine, guanosine, inosine, cytidine, and uridine monophosphates. Generally, there are three sources of nucleotides: de novo synthesis, salvage pathways, and dietary nucleotides (4- 5).

De novo synthesis of nucleotides is a metabolically costly process requiring substantial amounts of energy in the form of ATP (2). Another mechanism for maintaining the cellular nucleotide pools is the salvage pathway. Since this pathway recycles 90% or more of the purine bases, it is thought to be dependent on the availability of free purine and pyrimidine bases (4). The salvage pathway requires less energy than the reactions needed for the de novo synthesis of nucleotides; it is characterized by linkage of a ribose phosphate moiety to free bases formed by the hydrolytic degradation of nucleic acid and nucleotides (6). Finally, since some tissues have limited capacity for the de novo synthesis of nucleotides, they require exogenously supplied bases that can be utilized by the salvage pathway (7).

For example, since the intestinal mucosa, hematopoietic cells of the bone marrow, leucocytes, erythrocytes, and lymphocytes are incapable of de novo nucleotide production (8), they all utilize the salvage pathway. This suggests that dietary supplementation

with nucleotides may be important for these rapid turnover cells (4).

Nucleotide supplementation has been variously studied in farm animals but much less in companion animals. Rutherfurd-Markvick et al. (9) demonstrated an increased proliferative response of post-vaccination lymphocytes in 43 cats fed with an integrated diet supplemented with nucleotides. Improvement in humoral and cellular immunity (IgG, IgA, IgM) was observed in puppies fed with supplemented food (10-11). The dietary effects of nucleotides were correlated with increased CD4+/CD8+ ratio, improved protein electrophoretic pattern and acute phase response in dogs suffering from leishmaniasis (12).

Dietary inclusion of nucleotides into commercial pet food has raised questions about whether and how nucleotides undergo alterations during pet food processing and storage. Commercial pet food is manufactured by extrusion/expansion (dry food) and retort sterilization (canned food). Conventional dry pet food is obtained by extruding a finely ground mixture of ingredients of animal and vegetable origin in variable percentages, depending on the species (dog or cat) and the age or size of the animal for which the food product is intended. Extrusion enhances starch digestibility, which is essential for cats and dogs since they lack a complete enzyme pattern for digesting starches (13). The mixture is treated with water and steam, followed by a short but intense heat and mechanical treatment. To guarantee storage at room temperature, dry pet food is dehydrated in ovens for 20-50 minutes. During this industrial process, the food components are exposed to high temperatures, which can degrade any heat-sensitive and easily oxidizable nutrients (14).

From a food technology perspective, canned pet food is simply a preserved food with a pH close to neutral that ensures its microbiological, chemical, and physical stability and that prolongs product's shelf life. Since it is subjected to intense heat treatment, overdosage of thermolable ingredients is necessary to guarantee adequate final recovery net of losses due to heating (15).

The aim of the present study was to compare dry and canned pet food supplemented with nucleotides, the recovery percentage at the beginning and after production and after storage for 12 months, and to determine whether or not heating and prolonged storage cause significant degradation in these components.

Material and Method

Preparation of dry food

A commercial dog kibble formulation was prepared with a percentage of inclusion of 0.14 g/100 g on dry matter (DM) of Prosol petMOD™, a *Kluyveromices fragilis* yeast cell derivative containing a high concentration of free nucleotides 5'-mono phosphate (> 40%) and total nucleic acids (> 80%) (PROSOL S.p.A, Madone/Bergamo, Italy). The supplemented nucleotides were adenosine (AMP), cytidine (CMP), uridine (UMP), and guanosine (GMP) (Table 1). The control was the same commercial dog kibble formulation without nucleotides.

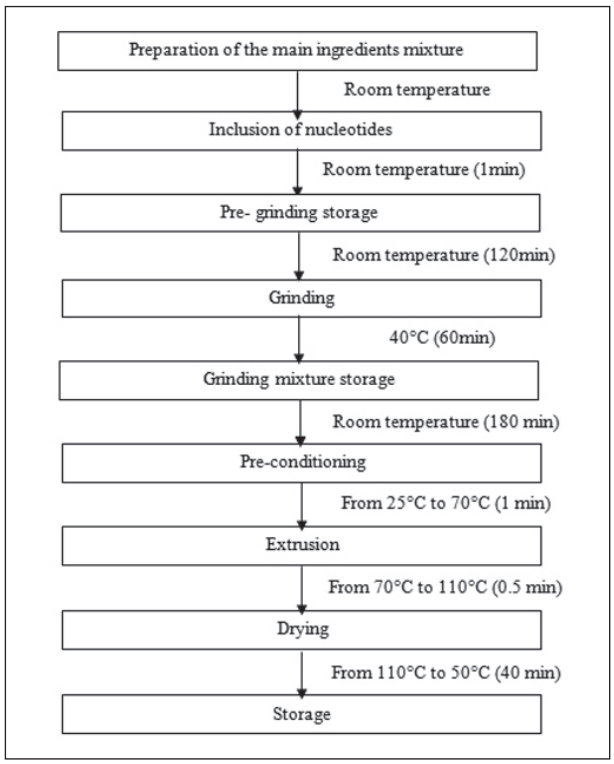
The tested dry pet food (with Prosol petMOD™) and the control food (without Prosol petMOD™) contained the same ingredients combined in a mixture of cereals, mixed poultry meal, oils and fat derivatives of vegetable origin, and minerals. Chemical composition was determined in triplicate according to methods approved by the European Pet Food Industry Federation (16). Table 2 presents the results (expressed on dry matter bases (DMB)). Moisture of finished product was 7.9% and activity water (aW) was 0.497 at 21° C.

The production flow for dry food preparation is illustrated in Flow chart 1. In detail, nucleotides were added during the mixing phase. The percentage of inclusion was calculated at the beginning and at the end of processing and after 12-month storage of the finished product: three vacuum packed lots of the dog kibble formulation. The products were stored at room temperature to simulate domestic environmental conditions.

Table 1. Free 5'-mono-phosphate nucleotide pool in Prosol petMOD ™.

Nucleotide	Concentration (g/100g)
GMP	10
AMP	20
CMP	40
UMP	30

GMP, guanosine; AMP, adenosine; CMP, cytidine; UMP, uridine.



Flow chart 1. Dry food processing, temperatures, and duration.

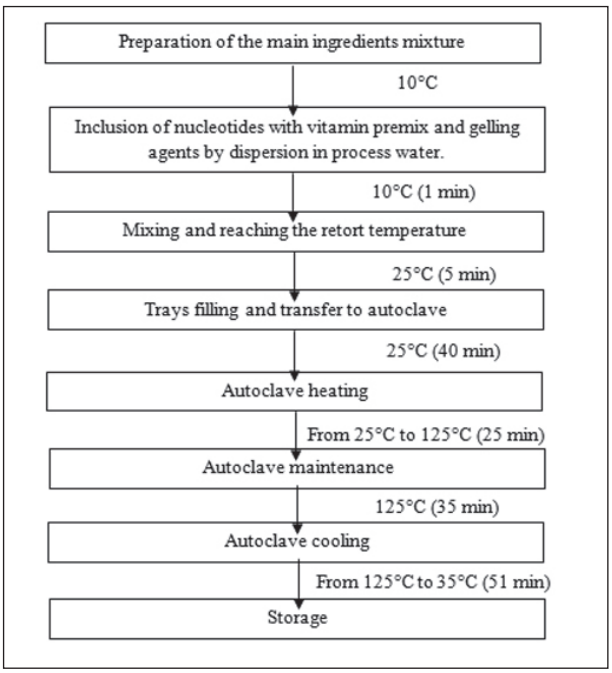
Preparation of canned food for cats

A commercial pâté formulation (final moisture 80%) was prepared containing a Prosol petMOD™ percentage of 0.4g/100g raw materials. The pâté was composed of uncooked chicken and rabbit meat, minerals with (tested food) or without (control food) the addition of nucleotides. Table 2 presents the chemical composition expressed on DMB. Flow chart 2 illustrates the production of the canned food. The product was packed in a 100 g aluminum tray, which was chosen because this form is the one usually subjected to the intense sterilization. The nucleotides were added immediately before packaging to minimize their permanence in the unsterilized batch and to prevent decay

Table 2. Chemical composition of dry and canned foodproducts (g/100 g DMB).

Chemical composition	Dry food	Canned food
Crude Protein	27	47.5
Ether Extract	12.4	30
Ash	7	9
ME (kcal)[a]	423	504

[a]Metabolisable energy (NRC, 1985).



Flow chart 2. Processing of canned food during phase 1, temperature, and duration.

due to fermentation of the nucleotides by the bacterial flora present before sterilization. As suggested by Sevenich et al., sterilization was performed based on the unwanted food processing contaminants (FPCs) value (17). The percentage of nucleotide inclusion was determined at the same time points and under the same atmospheric conditions as for the dry food. Measurements were performed in triplicate on three different lots of the same canned food for cats in the tested and the control formulations.

Method for food processing contaminants (FPCs)

The indicator we used to identify the intensity of heat treatment in pet food is the so-called sterilization value (FPCs). By convention, the FPCs value expresses the number of minutes necessary to obtain a lethal effect against a guide germ (i.e., a pathogen of reference) at a temperature of 121.1° C (i.e., classical sterilization). The FPCs value for a process is the number of minutes needed to kill a known population of microorganisms in a given food under specified conditions.

The FPCs value was calculated using the following equation, where Dr is the decimal reduction time

of a bacterium at 121.1° C (250° F) and Dn is the number of decimals that will be obtained with the heat treatment. The equation is:

$$FPCs = Dr_{121.1} \times Dn$$

Given that the $D_{121.1}$ value for *Clostridium botulinum* spores is 0.21 minutes (min) and that, by convention, a decimal reduction of 12 is considered acceptable, we will have:

$$FPCs = 0.21 \times 12 = 2.52 \text{ min (3 min)}$$

The F0, or lethality value designation, is the number of minutes required to destroy a specific number of *Clostridium botulinum* spores at 250° F. The F0 value was defined as the equivalent number of minutes at 250° F (121.1° C) when no time is required to heat to 250° F or cool to sublethal temperatures when the thermal death time curve slope (Z) is equal to 18° F. The minimum time to obtain commercial sterility is 2.78 minutes at 250° F or its equivalent, or in other words, an F0 of 2.78. With these parameters, assuming a very high initial contamination (1000 spores / container), the probability of survival is reduced to 1 spore per 100 mln of containers. Fc represents the calculation of this parameter at the core of the product. According to EU legislation, wet pet food must undergo a core treatment of at least 121.1° C for at least 3 minutes.

Method for nucleotide determination

Nucleotide determination was modified from Gill et al. (18). Briefly, 50 mg of each standard sample (AMP free acid, CMP free acid, GMP Na2, IMP Na2, and UMP Na) were weighed and diluted in high-performance liquid chromatography (HPLC)-grade water to reach a final volume of 100 ml. After solubilization, 10 ml of this solution were diluted in 50 ml of the solvent. A volume of 10 µl of the final solution was injected into the HPLC system.

For the dry food samples, 50 g of kibbles were weighed and ground with pestle and mortar. A precise quantity of 1 g of ground kibbles was weighed and dissolved in 100 ml of HPLC-grade water, stirred for 15 min, and filtered through 30-micron filter paper. A filtrated volume of 10 µl was injected into the HPLC system. For the canned food samples, 50 g of pâté were weighed, crushed with a few drops of water, and dissolved in 100 ml of HPLC-grade water. The emulsion was settled for 30 min, and 2 ml of supernatant were collected for HPLC injection. The retention time of the standards and the sample were compared. The coefficient of variation (CV) was calculated and the data with a CV ≤ 5% were validated.

The fraction of nucleotides from the raw materials included in the dry and the canned food of the same analytic composition was measured during previous experiments (data not shown). These quantities were then deducted from the values obtained at each time point.

Statistics

Statistical analysis was performed using SPSS, version 17.0 (SPSS Inc., Chicago, IL, USA). Nucleotide inclusion data were processed using one-way ANOVA on ranks (rank-based nonparametric test) for the time points (T0 vs. T1 vs. T12). Statistical significance was set at $p<0.05$. The results are reported as mean percentage of nucleotides in dry and canned pet foods ±standard deviation (SD).

Results and Discussion

Nowadays, a growing trend in nucleotides supplementation is occurring in pets with the final goal of enhancing immune function and gut health, especially in young and debilitated animals. In these subjects, indeed, the cellular turnover is aximized and this aspect leads to an increased demand in dietary nucleotides as well. Segarra et al. (12) demonstrated that nucleotides improve biological markers of immune response in dogs and helped to a better- health status. This is particularly true for puppies since all tissues are in rapid growth and moreover the immune system is still incomplete (10-11). The best way to achieve a dietary integration of nucleotides is to add them directly to a commercial food, even if the main source of these elements for healthy subjects is usually represented by meat and its by-product, which represent one of the main components of their maintenance diet. Despite the beneficial function of nucleotides administration

is well recognized, little is still known about the capability of these elements to resist to technological processes. The present study was conducted to improve the knowledge in this field.

The recovery percentage of nucleotides in the dry food after processing was 75% and 74% after 12 months of storage, respectively, and 43% and 41%, respectively, in the canned food (Fig. 1). The difference in recovery percentages between the dry and the canned food is due to the fact that the much higher temperatures for sterilization of the canned food mixture led to greater deterioration of all the ingredients (16). Furthermore, the recovery percentage of nucleotides is related to FPCs: the higher the FPC value the longer the duration of sterilization. The FPC values we estimated for sterilization of the moist food was 69, which is a standard value for canned pet food. It should be said, however, that the intensity of heat treatment varies according to the volume of the container and the resistance of its contents to the penetration of heat. Pâté was chosen precisely because, unlike chunks in sauce, it is more resistant to heat penetration and so requires more thermal energy to achieved the same sterilizing effect. Nonetheless, despite the high percentage of degraded nucleotides, their concentration remained stable after 12 months of storage.

N denotes nucleotides, T0 before processing, T1 after processing, T12 after 12-month storage.

Processing of the dry food did not significantly degrade the raw materials, leading to a higher recovery percentage as compared with the canned food. The concentration remained stable during the 12 months of storage. A plausible explanation for this finding is that the temperatures reached at the product core during processing were lower than those in the canned food.

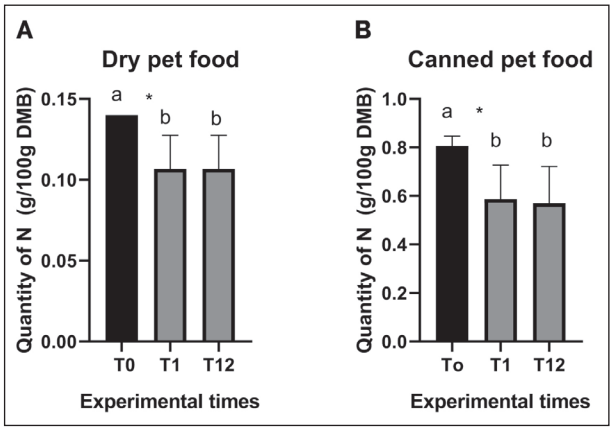


Figure 1. Nucleotides in dry (A) and canned (B) pet food before and after processing and after 12 months of storage. Data are expressed as mean ±SD. ($p < 0.05$)

Conclusions

Nucleotides for dietary supplementation in pet food differ in their resistance to processing for dry or canned food. Pet food manufacturers should be aware of this difference and ensure that sufficient levels of nucleotides are present in the finished product so that it exerts the expected beneficial effects on the immune system and the gastrointestinal tract.

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Ideal extraction temperature for antioxidants from holy basil and bunching onion

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Summary. This study aimed to determine ideal temperature for antioxidants from holy basil and bunching onion. Holy Basil *(Ocimum Tenuiflorum)* and Onion *(Allium Fistulosum)* were extracted with various temperatures ranging from 75 – 100 °C with two solvents i.e. methanol and water (room temperature & boiling). Total phenolic contents (TPC) and total flavonoid contents (TFC) were determined in the extracts by using the Folin-Ciocalteau and Aluminum chloride complex formation assays respectively. Extracts were analyzed in triplicates statistically compared using one-way analysis of variance (ANOVA) and the difference between the mean was ascertained at 95% confidence interval ($P<0.05$) using Tukey›s honest significance test. In both holy basil and bunching onion, the TPC and TFC values for methanolic extracts were significantly ($P<0.01$, $P<0.001$) higher than the water extracts. The best temperature among the various temperature used was 85°C where maximum TPC and TFC were observed in the extracts. This study shows that using optimum temperature helps in extraction of maximum antioxidants with methanol.

Keywords: culinary herbs, extraction temperature, antioxidants concentration

Introduction

The interest in the potential uses of natural antioxidants as food preservatives and for health reason has shown an increasing trend (1). Over the past 20 years, synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytolulene (BHT) and tert-butyl hydroquinone (TBHQ) are being used as food preservatives due to their low cost. However, several researchers have found that there are possible unhealthy effects of synthetic antioxidants and developed countries like Japan, Europe and Canada have banned the use of synthetic antioxidants used in food (2). There are numerous types of natural antioxidants which act as reducing agents to stabilize the free radicals in human body (3) e.g. polyphenols (4). Phytosterols, (5-7), flavonoids (8), vitamin C (1, 9-12). Apart from health benefits, antioxidants also have many industrial uses, such as preservatives in cosmetics and to prevent the degradation of rubber and gasoline (12). However, synthetic antioxidants used in the food and pharmaceutical industries may be toxic (2) compared to natural sources (13). Holy basil *(Ocimum tenuiflorum or Ocimum sanctum)* is an aromatic plant that is native to Eastern World. It is also called tulsi in India and is considered as a holy religious plant among Hindus. Apart from this, it is used as therapeutic ingredients for various purposes (14-16). Furthermore, commonly used as a condiment and garnishing agent in variety of cuisines (17). According to (18), the antioxidants

found in herbs have been identified to have multiple biological effects, including antioxidant activity. The most important chemical constituents in O. tenuiflorum extract (mainly in leaf) are eugenol, carvacrol, tannins, methyl eugenol and caryophyllene (19-22). However, other researchers have observed that other essential antioxidants, such as ascorbic acid, carotenoids, tocopherol, tocotrienols, glutathione, phenolic compounds (like flavonoid) and cichoric acid are also present in it (1, 10, 23). Furthermore, O. tenuiflorum also has showed to exhibit a hepatic protective effect and can be used in the treatment of hepatic disorders anti-stress, immune modulator, anti-inflammatory, mast cell stabilization, anti-histamine (14) and (24). Onion (A. fistulosum) also possesses antioxidants which are characterized by its higher contents of thiosulfunates (allicin), an antioxidant useful for disease condition (25, 26). Allicin also has anti-bacterial, antiviral, and anti-fungal properties (9, 25, 27, 28). Various studies report that cooking temperature may affect antioxidants concertation/activity (1, 3, 13, 29-33). The optimum release depends on the types and nature of the material and antioxidants (34). Various methods of extraction are used but little attention has been paid to have ideal temperature for extractions (35). Similarly, aqueous and organic solvents would also have positive or negative effect on extraction (36). Therefore, it is would be excellent to have optimal solvent and ideal temperature for extraction of antioxidants (37)

Despite of the wide uses of these two common herbs in cooking, the optimum temperature that would optimize the retention of antioxidant contents of these herbs in cooking is still unknown. Thus, there is a need to study the effect of temperature to identify the suitable temperature that can maximize the retention of the antioxidants in the extract.

Materials and Methods

This study involved two famous Asian culinary herbs, namely holy basil and bunching onion. Both herbs were purchased from the local market in Kuantan, Pahang, Malaysia. The herbs were then cleaned with distilled water, dried and ground. After grinding, extracted separately with methanol at various temperatures (65, 75, 85, 95 and 100°C), water (room temperature) and hot water (65, 75, 85, 95 and 100°C). Two assessments were performed namely Folin-Ciocalteau and Aluminium chloride complex formation for total phenolic contents (TPC) and total flavonoid (TFC) contents. For phenolic contents, a calibration curve of the Gallic acid standards (5, 2.5, 1, 0.5, 0.1 mg/l) was prepared; the absorbance was measured at 760 nm using UV-Vis spectrophotometer and using pure methanol as a blank as described previously by (38). The concentration of phenolic contents in samples was estimated using the formula below:

Phenol content mg GAE/g = [(Slope x absorbance) + c] / Sample concentration
*c is the y-intercept

Total flavonoid contents were determined by Aluminium chloride complex formation as described by (39). A calibration curve of the quercetin standards (5, 2.5, 1, 0.5, 0.1 mg/l) was prepared and the absorbance was measured at 425 nm using UV-Vis spectrophotometer and using pure methanol as a blank. The concentration of total flavonoid in samples was estimated using the formula below:

Flavonoid contents (QE mg/g) = [(Slope x absorbance) + c] /Sample concentration
*c is the y-intercept

Statistical analysis

Statistical analysis of the data was performed by using SPSS (Version 12.1), statistically compared using one-way analysis of variance (ANOVA) and the difference between the mean was ascertained at 95% confidence interval ($P<0.05$) using Tukey's honest significance test.

Results and Discussion

The present study demonstrated some interesting findings for the antioxidant concentration of extracts with different temperatures. As mentioned earlier, the antioxidant concentrations of these two herbs were determined in the form of total phenolic contents (TPC)

and total flavonoid contents (TFC) and extracted with two different solvent namely methanol and water. The TPC of three different procedure extracts of the two herbs i.e. are presented in the Figure 1 & 2 and Table 1.

Highest ($P < 0.001$) concentration was obtained for the total phenolic contents at 85°C for both herbs compared to the rest of the temperatures used. From these results, it can be deduced that hot water extracts at 85°C exhibits higher content of TPC (Fig. 1). General concept of the effect of the temperature on the antioxidant's concentration is that higher temperature would lead to lower antioxidant concentration (40). The results of this study reveal that TPC at 85°C was higher than at 65, 75, 95 and 100 °C which might be the suitable temperature. Furthermore, increase in temperature beyond 85 °C would lead to a decrease in TPC yield as it has been observed at 95 and 100°C (Fig 1). Similar, effect has been suggested by (13) that an increase in temperature will lead to an increase in TPC to the maximum concentration whereas there will be a decrease at further increase of temperature and that is what we observed (85 °C). The present study reveals that the effect of tem-

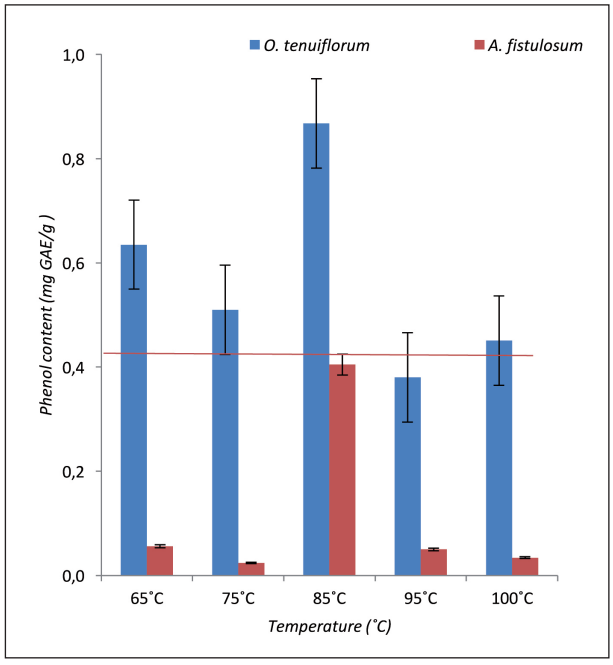

Figure 1. Alteration in cooking temperature affects total phenol contents of holy basil and onion. Each bar represents the mean values ± standard deviation, where n = 3.

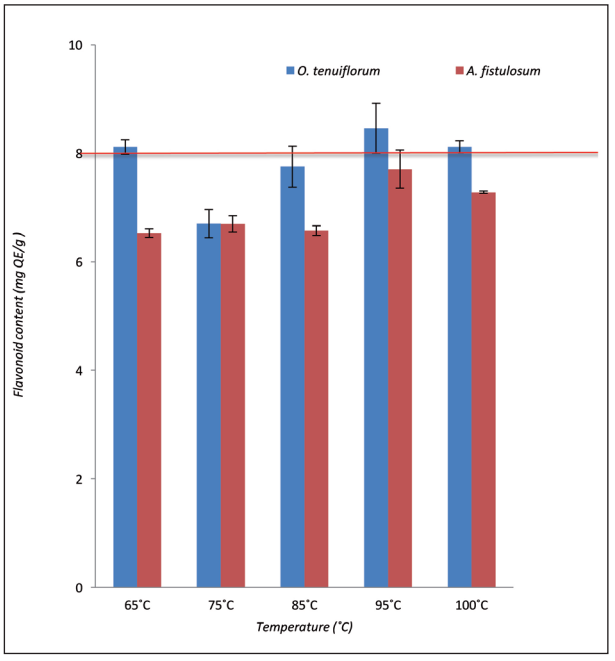

Figure 2. Alteration in cooking temperature affects total flavonoid contents of holy basil and onion. Each bar represents the mean values ± standard deviation, where n = 3.

Table 1. The phenolic contents of these extracts were estimated using a standard curve of Gallic acid and expressed as milligrams of Gallic acid equivalents (GAE), the flavonoid contents that were projected using a standard curve of quercetin and expressed as mg of Quercetin equivalents (QE) over weight of sample in gram and

Holy Basil Extracts			Onion Extracts		
Methanol	Water (room Temperature)	Hot Water	Methanol	Water (room Temperature)	Hot Water
Total Phenol Contents (TPC) (mgs GAE/g)					
0.83 ± 0.17^{a}	0.02 ± 0.04^{b}	0.11 ± 0.01^{b}	4.16 ± 0.63^{c}	0.01 ± 0.03^{b}	0.11 ± 0.03^{b}
Total Flavonoid Contents (TFC) (mg of Quercetin equivalents (QE)					
5.04 ± 0.06^{a}	1.57 ± 0.01^{c}	3.77 ± 0.05^{b}	4.91 ± 0.12^{b}	2.14 ± 0.04^{c}	4.81 ± 0.11^{a}

Each value represents the mean ± standard deviation, where n = 3. The vales with different superscripts are significantly ($P<0.05$, $P<0.01$) different.

perature on the antioxidant contents of Holy basil and Onion were different from one temperature to another which is due to the thermal effects which varies depending on the type of plants, species (33, 41, 42).

The TFC was higher at 95 and 100°C of hot water extracts as compared to other temperatures for both herbs (Figure 2). Similar result was found by (10) who reports that heating at a higher temperature gave a higher flavonoid content compared to heating at a lower temperature. This has been attributed to the hydroxyl structure of flavonoids that is effectively extracted at 95°C (33). Surprisingly, the results of this study showed that the highest content of TFC was obtained at higher temperature compared to TPC. This was similar to (13) who found that the highest yield of TFC higher temperature (63°C) while the highest yield of TPC were observed at lower temperature (53°C). These authors attribute the results to the differences in the hydroxyl group in the phenolic compounds which responsible for responding differently towards thermal treatment. The specific hydroxyl structure in flavonoids and are acting in a specific different mechanism (43). The variation may be due to the differences in cultivar which may be influenced by genetic factors (41) and varied from one region to another (44).

From Figure 1 Holy basil expressed the highest phenolic contents at temperature of 85°C. As suggested by (44), the total phenolic content which mainly affects the antioxidant activity is a main parameter to determine the overall concentration and activity of antioxidants in the herbs. Therefore, it is considerably valid to conclude that the temperature which resulted in the highest phenolic compounds was the optimum temperature for retention of the antioxidant contents in the tested samples. Figure 1 has revealed that 85°C was the optimum temperature for the optimization of antioxidants concentration in Holy basil.

These results show that both tested herbs possess significant amount of phenolic compounds. The content of TPC was highest in methanol compared to cold and hot water extract similar to the other studies of Holy basil (45) and Onion (44). It can be observed from these results that methanol extracts of both herbs yielded the highest concentration of TFC compared to hot and cold water extracts respectively. This has been reported elsewhere in a study conducted by a group of (46), who found that the phenolic compounds yield 30% more with methanol compared to the other forms of solvents. The content of holy basil and onion show that both herbs are important for antioxidant activities (10). However, the concentration of the TFC is varied depending on the type of extraction solvent. Similar to the TPC, the highest concentration of TFC was observed in the methanol extracts of Holy basil and Onion. A similar observation has been reported for spices with methanol to be the most effective extraction agent for TFC compared to the other solvents (10).

Phenolic compounds are good sources of antioxidants and have excellent potential in elucidating antioxidants scavenging activity (18, 44, 45). However, the concentration of the phenolic compounds varies depending on the type of extraction (46, 47) and (10). The observed concentration TPC and TFC may have the antioxidant activity and free radical-scavenging capacity in the tested herbs (44, 48, 49) and (44). In fact, the higher the phenolic contents, the higher the free radical-scavenging activity and antioxidant capacity of the sample, and vice versa (49, 50). In the present study, it was found that extraction solvent also influenced the antioxidant contents of these two herbs. Methanol possessed highest yields of total phenols as compared to hot and cold water. Interestingly, the present study revealed that the effect of temperature on the antioxidant contents of both herbs were unique from one temperature to another. Hot water extracts at 85°C had the highest phenolic compounds followed by 65, 95, 100 and 75°C for Holy basil while for Onion; the phenolic compounds yield ranged from highest to lowest was at 85, 65, 75, 95 and 100°C. In fact, the antioxidant activity elucidated by Holy basil of hot water extracts at all manipulated temperatures was considerably higher except for 95°C. Thus, it appears that 85°C was the optimum temperature for the optimization of antioxidants contents for both herbs.

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Effect of the heart of date palm aqueous extract administration on antioxidant enzymes and obesity-related hormones levels in rats

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Summary. The aim of this study was to determine the levels of antioxidant enzymes and some hormones in rats samples after administration of aqueous extracts of heart of date palm (HDP). HDP extracts were prepared from three Saudi date palm cultivars (Sukkari, Naboat Saif and Solleg) and administered orally every day for eight weeks. Activities of glutathione peroxidase (GPx) superoxide dismutase (SOD) and Catalase (CAT) were determined in rat liver and plasma, while Leptin, Ghrelin, Insulin and glucose were determined only in plasma. Animals in all groups had normal growth and health through the experiment. The results showed significant increase in activities of GPx, SOD and CAT in both rat liver and plasma. Circulating levels of leptin and insulin decreased significantly after administration of the three HDP extracts to the animals. Only the level of ghrelin in rat plasma increased significantly ($p< 0.05$) in case of Sukkari HDP extract. Although HDP have significant effects on leptin, ghrelin and insulin in rats, relationship of HDP administration to obesity could not be confirmed, and further research is needed to investigate the effect of HDP on human body weight control.

Key words: Heart of date palm, antioxidant enzymes, leptin, ghrelin, insulin, obesity.

Introduction

Date palm (*Phoenix dactylifera L.*) is a well-known tree in Saudi Arabia, and mainly cultivated for its fruit (1), the heart of date palm (HDP) or the apical meristem of the date palm tree is edible, and produced for commercial purposes (2, 3). In Saudi Arabia, the heart of date palm (*Al-Guomar*) is extracted from date palm tree and consumed fresh by some Saudi people (4). HDP was considered as a rich source of protein, dietary fiber, minerals and antioxidants (2, 4, 5). Antioxidant enzymes activities in embryonic stages of date palm were studied by Zein El din and Ibrahim (6), and these enzymes included peroxidase and polyphenol oxidase. Endogenous antioxidants in the body deactivate free radicals and minimize the risk of oxidation and damage of cells in the body. Some natural antioxidants from plants (e.g. antioxidant enzymes from fruits and vegetables) were investigated as protective agents against free radical's damage (7). Glutathione peroxidase and superoxide dismutase activities were examined in the plasma of rats before and after date palm seed steeping treatment, and these enzymes activities were significantly increased after the treatment (8).

Camel thorn plant (*Alhagi maurorum*) was described by Sheweita et al. (9) as promising medicinal plant because it has high contents of flavonoids and phenolic compounds which are antioxidant phytochemicals, aqueous and methanolic extracts, of this plant when administered to induce diabetes in rats lead to decreased oxidative stress, also these treatments lead to changes in antioxidant enzymes activities in the rat's plasma (9).

Leptin and ghrelin in humans are hormones that seem to have roles in food intake, energy balance and body weight regulation (10). Leptin is produced mainly from adispose tissue (11). Leptin decreases appetite

and food intake (12), while ghrelin, a peptide produced mainly from the stomach, seems to stimulate appetite and induces food intake (13, 10). Ghrelin in rats has vital roles as stimulator of growth hormone secretion, energy balance and body weight regulation (14), while leptin in rats has different effects on body weight, appetite, liver and kidney functions, and plasmatic glucose level (15). Leptin has been suggested to play causative role in insulin resistance associated with obesity in humans (16). In their study of leptin levels in obese Nigerian women, Obsegbe et al. (17) found that in these women, leptin Serum levels were positively correlated with insulin resistance which is a characteristic of types 2 diabetes mellitus. Also, Leptin and its relation to insulin and obesity were investigated, in type 2 diabetes mellitus model rats, by Velasquez et al. (18). They reported that a significant positive correlation between plasma leptin and plasma insulin was observed in the entire groups of rats, plasma leptin as well was positively correlated with fasting plasma glucose (18).

The present study may be considered as a continuation of the previous study (4), and aims at assessing the levels of antioxidant enzymes in rat's liver and plasma, and Insulin, Leptin, Ghrelin in rat's plasma after administration of aqueous extracts of HDP. The results of the analyses of this work may highlight a possible role of HDP as a natural product, in applications of food science and human nutrition.

Material and methods

Preparation of heart of date palm aqueous extract: HDP samples from three Saudi date palm cultivars (Sukkari, Naboat Saif and Solleg) were prepared as previously described (4). Five grams of finely powdered freeze dried HDP samples flours were mixed with 50 ml distilled water, and the mixture was stirred using a magnetic stirrer for one hour at room temperature, and then centrifuged for 20 minutes at 4000g to obtain clear aqueous extract. The extract was prepared fresh daily and used immediately in the rat bioassays.

Animals: A total of twenty-four male rats (Wistar strain) weighing 180-200 grams were obtained from Experimental Animals Care Center, College of Pharmacy, King Saudi University, Saudi Arabia. Animals were maintained in a controlled environment at 25^{0}C and 12/12 light / dark cycle, and animals had free access to water and chow diet throughout the experimental period. Rat chow diet was obtained from the grains silos and flour mills, Riyadh, Saudi Arabia. All animals' experiments were carried on according to the ethical Guidelines of Experimental Animals Care Center, College of Pharmacy, King Saudi University, Riyadh, Saudi Arabia.

Rat bioassays: Animals were kept individually in single rat cages for an adaptation period of a week, and then divided randomly into four groups of six rats each, as follows:

a. Group 1: Animals consuming 3 ml Sukkari extract
b. Group 2: Animals consuming 3 ml Naboat Saif extract
c. Group 3: Animals consuming 3 ml Solleg extract
d. Group 4: (Control group) received 3 ml distilled water.

For all groups of rats the specified HDP extract was administered by oral gavage for each rat at fixed time of the day.

Preparation of plasma and liver samples: At the end of the eight weeks experimental period, rats were anesthetized with pentobarbital sodium (60 mg/kg body weight). Blood samples were collected from each rat from the heart into EDTA Tubes, and then centrifuged for 15 minutes. Plasma samples were stored at -80^{0}C and used for glucose, insulin, Leptin, Ghrelin and antioxidant enzymes analyses. Liver samples were removed and cleaned from other tissues, then stored at -80^{o}C, to make liver homogenates, portions of liver samples were homogenized with phosphate buffer (pH 7.4) and the homogenates centrifuged to obtain clear supernatants which were used for antioxidant enzymes analyses.

Biochemical analyses

Glutathione peroxidase: Glutathione Peroxidase was analyzed by using diagnostic kits from Bio-diagnostic (Egypt). The ultra violet (UV) method as described by Goldberg and Spooner (19) was followed.

Superoxide dismutase: Superoxide dismutase was analyzed using diagnostic kits from Bio-diagnostic (Egypt). Superoxide dismutase activity was determined by measuring the inhibition in photo reduction

of nitro blue tetrazolium by superoxide dismutase enzyme and as determined by Kumar, et al. (20).

Catalase: Catalase was analyzed by using diagnostic kits from Bio-diagnostic (Egypt). Catalase activity was measured spectrophotometrically according to the method of Aebi (21).

Leptin: Leptin ELISA kit was purchased from Abcom. (UK). For quantitative measurement of Leptin in the samples, enzyme Linked immure sorbent assay was followed according to the manufacturer's instructions.

Ghrelin: Ghrelin EA kit was obtained from sigma- Aldrich (USA). Enzyme immune assay was used for *in vitro* quantitative assay for detecting ghrelin peptide (22).

Insulin: Insulin was determined by diagnostic kits from Diasorin (Italy) *in vitro* quantitative determination of insulin in the samples was carried on by using chemiluminesence immune assay technology.

Glucose: Glucose diagnostic kit was abstained from Human (Germany). For quantitative determination of glucose in the samples, the enzymatic colorimetric test for glucose as described by Barham and Trinder (23) was followed.

Statistical Analysis: Statistical package for social sciences (SPSS) program version 16.0 was used to obtain all statistical parameters. All analyses were done in triplicate and the results expressed as mean ± standard deviation. Significant differences ($P<0.05$) between treatments and control groups, or rats, were determined by pair sample T-test, and correlations between insulin and Leptin were calculated using Pearson's Test.

Results and discussion

Aqueous extracts of the three hearts of date palm (HDP) cultivars were administered orally to the experimental groups of rats, for eight weeks which was the experimental period. No negative signs regarding animals' growth or health were observed.

Levels of antioxidant enzymes: Levels of catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD) in liver and rat plasma was shown in figure 1 (A-D). Administration of Naboat Saif and Solleg HDP extracts lead to significant increase ($p<0.01$) in CAT level in the liver. The level of CAT for Sukkari HDP was 0.568 U/L/g tissues; a little lower than that of the control 0.614 U/L/g tissue. GPx levels in liver of rats received HDP extracts of Sukkari, Naboat Saif and Solleg were significantly higher ($p<0.01$) compared to the control (Figure 1 A). Rats in the control group have higher level of SOD compared to HDP groups of Sukkari and Naboat Saif ($p>0.05$), and also higher than that of Solleg ($p>0.01$) (Figure 1 B). Some studies have reported increase in antioxidant enzymes activities in rat liver, when these rats received different dietary treatments, e.g. *Hibiscus Sabdarrifa* extract (24), *Azdirachta indica* leaf extract (25) and Gum Arabic extract (26).

Administration of HDP extracts lead to significant increase in CAT values in rat plasma in case of Sukkari ($p<0.01$) and Solleg ($p<0.05$). CAT activity in case Naboat Saif was found to be 0.200 U/L almost similar to control (0.204 U/L). GPx activity increased significantly ($p<0.01$) when Sukkari and Naboat Saif HDP extract was administered to rats and also increased ($p<0.05$) in case of Solleg HDP extract (Figure 1 C). Significant increase in SOD level, in case of Sukkari and Solleg ($p<0.01$), and in case of Naboat Saif ($p<0.05$) has been observed after treatment with HDP extracts (Figure 1 D). It could summarize that the levels of CAT, GPx and SOD in rat plasma significantly increased after administration of

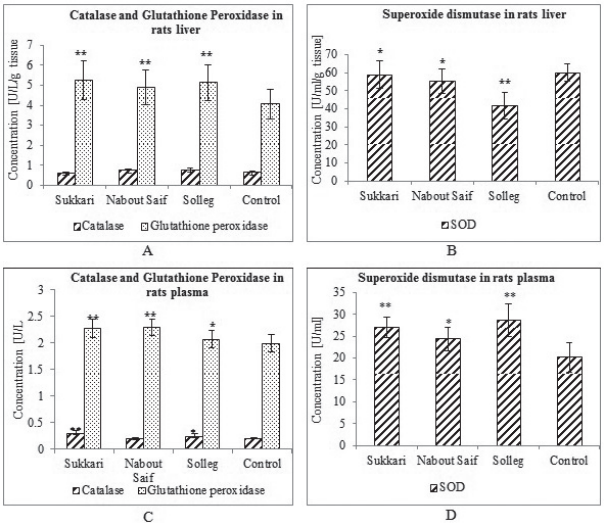

Figure 1. Effect of heart of date palm aqueous extract administration on Catalase, Glutathione peroxidase and Superoxide dismutase level in rat's liver (A-B) and plasma (C-D).

aqueous extracts of the three cultivars. This may due to the capacity of HDP itself as antioxidant, because of its, content of total phenols and flavonoids (4). Date palm seeds steeping treatment results in significant increase in GPx and SOD activities in rat plasma (8). Also another study (27) reported that soy isoflavones supplementation significantly enhances activities of SOD, CAT in liver tissue and serum in exercised rats. Increasing the body levels of antioxidant enzymes by increasing intake of foods rich in antioxidant enzymes is a therapeutic approach to protect the body from oxidative injury and its associated disorders (28).

Levels of hormones: Figure 2 showed the levels of Leptin and Ghrelin in Plasma of rats after administration of HDP extracts. These hormones are involved in regulating appetite and energy balance in human subjects, and rats (29). Treatment with HDP extracts resulted in significant decrease in levels of Leptin in rats receiving Sukkari and Solleg extracts ($p<0.01$) and Naboat Saif extract ($p<0.05$).

Levels of Ghrelin in rats receiving Sukkari extract, increased significantly ($p<0.05$). Contrary to this levels of Ghrelin in rat's plasma receiving Naboat Saif extract (0.661 pg/ml) and Solleg (0.660 pg/ml) were not significantly different compared to the control group (±0.667 pg/ml). It can be concluded that the levels of Leptin decreased and level of Ghrelin increased to some extend in the rats plasma due to HDP extracts treatments.

Type and composition of the diet have significant effects on circulating Leptin and Ghrelin levels in humans (10) and in rats (29). In the same context, Azizi (13) reported that Leptin level and body mass index (BMI) decreased, while Ghrelin level increased in female subjects after 8 weeks aerobic training program. Levels of glucose and insulin in rat's plasma after treatment with HDP extracts are shown in figure 3 (A-B). The levels of random Glucose in rat's plasma after treatment of HDP extracts were significantly increased compared to the control group figure 3A, this may be due to the presence of different amounts of fructose, glucose and sucrose in HDP extracts (4). Treatments of HDP extracts lead to significant decrease in Insulin levels in rats receiving Sukkari ($p>0.01$), Naboat Saif extracts ($p>0.01$), and Solleg extract ($p>0.05$) figure 3B. The decrease in insulin levels was reported after dietary treatment for diabetic rats (9) and decrease in Insulin levels was also observed by Azizi (13) after aerobic physical exercise in humans.

Correlations between insulin and Leptin, in rat's plasma have been reported in Table 1. Insulin and Leptin in levels were positively correlated in case of the three HDP samples, although to different correlation coefficient values. Leptin and Ghrelin are associated with body weight control in case of weight reduction or

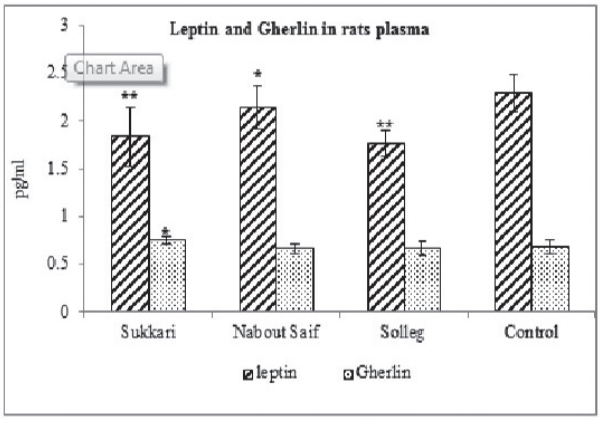

Figure 2. Effect of heart of date palm aqueous extract administration on Leptin and Gherlin level in rat's plasma

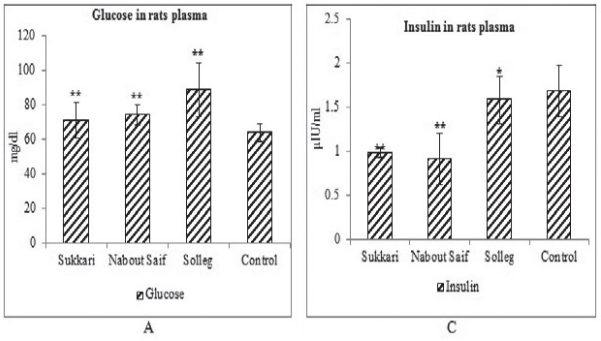

Figure 3. Effect of heart of date palm aqueous extract administration on glucose (A) and insulin (B) in rat's plasma

Table 1: Pearson correlations between insulin and leptin in plasma of the rat after treatment of heart of date palm aqueous extract

Sample (n = 18)	**Leptin x Insulin**
Sukkari	0.279
Naboat Saif	0.834**
Solleg	0.624**
** Correlation is significant at the 0.01 level.	

obesity, also there may be relationship between Insulin resistance and obesity, as study reported that high levels of circulating Leptin and Insulin are characteristics signs of obesity in humans and animals (30). Diet-induced obesity rats when fed on high-energy diet for 10 days gained more body and fat weights and had higher Leptin and Insulin levels (31). The correlation between leptin and insulin in rat plasma as observed in this study was confirmed by Rossetti et al. (32) for rodents and also Antuna-Puente et al. (33) reported that serum Leptin levels were significantly and highly correlated with body weight and Insulin levels in human subjects.

Conclusion

HDP is a secondary product of date palm tree, and is known to be a rich source of minerals and antioxidants. The results of the present study confirmed the significant effect of HDP in increasing of antioxidant enzymes levels in rats; also the results revealed that HDP treatments have significant effects on the levels of the hormones leptin, ghrelin, and insulin in rat plasma. However, the clinical interpretation of these findings to human diseases (e.g. obesity) cannot be made at this stage. Further investigation may be needed to test the potential of HDP as therapeutic agent against nutrition-related disorders in humans.

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The correlation between body mass index and body image dissatisfaction and body image perception in young saudi women

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Summary. *Background/ aims:* Staying healthy may become a challenge, especially for young women, as they tend to focus on their body image and weight gain. Greater body size links to body image dissatisfaction, among young women. The prevalence of BID influences by many factors including culture. Therefore, this research aimed to; (1) explore the prevalence of Body Image Dissatisfaction (BID), and body image perception across Body Mass Index (BMI) categories; and (2) explore the correlation between BMI and BID among a sample of Young Saudi women (n=226) who live in Makkah region. *Methodology:* A self-administrative questionnaire was used for data collection on social-demographic and anthropometric measurements, and a figure rating scale of 9 graphics of body shape by Stunkard et al. was used to measure the BID and body image perception. The BID score was calculated as the differences between the perceived current image and ideal image. Body image perception was calculated as the differences between perceive body image and actual BMI. BMI for each participant was calculated from self-reported weight and height. *Results:* The participants were 226 young women with an age range from 18 to 35 years. The findings indicated that BMI positively correlated with BID, but this correlation was small with r=0.135, P value > 0.01. Even though the prevalence of overweight and obesity was low (23%), the prevalence of BID was high (80.5%). Of those who classified as healthy weight, 57.6% wanted to lose weight, while 17.3% wanted to add weight. The majority of participants (61.5%) adequately estimated their BMI, while 28.3% underestimated their weight and 10% overestimated it. All the obese and 32% of the overweight underestimated their BMI, while 20% of underweight participants overestimated their BMI. *Conclusion:* The findings indicated that most of the young women were dissatisfied with their weight, even if it is within normal BMI, and had a misconception about their healthy weight.

Keywords: BMI, dissatisfaction, body image perception, women, Saudi, perceive body image.

Introduction

Maintaining a healthy weight is challenge, and it lowers future risks of diabetes, cardiovascular diseases, various cancers, and other chronic illnesses (1, 2). In communities like Saudi Arabia where there is a high prevalence of obesity and a burden of type 2 diabetes, aspects of Body Mass Index (BMI) and body image are critical to ensure that the young women adopt healthy alternatives to maintain a healthy weight (3-7). BMI is often used as an assessment tool in estimating a person's weight status. The BMI refers to the ratio of one's weight to his or her height (8). It can be a predictor of body image dissatisfaction (BID) in some cases. Moreover, young women are at greater risk of body image and eating disorders concerns compared to other age group. A negative body image concern links to an adverse effect on an

individual's physical and mental health and on public health as well (9-12).

Body image is a biased concept of an individual's physical look that is based on both self-perception and the perception of peers (10). In addition to perception, body image encompasses behavioural, cognitive, and affective body aspects (9). The concept of body image involves multiple dimensions like psychological, neurological, and sociocultural elements (13-17). Body Image Dissatisfaction (BID) occurs when an individual has negative feelings, thoughts about their own body and can't appreciate, respect, and accept their body as it is (11, 18). BID has an impact on individuals' self-esteem and confidence as one may not be comfortable among their peers. Body image since is a factor that can make an individual more to eating disorders (9).

While BID can cause eating disorders and other adverse health outcomes on individual or public health levels (9-11, 19). Those who are experiencing body image dissatisfaction may become fixated on changing the shape and size of the body, which may trigger unhealthy practices with nutrition and physical exercise, that can damage physical and psychological wellbeing (12, 19, 20). However, body image dissatisfaction is an internal process and can be impacted by several external factors. Preference for given body weight as well as attitude towards one's body image can be influenced by physical, cultural, emotional, media, and interpersonal factors (21-25). Culture may encourage various body shapes and composition. Since culture and social factors impact BID, it is important to explore BID and body image perception among populations in different areas. Few Saudi studies have been carried out to measure BID or body image perception in Taibah (26) Riyadh (27) and Hofuf (28).

However, the current study explored the correction between BMI and BID; and to investigate the prevalence of BID and body image perception across BMI categories among a sample of Young Saudi women in Makkah region. This study will give insights on intervention approaches to decrease body image dissatisfaction. Unlike the three studies referenced above, the current study incorporated both the prevalence of BID and the correlation between BMI and BID among young Saudi women, with the age of 18 to 35, who live in Makkah Region.

Materials and Methodology

Sample Size and Procedure

The study was descriptive, and carried out in Makkah region at 2019. The size of the sample was 200 people based on the argument that the number of participants should be about 10-20 times the items in the study questionnaire (29). The exclusion criteria of participants were pregnant women or those with age 36 or older, and if had missing data on weight, or height, or the BID or body image perception. The criteria for inclusions in the study were females, aged 18 to 35 years with Saudi nationality and completed data on the BMI and BID. 247 women completed the survey, but only 226 women were included in the study. Four participants were excluded from the study due to the extreme values of weight and height. The number of participants who were excluded for missing data on weight and height was 15. Two others were excluded due to the age one were 51 years old and the other was 49 years old. This study followed the research policies of the King Abdulaziz University Research Centre. The survey included a written consent for the participants to participate in the study voluntarily. The researchers assured the participants that the information provided would only be exclusive to the scientific purposes, and be kept confidential.

The Tool and Anthropometric Data

A self-administrative questionnaire containing social-demographic, anthropometric data and a figure rating scale of 9 graphics of body shape, was used for data collection. The survey asked participants about gender, age, health status, marital status, weight in kg, and height in cm. Body weight and height were self-reported. A study conducted on young adults shows good agreement between self-reported data on BMI and measured BMI with r=0.99; P<.001(29). Other studies also came up with same conclusion, that self-reported data on weight and height are sufficient to obtained BMI values in public health studies (30, 31). The BMI for all the participants was calculated from the self-reported weight and height data. Participants classified into one of the four categories of the BMI values by the World Health Organization as following; (a) underweight (BMI <18.5), (b) healthy weight

(BMI 18-24.9), (c) overweight (BMI 25.0-29.9), and (d) obesity (BMI 30.0 or more) (8).

Body Image Dissatisfaction and Body Image Perception

The Figure Rating Scale (FRS) were used for the assessment of Body Image Dissatisfaction (BID) and body image perception. Stunkard *et al.* (1983) (32) developed the FRS tool as a simple and visual tool to help in assessing individuals' dissatisfaction with body image and perceived body size. The tool comprises nine body image drawings depicting different sizes as indicated in figure 1 below. The drawings range from slim (1) to very big or plus size (9). The assessment was done by asking the participants to select their body size they resembled (perceived body size) and the one they liked to look like (ideal body size). The subjects' BID was assessed by determining the difference between their perceived and ideal body types. Those who scored zero would be satisfied with their body sizes while negative and positive scores indicated that the subjects were dissatisfied. While negative scores depicted that the subjects desired to gain weight, the positive scores would indicate the desire to lose weight. Body Image percrption was assessed by comparing the perceived body size with the actual body size according to the subjects' BMI levels. In this case, zero scores would indicate a correct estimation with current body image, while negative or positive scores would depict underestimation or overestimation. The numbers on the FRS were classified based on BMI categories as follows; underweight (1 and 20), healthy weight (3 and 4), overweight (5-7), and obese (8 and 9). This tool approved to be validity and reliability in different populations (30).

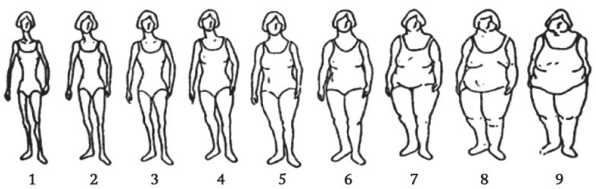

Fig. 1: Body image figures.
FRS tool for the assessment of body image perception among the subjects. Adapted from Stunkard et al. (1983).

Statistical Analysis Procedure

The data were analysed using SPSS version 21, the frequencies for category variables presented as percentages (%), means ± standard deviation presented for continuous variables. Also, participants were grouped according to their actual BMI to present the percentages of BID for each group. To test the relationship between the BMI and BID, we used Spearman Rank Correlation. The normality was tested using the Kolmogorov-Smirnov test, and the data were normally distributed ($p \geq 0.05$).

Results

Participant Measurements and Participants

Table 1 below outlines the characteristics of the 226 participants. Mean age of participants was 21.8 years ± 3.2 SD. The mean weight for participants was 55.28 kg with SD=5.5, and the BMI means was 22.57kg/m^2. The majority of participants were single with 88.5 % (n=200), married was only 10.6% (n=24) of the whole sample. Most of the participants had an income of 5000 SAR-12999 SAR with 38.1% (n=86); the rest income category ranged between 17.3% and 24%. Almost all the participants (99.1%; n=224) reported healthy status with no diseases, and only 2 had type 1 diabetes. The majority of participant (61.5%, n= 139) classified with a healthy weight (BMI 18 To 24.9 kg/m^2), while 17.7% (n=40) were overweight and 15.3% (n=35) were underweight and only 5.3% (n=13) were obese. Of 226 participants, 182 (80.5%) were dissatisfied with body image. Most of them desired to lose weight, 135 (59.7%), while 47 (20.8%) desired to gain weight. Only 19.5% (44) of total participants were satisfied with body image.

Fig. 2 below presents the percentage between BMI with its four categories and body image dissatisfaction. Of those who were with a healthy weight only 19.7% were satisfied with their current body size. 57.5% of healthy weight women wished to lose weight, while 17.3% of healthy weight want to gain weight and wished for larger body size. The underweight category has 14.3 % (n=35) dissatisfied participants, and want to lose weight. 2.5% and 8.3% of those who category as overweight and obese, respectively, wished for larger body size.

Table 1: Participant's characteristics (N=226)

Variables	Mean	SD
Age (years)	21.8	3.2
Height (cm)	157.23	5.66
Weight (kg)	55.28	5.05
BMI (kg/m^2)	22.57	4.41
Dissatisfaction with body Image	**Frequency**	**Percent**
Satisfied with Body image	44	19.5
Dissatisfied with body image	182	80.5
desired to lose weight	135	59.7
desired for gain weight	47	20.8
BMI categories		
Underweight	35	15.5
Healthy weight	139	61.5
Overweight	40	17.7
Obese	12	5.3
Marital status		
Single	200	88.5%
Married	24	10.6%
Divorce	2	0.9%
Income		
Below 5000 SAR	53	23.5%
5000 SAR - 12999 SAR	86	38.1%
13000 SAR - 17000 SAR	39	17.3%
More than 17000 SAR	48	21.2%
Health status		
No diseases	224	99.1%
Diabetes	2	0.9%

Table 2: The distribution of body image perception was as illustrated in Table 2. Overall, 64.6% of participants perceived their body adequately, while 35.4% estimated their weight either under or over than their actual body size. All obese and almost one third (32.3%) of the overweight participants underestimate

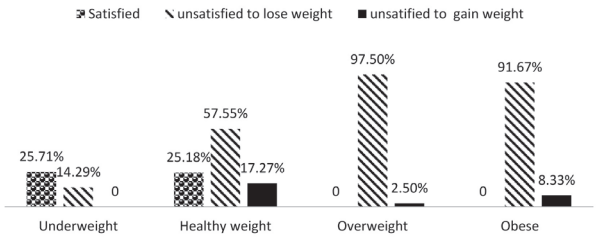

Fig. 2: The distribution of participants in different BMI by BID (N=226)

their body size. 11.5% and 20% of those with underweight and healthy weight respectively overestimate their actual BMI.

Correlation between BMI and Body Image Dissatisfaction

The BMI and BID correlated positively (r=0.135, p<0.05). Those with greater BMI were less satisfied with their current body image. While there was no correlation between the BMI and body image perception.

Discussion

This study investigated the prevalence of body image dissatisfaction (BID) and the perception of body image across BMI categories among young women age 18 to 35 years in Makkah region, and it explored the relationship between actual BMI and BID in these women (n=226). Overall, the prevalence of BID was high (80.5%), while only 19.5% of these women were satisfied with their body image. Similar to our results, an Emirate study that recruited both men and women with age 18 to 25 years old, found that 80.9% were dissatisfied with body image (33). In comparison to a Saudi study that used the same methodology but conducted in a different area in Saudi Arabia, it was

Table 2: Distribution of body image perception across actual BMI among young women (n=226)

Body image perception		BMI categories Frequency (percent)				Total	X^2
		Underweight	Healthy weight	Overweight	Obese		
Adequate		28 (80)	93(66.9)	25(62.1)	0	146(64.6%)	0.04
Misconception	Underestimate	0(0)	30(21.6)	13(32.5)	12 (100)	55(24.3%)	
	Overestimate	7(20)	16(11.5)	2(5.0)	0	25(11.1%)	
Total		35(15.5)	139(61.5)	40(17.7)	12(5.3)	226	

found that the prevalence of BID was 73.6% amongst Saudi women who lived in Taibah (26), which is lower than our finding. In a different Saudi study that used a different methodology in assessing the BID by Body shape Questionnaires, it found that around 33% of young women were dissatisfied with their body shape (28), while in Pakistani almost 100% of the sample were dissatisfied with body image (34). Studies conducted in Brazil (35) and Poland (36) showed a lower prevalence of BID with 47.3% and 65.6% respectively.

Regarding the relationship between actual BMI and BID, the study established that BMI was positively correlated with BID amongst these young Saudi women (n=226). However, the degree of the positive correlation between BMI and BIS was shown to be small for young Saudi women. A straightforward explanation of why the association was small (r=0.135) was because even the young women who had healthy weight were not pleased with their body appearance. The findings indicated that the percentage of participants with a healthy weight and satisfied with their current body image was low (19.7%), taking into consideration that the prevalence of healthy weight was high (61.5%). Despite the prevalence of overweight and obesity being low (23%), it was found that more than half of the participants (59.7%) were eager to lose their weight. The finding could be attributable to the Western media effect (25, 37), advocating for certain body images as beautiful and good looking, since 57.6% of those with a healthy weight wished to lose weight. This aspect of the need to lose weight even when some has ideal weight may be as a result of body image dissatisfaction. Although their BMI indicated that their weight was ideal, they may be dissatisfied with some aspects of their body, such as shape, size, and figure.

The BID could also be associated with peer contact influence and media portrayal of the ideal female body image as very thin. 14.3% of those who desired to lose weight was underweight, and such percentage is considered to be too high posing a significant problem. This finding shows that even those who are underweight have body image dissatisfaction and that they wish to lose more weight rather than gain weight to attain a healthy weight mark. The root cause could be considered to be as a result of women's unhappy mood with their body shape or composition. The reason for the unhappiness may be due to media portrayal and societal perception of ideal image as extremely thin. Even though a young female may be underweight, she may wish to lose more weight to achieve the body shapes and sizes that are considered ideal in the current society or by their peers. For this group, the traditional cultural influence on body shape may have been outdone by the media influences, when it comes to ideal body image and body image dissatisfaction. This aspect indicated that body image distortion might lead to poorer psychological outcomes, unhealthy eating habits, and over exercising to achieve the body image that they consider ideal (11, 12, 19, 38).

Another explanation for this group of participants who wished to lose weight especially those who were at a healthy weight and being unsatisfied with their body image, maybe because they are unhappy with specific parts of their body which may have high fat, and low muscle mass even if they classified with the healthy weight based on the BMI measured. BMI is a simple measure of height and weight and does not include a fat percentage or other anthropometrics in consideration (8). Additionally, a study showed that most of the women in Saudi Arabia are physically inactive (39). A sedentary lifestyle may result in high accumulation of fat and low muscle mass in physically inactive people, causing BID in this participant group. However, the study shows that there is a misconception of the ideal body image and the healthy weight amongst the young Saudi females.

On the contrary, the percentage of those at a healthy weight and wish to gain weight is also alarming at 17.3%. Other results that were worth reporting were that 2.5% and 8.3% of those who were classified as overweight and obese respectively, desired to gain weight despite having an unhealthy weight. However, it is essential to indicate that if the same women go ahead and manage to gain weight, they become overweight and/or obese, exposing themselves to the risks associated with weight-related chronic illnesses like diabetes. Obese and overweight young women who prefer to gain more weight to conform to the community expectations may risk overweight issues at some point in their lives (2, 40). Overall, the study underscores the need for advice and sensitisation of people about the need to appreciate their body image, size, and shape,

as well as the benefits of maintaining a healthy weight. Such programs may touch on the negative impacts of unhealthy eating habits, being overweight, or underweight (40).

The reason behind yearning to gain more weight is their unhappy mood with their body composition, especially their figure. Also, for those at healthy weight and desiring to add more weight could be due to culture and society effect as they think that plump women are more beautiful and attractive (37). Another explanation would be the misconception of a healthy weight. The findings revealed that the majority of participants (61.5%) correctly perceived their weight while all obese, 32% of overweight and 21.3% of healthy weight underestimated their BMI. This finding is coherent with the conclusion by Albeeybe *et al.* (26), whereby, a notable proportion of young females in college, especially the obese or overweight, tend to underestimate their perceived body weight. The trend indicates the prevalence of body image distortion among young females. Generally, most of the participants in the current study saw their BMI adequately. The finding of our study can also imply that a section of the Saudi females has failed to subscribe to the portrayal of the thin body as the ideal body image but are also in danger of becoming overweight. The finding also appears to contradict with results of studies that a higher BMI leads to a panic of being negatively judged by others (26). The outcome is contradictory, due to the pervasiveness of overweight and obesity in Saudi Arabian population. According to (26), underestimation of bodyweight amongst young Saudi females with overweight or obesity are alarming.

Taking into consideration the cultural, societal, and media impact on the satisfaction of body size may help avoid future problematic issues such as eating disorders and public health concerns regarding an increase in chronic illnesses. According to (20, 41), culture, ethnicity, and socioeconomic factors have been shown to have protective impacts against body image dissatisfaction and worrying disorders in women. Body mass index is an essential destructive factor regarding the increment of negativity about body image. In line with Albeeybe, *et al.* (27) study, obese and overweight participants showed considerably advanced intensities of body image dissatisfaction, more shape, and weight worries than underweight and normal participants did. Young women with obesity and overweight issues reported disconsolate feelings about the shape and appearance of their bodies (42). For female students in colleges, higher BMI is linked with body image dissatisfaction (33) and poorer psychosocial outcomes (43). Two studies conducted amongst young women in Saudi Arabia who had greater weight showed that they had a high frequency of depressive disorders (43) and were highly concerned about their body image (26, 27).

Obesity and body image dissatisfaction has been associated with a decline in self-esteem levels (44). Also, the preference for thinner bodies amongst young women in Saudi Arabia may be associated with exposure to the Western way of life through social media and online magazines that promote a certain body type as the most acceptable and beautiful. Similar to the finding by Muasiger (37), more underweight and overweight women tend to express dissatisfaction with their current body weight compared to normal-weight females (26, 27). Amongst females, overweight status was linked with eating disorders, unhealthy eating behaviours, emotional problems, and hopelessness (9, 38).

A major limitation of this study that should be addressed is that data on anthropometric measurements were self-reported, few studies concluded that data of self-reported weight and height can be used to obtained BMI values in public health studies (29-31, 45). However, a review concluded that the calculation of BMI from self- reported weight and height data may lead to misestimating of the BMI categories (46). Therefore, results of this study should be interpreted with caution.

Conclusion and Recommendations

This study showed that BMI is negatively correlated with body image satisfaction. Besides, body image dissatisfaction was proven to be a great challenge that may lead to adverse health problems, psychological issues, and public health concerns amongst young Saudi women. As such, the need for a systematic approach towards the problem of body image dissatisfaction is an essential element in the prevention of public health problems, such as increases chronic illnesses. Self-esteem can help protect people, especially the obese and the overweight, from the harmful effects of

high body mass index on the body image. The misconception of the ideal or healthy body image among a section of these young women was also associated with BID. It is important to note that cultural factors may also play an important role in body image satisfaction and body image misconception of the ideal body image. However, this study did not explore the cultural impact or the reasons behind this.

The recommendations focused on the need for further studies to validate self-reported weight in young Saudi women. Further public health interventions to address the problem of the BID. Future studies are needed to explore the underneath reasons behind BID and the cultural roles. Also, future studies to address lifestyle and psychological factors that may cause misconceptions about body weight and size should be considered. As a public health issue, it would be essential to focus on the factors that influence BID as a result of high or low BMI. In addition, the call to educate people about the importance of keeping fit and maintaining a healthy weight is an essential aspect. The current study findings indicate the need for intervention programs amongst young females about the importance of maintaining an ideal weight. Boosting women's self-esteem and minimize the chance of being negatively impacted by the media should be considered.

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Conflict of Interest

The writer declared that there is no potential conflict of interest relevant to this article.

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Predictive ability of waist-to-hip-ratio and waist-to-height-ratio in relation to overweight/obesity in adolescents from Vojvodina (the Republic of Serbia) predictive ability of waist-to-hip-ratio and waist-to-height-ratio

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Summary. *Introduction/Aim* Overweight in childhood is a risk factor for later diseases in adulthood. It is necessary to identify thresholds for anthropometric indexes for assessing obesity. The aim of our study was to explore the accuracy of waist-to-height ratio (WHtR) and waist-to-hip ratio (WHR) and for the first time proposes the optimal thresholds of these indices for identifying overweight/obesity in Serbian adolescents aged 11-15 years. *Methods* The cross-sectional study was conducted with 2391 adolescents. Anthropometric measurements included body height, weight, waist circumference and hip circumferences. The relation between WHR and WHtR and general obesity as defined by the International Obesity Task Force IOTF was investigated with nonparametric receiver operating characteristic (ROC) analysis. *Results* Both of the indicators of central adiposity showed a higher mean in the group of children with exceeded weight. The correlation of WHtR and body mass index (BMI) was considerably greater than the correlation between WHR and BMI in both sexes. The WHtR was a better predictor of general obesity than the WHR in both boys and girls. The WHtR cut-offs of 0.464 for boys and 0.465 for girls, and 0.510 for boys and 0.504 for girls have been proposed to identify overweight and obesity, respectively, in Serbian adolescents. *Conclusion* WHtR is an accurate index with high applicability to screening adolescents with excess weight.

Key words: waist-to-height ratio, waist-to-hip ratio, body mass index, adolescents, Serbia

Introduction

In the period of adolescence excess body fat, especially in abdominal region, can be related to a number of metabolic disorders, such as dyslipidemia, hypertension and hyperinsulinemia, all of them reflecting metabolic syndrome (MetS) (1). Considering this, efficient diagnostic tools to identify children and adolescents with excess fatness are becoming very important. Waist-to-height ratio is receiving increasing attention as a measure of children and adolescents' abdominal obesity (2). It has been suggested that the same cut-off value of 0.5 could be used across all age groups in children and adolescents (3), although some studies indicate that the cut-point of WHtR of 0.5 is not ideal for all ages (4), suggesting the need for age-related references. Also, small variance may be present according to ethnic backgrounds (5). Beside WHtR, the WHR has been suggested for use instead of the BMI in predicting the health risk in adults and adolescents (6). The WHR is also one of the indicators of central obesity in adolescents (7,8).

Since abdominal obesity tends to increase more intensively than overall obesity in adolescents (9), it is important to determine specific values of indicators of abdominal obesity in various populations of children

and adolescents. In Serbia, to the best of our knowledge, no population-based studies have evaluated the validity of WHtR and WHR as indicators of overweight/obesity in schoolchildren. The present study aimed to explore, for the first time, the feasibility and accuracy of WHtR and WHR and propose the optimal thresholds of these indices for identifying overweight and obesity in Serbian children aged 11-15 years.

Methods

The target population were schoolchildren attending primary schools in some regions of North and West Serbia. The cross-sectional study was performed in the period between 2012 and 2016 and included 2391 adolescents (1112 boys and 1279 girls) aged 11-15 years. The age was calculated as the difference between the date of birth and the date of data collection. The subjects were grouped into five age categories (10.50-15.49). Informed consent was obtained from participants and their parents before data collection and the inclusion of subjects was on voluntary basis. The research protocol was approved by the Scientific Committee of the Department for Biology and Ecology, University of Novi Sad and primary school principles. Anthropometric measurements were taken on standing participants wearing light clothing and without shoes using standard techniques. The same measurement protocol, as described by Weiner and Lourie (10) and Lohman et al. (11) was used in all measurements. Anthropometric measurements included body height, weight, waist circumference and hip circumferences. Height was measured with anthropometer (±1mm; SieberHegnerMaschinen AG Zürich Switzerland) with the head positioned in the Frankfurt plane, and portable electronic digital scale was used to measure weight with accuracy ± 0.1kg. Waist circumference (WC) was measured above the iliac crest and below the lowest rib margin at minimum respiration with an inelastic flexible tape in a standing position. Hip circumference (HC) was measured at the maximum protuberance of the buttocks. Body mass index was calculated from the ratio of weight/height^2 (kg/m^2). The subjects were classified into underweight, normal weight, overweight (OW) and obese (OB) categories according to age- and sex specific cut-off points proposed by the International Obesity Task Force (IOTF) (12). WHR was calculated as waist circumference (cm) divided by hip circumference (cm), and WHtR as waist circumference (cm) divided by height (cm). Abdominal obesity (AO) was defined according to WHR ≥ 0.9 (13) and WHtR ≥ 0.5 (3).

Statistical Analysis

Data were analyzed with SPSS software for Windows version 21 (SPSS Incorporation, Chicago, USA). Abdominal indices WHR and WHtR were expressed as mean ± standard deviation (SD). The correlation between the indicators of general (BMI) and abdominal obesity (WHtR and WHR) was determined by Spearman correlation coefficient. The overall significance level was set at $P < 0.05$. The relation between WHR and WHtR and general obesity as defined by the IOTF was investigated with nonparametric receiver operating characteristic (ROC) analysis. The discriminating power of the WHR and the WHtR was expressed as area under the curve (AUC) and 95% confidence intervals (CI). An AUC value of ≥ 0.90 was considered an excellent accuracy; 0.80-0.89 good; 0.70-0.79 satisfactory and <0,70 bad accuracy. The sensitivity and specificity of WHtR and WHR as indicators of overweight/obesity were determined with cut-off values. The Youden index was used to determine optimal cut-off values WHtR and WHR for identification of overweight/obesity (maximum value of (sensitivity + specificity -1)) (14).

Results

Overweight (OW) and obesity (OB) prevalence is nearly identical in boys (10.3% and 4.6%) and girls (10.1% and 4.7%, respectively). Higher percentages of abdominal obesity based on WHtR (14.9%) and WHR (13.5%) are also observed in boys than it is the case with girls (10.2%; 5.7%) (Table 1).

Means of WHR and WHtR in relation to nutritional status based on BMI, as well as the correlation of WHR and WHtR with BMI in both boys and girls are shown in Table 2. The means of WHR and WHtR in the nutritional status groups show a linear increase

Table 1. Prevalence of general and abdominal obesity (in boys and girls and total) [%(n)]

	BMI (kg/m2)			
	Overweight	**Obesity**	**WHR≥0.90**	**WHtR≥0.50**
Boys (N=1112)	10.3 (114)	4.6 (51)	13.5 (150)	14.9 (166)
Girls (N=1279)	10.1 (129)	4.7 (60)	5.7 (73)	10.2 (131)
Total (N=2391)	10.2 (243)	4.6 (111)	9.3 (223)	12.4 (297)

WHR: waist-to-hip ratio, WHtR: waist-to-height ratio, BMI: Body mass index

Table 2. Means of waist-to-hip ratio (WHR) and waist-to-height ratio (WHtR) in relation to nutritional status (BMI) and correlation of WHR and WHtR with BMI in boys and girls

		Boys		**Girls**		**Total**	
		WHR	**WHtR**	**WHR**	**WHtR**	**WHR**	**WHtR**
Underweight	n	128	128	181	181	309	309
	Mean	0.80	0.39	0.79	0.39	0.80	0.39
	SD	0.04	0.02	0.05	0.02	0.05	0.02
Normal weight	n	819	819	909	909	1728	1728
	Mean	0.83	0.44	0.80	0.43	0.81	0.43
	SD	0.07	0.04	0.07	0.03	0.07	0.04
Overweight	n	114	114	129	129	243	243
	Mean	0.86	0.52	0.83	0.50	0.84	0.51
	SD	0.06	0.04	0.07	0.04	0.07	0.04
Obesity	n	51	51	60	60	111	111
	Mean	0.88	0.58	0.84	0.56	0.85	0.57
	SD	0.05	0.05	0.09	0.06	0.08	0.06
BMI#	Rho	0.267	0.758	0.108	0.737	0.195	0.798
	P	0.000	0.000	0.000	0.000	0.000	0.000

Spearman correlation, Rho: Correlation Coefficient, P: Significance, BMI: Body mass index;

in both sexes. The WHR and WHtR are in significant positive correlation with BMI in both boys and girls. The correlation between WHtR and BMI is significantly greater than the correlation between WHR and BMI in all subjects.

Figures 1 and 2 show the ROC curves of WHR and WHtR for predicting OW and OB in both boys and girls. WHtR index shows greater predictability of exceeded weight overweight/obesity than WHR.

Table 3 presents the area under the ROC curve

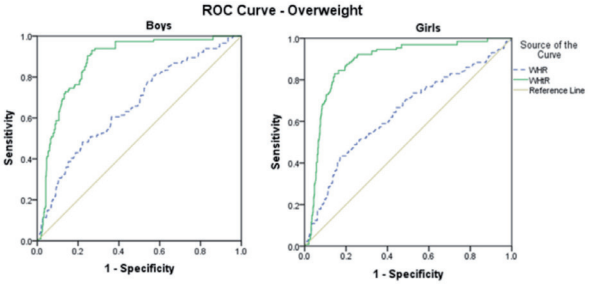

Figure 1. Receiver operating characteristic (ROC) curve for prediction of overweight from waist-to-hip ratio (WHR) and waist-to-height ratio (WHtR) in both sexes.

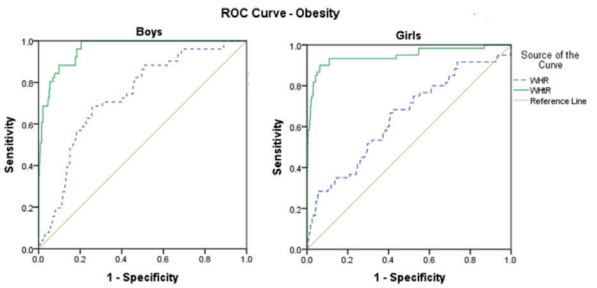

Figure 2. Receiver operating characteristic (ROC) curve for prediction of obesity from waist-to-hip ratio (WHR) and waist-to-height ratio (WHtR) in both sexes.

(AUC) of WHR and WHtR. The two indices show a satisfactory prediction capacity for identifying OW and OB in children of both sexes (95% CI > 0.5) in all ages referring to WHtR, and in most ages when it comes to WHR. ROC analysis shows that WHtR has higher discriminating power to detect IOTF obesity than WHR. In both boys and girls, WHtR proves to be a better predictor of general obesity than the WHR.

Optimal cut-off values of WHR and WHtR as indicators of overweight and obesity in boys and girls are shown in Table 4. Cut-off values of WHR for detecting OW equal 0.846 in case of boys and 0.803 in girls. Sensitivity is 60.5% and 66.7% and specificity 63.6% and 55.9% in boys and girls, respectively. In boys, WHR shows higher cut-off values for detecting obesity (0.866) than in girls (0.808). Sensitivity values are approximately identical in both sexes, while those referring to specificity are greater in boys. As for overweight, the obtained WHtR cut-off values of 0.464 in boys and 0.465 in girls give sensitivity of 90.4% and specificity of 75.6% in case of boys, and 82.9% and 85.5% in case of girls. For assessing overweight, highest cut-off values of WHR and WHtR are detected in boys aged 12 and girls aged 11. For assessing obesity in boys, the highest WHR and WHtR cut-off values are observed at the age of 12 and 13, respectively. In girls, the highest cut-offs of both indices are observed at the age of 11.

Discussion

This study presents the first gender specific optimal thresholds for WHR and WHtR for Serbian adolescents aged 11 to 15 years. The relationship between BMI and abdominal adiposity observed in the present study is consistent with previous study reports. Body mass index correlates with total and visceral body fat, which is considered an important risk factor for chronic-degenerative diseases (15). In our study, the correlation between WHtR and BMI is significantly higher than the correlation between WHR and BMI. This might seem as an expected result because both parameters take into account the body height. In the Tuscany (Italy) study (16) the correlation between WHtR and BMI was also high for the overall sample. Both indicators of central adiposity show a higher mean in the group of subjects with exceeded weight, and this re-

Table 3. Area under the ROC curve (AUC) for identification of optimal waist-to-hip ratio (WHR) and waist-to-height ratio (WHtR) for predicting overweight and obesity defined by BMI in relation to age and sex

Age (years)	Boys		Girls	
	WHR AUC (95% CI)	WHtR AUC (95% CI)	WHR AUC (95% CI)	WHtR AUC (95% CI)
Owerweight				
11	0.520 (0.401-0.671)	0.843 (0.738-0.908)	0.610 (0.506-0.790)	0.917 (0.856-0.979)
12	0.766 (0.689-0.843)	0.913 (0.880-0.947)	0.629 (0.484-0.717)	0.840 (0.774-0.906)
13	0.663 (0.566-0.761)	0.835 (0.747-0.918)	0.615 (0.517-0.733)	0.912 (0.878-0.946)
14	0.578 (0.409-0.682)	0.893 (0.848-0.938)	0.673 (0.559-0.787)	0.862 (0.787-0.941)
15	0.781 (0.617-0.846)	0.891 (0.832-0.950)	0.666 (0.538-0.795)	0.881 (0.846-0.953)
Total	0.662 (0.609-0.715)	0.875 (0.846-0.904)	0.639 (0.585-0.693)	0.882 (0.853-0.911)
Obesity				
11	0.821 (0.781-0.941)	0.955 (0.910-1.000)	0.594 (0.491-0.798)	0.988 (0.963-1.000)
12	0.775 (0.723-0.892)	0.996 (0.990-1.000)	0.603 (0.507-0.834)	0.892 (0.803-0.986)
13	0.776 (0.679-0.873)	0.983 (0.969-0.998)	0.651 (0.505-0.768)	0.909 (0.782-0.935)
14	0.698 (0.609-0.837)	0.929 (0.918-0.989)	0.648 (0.543-0.812)	0.981 (0.977-1.000)
15	0.607 (0.504-0.770)	0.941 (0.819-0.997)	0.739 (0.612-0.866)	0.961 (0.921-1.000)
Total	0.735 (0.672-0.799)	0.961 (0.943-0.979)	0.647 (0.571-0.723)	0.946 (0.907-0.985)

AUC: area under the curve, ROC: receiver operating characteristics, CI: confidence interval,

Table 4. Optimal cut-off values of waist-to-hip ratio (WHR) and waist-to-height ratio (WHtR) as indicators of overweight and obesity in boys and girls

		WHR				WHtR			
		Cut-off Value	Se (%)	Sp (%)	Jouden Index	Cut-off Value	Se (%)	Sp (%)	Jouden Index
Boys									
OW	11	0.809	59.8	75.3	0.35	0.461	95.4	71.9	0.67
	12	0.888	61.4	62.9	0.24	0.469	95.3	81.3	0.77
	13	0.847	60.2	65.5	0.26	0.464	89.4	72.8	0.62
	14	0.821	60.1	50.5	0.11	0.460	79.3	76.4	0.56
	15	0.865	61.2	63.8	0.25	0.466	92.5	75.5	0.68
	Total	0.846	60.5	63.6	0.24	0.464	90.4	75.6	0.66
OB	11	0.894	71.0	80.0	0.51	0.513	91.0	92.5	0.83
	12	0.898	69.3	79.3	0.49	0.519	96.6	94.1	0.91
	13	0.858	74.6	73.3	0.48	0.521	90.0	93.1	0.83
	14	0.849	64.9	69.9	0.35	0.486	81.3	88.8	0.70
	15	0.829	63.0	68.7	0.32	0.489	82.0	82.8	0.65
	Total	0.866	68.6	74.2	0.43	0.510	88.2	90.2	0.78
Girls									
OW	11	0.810	67.4	56.3	0.24	0.484	91.4	81.6	0.73
	12	0.805	70.4	53.9	0.24	0.457	78.6	77.8	0.56
	13	0.801	68.4	47.9	0.16	0.467	84.5	90.4	0.75
	14	0.800	64.9	60.0	0.25	0.449	77.5	86.7	0.64
	15	0.801	62.6	61.3	0.24	0.466	82.6	90.9	0.74
	Total	0.803	66.7	55.9	0.23	0.465	82.9	85.5	0.68
OB	11	0.821	62.1	59.1	0.21	0.520	100	94.0	0.94
	12	0.812	65.7	57.9	0.24	0.480	79.0	82.3	0.61
	13	0.803	71.9	53.1	0.25	0.504	91.3	86.6	0.78
	14	0.804	60.1	65.2	0.25	0.510	100	96.1	0.96
	15	0.801	81.8	55.0	0.37	0.506	98.0	91.1	0.89
	Total	0.808	68.3	58.0	0.26	0.504	93.7	90.0	0.84

Se: Sensitivity, Sp: Specificity; OW: Overweight; OB: Obesity

sult is in agreement with other studies on children and adolescents (17-19).

The results of the study point out that WHtR is a better predictor of general obesity than WHR in both boys and girls. According to AUC values WHtR appears to be good in predicting overweight and excellent for obesity prediction. In both sexes, the accuracy of WHtR ranged from 0.875 to 0.961 in identifying overweight and obese children. The accuracy of WHR, however, is lower (overweight: 0.875 vs. 0.662 in boys, 0.882 vs. 0.639 in girls; obesity: 0.961 vs. 0.735 in boys, 0.946 vs. 0.647 in girls). The results of research in other countries (20,21), also show that WHtR appeared as the best predictor to classify the nutritional status of children. In our investigation, the area under the curve for WHtR was greater in boys than in girls in defining obesity, the result being in line with other reported findings (20,22-25).

In case of WHR, the cut-offs to identify overweight were higher in boys than in girls, 0.846 vs 0.803. The same holds true for identification of obesity: WHR of 0.866 vs 0.808, and WHtR of 0.510 vs 0.504, respectively. The only exception refers to the cut-off values of WHtR for overweight, where the val-

ues were almost the same (0.464 and 0.465 for boys and girls, respectively). The present study shows that the optimal WHtR cut-off value of 0.46 for identifying overweight in adolescents aged 11-15 gives the sensitivity of 90.4% and specificity of 75.6% in case of boys, while in girls the values equal 82.9% and 85.5%, respectively.

In case of obesity, the best combination of sensitivity and specificity is detected in the optimal cut-off value of 0.510 in boys (88.2% and 90.2%, respectively) and 0.504 in girls (93.7 % and 90.0%, respectively). Similar values were obtained in children aged 6 to 14 in Spain (26) for overweight (0.47 and 0.48 both males and females) and obesity (0.51 males; 0.50 females). Slightly higher values have been observed in Korean children aged 6-18 years, where the WHtR cut-off value for discriminating OW has been reported to be 0.49 in case of boys and 0.48 in girls. As for obesity, similar values to ours are reported by Gil et al. (22), 0.51 in boys and 0.49 in girls. Hovewer in recent investigations in Korea (21) the cut-offs for obesity are lower than ours (boys 0.50; girls 0.47). Lower values were observed in Chinese (20), Brazil (27) and South India population (28).

Differences detected between age groups suggest age-specific WHR and WHtR values, i.e. WHR ranging from 0.809 to 0.888 among boys and 0.800 to 0.810 among girls for overweight and from 0.829 to 0.898 among boys and 0.801 to 0.821 among girls for obesity. As for WHtR, the values range from 0.460 to 0.469 among boys and 0.449 to 0.484 among girls for overweight and from 0.486 to 0.521 among boys and 0.480 to 0.520 among girls in case of obesity. The different cut-offs are maybe due to physical changes during adolescence. The same trend was noticed in the investigation of adolescents aged 10 to 15 years in Brazil (27) where differences in WHtR were observed in different age groups. However, among Spanish children and adolescents aged 6-16 years, no differences in WHtR cut-offs by pubertal stage were found (29).

The limitation of the present study lies in the fact that it was a cross-sectional and not a population-based survey with a representative sample. Therefore, the obtained results should be extrapolated with caution.

Conclusion

These data refer to one part of the adolescent population, however, the present results can be used as a starting point for future research in this field. The WHtR cut-off points of 0.464 in boys and 0.465 in girls, as well as those of 0.510 in boys and 0.504 in girls can be suggested to identify overweight and obesity, respectively, in Serbian adolescents.

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Changes in anthropometric, biochemical, hematological, hormonal and cardiac markers in a group of late-adult amateur cyclists, after continuous and prolonged exercise on an uncontrolled diet

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Summary. *Purpose:* to describe and compare the main biochemical and hematological parameters, markers of cardiac and stress suffering (cortisol), in an amateur group of 8 late-adult cyclists (average age 60.9 years s.d. 4.1 years) before and after a continuous bicycle course of 9 days with an actual duration of 7 days and a daily average of 103.5 Km (total of 725 km) on an uncontrolled diet. *Results:* body weight, BMI, systolic and diastolic pressure did not vary significantly in pre- and post-cycling performance ($p > 0.05$). There was no significant change in the pre- and post-red blood cell count in the hemochromocytometric hematological parameters ($p = 0.57$), while hemoglobin values decreased significantly after pedaling ($p = 0.03$), as did the average cellular hemoglobin values and the average cell concentration of hemoglobin ($p = 0.002$ and $p = 0.0006$, respectively). The number of platelets, white blood cells, the absolute number of neutrophils, lymphocytes, eosinophils, basophils and monocytes, and the percentage of neutrophils, eosinophilic lymphocytes, basophils and monocytes did not change significantly ($p > 0.05$). With regard to basic biochemistry, there was no significant variation in the values of glucose, urea, creatinine, alanine amino transferase, alkaline phosphatase, cholinesterase and creatinine kinase ($p > 0.05$). Aspartate amino transferase was found to be significantly greater after pedaling ($p = 0.03$). The values of albumin, total proteins, lactate dehydrogenase, total calcium, inorganic phosphorus, total magnesium, total iron, sodium and potassium were statistically non-significant between pre and post phases. The lipid profile, total cholesterol, triglycerides, lipases, HDL and LDL were also statistically non-significant even if HDL values increased on average after cycling performance (before 48.9 ± 9.5 and after 53.8 ± 12.4) while LDL values decreased on average (before 118.5 ± 28.8 and after 101.6 ± 10.3). In the hormone-labeling and vitamin group, ferritin was statistically non-significant. Pre and post changes in the stress hormone cortisol, PSA, vitamin B12 and natriuretic B-type NT-proBNP peptide were statistically non-significant. Instead, folate decreased significantly following the cycling performance ($p = 0.017$). In protein biochemistry, apolipoprotein A1 was statistically significant ($p = 0.038$) increasing after pedaling, while apolipoprotein B, C-reactive protein and transferrin were statistically non-significant. CK MB mass and troponin I in the cardiac markers did not undergo significant changes between pre and post phases. *Conclusions:* despite the small size of the chosen sample, parameters analyzed between pre and post continuous physical effort lead to the conclusion and confirmation of many data in the literature and, that is, that sporting activity conducted in an important way can improve the biochemical/functional state and, therefore, the health of practising subjects even in late adults and/or the elderly. This could postpone physical psychic decline caused by the natural progression of years.

Key words: older adults, physical exercise, cycling

Introduction

Physical inactivity associated with a high calorie and unhealthy diet are often the cause of obesity and type 2 diabetes, and chronic and degenerative diseases that are very widespread, ubiquitous and constantly increasing (1).

It is proven that physical activity should therefore be increased together with an adequate caloric intake and micronutrients. This leads to benefits in the health of individuals and society. Today, more and more people are dedicated to long-distance sports such as marathons, long bike rides and triathlons. However, both the positive and negative health mechanisms remain unclear.

A high level of physical activity is associated with a reduction in low-grade inflammatory states and therefore less likely to develop obesity and type 2 diabetes (2-4).

There is evidence that physical activity combined with weight loss attenuates low-grade inflammation (5) and it has also been shown that 15 weeks of lifestyle change consisting in a low-calorie diet and physical activity in obese subjects decrease low-grade inflammation and the density of macrophages in adipose tissue together with an increase in insulin sensitivity and an improvement in metabolic activity (6). From a meta-analysis study in patients with coronary artery disease, it appears that C-reactive protein and fibrinogen undergo a strong reduction as a result of exercise (7)

Cycling is considered to be a physical activity that has the ability to improve the quality of life of those who practise it. All this is particularly true in those who practise it at amateur level. However, performance decreases with longevity, and particularly in relation to prolonged efforts in resistance performance. There are few data in literature that represent the biochemical state of repeated and prolonged exercises in elderly people over time (8).

The aim of this study was to describe the main biochemical parameters, markers of cardiac and stress suffering (cortisol) in an amateur group of 8 late adult cyclists (mean age 60.9 years sd 4.1 years) before and after a continuous cycling route of a total of 725 km.

Materials and methods

Study design

8 amateur cyclists (7 males and 1 female aged 60.9 ± 4.1 years and height of 174.5 ± 14.4 cm) participated in the project which included a total route of 725 km divided into 7 stages whose characteristics are shown in Table 7. All participants had carried out cycling activities 2-4 times a week in the months prior to departure and no special dietary measures were taken during the trial. Of the 8 participants, 3 participants took medications for: heart disease, arterial hypertension, epilepsy and hypothyroidism. Dietary bars and saline supplements were taken before, during and after the ride. All subjects were tested by means of fasting venous blood on the day before and after the cycling performance. Analytical tests included main anamnestic data, blood count, basic biochemistry, some hormone-markers and vitamins, biochemistry of proteins and cardiac markers (Tables 1-6)

Ethical Approval

All participants gave their informed consent.

Analytical procedures

Serum glucose, creatinine, total cholesterol, HDL and LDL cholesterol, ALT, AST, testosterone, prolactin and 17-beta-estradiol were commercially available using Vitros 5500 (Ortho Clinical Diagnostic, Milan, Italy). Cortisol was measured by Radio Immuno Assay, Beckman Coulter S.P.A. Cassina de Pecchi, Milan Italy.

Statistical analysis

Data were analyzed using the IBM SPSS Statistics 24 statistical program. The two-tailed t-test was used to verify any changes before and after the study, taking $P \leq 0.05$ as significant.

Results

The results of the various biochemical parameters and anthropometric data are shown in Tables 1-6 (see online for Tables as supplementary files). With regard to anthropometric data (See online Tab. 1),

body weight and BMI did not change significantly (p = 0.29 and p = 0.31, respectively), nor did the physiological values of diastolic and systolic blood pressure (p = 0.66 and p = 0.74, respectively). Analyzing hematology parameters (See online Tab. 2), the pre and post red blood cell count did not change significantly (p = 0.57), while hemoglobin values decreased significantly after pedaling (p = 0.03), as did the average cellular hemoglobin values and the average cell concentration of hemoglobin (p = 0.002 and p = 0.0006, respectively). There was no significant variation in the number of platelets, white blood cells, the percentage of neutrophils, eosinophilic lymphocytes, basophils and monocytes (p> 0.05). There was also no significant variation with regard to the absolute number of neutrophils, lymphocytes, eosinophils, basophils and monocytes (p> 0.05).

There was no significant variation in the values of glucose, urea, creatinine, alanine amino transferase, alkaline phosphatase and cholinesterase (p> 0.05) in the basic biochemistry (See online Tab. 3). Aspartate amino transferase was found to be significantly greater after pedaling (p = 0.03). Creatinine kinase increased after cycling (before 134.6 ± 31.4 and after 159.1 ± 40.1), although there is no statistically-significant difference (p = 0.09). The pre and post-values of albumin, total proteins, lactate dehydrogenase, total calcium, inorganic phosphorus, total magnesium, total iron, sodium and potassium were statistically non-significant. Iron-binding capacity and chloride are at the limit of statistical significance (p = 0.069 and p = 0.07, respectively). With regard to the lipid profile, total cholesterol, triglycerides, lipases, HDL and LDL, these were statistically non-significant even if HDL values increased on average (before 48.9 ± 9.5 and after 53.8 ± 12.4) while LDL values decreased on average (before 118.5 ± 28.8 and after 101.6 ± 10.3). In the hormone-markers and vitamins group analysis (See online Tab. 4), ferritin was statistically non-significant, although it showed a significant increase after pedaling (ferritin before 134.7 ± 112.8 and after 158.7 ± 108.2). The stress hormone cortisol was not significantly different pre and post-pedaling. Instead, there was a significant post-pedaling decrease in folate (p = 0.017). Vitamin B12 remained unchanged in the pre and post-pedaling phases. The B-type natriuretic peptide (NT-proBNP) did not differ statistically even if there is a marked decrease in post-pedaling values (before 84.9 ± 66.5 and after 46.6 ± 35.3). The prostatic activity marker (PSA) was not significantly different pre and post-pedaling.

As regards the biochemistry of proteins (See online Tab. 5), apolipoprotein A1 structural component of HDL was significantly different (p = 0.038) in structural proteins of the lipid component, which increased after pedaling. However, apolipoprotein B structural component of VLDL and LDL was not statistically different. C-reactive protein increased after cycling while remaining statistically insignificant. Transferrin was not significantly different even though there was an increase between pre and post-pedaling (before 220.4 ± 51.4 after 260.4 ± 12.7).

In cardiac markers (See online Tab. 6), pre and post muscle damage measured by CK MB mass and troponin I were not statistically different.

Conclusions

Overall data (See online Tables 1-6) conclude that there are no major differences between pre and post physical exertion which demonstrates that late adults and/or elderly people can also undertake a good level of physical activity. This can lead to improved metabolism with a possible slowing down of the pathophysiological processes typical of aging. Participants' mood also definitely improved thus proving that physical activity helps to deal with problems related to depression and the decline of psycho-physical energies (9).

The uncontrolled diet did not result in any significant weight loss as pressure values and pulsations per minute did not change significantly pre and post-pedaling.

From the data it emerges that there was a slight statistically-significant decrease between pre and post-pedaling in hemoglobin (p = 0.035), in the average cellular hemoglobin (p = 0.006) and in the mean cellular hemoglobin concentration (p = 0.002). This may be due to the so-called "sports anemia" which some authors consider to be a false anemia and, rather, a pseudoanemia which is an adaptation of the athlete's organism linked to sports activity; it can also be ex-

plained as an anemia by dilution; in fact, hemoglobin levels are lower than normal because the aerobic activity causes an expansion of the blood volume which results in a decrease in the concentration of erythrocytes (10).

As regards enzymes of hepatic origin, only aspartate amino transferase showed a significant increase in values ($p = 0.03$) although all the other enzymes (ALT, γGT, alkaline phosphatase, cholinesterase and albumin) show a compatible increase tendency with prolonged effort but not at statistically-significant levels. Creatinine kinase, which is a marker of tissue damage in skeletal muscle, showed an increase in values (before 134.6 ± 31.4, after 159.1 ± 40.1) while not achieving statistical significance ($p = 0.09$). With regard to folic acid, this resulted in a significant decrease ($p = 0.017$) which can be explained because folic acid is more metabolized in athletes.

As for the lipid profile, there is a significant increase in apolipoprotein A1, the main protein component of high-density lipoproteins (HDL, called «good» cholesterol) ($p = 0.037$). The relationship between apolipoprotein A1 and apolipoprotein B increases after cycling (from 1.82 to 1.91) and this is correlated with a reduction in cardiovascular risk. LDL values decrease after pedaling (from 118.5 ± 28.8 to 101.6 ± 10.3) and HDL increases (from 48.9 ± 9.5 to 53.7 ± 12.4), while not presenting a significant difference. This demonstrates that the lipid profile improves significantly after physical activity even in the elderly.

As far as cardiac markers (troponin I and PCK-MB) are concerned, no significant differences are noted between pre and post-pedaling. Values remained approximately unchanged even in the cardiopathic subject. No significant difference was shown in all other parameters. Despite the small chosen sample, the general framework of analyzed parameters leads to the conclusion and confirmation of many data in the literature and, that is, that sporting activity conducted in an important way can improve the biochemical/functional state and, therefore, the health of the practising subjects even in late adults and/or the elderly. This could postpone the physical psychic decline caused by the natural progression of years.

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Influence of food behavior and physical activity in relation to the overall physical condition of Romanian students

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Summary. The aim of the present study was to determine the influence between food behavior and physical activities in relation to the overall physical condition of Romanian students. The research study was conducted on a group of athletic and non-athletic students from one college and two faculties from Galati County, in Romania. For this purpose, a cross-sectional analysis of data was processed from a representative sample of 1,214 students aged between 19 and 25 years old who answered a self-reported diet history questionnaire (DHQIII) questionnaire to establish their food habits quantified in healthy eating index (HEI) 2015 score and self-administered international physical activity questionnaire (IPAQ) questionnaire to assess their physical activity. The EUROFIT test battery was used to determine the overall physical condition of the subjects. Within this study, after 3 weeks of the initiation of the main study, a different subgroup of 228 subjects aged 19 ± 25 years old also participated in an ancillary study including an interview about their physical activities and an interview with a dietician before completing IPAQ and DHQIII questionnaires. The objective was the determination of the overall physical condition of the subjects that have used EUROFIT tests battery. To express the students' overall performance in one score, it was calculated a total test score. The percentage of athletic male students overall involved in intense sports activities was 26.72% which was higher with 3.8% than the percentage of athletic female students (22.92%) tested in the main study. Median values of combined activities were expressed in metabolic equivalent min/week and ranged from 394.12 to 5586.44 for male students and from 412.53 to 4318.81 for female students. For the food habits, the median values for the HEI) score ranged from 50.4 to 55.1 for male students and from 51.3 to 54.2 for female students surveyed in the main study. Body fat percentage median values ranged from 22.6 to 16.2 for male students and from 28.6 to 20.1 for female students and body mass index median values ranged from 23.64 to 21.02 for male students and from 22.14 to 20.19 for female students that participated in the main study. The multivariate analysis of the results showed a strong correlation between their food habits and their overall physical conditions. The Pearson correlation coefficient between the HEI and total Eurofit test score values had the value $p = 0.046 < 0.05$ for male students and $p = 0.044 < 0.05$ for female students tested in the main study. The statistical analysis showed that there are strong positive correlations between the indices calculated in the main study and those in the ancillary study. Students with a healthy diet and high physical activity have had a higher overall physical condition than sedentary students or those with less healthy eating habits.

Key words: Physical activity, food habits, HEI 2015 score, BMI

Introduction

Physical activity and eating behavior are the most important factors that influence the health of people (1–3). The technical progress of the last decades has decreased considerably the physical activity of the students and increased the risk of developing cardiovascular diseases (3,4). Although most studies concluded that sustained physical activity contributed to good health, the assessment of physical activity and quantifying the effect on health is still in the researchers' attention (5–7). Another decisive factor in ensuring good health is nutrition (8–10). For various reasons, many students adopt an unhealthy diet and gain weight (11,12). The most frequently cited reasons, why students have an unhealthy diet, are lack of time, limited financial resources, culinary preferences, and the ability to cook on their own (13–15). In some cases, students would like to adopt a healthy diet, but the lack of the necessary knowledge does not give them the possibility of doing so (15). In many cases, nutrition information reached to the students through media channels, especially TV channels, but these shows have had a commercial side and have not provided sufficiently nutritionally accurate information (16,17). These things suggest the need to promote knowledge among students about the effect and importance of eating habits and physical activity on health.

The objective of the present study was to determine the influence between food behavior and physical activities in relation to the overall physical condition of a group of Romanian students from Galati University.

Materials and Methods

In this survey, anonymous self-administered questionnaires were distributed to a representative sample of 1,214 athletic and non-athletic students, males and females, aged between 19 and 25 years old (with an age median value of 22.6 years old) from one college and two faculties from Galati county, in Romania. Three kinds of variables were recorded during the study: anthropometric measures, fitness stage, assessed by EUROFIT tests (18), and self-reported data including food habits, various types of physical activities, health status, and lifestyles. After 3 weeks of the main study, a subgroup of 228 subjects aged 19 ± 25 years old (with an age median value of 22.3 years old) also participated in an ancillary study including an interview about physical activities and an interview with a dietician before completing an anonymous questionnaire called International Physical Activity Questionnaire (IPAQ) (19) to assess their physical activity and a self-reported anonymous diet history questionnaire (DHQIII) (20) to establish their food behavior and the determination of overall physical condition of the subjects was assessed using Eurofit Physical Fitness Test Battery (EUROFIT).

Anthropometric measurements

Anthropometric measurements were used in this study to calculate the body mass index (BMI) and body fat percentage of the subjects. To calculate the BMI index, the height and weight of the subjects were measured (21). A stadiometer device Seca 217 (Seca, Germany) was used to measure body height. The measurement was made with a precision of ± 0.1 cm, the subjects found in bare feet. The weight of the subjects was measured by weighing with an accuracy of ± 0.1 kg and was performed with an electronic medical scale Wunder (Wunder, Italy).

Skinfold test was used to measure body fat percentage. Measurements were performed with a Holtain skinfold caliper (Holtain, UK) by measuring subcutaneous tissue at four points (biceps, triceps, subscapular, and suprailiac skinfold). The measurements were performed on the right side of the body (21).

Food behavior

The evaluation of the students' eating habits was made using the healthy eating index (HEI) 2015 index proposed by the US Department of Agriculture (22). The HEI 2015 score evaluates 13 food groups from the diet components resulting in a score ranging from 1 to 100. The closer the score from the calculation is to 100, the more nutritionally appropriate the diet. To calculate the HEI 2015 index, the subjects completed DHQIII self-reported questionnaires.

Physical activity (PA)

The physical activity of the subjects was determined based on the IPAQ questionnaire, the short

version, translated into Romanian. Physical activity was expressed in metabolic equivalent (MET) min/week. According to the number of METs resulting from the sum of the activities declared in the questionnaire, the participants in this study were divided into three categories: **a**. Students with physical activity <600 MET min/week (low sports activity level), **b**. Students with physical activity between 600 and 2,999 MET min/week (moderate physical activity level), and **c**. students with physical activity> 2,999 MET min/week (high physical activity level).

Overall physical condition

EUROFIT tests were used to determine the overall physical condition of the subjects.

Eurofit test battery was performed according to the protocols in the Eurofit manual (18). The following tests were used: flamingo balance, plate tapping, sit-and-reach, standing broad jump, standing broad jump, handgrip strength, sit-ups bent arm hang, 10×5 m agility shuttle run, and the 20 m shuttle run (18). All tests were performed on the same day between 8.00 am and 2.00 pm.

Statistical analysis

In order to express the results from all Eurofit tests performed in a single score, the method described by Fjørtoft et al. (23) was applied. Moreover, by applying the Kolmogorov–Smirnov tests, it was determined that the scores obtained after the conversion of the results have a normal distribution. For each test to have the same weight in the overall score, all scores were transformed into z scores based on the sample mean calculation and the standard deviation. The total score was then calculated for each student as the average of the z scores of all the tests performed (23). All analyses were conducted with the use of SPSS Version 23 for Windows (SPSS, USA). For continuous variables, it was calculated the means and standard deviation and percentages for definite outcomes. The significance level was set to 0.05. The difference in results was considered statistically significant when a p value obtained was less than or equal to 0.05.

Results and Discussions

One of the results of this present survey was that the male students participated in a higher percentage of intense physical activity than female students.

The percentage of athletic male students overall involved in intense sports activities was 26.72% which was higher with 3.8% than the percentage of athletic female students (22.92%) tested in the main study (Table 1). The same tendency of male subjects to engage more in sports activities than female subjects was found in other studies (24,25).

Eurofit tests results

In order to estimate the internal consistency of the Eurofit test battery for the main study, Cronbach alpha value was calculated and the value 0.788 was obtained. This value confirmed the correctness of the test set based on which the final score was calculated.

Because the Cronbach alpha value is based on the correlations between the values of the items that make up the battery of samples administered to the subjects, in Table 2 are presented the values of the correlation coefficients and the associated probabilities for each pair of items, but also for the final score with each item. Analyzing Table 2, it can be seen a very good correlation between the variables analyzed for most variables with ($| r |> = 0.610$ and $p < 0.05$).

The internal consistency of the test set was also confirmed for the ancillary study (Cronbach's alpha =

Table 1. Distribution of students participating in the main and ancillary study by gender and PA levels.

		Main study			Ancillary study		
		Male students	**Female students**	**Total**	**Male students**	**Female students**	**Total**
		625 (51.5%)	**589 (48.5%)**	**1,214**	**127 (55.70%)**	**101 (44.30%)**	**228**
Sport activity level	Low	194 (31.04%)	198 (33.62%)	392 (32.29%)	38 (29.92%)	34 (33.66%)	72 (31.58%)
	Moderate	264 (42.24%)	256 (43.46%)	520 (42.83%)	54 (42.52%)	44 (43.57%)	98 (42.98%)
	Intense	167 (26.72%)	135 (22.92%)	302 (24.88%)	35 (27.56%)	23 (22.77%)	58 (25.44%)

Table 2. Main study Pearson correlation coefficients between individual test item scores and total test scores.

		Score	Flamingo Balance, number	Plate tapping, sec	Sit and reach, cm	Standing broad jump, cm	Grip strength, kg	Sit-ups, number	Bent arm hang, sec	Shuttle run 10 × 5 m, sec	VO2, max ml.kg^{-1} min−1
Score	Pearson Correlation	1									
	p										
Flamingo Balance, number	Pearson Correlation	0.868*	1								
	p	0.025									
Plate tapping, sec	Pearson Correlation	0.753	0.895*	1							
	p	0.048	0.016								
Sit and reach, cm	Pearson Correlation	−0.642	−0.610	−0.870*	1						
	p	*0.040*	*0.038*	*0.024*							
Standing broad jump, cm	Pearson Correlation	−0.947**	−0.957**	−0.806	0.583	1					
	p	0.004	0.003	0.043	0.029						
Grip strength, kg	Pearson Correlation	−0.839*	−0.823*	−0.515	0.192	0.878*	1				
	p	0.037	0.044	0.029	0.046	0.021					
Sit-ups, number	Pearson Correlation	−0.883*	−0.891*	−0.936**	0.855*	0.898*	0.647	1			
	p	0.020	0.017	0.006	0.030	0.015	0.045				
Bent arm hang, sec	Pearson Correlation	−0.886*	−0.908*	−0.787	0.572	0.940**	0.865*	0.912*	1		
	p	0.019	0.012	0.044	0.023	0.005	0.026	0.011			
Shuttle run 10x5m, sec	Pearson Correlation	0.899*	0.981**	0.943**	−0.740	−0.952**	−0.764	−0.960**	−0.924**	1	
	p	0.015	0.001	0.005	0.047	0.003	0.046	0.002	0.008		
VO2, max ml.kg -1min-1	Pearson Correlation	−0.900*	−0.959**	−0.939**	0.766	0.946**	0.748	0.982**	0.946**	−0.994**	1
	p	0.014	0.002	0.006	0.044	0.004	0.037	0.000	0.004	0.000	

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed).

0.783). This conclusion also resulted from the values of the correlation coefficients calculated for the total score and each variable entering its calculation, or for each pair of variables separately ($p < 0.05$) (Table 3).

Food behavior and overall physical conditions

Students evaluated in the main study who reported a healthier diet represented by the HEI index obtained better results in the physical evaluation represented in total score (Fig. 1). Similar results have been reported by Croll et al. [26] and Georgiou et al. [27]. For the food habits, the median values for the Healthy Eating Index (HEI 2015) score ranged from 50.4 to 55.1 for male students and from 51.3 to 54.2 for female students surveyed in the main study (Fig. 1).

The Pearson correlation coefficient between the HEI index and total Eurofit test score values had the value r = 0.56, p = 0.046 < 0.05 for male students and r=0.58, $p = 0.044 < 0.05$ for female students tested in the main study.

In the ancillary study, the median values of the HEI index score ranged from 51.2 to 54.6 for male students and from 50.8 to 54.6 for female students (Fig. 2).

The Pearson correlation coefficient between the HEI and total test score values had the value r = 0.54,

Table 3. Ancillary study Pearson correlation coefficients between individual test item scores and total test scores.

		Score	Flamingo Balance, number	Plate tapping, sec	Sit and reach, cm	Standing broad jump, cm	Grip strength, kg	Sit-ups, number	Bent arm hang, sec	Shuttle run 10 × 5 m, sec	VO2, max ml.kg^{-1} min^{-1}
Score	Pearson Correlation	1									
	p										
Flamingo Balance, number	Pearson Correlation	0.893*	1								
	p	0.017									
Plate tapping, sec	Pearson Correlation	0.833*	0.889*	1							
	p	0.039	0.018								
Sit and reach, cm	Pearson Correlation	−0.792	−0.633	−0.781	1						
	p	0.046	0.041	0.047							
Standing broad jump, cm	Pearson Correlation	−0.948**	−0.956**	−0.863*	0.731	1					
	p	0.004	0.003	0.027	0.049						
Grip strength, kg	Pearson Correlation	−0.703	−0.866*	−0.648	0.239	0.833*	1				
	p	0.049	0.026	0.044	0.048	0.039					
Sit-ups, number	Pearson Correlation	−0.837*	−0.776	−0.903*	0.957**	0.849*	0.457	1			
	p	0.038	0.048	0.014	0.003	0.033	0.036				
Bent arm hang, sec	Pearson Correlation	−0.898*	−0.954**	−0.918**	0.719	0.982**	0.835*	0.870*	1		
	p	0.015	0.003	0.010	0.047	0.000	0.038	0.024			
Shuttle run 10x5m , sec	Pearson Correlation	0.804	0.889*	0.868*	−0.788	−0.923**	−0.691	−0.912*	−0.946**	1	
	p	0.044	0.018	0.025	0.042	0.009	0.036	0.011	0.004		
VO2, max ml.kg -1min-1	Pearson Correlation	−0.897*	−0.955**	−0.944**	0.782	0.970**	0.771	0.913*	0.991**	−0.966**	1
	p	0.015	0.003	0.005	0.032	0.001	0.034	0.011	0.000	0.002	

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed).

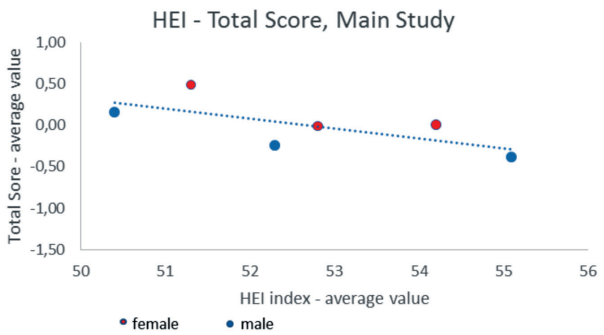

Figure 1. Main study. Male and female students HEI index and total score variation.

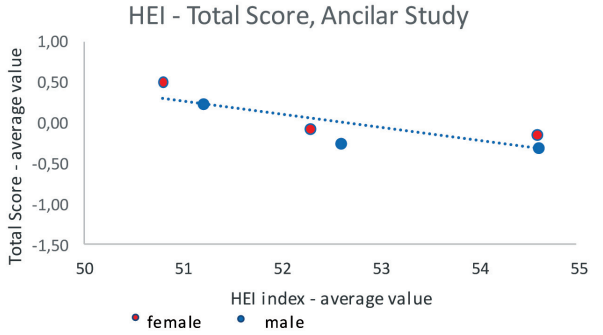

Figure 2. Ancillary study. Male and female students HEI index and total score variation.

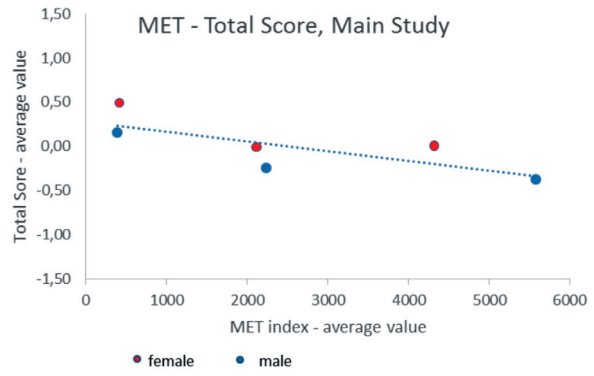

Figure 3. Main study. Male and female students MET index and total score variation.

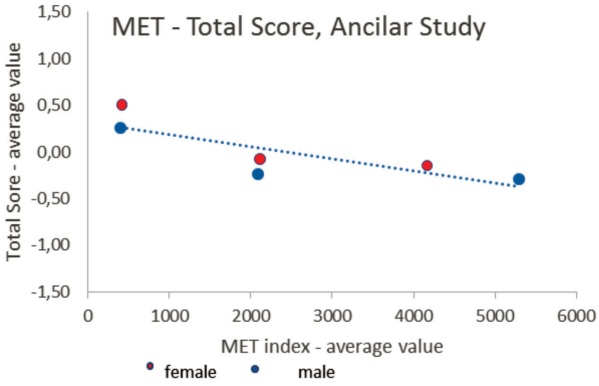

Figure 4. Ancillary study. Male and female students MET index and total score variation.

p = 0.042 < 0.05 for male students and r=0.57, p = 0.045<0.05 for female students tested in the main study.

The values of r >0.5, p < 0.05 obtained for both the main study and the ancillary study certified that it was obtained a good correlation between the scores obtained for HEI score and total score for both male and female students.

Physical activity

As expected, students who practiced more intense physical activity achieved better results in physical tests from the Eurofit battery. Similar results were reported by Cavadini et al. (28), Lee et al. (29), and Carraro et al. (30).

In the main study, median values of combined physical activities were expressed in MET min/week and ranged from 394.12 to 5586.44 for male students and from 412.53 to 4318.81 for female students (Fig. 3).

The Pearson correlation coefficient between the PA expressed in MET and total test score values had the value r=0.72, p = 0.048 < 0.05 for male students and r.6, p = 0.046 < 0.05 for female students tested in the main study.

In the ancillary study, median values of combined physical activities expressed in MET min/week ranged from 409.22 to 5296.44 for male students and from 421.53 to 4162.81 for female students (Fig. 4).

The Pearson correlation coefficient between the PA expressed in MET and total test score values had the value r= 0.75, p = 0.047 < 0.05 for male students and r=0.77, p = 0.045 < 0.05 for female students tested in the ancillary study.

BMI and body fat percentage

Body fat percentage median values ranged from 22.6 to 16.2 for male students and from 28.6 to 20.1 for female students and BMI median values ranged from 23.64 to 21.02 for male students and from 22.14 to 20.19 for female students that participated in the main study (Table 4).

In the ancillary study, body fat percentage median values ranged from 21.9 to 16.3 for male students and from 27.3 to 20.7 for female students and BMI median values ranged from 24.42 to 20.82 for male students and from 25.94 to 20.66 for female students (Table 5).

Table 4. Main study. BMI index and Body fat percentage by gender and PA levels of students.

PA Levels	BMI index, Median value (SD)		Body fat percentage Median value (SD)	
	Male students	Female students	Male students	Female students
Low	23.64 (1.67)	22.14 (1.69)	22.6 (1.69)	28.6 (1.51)
Medium	22.47 (1.53)	21.46 (1.55)	20.3 (1.58)	25.7 (1.61)
High	21.02 (1.57)	20.19 (1.57)	16.2 (1.54)	20.1 (1.48)

Table 5. Ancillary study. BMI index and Body fat percentage by gender and PA Levels of students.

PA Levels	BMI index, Median value (SD)		Body fat percentage Median value (SD)	
	Male students	Female students	Male students	Female students
Low	24.42 (1.61)	25.94 (1.44)	21.9 (1.61)	27.3 (1.54)
Medium	22.85 (1.56)	23.24 (1.65)	19.4 (1.54)	24.5 (1.64)
High	20.82 (1.65)	20.66 (1.53)	16.3 (1.64)	20.7 (1.55)

Similar results have been reported by Brener et al. (31) and Huang et al. (32).

It has been calculated the correlation between coefficients from the main study and the ancillary study. For physical activity (MET index), it was found a very strong positive correlation $r = 0.776$, $p < 0.001 < \alpha = 0.05$. For HEI score for the main study vs ancillary study has resulted in $r = 0.765$, $p < 0.001 < \alpha = 0.05$, also being with a very strong positive correlation. For body composition (BMI index), the results obtained were in the same trend $r = 0.728$, $p < 0.001 < \alpha = 0.05$, also having a very strong positive correlation. For total score, it was found a very strong positive correlation $r = 0.779$, $p < 0.001 < \alpha = 0.05$. These results validate the results obtained in the main study.

Conclusion

These results can be taken into consideration while designing educational programs and interventions. Both male and female athletic students' involvement in PA was associated with better food habits and better BMI indexes for both gender students compared with non-athletic students. The results obtained in the present study confirmed once again that healthy eating and physical activity ensured a good physical form of the students. The percentage of male students involved in sports activities is higher than that of female students. In terms of eating habits, no notable differences were observed between the genders, HEI index falling within the reported limits and in other similar studies. Considering that both eating habits and active or sedentary lifestyles are formed from the period when young people are students, it is recommended to develop educational programs that improve the knowledge about the importance of nutrition and physical activity in students.

Limitations

The survey was limited to samples of 19- to 25-year-old Romanian students. Larger samples in each age and country group are essential for establishing age and sex-specific indexes and correlations

Conflict of interest

The authors declare that they have no competing interests.

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Authors' contributions

All authors contributed equally to this manuscript. All authors read and approved the final manuscript.

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Prevalence of malnutrition and its association with activities of daily living in older adults attending primary health care centers: a multistage cross-sectional study

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Summary. Objective: Nutritional status plays a vital role in the quality of health of older adults. Thus, we aim to assess nutritional status and its association with functional status in older adults. **Methods:** A cross-sectional descriptive study with a multistage stratified sampling strategy was carried out in primary health care centers (PHCCs). Community-dwelling older adults ≥ 60 years of age (n=2045) participated in the study. Nutritional status was assessed using the mini-nutritional assessment tool, and BMI was measured. Functional status was measured by Katz index of independence in activities of daily living (ADL) and its relationship to nutritional status was examined and assessed using chi-square test and binary logistic regression. **Results:** Obesity prevalence was high (43.2%) while 20.9% were classified as at risk of malnutrition or were malnourished. There was a significant association between nutritional status and ADL. **Conclusions:** Assessing nutritional status of older adults identified a high prevalence of both undernutrition and obesity. Such assessments should be routine practice in PHCCs.

Keywords: Nutritional status, body mass index, older adults, primary health care centers, activities of daily living

Introduction

Aging of the population is emerging as a major demographic trend worldwide. In the Kingdom of Saudi Arabia (KSA), older adults (i.e., aged 60 years and over) made up about 5.6% of the total population in 2017, and this percentage is expected to reach 22.9% by the year 2050 (1). The age-related demographic transition is associated with a concomitant increase in the rates of chronic diseases. Globally, behavioral risk factors (e.g., unhealthy diet, physical inactivity, tobacco use) are responsible for about 80% of diagnosed coronary heart disease and cerebrovascular disease (2). Amongst these risk factors, aging may result in reduced physical activity and in a poor diet.

In older adults, malnutrition has been associated with increases in the risk of falls, loss of mobility, and poor wound healing (3), increased healthcare costs (4), and impaired quality of life (5). Nutritional status assessment is necessary because it can identify malnutrition, which is a potential cause of morbidity and mortality (6). Older adults who are malnourished when admitted to hospital tend to have a longer hospital stay and have more complications than well-nourished patients having the same disease (7). Thus, prevention of malnutrition in older subjects is likely to have benefits both while they are in the community and also if they need to be hospitalized. On the other hand, two national sur-

veys conducted in the KSA showed that overweight and obesity exceeds 60% of the adult population (8,9).

Previous research showed that declined functional ability is associated with malnutrition (10,11). Functional ability can be assessed using the Katz index of independence in activities of daily living (ADL) (12), which measures independence in six domains: feeding, transferring, bathing, continence, dressing, and using the toilet.

In KSA, there is limited data available on nutritional status, body mass index (BMI) and ADL in community-dwelling older subjects. Thus, the study was designed with the aim to have an overview of the nutritional status and its association with inability to perform ADL in Saudi-older adults attending PHHCs in Riyadh city, the capital of KSA.

Materials and Methods

Participants and design

The present study was conducted as a part of a research project evaluating the internal environment of primary health care centers (PHCCs) and assessing the health status of older people attending these centers in Riyadh city, KSA. The study was approved by the Ethical Committee at the Ministry of Health, KSA. It was designed as a cross-sectional descriptive study with a multistage stratified sampling strategy carried out in PHCCs in Riyadh city between January 2015 and April 2017. The inclusion criterion was older adults (≥ 60 years of age) attending the selected PHCCs for routine primary care services.

In the KSA, PHCCs provide preventive and curative primary care services, and these services are offered free of charge to Saudi Citizens. The PHCCs were randomly selected, according to geographical location (north, south, central, east and west) to represent the geographic sectors of the city of Riyadh. Three PHCCs were selected from each sector using the simple random sampling method (total: 15 PHCCs were chosen from the five sectors). Within each sector, the size of the older adults' sample was determined proportional to the population attending the three selected PHCCs, and the samples from each center were stratified according to the sex. The older adults from each PHCC were selected consecutively.

Older adults or their caregivers signed a written consent form before participating in the study.

Physicians (one man and one woman) selected from each PHCC received training sessions in order to conduct the data collection, including the completion of the mini-nutritional assessment (MNA®) and ADL forms. The MNA and ADL forms were answered directly by older persons. In some cases, caregivers helped to complete the forms.

Nutritional status

The nutritional status of older adults was assessed using the MNA® tool. The answers can give a maximum of 30 points {well-nourished (24-30 points), at risk of malnutrition (17-23.5) points, and undernourished (< 17 points)}(13). About 15% of older adults were excluded due to inability to complete the MNA form, mainly due to being unable to provide necessary information or a communication problem.

Functional status

Functional status was measured by the Katz ADL scale (12). A score of 6 indicates fully functional, 3-5 moderate functional impairment, and 0-2 severe functional impairment.

Body mass Index

Body mass index (BMI) [weight in kg/(height in m)^2] was used to classify participants as underweight (< 18.5 kg/m^2), normal weight (18.5–24.9 kg/m^2), overweight (25.0–29.9 kg/m^2) and obese (≥30 kg/m^2), based on the WHO international standard (14). Based on older adults-BMI classification (15,16), a BMI between 22-27 kg/m^2 is considered normal range for older adults aged ≥ 65 years. Therefore, the following cutoff points were also been used (< 22 kg/m^2 underweight, and > 27 kg/m^2 for overweight).

Statistical analysis

Data were analyzed using Statistical Package for Social Science (SPSS) software, version 22 (IBM Company, USA). Categorical data are expressed as frequency (number and percentage). Mann-Whitney U test was used for non-normally distributed variables and chi-square test was used for categorical variables. Binary logistic regression analysis was performed to

assess the impact of difficulties to perform ADL (the independent variable) on the nutritional status (dependent variable). Odds-ratios with 95% confidence intervals were calculated. Kolmogorov - Smirnov test was used to assess the normality of the data. Results were considered to be statistically significant at $p<0.05$.

Results

Table 1 shows the characteristics of the subjects. The total number of older adults (aged ≥ 60 years) who participated in the study was 2045. There were 55.6% men and 44.4% women. The data showed that 36.5%

Table 1. Characteristics of older adults attending primary health care centers in Riyadh city, KSA

Characteristics	Males (1138)		Females (907)		Total (2045)		P value
	N	%	N	%	N	%	
Sex	1138	55.6	907	44.4	2045	100	
Age (Mean, SD)	67.0	6.8	65.1	6.5	66.1	6.8	<0.001
60 – 69 y	767	67.4	708	78.1	1475	72.1	<0.001
70 – 79 y	298	26.2	158	17.4	456	22.3	
80 y and above	73	6.4	41	4.5	114	5.6	
Marital status							
Married	1084	95.2	540	59.5	1624	79.4	<0.001
Single	6	0.5	9	1.0	15	0.7	
Widow	45	4.0	304	33.5	349	17.1	
Divorced	3	0.3	54	6.0	57	2.8	
Education levels							
Illiterate	224	19.7	522	57.6	746	36.5	<0.001
Primary	337	29.6	214	23.6	551	26.9	
Intermediate & Secondary	416	36.6	137	15.1	553	27.0	
University or above	161	14.1	34	3.7	195	9.5	
Occupation							
Employed	259	22.8	74	8.2	333	16.3	<0.001
Not Employed	879	77.2	833	91.8	1712	83.7	
living conditions							
Alone	73	6.4	32	3.5	105	5.1	<0.003
With your children / spouse / other	1065	93.6	875	96.5	1940	94.9	
Weight (Mean, SD)	78.0	15.1	74.7	14.9	76.5	15.1	<0.001
Height (Mean, SD)	165.4	6.8	155.2	6.6	160.9	8.4	<0.001
BMI (Mean, SD)	28.5	5.3	30.9	5.7	29.6	5.6	<0.001
BMI (WHO)[+]							
Underweight	18	1.6	7	0.8	25	1.3	<0.001
Normal	251	22.8	102	12.0	353	18.1	
Overweight	450	40.9	280	32.8	730	37.4	
Obese	380	34.6	464	54.4	844	43.2	
BMI[++]							
Underweight	94	8.6	29	3.4	123	6.3	<0.001
Normal	376	34.2	187	21.9	563	28.8	
Overweight	629	57.2	637	74.7	1266	64.9	
Activities of daily living (ADL) (Mean, SD)	5.8	0.7	5.7	0.8	5.7	0.8	0.077

Values are presented as number (N) and percentage (%) or mean and standard deviation (SD). Chi-square test for categorical variables. Mann-Whitney U test to compare men and women mean values. *P* values are significant at $p< 0.05$.
[+]BMI (WHO): World health organization classification. [++]BMI**:** Older adults-BMI classification (15, 16).

of the included older adults were illiterate, 83.7% were not employed, and only 5.1% lived alone.

Older women had significantly higher BMI (30.9 kg/m^2) than older men (28.5 kg/m^2). However, based on the WHO-BMI classification, overweight prevalence was higher in the older men (40.9%), while obesity prevalence was higher in the older women (54.4%). Based on older adults-BMI classification, the prevalence of underweight was 8.6% and 3.4% for older men and older women, respectively, while the prevalence of overweight reached 57.2 % in older men and 74.7% in older women.

There was no significant difference in the ADL-mean score between older men and older women (p=0.077).

Table 2 presents the nutritional status of older adults by socio-demographic characteristics, functional status and BMI. The prevalence of malnutrition or at risk of malnutrition was higher in older women (24.9%) than in older men (17.7%). However, there were very few older adults classified as malnourished (1.4%) while 19.5% were at risk of malnutrition. In the age group ≥ 80 years, the % classified as at risk of malnutrition or malnourished increased to 47.1%.

The percent of older adults classified as at risk of malnutrition was higher in those who were single (33.3%) or widowed (34.8%) compared to those with another marital status. Those who were illiterate had a higher percent classified as at risk of malnutrition (27.3%) or malnourished (2.5%) compared to other educational levels.

There was a significant association between nutritional status and ADL. Among those who were classified as fully functional, only 15.2% were classified as at risk of malnutrition or malnourished. Among those classified as moderately functional impairment or severe functional impairment, 55.3% and 73.9% were classified as at risk of malnutrition or malnourished, respectively.

About 38% and 47.6% (total 85.7%) of older adults classified as underweight, based on the WHO-BMI classification, were at risk of malnutrition and malnourished, respectively. Based on BMI older-adults' classification, 53.4% and 13.6% (total 67%) of those classified as underweight were at risk of malnutrition and malnourished, respectively.

Binary logistic regression analysis was performed to assess the impact of difficulties to perform ADL (the independent variable) on the nutritional status (dependent variable), dichotomized as at risk of malnutrition or malnourished vs normal nutritional status (table 3). There was a significant influence of functional status using ADL index on the nutritional status of older adults. In the crude model (model 1), the results indicated that the reduction in one unit of ADL index, significantly increased the OR of being at risk of malnutrition or malnourished (OR: 2.64, CI: 2.19 – 3.17; p<0.001). After adjusting for sex and age in model 2, the relationship remained significant (OR: 2.09, CI: 1.73 – 2.53; p<0.001). It is worth mentioning that most older adults in the at risk of malnutrition or malnourished group, were categorized as at risk of malnutrition.

Discussion

The current study identified a high prevalence of both obesity (43.2%), and risk of malnutrition or malnutrition (20.9%) among older subjects attending PHCCs in Riyadh city. Individuals who were unemployed, illiterate, single and widowed were at a higher risk of malnutrition. Finally, there was a significant association between nutritional status and ADL. This study provides valuable new information of relevance to public health policy in KSA.

In the present study, there were more men classified as overweight compared to women, while there were more women classified as obese compared to men. The current study demonstrates that the picture of high prevalence of overweight and obesity in older adults and the sex differences in pattern, is not different when compared to studies conducted in adults in the KSA (8,9,17).

The current study identified that 17.7% of older men and 24.9% of older women were categorized as either at risk of malnutrition or as malnourished. However, most of these older adults were classified as at risk of malnutrition. These findings are in accordance with several studies in the literature from different countries. Winter et al. reported that 17% of older subjects attending a general practice clinic in Victoria, Austral-

Table 2. Nutritional status of older subjects attending primary health care centers by socio-demographic characteristics, functional status, and body mass index (row-wise)

	MNA								
Characteristics	**Normal nutritional status**		**At risk of malnutrition**		**p-value***	**Malnourished**		**p-value****	**p-value*****
Sex	**N**	**%**	**N**	**%**		**N**	**%**		
Male	791	82.4	157	16.4	< 0.001	12	1.3		
Female	586	75.0	183	23.4		12	1.5	0.465	< 0.001
Total	1377	79.1	340	19.5		24	1.4		
Age groups									
60 - 69 y	1095	84.8	186	14.4	< 0.001	11	0.9		
70 – 79 y	236	65.2	118	32.6		8	2.2	< 0.001	< 0.001
≥ 80 y	46	52.9	36	41.4		5	5.7		
Marital status									
Married	1145	82.9	224	16.2	< 0.001	12	0.9		
Single	8	66.7	4	33.3		0	0.0	< 0.001	< 0.001
Widow	184	61.5	104	34.8		11	3.7		
Divorced	40	81.6	8	16.3		1	2.0		
Occupation									
Employed	261	90.6	27	9.4	< 0.001	0	0.0	< 0.007	< 0.001
Not employed	1116	76.81	313	21.5		24	1.7		
Education levels									
Illiterate	444	70.1	173	27.3	< 0.001	16	2.5		
Primary	389	83.5	70	15.0		7	1.5	< 0.001	< 0.001
Intermediate & Secondary	407	85.0	71	14.8		1	0.21		
University or above	137	84.0	26	16.0		0	0.00		
Living conditions									
Alone	73	77.7	20	21.3	0.670	1	1.1	0.635	0.881
With your children/Spouse/Other	1304	79.2	320	19.4		23	1.4		
Activities of daily living (ADL)									
Fully functional	1275	84.8	217	14.4	<0.001	12	0.8		
Moderate functional impairment	92	44.7	103	50.0		11	5.3	<0.001	<0.001
Severe functional impairment	6	26.1	16	69.6		1	4.3		
BMI (WHO)⁺									
Underweight	3	14.3	8	38.1	<0.001	10	47.6		
Normal	172	58.3	116	39.3		7	2.4	<0.001	<0.001
Overweight	526	84.2	96	15.4		3	0.5		
Obese	638	87.4	91	12.5		1	0.1		
BMI⁺⁺									
Underweight	34	33.0	55	53.4	<0.001	14	13.6		
Normal	358	73.8	123	25.4		4	0.8	<0.001	<0.001
Overweight	947	87.4	133	12.3		3	0.3		

Nutrition status was assessed using the Mini-nutritional assessment (The MNA®) tool. Analysis by Chi-square test. P-value < 0.05 is considered statistically significant. * P-value between normal nutritional status & at risk of malnutrition; ** P-value between normal nutritional status & malnourished; *** p-value among all the groups. ⁺BMI (WHO): World health organization classification. ⁺⁺BMI: Older adults-BMI classification (15, 16).

Table 3. Binary logistic regression: The impact of reduction in the activities of daily living (ADL) on the odds ratio of being malnourished or at risk of malnutrition in comparison to normal nutritional status

	OR for nutritional status*	
	OR (*C.I.* 95%)	*P value*
Model 1		
ADL	2.64 (2.19-3.17)	<0.001
Model 2		
ADL	2.09 (1.73-2.53)	<0.001

*The reference category of the outcome is well nourished group (At risk of malnutrition group and malnourished group were merged). Model 1: crude. Model 2: adjusted for age & gender. OR=Odds ratio; C.I.=confidence interval. *P*-value < 0.05 is considered statistically significant.

ia were at risk of malnutrition or malnourished, using the MNA Short Form (18). A cross-sectional study conducted on community-dwelling older adults living in Lebanon, in which nutritional status was assessed by MNA®, and its association with socio-demographic characteristics, showed that the proportion classified as at risk of malnutrition or malnourished was significantly higher in older subjects aged >85 years, in women, and in widowed and illiterate individuals (19). This is consistent with the current study, in which the percentage classified as at risk of malnutrition was higher in women than in men, and the frequency of subjects classified as at risk of malnutrition or malnourished was higher in the group aged ≥ 80 years, in widowed and in illiterate individuals compared to other corresponding categories. Furthermore, Rosa and her colleagues have shown that the frequency of malnutrition and risk of malnutrition determined using the MNA® was 18.6% in non-hospitalized older adults (20).

A study conducted on a community-dwelling older Americans (aged 60 years and older) to determine the prevalence of malnutrition (21) showed that 14.2% of those who were categorized as malnourished were more likely to have more than two difficulties in performing ADL compared to only 1.6% of those categorized as well-nourished. The results of the above study are consistent with the present study, in which the percentage of older people classified as at risk of malnutrition or malnourished was higher among those with moderate functional impairment or severe functional impairment compared with those classified as fully functional. This is also supported by the binary logistic regression, which indicates that the reduction in the ADL significantly increased the OR of being at risk of malnutrition or malnourished.

In a cross-sectional study of elderly Japanese patients, Kuzuya and colleagues (22) found a significant positive correlation between MNA score and BMI. A population based-study conducted in a very old adults (>85 years) to investigate the association between nutritional status, assessed using MNA and BMI, and five years mortality (23) showed that the prevalence of at risk of malnutrition and malnourished was 40.3% and 13%, respectively. Sixty-five percent of those classified as malnourished and 28% of those classified as at risk of malnutrition had a BMI < 22.2 kg/m^2. In addition, they found that a BMI <22.2 kg/m^2 and a MNA score <17 were associated with lower survival. In our study, 66.6% (14/21) of those classified as malnourished and 17.7% (55/311) of those classified as at risk of malnutrition had a BMI <22 kg/m^2. When the nutritional status, using MNA and nutritional risk screening 2002, and other nutritional biochemical parameters of inpatient older adults aged ≥ 70 years was assessed within 24 hours of admission (24), those who were undernourished (malnourished and at risk of malnutrition group) had a lower BMI, hemoglobin, albumin and prealbumin and longer length of stay. Furthermore, the study showed that the nutritional and biochemical parameters were positively correlated with the MNA score. Thus, detecting malnourished older persons in PHCCs using an appropriate screening protocol followed by an appropriate nutrition intervention may reduce the risk of malnourished older people requiring hospital admission and may reduce the length of hospital stay.

The main limitations of the study were being a cross-sectional study and that it is not nationally representative, as it involved older adults from the capital of KSA, who may not represent older adults from other geographical regions of KSA.

Conclusions

To conclude, the current study provides health-policy makers in the KSA with the necessary data on

the current nutritional status of older adults in order to take appropriate measures to improve the existing health care services provided for older adults. Furthermore, there is an association between poorer nutritional status and low ADL in the older population of the KSA.

Conflict of interest
The authors have no conflicts of interest to declare.

Ethical approval
The study was approved by the Ethical Committee at the Ministry of Health, KSA.

Contribution of the authors
Study design, concept & acquisition of data: Adel Alhamdan, Saad Bindawas, Sulaiman Alshammari, Sadaa Al-Orf, May Al-Muammar, and Maysoon Al-Amoud; drafting of the manuscript: Adel Alhamdan; Analysis and interpretation of data: Adel Alhamdan, Saad Bindawas, Sulaiman Alshammari; Revising of the manuscript: Philip Calder, Saad Bindawas, Sulaiman Alshammari, Sadaa Al-Orf, May Al-Muammar, and Maysoon Al-Amoud; Study supervision, and Critically review and edit the manuscript: Philip Calder.

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Body composition and torso muscle strength relationship in athletes

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Summary. The purpose of this study is to investigate the relationship between body composition and torso isokinetic muscle strength among athletes and compare the parameters between both genders. The study was conducted on 76 female and 162 male athletes from various sports branches. Their body composition measurements were taken by the multifrequency bioelectric impedance analysis (Tanita MC-980, 1000 kH, Tokyo, Japan), whereas the torso flexor-extensor and the torso right-left rotator muscle strength by Isokinetic Dynamometer (D.& R. Ferstl GmbH, Hemau, Germany). The body fat percentage, fat mass, torso fat mass and torso fat percentage in female athletes were measured to be higher than the male ones, whereas their lean mass, muscle mass, torso muscle mass and whole torso isokinetic muscle strength values were lower (p>0.05). It was determined that there was a medium to high level of inverse relationship between fat percentage, fat mass, torso fat mass, torso fat percentage and torso muscle strength in all athletes (p<0.05). Moreover, there was a medium to high level of direct relationship between lean body mass, muscle mass, torso muscle mass and torso muscle strength (p<0.05). Female athletes had higher rate of body fat, and lower rate of muscle mass and torso muscle strength than male athletes, and the body composition parameters in all athletes were associated with the torso muscle strength. Therefore, we suggest that the athletes' training should not only focus on increasing the body muscle strength, but also include special sessions for increasing the muscle mass and optimizing the body fat percentage of male and female athletes. Body composition of the athletes should be monitored regularly with a focus on these parameters.

Keywords: Bioelectrical Impedance, Gender, Isokinetic, Nutrition, Performance, Sport.

Introduction

Body composition is one of the most important parameters that affect athletic performance, and is composed of various components such as lean body mass, body fat mass and body fat percentage. While the lean body mass being an important parameter for increasing athletic strength, power and speed; the body fat mass is one of the main components impacting athletic performance particularly in strength and agility category sports involving antigravity exertion (1, 2).

Body composition parameters are affected by many factors such as age, height and gender, and, it is suggested that this is associated with the normal growing/development process (3,4,5). Besides, body composition and athletic performance are related, and the role of body composition varies depending on the type of sports that require certain energy usage. Various studies suggest that there is a relationship between body composition parameters and aerobic and anaerobic capacity (5-8).

Muscle strength is another important parameter that affects athletic performance (1,9,10). While the entire body muscle strength contributes to the athletic performance, torso muscles which are composed of anterior, posterior and oblique core muscles are im-

portant for both postural stability and sports performance. It has been stated that there is a relationship between the strength of the aforementioned muscles and extremity muscles. This relationship contributes to the transmission of strength to the lower and upper extremities of the body, and directly affects sports performance (11-13).

Body composition and muscle strength not only affects each other, but they also directly affect sports performance (14,15). In one of the papers studying the relationship between body composition and muscle strength, it is suggested that there is a negative relationship between body fat mass and knee isokinteic muscle strength, and a positive relationship between lean body mass and knee isokinetic muscle strength (14). Since it is known that lean body mass is composed of muscles, bones, tendon tissues and water; a relationship between lean body mass and muscle strength is deemed likely (15). In the light of this information, we hypothesize that the entire body fat and lean mass as well as segmental fat and muscles mass that are obtained from body composition measurements should affect partial muscle strength. Despite studies investigating the relationship between entire body fat and lean mass, and muscle strength (13,16,17), and between the anthropometric variables with the upper and lower extremities muscle strength (18,19), we have not come across any papers studying this relationship with a focus on the torso in elite athletes. Therefore, the main purpose of our study is to investigate the relationship between the entire and segmental body composition parameters, and torso isokinetic muscle strength in elite athletes. The secondary purpose is to make the same comparison based on gender.

Material and methods

This study was conducted at the Athlete Health Training and Research Center. A total of 417 athletes from different sports branches were invited to this study. 238 elite athletes who are competing at an international level, 76 of which were female and 162 male met the selection criteria and were included in the study (Athletics=60, Gymnastics=19, Wrestling=37, Weight Lifting=27, Archery=19, Tekwandoo=47, Tennis=9, Triathlon=5, Swimming=32). The selection criteria were as follows; not to have any systemic problems, to be doing her/his sport branch at least for three years and at least one hour-five days a week, to cooperate in following the study parameters and to volunteer to participating in the study. The exclusion criteria were; not meeting any of the selection criteria, having sustained acute or chronic sports injuries/disease, having prosthetics, being or possibility of being pregnant, menstruating, and having an acute or chronic disease related to the muscle/skeleton system. The athletes who have volunteered to participate in the study have signed consent forms following a briefing on the details of the tests. Approvals for conducting the tests were received from University Social and Human Sciences Ethics Committee (2018/07;304). The tests were in compliance with the Helsinki Declaration 2008 Regulations.

During the first day of the study, the demographic profiles of the participants were recorded. Then, multifrequency bioelectric impedance analysis (MF-BIA) measurements were taken after a minimum 8 hour fasting period. During the second day, the isokinetic muscle strength measurements of torso flexor-extensor and right-left rotator muscles were taken minimum 2 hours after breakfast.

The general characteristics and body composition measurements of the athletes based on gender distribution is provided in Table 1. The all athletes' characteristics have been recorded as: age 18.1±1.8 years, weight 68.0±13.0 kg, height 172.9±8.7 cm and body mass index 22.7±3.4 kg/m^2. The male athletes had an age range of 18.3±1.8 years, weight of 72.0±12.4 kg, height of 176.2±6.9 cm and body mass index of 23.2±3.5 kg/m^2, whereas the female athletes had the age of 17.7±1.7 years, weight of 59.5±10.0 kg, height of 166.0±8.0 cm and body mass index of 21.5±3.1 kg/m^2 (Table 1). The distribution of these characteristics based on the sports branches are provided in Table 2.

Evaluation of Body Composition

The evaluation of the body composition of athletes was done by MF-BIA (Tanita MC-980, 1000 kH, Tokyo, Japan; 0.1 accuracy). 24 hours prior to testing, the athletes were asked not to perform intense physical activities and not to consume excessive diuretic beverages such as tea or coffee. Any metal objects on the ath-

Table 1. Distribution of Antropometric Characteristics and Body Composition measurements of Athletes based on gender

	Female (n=76)	Male (n=162)	z	p	Total (n=238)
Age (Years)	17.7±1.7	18.3±1.8	-1.956	0.052	18.1±1.8
Body Weight (kg)	59.5±10.0	72.0±12.4	-7.715	**0.000****	68.0±13.0
Height (cm)	166.0±8.0	176.2±6.9	-10.086	**0.000****	172.9±8.7
BMI (kg/m²)	21.5±3.1	23.2±3.5	-3.461	**0.001****	22.7±3.4

*Values are given by Mean± Standard Deviation, BMI: Body Mass Index, **p*:<0.01.

Table 2. Distribution of Sports Branches Based on Gender

	Female (n=76)	Male (n=162)	Total (n=238)
	Number (%)	Number (%)	Number (%)
Athletics	33 (20.4)	44 (16.8)	60 (14.4)
Gymnastics	7 (9.2)	12 (7.4)	19 (8.0)
Wrestling	-	37 (22.8)	37 (15.5)
Wight lifting	10 (13.2)	17 (10.5)	27 (11.3)
Archery	3 (3.9)	16 (9.9)	19 (8.0)
Taekwondo	27 (35.5)	20 (12.3)	47 (19.7)
Tennis	8 (10.5)	1 (0.6)	9 (3.8)
Triathlon	1 (1.3)	4 (2.5)	5 (2.1)
Swimming	10 (13.2)	22 (13.6)	32 (13.4)
Total	**76 (100)**	**162 (100)**	**238 (100)**

letes had to be removed for the testing. The tests were performed after a minimum 8 hours of fasting and the athletes that did not meet this requirement were excluded. The testing was done barefoot with the participant standing up with the entire bottom of the feet in contact with the metal plates of the device. The testing gave out measurements' data related to body weight, body fat percentage, fat mass, lean body mass and muscle mass parameters; and moreover, torso muscle mass, torso fat mass and torso fat percentage parameters within the category of segmental body analysis.

Isokinetic Torso Muscle Strength Testing

Torso flexor and extensor strength assessment was performed by an isokinetic dynamometer (IsoMed 2000; D&R Ferstl, Hemnau, Germany). Prior to each testing, all participants were asked to warm-up for 10 minutes on a stationary bicycle ergometer by the cadence was kept at a constant 60-70 rpm. Subsequently, a submaximal warm-up on the isokinetic device was completed for each task by started with five concentric repetitions at a speed of 120º/s and followed by five repetitions of 90º/s for familiarization. Torso flexor and extensor strength assessment was performed by five repetitions at a speed of 60º/s and 150º/s isokinetically. The participants were fixed in sitting position wearing girdles at the shanks, thighs, and shoulders. The point of rotation of the device was verified by a laser pointing at the upper part of the iliac crest. Each participant has completed two trials of testing with five repetitions, starting with the torso flexion and a subsequent torso extension ranging between 30º felxion to 30º extension. The torso was in the upright position with the hip angled at 90º, which afterwards ranging between 55º- 115º. Similarly, there were two trials with five repetitions for the isokinetic testing of torso rotation. Range of trunk rotation movement assessment was based on a range of 35º left-35º right, corresponding to a longitudinal axis, neutral zero method. Participants were securely fixed on the knee and hip angled at 90º, respectively. Thus, the movement of the hips or knees was restricted. Data was exported and processed by an external software. Total amount of work (TW) (Joule), peak torque (PT) [Newtonmeter-(Nm)] for each isokinetic assessment was analyzed and the data were normalized for lean body mass (PT/W) [Nm/(kg)] (20).

Data Analysis

SPSS 20 (Statistical Package for Social Sciences Inc. Chicago, IL, ABD) statistics software was used for the evaluation of the data obtained from our study. The definition of the distribution of variables was made using visual (histogram, probability graphs) and analytic (Kolmogorov-Smirnov test) methods. For the comparison of genders, Independent Samples t test was used for normal distribution of variables, whereas Mann Whitney U test was used for the abnormal distribution. For the assessment of the relationship between variables, Pearson Correlation Analysis was

used for normal distribution and Spearman Correlation Anaysis was used for cases where at least one variable was not within the normal distribution. The definitive statistical analyses were conducted for all variables and the variables were presented as mathematical average±standard deviation. The significance rate of the statistical analysis was defined as p<0.05.

Results

The antropometric characteristics and body composition measurements of the athletes including their distribution by gender is provided in Table 1. Accordingly, the body weight, height and body mass index values of female athletes were lower than male ones (p<0,05), whereas the ages in both gender groups were similar (p>0,05) (Table 1).

The distribution of body composition, segmental body analysis and isokinetic muscle strength measurements of athletes based on gender are provided in Table 3. Regarding the body composition parameters and segmental body analysis parameters in female athletes, body fat percentage, fat mass, torso fat mass and torso fat percentage values were higher that male athletes, whereas lean mass, muscle mass and torso muscle mass as well as entire torso isokinetic muscle strength values were lower in comparison (p>0,05) (Table 3).

The muscle strength and body composition relationship of the all athletes is provided in Table 4. A negative relationship at a medium to high rate between the body fat percentage, fat mass, torso fat mass and

Table 3. Distribution of Body Composition, Segmental Body Analysis and Isokinetic Muscle Strength Measurements of Athletes Based on Gender

		Female (n=76)	Male (n=162)	*z*	*p*	Total (n=238)
Body Composition Parameters	**Body fat percentage (%)**	23.6±5.5	13.9±5.1	13.081	**0.000****	17.0±6.9
	Fat Mass (kg)	14.3±5.1	10.4±5.3	5.374	**0.000****	11.6±5.6
	Fat Free Mass (kg)	45.1±6.2	61.6±8.8	-14.711	**0.000****	56.3±11.1
	Muscle Mass (kg)	42.8±5.9	58.5±8.3	-14.698	**0.000****	53.5±10.6
Segmental Body Analysis Parameters	**Torso Muscle Mass (kg)**	24.8±3.1	31.7±4.0	-13.183	**0.000****	29.5±4.9
	Torso Fat Mass (kg)	6.1±2.5	4.9±3.1	2.791	**0.006****	5.3±2.9
	Torso Fat Percentage (%)	18.3±5.4	12.4±5.4	7.885	**0.000****	14.2±6.1
Isokinetic Torso Muscle Strength Measurement Parameters	**60˚/sec Flexion PT/W (Nm/kg)**	2.10±0.50	2.70±0.60	-7.661	**0.000****	2.60±0.50
	60˚/sec Flexion TW (Joule)	341.6±88.1	505.8±120.8	-10.587	**0.000****	453.4±135.1
	60˚/sec Extension PT/W (Nm/kg)	3.2±0.8	4.1±1.1	-7.348	**0.000****	4.10±1.0
	60˚/sec Extension TW (Joule)	639.0±193.6	1016.9±354.2	-8.703	**0.000****	896.2±358.0
	150˚/sec Flexion PT/W (Nm/kg)	2.2±0.5	2.3±0.4	-2.615	**0.010****	2.2±0.4
	150˚/sec Flexion TW (Joule)	192.2±74.1	505.8±120.8	-10.532	**0.000****	287.0±115.0
	150˚/sec Extension PT/W (Nm/kg)	2.5±0.8	3.6±1.1	-8.691	**0.000****	3.5±1.2
	150˚/sec Extension TW (Joule)	408.0±179.8	760.1±321.0	-8.921	**0.000****	647.7±327.5
	60˚/sec Right Rotation PT/W (Nm/kg)	2.0±0.5	2.5±0.5	-9.639	**0.000****	2.3±0.5
	60˚/sec Right Rotation TW (Joule)	351.0±86.9	577.5±157.0	-11.747	**0.000****	505.2±174.1
	60˚/sec Left Rotation PT/W (Nm/kg)	1.9±0.4	3.5±16.29	-9.441	**0.000****	2.3±0.5
	60˚/sec Left Rotation TW (Joule)	329.0±82.2	547.9±142.9	-12.410	**0.000****	478.0±162.6
	150˚/sec Right Rotation PT/W (Nm/kg)	1.7±0.4	2.3±0.5	-12.871	**0.000****	2.2±0.6
	150˚/sec Right Rotation TW (Joule)	277.8±81.0	509.9±143.0	-13.178	**0.000****	277.8±81.0
	150˚/sec Left Rotation PT/W (Nm/kg)	1.6±0.4	2.9±12.1	-12.210	**0.000****	2.1±0.6
	150˚/sec Left Rotation TW (Joule)	252.8±79.0	464.6±125.2	-13.525	**0.000****	397.0±149.7

*Values are given by Mean± Standard Deviation, PT: Peak Torque; W: Weight, TW: Total Work, **p:<0.01.*

torso fat percentage; and torso muscle strength has been identified (p<0,05). A positive relationship at a medium to high rate between lean body mass, muscle mass and torso muscle mass; and torso muscle strength has been identified (p<0,05) (Table 4).

The muscle strength and body composition relationship of the female athletes is provided in Table 5. A positive relationship at a low to high rate between the body fat percentage, fat mass, torso fat mass, torso fat percentage, lean body mass, muscle mass and torso

Table 4. Correlation Between the Isokinetic Muscle Strength / Body Composition and Segmental Body Analysis in all Athletes

Isokinetic Torso Muscle Strength Measurement Parameters of all athletes		*Body composition and segmental body analysis data of all athletes*						
		Body fat percentage (%)	**Fat Mass (kg)**	**Fat Free Mass (kg)**	**Muscle Mass (kg)**	**Torso Muscle Mass (kg)**	**Torso Fat Mass (kg)**	**Torso Fat Percentage (%)**
60°/sec Flexion PT/W (Nm/kg)	**p**	0.000	0.000	0.000	0.000	0.000	0.005	0.000
	r	-0.404**	-0.248**	0.369**	0.369**	0.356**	-0.183*	-0.306**
60°/sec Flexion TW (Joule)	**p**	0.000	0.799	0.000	0.000	0.000	0.092	0.049
	r	-0.285**	0.017	0.787**	0.787**	0.787**	0.110	-0.128*
60°/sec Extension PT/W (Nm/kg)	**p**	0.000	0.000	0.000	0.000	0.000	0.004	0.000
	r	-0.402**	-0.226**	0.800**	0.463**	0.437**	-0.187*	-0.331**
60°/sec Extension TW (Joule)	**p**	0.000	0.159	0.000	0.000	0.000	0.002	0.045
	r	-0.278**	0.092	0.760**	0.761**	0.760**	-0.197**	-0.130*
150°/sec Flexion PT/W (Nm/kg)	**p**	0.000	0.000	0.486	0.484	0.354	0.000	0.000
	r	-0.430**	-0.480**	-0.045	-0.046	-0.060	-0.444**	-0.422**
150°/sec Flexion TW (Joule)	**p**	0.000	0.303	0.000	0.000	0.000	0.329	0.011
	r	-0.305**	0.067	0.746**	0.746**	0.746**	0.064	-0.164*
150°/sec Extension PT/W (Nm/kg)	**p**	0.000	0.001	0.000	0.000	0.000	0.025	0.000
	r	-0.414**	-0.208**	0.534**	0.534**	0.511**	-0.146*	-0.311**
150°/sec Extension TW (Joule)	**p**	0.000	0.229	0.000	0.000	0.000	0.004	0.015
	r	-0.290**	0.078	0.718**	0.718**	0.718**	-0.185**	-0.158*
60°/sec Right Rotation PT/W (Nm/kg)	**p**	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	r	-0.453**	-0.306**	0.380**	0.380**	0.344**	-0.264**	-0.385**
60°/sec Right Rotation TW (Joule)	**p**	0.000	0.214	0.000	0.000	0.000	0.213	0.011
	r	-0.308**	0.081	0.809**	0.809**	0.809**	0.081	-0.165*
60°/sec Left Rotation PT/W (Nm/kg)	**p**	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	r	-0.459**	-0.303**	0.435**	0.435**	0.401**	-0.264**	-0.389**
60°/sec Left Rotation TW (Joule)	**p**	0.000	0.361	0.000	0.000	0.000	0.231	0.012
	r	-0.308**	0.059	0.804**	0.804**	0.804**	0.078	-0.162*
150°/sec Right Rotation PT/W (Nm/kg)	**p**	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	r	-0.537**	-0.336**	0.534**	0.534**	0.534**	-0.276**	-0.440**
150°/sec Right Rotation TW (Joule)	**p**	0.000	0.631	0.000	0.000	0.000	0.633	0.001
	r	-0.368**	0.031	0.812**	0.812**	0.812**	0.031	-0.214*
150°/sec Left Rotation PT/W (Nm/kg)	**p**	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	r	-0.506**	-0.323**	0.512**	0.512**	0.470**	-0.265**	-0.417**
150°/sec Left Rotation TW (Joule)	**p**	0.000	0.724	0.000	0.000	0.000	0.036	0.002
	r	-0.355**	0.023	0.811**	0.811**	0.811**	-0.136*	-0.204*

*p<0.05, **p<0.01, (PT: Peak Torque; W: Weight, TW: Total Work)

muscle mass; and torso muscle strength has been identified (p<0,05) (Table 5).

The muscle strength and body composition relationship of the male athletes is provided in Table 6. A negligible relationship at low rate between the body fat percentage, fat mass, torso fat mass and torso fat percentage; and torso muscle strength has been identified. A positive relationship at a medium to high rate between lean body mass, muscle mass and torso muscle mass; and torso muscle strength has been identified (p<0,05) (Table 6).

Discussion

At the end of our study focusing on the comparison of the entire and segmental body composition parameters with torso muscle strength based on

Table 5. Correlation Between the Isokinetic Muscle Strength / Body Composition and Segmental Body Analysis in Female Athletes

Isokinetic Torso Muscle Strength Measurement Parameters of female athletes		*Body composition and segmental body analysis data of female athletes*						
		Body fat percentage (%)	**Fat Mass (kg)**	**Fat Free Mass (kg)**	**Muscle Mass (kg)**	**Torso Muscle Mass (kg)**	**Torso Fat Mass (kg)**	**Torso Fat Percentage (%)**
60°/sec Flexion PT/W (Nm/kg)	**p**	0,002	0,000	0,000	0,000	0,000	0,000	0,001
	r	0,356**	0,550**	0,604**	0,604**	0,617**	0,523**	0,376**
60°/sec Flexion TW (Joule)	**p**	0,650	0,811	0,796	0,798	0,836	0,765	0,712
	r	-0,053	-0,028	0,030	0,030	0,024	-0,035	-0,043
60°/sec Extension PT/W (Nm/kg)	**p**	0,001	0,000	0,000	0,000	0,000	0,000	0,000
	r	0,387*	0,579**	0,612**	0,612**	0,625**	0,563**	0,428**
60°/sec Extension TW (Joule)	**p**	0,039	0,001	0,000	0,000	0,000	0,005	0,058
	r	0,237*	0,390**	0,506**	0,506**	0,537**	0,319**	0,218
150°/sec Flexion PT/W (Nm/kg)	**p**	0,088	0,101	0,496	0,497	0,580	0,042	0,031
	r	-0,197	-0,190	-0,079	-0,079	-0,065	-0,234*	-0,247*
150°/sec Flexion TW (Joule)	**p**	0,041	0,001	0,000	0,000	0,000	0,005	0,048
	r	0,235*	0,380**	0,467**	0,467**	0,474**	0,317**	0,228*
150°/sec Extension PT/W (Nm/kg)	**p**	0,015	0,002	0,007	0,007	0,005	0,003	0,001
	r	0,279*	0,358**	0,307**	0,307**	0,316*	0,338**	0,367*
150°/sec Extension TW (Joule)	**p**	0,004	0,000	0,000	0,000	0,000	0,000	0,000
	r	-0,323**	-0,429**	-0,415**	-0,415**	-0,428**	-0,400**	-0,478**
60°/sec Right Rotation PT/W (Nm/kg)	**p**	0,002	0,000	0,000	0,000	0,000	0,000	0,003
	r	0,347**	0,579**	0,513**	0,513**	0,547**	0,474**	0,339*
60°/sec Right Rotation TW (Joule)	**p**	0,033	0,001	0,001	0,001	0,000	0,004	0,056
	r	0,245*	0,380**	0,367**	0,367**	0,394**	0,326**	0,220
60°/sec Left Rotation PT/W (Nm/kg)	**p**	0,923	0,949	0,916	0,916	0,774	0,813	0,637
	r	-0,011	-0,007	0,012	0,012	0,034	-0,028	-0,055
60°/sec Left Rotation TW (Joule)	**p**	0,064	0,001	0,009	0,009	0,003	0,007	0,150
	r	0,213	0,365**	0,296**	0,296**	0,339**	0,305**	0,167
150°/sec Right Rotation PT/W (Nm/kg)	**p**	0,047	0,001	0,000	0,000	0,000	0,005	0,000
	r	0,228*	0,376**	0,430**	0,430**	0,473**	0,319**	0,465**
150°/sec Right Rotation TW (Joule)	**p**	0,176	0,154	0,240	0,241	0,413	0,061	0,188
	r	-0,157	-0,165	-0,136	-0,136	-0,095	-0,216	0,153
150°/sec Left Rotation PT/W (Nm/kg)	**p**	0,002	0,000	0,000	0,000	0,000	0,000	0,005
	r	0,263**	0,476**	0,458**	0,458**	0,468**	0,430**	0,322**
150°/sec Left Rotation TW (Joule)	**p**	0,035	0,001	0,000	0,000	0,000	0,011	0,073
	r	0,242**	0,359**	0,415**	0,415**	0,447**	0,291*	0,207

*p<0.05, **p<0.01, (PT: Peak Torque; W: Weight, TW: Total Work)

genders, and evaluation of the relationship between the obtained parameters; we have determined that the female athletes have higher fat content as compared to the male ones, whereas have lower muscle mass and torso muscle strength. Moreover, a relationship between the body composition parameters and torso muscle strength of athletes has been identified. As the body fat percentage, fat mass, torso fat mass and torso fat percentage increases, the isokinetic muscle strength decreases. Whereas, as the lean body mass, muscle mass and torso muscle mass increases, torso isokinetic muscle strength increases as well. When this relationship investigates based on genders, while the body fat percentage, fat mass, torso fat mass and torso fat percentage relate with the torso muscle strength positively in female athletes; there is not any relationship in male

Table 6. Correlation Between the Isokinetic Muscle Strength / Body Composition and Segmental Body Analysis in Male Athletes

Isokinetic Torso Muscle Strength Measurement Parameters of male athletes		*Body composition and segmental body analysis data of male athletes*						
		Body fat percentage (%)	**Fat Mass (kg)**	**Fat Free Mass (kg)**	**Muscle Mass (kg)**	**Torso Muscle Mass (kg)**	**Torso Fat Mass (kg)**	**Torso Fat Percentage (%)**
60°/sec Flexion PT/W (Nm/kg)	**p**	0,018	0,000	0,000	0,000	0,000	0,000	0,001
	r	0,186*	0,314**	0,599**	0,599**	0,601**	0,361**	0,248*
60°/sec Flexion TW (Joule)	**p**	0,001	0,044	0,730	0,730	0,698	0,142	0,040
	r	-0,249*	-0,158*	0,027	0,027	0,031	-0,116	-0,162*
60°/sec Extension PT/W (Nm/kg)	**p**	0,410	0,010	0,000	0,000	0,000	0,001	0,100
	r	0,065	0,202*	0,624**	0,624**	0,621**	0,251*	0,130
60°/sec Extension TW (Joule)	**p**	0,071	0,001	0,000	0,000	0,000	0,000	0,000
	r	0,142	0,260**	0,693**	0,693**	0,664**	0,374**	0,347**
150°/sec Flexion PT/W (Nm/kg)	**p**	0,077	0,651	0,000	0,000	0,001	0,722	0,204
	r	-0,139	-0,036	0,279**	0,280**	0,250*	0,028	-0,100
150°/sec Flexion TW (Joule)	**p**	0,370	0,000	0,000	0,000	0,000	0,000	0,058
	r	0,071	0,302**	0,658**	0,658**	0,623**	0,340**	0,149
150°/sec Extension PT/W (Nm/kg)	**p**	0,635	0,048	0,000	0,000	0,000	0,002	0,602
	r	-0,038	0,155*	0,527**	0,527**	0,519**	0,247**	0,041
150°/sec Extension TW (Joule)	**p**	0,000	0,000	0,038	0,038	0,023	0,000	0,000
	r	-0,475**	-0,459**	-0,163*	-0,163*	-0,179*	-0,403**	-0,403**
60°/sec Right Rotation PT/W (Nm/kg)	**p**	0,694	0,052	0,000	0,000	0,000	0,014	0,321
	r	0,031	0,153	0,553**	0,552**	0,553**	0,193*	0,079
60°/sec Right Rotation TW (Joule)	**p**	0,260	0,000	0,000	0,000	0,000	0,000	0,067
	r	0,089	0,309**	0,648**	0,648**	0,637**	0,347**	0,144
60°/sec Left Rotation PT/W (Nm/kg)	**p**	0,073	0,653	0,000	0,000	0,000	0,881	0,277
	r	-0,141	-0,036	0,329**	0,329**	0,309**	-0,012	-0,086
60°/sec Left Rotation TW (Joule)	**p**	0,458	0,000	0,000	0,000	0,000	0,000	0,156
	r	0,059	0,291**	0,615**	0,615**	0,623**	0,331**	0,112
150°/sec Right Rotation PT/W (Nm/kg)	**p**	0,002	0,000	0,000	0,000	0,000	0,000	0,000
	r	0,245*	0,376**	0,611**	0,611**	0,595**	0,386**	0,283**
150°/sec Right Rotation TW (Joule)	**p**	0,079	0,133	0,889	0,890	0,646	0,144	0,119
	r	-0,138	-0,119	-0,011	-0,011	-0,036	-0,115	-0,123
150°/sec Left Rotation PT/W (Nm/kg)	**p**	0,021	0,000	0,000	0,000	0,000	0,000	0,008
	r	0,182*	0,325**	0,664**	0,664**	0,645**	0,324**	0,207**
150°/sec Left Rotation TW (Joule)	**p**	0,010	0,000	0,000	0,000	0,000	0,000	0,000
	r	0,202*	0,392**	0,692**	0,692**	0,669**	0,410**	0,279**

*p<0.05, **p<0.01, (PT: Peak Torque; W: Weight, TW: Total Work)

athletes. Also, a positive relationship between the fat free mass, muscle mass and torso muscle mass; and the torso muscle strength of athletes has been identified.

The antropometric parameters may vary based on gender. The difference associated between the genders are attributed to normal growth process, and it is accepted that antropometric, morphological and functional performances generally increase with age (16,21). In a study performed on Fencing athletes, it was determined that female athletes having same ages of their male peers, were shorter and weighed less. Similarly, in a study on soccer players, female athletes were also shorter and weighed less than male ones, but both genders had similar body mass indices (22). Smiliar to other studies, our study indicates that female athletes have less height and weight measurements than males, however, we also determined that their body mass index was lower than male athletes as opposed to other studies. We think that this difference in the body mass index values between the genders is related to the type of sports they participate in.

Other studies on athletes and people with sedentary lifestyles suggest that the females have higher average body fat percentage than male individuals (3,23). We have also determined in our study that female athletes have higher body fat percentage and fat mass than male ones. Studies show that fat storage regions between genders vary; women store more fat in the gluteal-femoral region, whereas men store more fat in the visceral depot (3). Our study suggests that female athletes have higher torso fat mass and torso fat percentage than male ones. We did not collect any data related to the gluteal region; however, we think that the reason in higher values of torso fat mass and torso fat percentage in women as opposed to the other literatures' findings are due to the fact that the participants in our study are athletes. The increase in body fat percentage and consequently the body fat mass negatively impacts sports performance as they are retained as "dead weight" in athletes particularly in sports requiring agility and antigravitational movements (1,2). Therefore, it might be interpreted that female athletes are expected to have lower sports performance than male ones (24).

Muscle strength is correlated with age, height, body weight and gender (25). In a study performed on elite athletes, it was determined that the physical performance of female athletes was lower than male ones (24). Another study suggests that the lateral core muscle strength in female athletes were lower than male ones as well (26). In our study, we have concluded that the lean mass, muscle mass and torso muscle mass as well as the entire torso isokinetic muscle strength values in female athletes were lower than male ones. We think that this is also the reason why the torso muscle strength in female athletes are lower than male ones as also suggested in other studies similar to ours.

Body composition parameters and muscle strength are the most important indicators of sports performance and there is a correlation between body composition parameters and muscle strength (16,17,27). Studies suggest that muscle strength tends to decrease as the body fat percentage increases (16,17). Our study reached conclusions similar to other studies suggesting that the torso muscle strength tends to decrease as the body fat percentage, fat mass, torso fat mass and torso fat percentage increase. Similarly, there are studies suggesting that muscle strength tends to increase as the lean body mass increases, and there is a positive medium level correlation between lean body mass / muscle mass and torso muscle strength. In a study performed on healthy individuals with a sedentary lifestyle, it was determined that there is a correlation between regional muscle mass and the muscle strength in that area. Moreover, the upper extremity muscle strength increases as the muscle mass in this area increases (28). Similary, our study concludes that the torso muscle strength in athletes increases in parallel to the torso muscle mass.

As a result of our study, as the body fat percentage, fat mass, torso fat mass and torso fat percentage increases, the isokinetic muscle strength also increases in female athletes. Also, nearly there is not any relationship between the body fat percentage, fat mass, torso fat mass and torso fat percentage with the torso isokinetic muscle strength in male athletes. These results are an interesting aspect of our study. We think that fat mass may have a weight effect and increase muscle strength in female athletes who have weak muscle strength (29). Also male athletes' strength may not have been associated with fat mass due to the high muscle mass and muscle strength.

As a limitation of our study, we have not evaluated this relationship on a control group of individuals with a sedentary lifestyle. However, we think that our study offers invaluable information as it was conducted on athletes, included an assessment of the torso muscles that directly affect athletic performance and included the identification of the correlation between body composition and torso muscles. More studies are needed in which various age groups and specific to sports branches are involved and, comparisons are made with a control group composed of healthy individuals.

Conclusion

As a result, it was determined that female athletes contain more body fat and have less muscle mass and torso muscle strength as compared to male athletes. Moreover, a correlation has been found between the torso muscle strength and body composition parameters, which are known to influence athletic performance. We suggest that athletic performance can be increased by lowering the body fat percentage, increasing lean body mass and increasing core muscle strength. Therefore, the exercise routines of athletes should also include special programs for male and female athletes tailored for increasing muscle mass and optimizing body fat percentage in addition to their standard torso muscle mass improvement trainings. Body composition measurements of the athletes should also be monitored at the same time.

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The relationship between physical activity and food habits of students. A case study

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Summary. The current study evaluated the relationship between physical activities and the food habits of a group of students from Galati County, in Romania. A cross-sectional study design was used to achieve the objectives. A total of 1,346 subjects, male and female students aged between 19 ± 25 years old answered a self-reported anonymous International Physical Activity Questionnaire to assess their physical activity and a self-reported anonymous Diet History Questionnaire III to establish their food habits quantified in Healthy Eating Index (HEI) 2015 score. Anthropometric measurements were used to calculate the body mass index (BMI) of the subjects. Within this study, randomly selected different subgroups of 246 subjects, also male and female students, aged between 19 ±25 years old also participated in an ancillary similar study with 1-day dietary tracking, evaluated by a dietician. The percentage of overall male students involved in intense sports activities was 28.02%, which was higher with 5.12% than the percentage of athletic female students (22.9%) tested in the main study. Median values of combined activities were expressed in metabolic equivalent task (MET)·minute/week, ranged from 421.86 MET minute/week to 5472.87 MET minute/week for male students and from 384.29 MET minute/week to 4506.62 MET minute/week for female students. For the food habits, the median values for the HEI 2015 score ranged from 51.3 to 54.7 for male students and from 52.2 to 55.3 for female students surveyed in the main study. Indices of fatness and body composition or BMI index values ranged from 24.38 to 21.18 for male students and from 23.87 to 20.64 for female students participated in the main study. The multivariate analysis of the results showed a strong correlation between a high level of physical activity and food habits. The Pearson correlation coefficient between the MET and HEI score values has the value r = 0.724 (p = 0.037) for boys students and $r = 0.748$ ($p = 0.028$) for girls tested in the main study. The statistical analysis showed that there are strong positive correlations between the indices calculated in the main study and in the ancillary study. The present study identified significant differences in the nutritional knowledge and food habits between the athletic students that had healthier food habits than non-athletic students. The results clearly support the need for developing programs for sports activity to be more accessible for everyone and better-disseminated information regarding health promotion and dietary habits, underlying the importance of prevention by a healthy behavior among students.

Key words: Physical activity; food habits; HEI 2015 score; BMI

Introduction

In the last decades, student's physical activity (PA) levels have radically changed (1). Students are increasingly being driven to school by car or bus before walking, and participation in organized sports is declining (2).

The importance of physical activity in modern daily life is very well established, and it is now considered as medicine for a vast majority of chronic diseases (3,4). Some studies underlined that food habits, physical performance, and the level of functional capacity of human beings are interrelated (4). As such,

any dietary deficiency that adversely affects the health of the individual is likely to impair his or her physical performance capacity (5–7). Universities may have the opportunity to form healthy habits that will improve the lifestyle and health of students' future lives (8,9).

There have been several worldwide publications in recent years on food habits, but few studies covered the relationship between the physical activity and nutritional needs in adolescents, particularly for students (10,11). Clearly, there is a need for effective and better understanding of how food habits and physical activity patterns differ between athletic and non-athletic students and hence the importance of this study.

Methods

A cross-sectional survey was used on a total group of 1,346 subjects, between 19 ± 25 year old, athletic and non-athletic students, males and females from faculties from the University of Galati, Romania. (Table 1) In this cross-sectional study, it has been compared their level of PA and their food habits.

The subjects answered a self-reported anonymous questionnaire called International Physical Activity Questionnaire (IPAQ) to assess their physical activity and a self-reported anonymous diet questionnaire history questionnaire (DHQIII) to establish their food habits quantified in Healthy Eating Index score (HEI 2015). Anthropometric measurements were used in this study to calculate the body mass index (BMI) of the subjects. Within this study, 2 weeks after the initiation of the main study, a different subgroup of 246 subjects also male and female students, aged between 19 ± 25 years old were randomly selected to participate in an ancillary study with 1-day detailed food record dietary tracking, evaluated by a dietician (Table 1) before completing IPAQ and DHQIII questionnaire, self-perception of body weight, and time watching TV or PC Games. Self-perception of body weight and time watching TV or PC Games were reported elsewhere.

Anthropometric measurements

Measurement of the weight of the subjects: The weight of the subjects were measured by a weighing machine which measures with a ±0.1 kg sensitivity using InBody 720 (Biospace Co. Ltd, Korea).

Measurement of Body Height: The measurements of the subjects were taken by a stadiometer device (Seka 217, Germany) having the subjects with bare feet and by taking the measurements between vertex point of the head and the feet with ± 0.1 cm sensitivity. Body composition: Indices of fatness was assessed by the BMI (12,13). Descriptive data were reported as proportions (%) and mean ± SD.

PA (Physical Activity). The students' PA was assessed based on the short version of the IPAQ questionnaire, translates into Romanian (14). The total weekly activity was expressed in metabolic equivalent task (MET) minute/week. One MET is equal to energy expenditure during rest and is approximately equal to 3.5 ml $O2kg^{-1}$ min^{-1} in adults. Students were classified into three main categories: Low physical activity (non-athletic Students) (<600 MET·minute/week), Moderate physical activity (600–2,999 MET·minute/week), and High physical activity (athletic students) (>2,999 MET·minute/week) (15).

Food habits

Food habits were assessed using the HEI 2015 score, a tool developed by the U.S. Department of Agriculture. The HEI 2015 score is a 13-component 100-point scale that assesses the adequacy and moderation components of the diet. Higher scores are associated with better dietary compliance (16). HEI 2015 score was calculated by means of self-reported DHQIII questionnaire (17).

Statistical analysis

Analyses of data were performed using SPSS 23 (SPSS Inc., USA). The level of significance $\alpha = 0.05$ was used to check the hypothesis. The difference in results was considered statistically significant when a *p*-value obtained was less than or equal to 0.05.

Results and Discussions

Sport activity: Median values of PA expressed in MET·min/week, ranged from 421.86 to 5,472.87 for boys and from 384.29 to 4,506.62 for girls for the

Table 1. Demographic data of students participating in the survey.

		Main study			Ancillary study		
		Boys	**Girls**	**Total**	**Boys**	**Girls**	**Total**
		621 (46.1%)	**725 (53.9%)**	**1,346**	**114 (46.3%)**	**132 (53.7%)**	**246**
Sport activity level	Low	198 (31.88%)	247 (34.07%)	445 (33.06%)	32 (28.07%)	48 (36.36%)	80 (32.52%)
	Moderate	249 (40.10%)	312 (43.03%)	561 (41.68%)	56 (49.12%)	56 (42.43%)	112 (45.53%)
	Intense	174 (28.02%)	166 (22.90%)	340 (25.26%)	26 (22.81%)	28 (21.21%)	54 (21.29%)

main study, and from 405.66 to 5,472.87 for male students and 365.29 to 4,674.32 for female students for ancillary study.

The percentage of athletic boys involved in intense sports activities (28.02%) is higher with 5.12% than the percentage of athletic girls (22.9%) testing in the main study (Table 1). Same results are also reported by previous observations from other studies. Similar trends were found by Pinto et al. 1995 (18). In the ancillary study, 22.81% of boys and 21.21% of girls report engaging in intense sport activities.

Food habits

Median value for HEI score range from 51.3 to 54.7 for boy's students (Fig. 1) and from 52.2 to 55.3 for girl s students surveyed in the main study (Fig. 2). In the ancillary study, HEI index range from 50.8 to 53.4 for boys and 51.24 to 54.9 for girls (Table 2).

Male athletic students had a median HEI score of 3.4 units higher than non-athletic students (Fig. 1). The Pearson correlation coefficient between the MET and HEI score values has the value r = 0.724, $p = 0.037 < 0.05$. This indicates that there is a strong positive

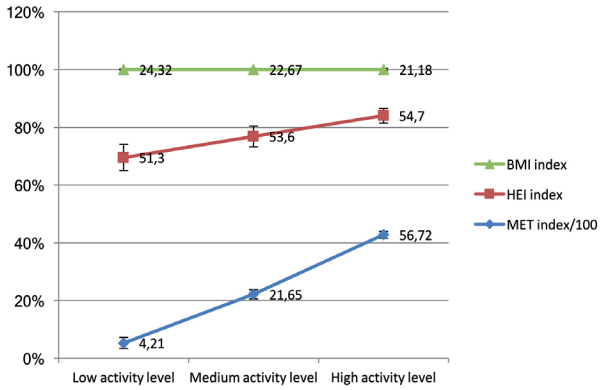

Figure 1. Comparison of BMI, HEI score, and MET for boy's student's participants at the main study.

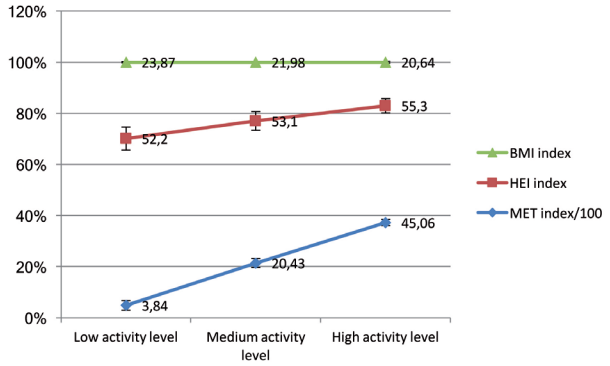

Figure 2. Comparison of BMI, HEI score, and MET for girl's student's participants at the main study.

Table 2. Ancillary study. MET, HEI score, and BMI index by gender and sport activity level of students.

Gender	**Boys**			**Girls**		
Sport activity level	**Low (non-athletic students)**	**Medium**	**High (athletic students)**	**Low (non-athletic students)**	**Medium**	**High (athletic students)**
MET, median value (SD)	405.66 (12.78)	2,215.63 (27.83)	5,472.87 (43.09)	365.29 (14.77)	2,132.68 (29.10)	5,472.87 (43.09)
HEI 2015 index, median value (SD)	50.8 (4.03)	52.5 (3.45)	53.4 (3.50)	50.8 (4.03)	52.5 (3.45)	53.4 (3.50)
BMI index, median value (SD)	25.12 (1.61)	23.42 (1.67)	22.11 (1.53)	25.12 (1.61)	23.42 (1.67)	22.11 (1.53)

correlation between the intensity of the physical activity and the increase of the HEI index which indicates a healthy diet.
The same result has reported by LaCaille et al. 2011 (19).

In the case of female students, there was the same tendency but the difference between the athletic and non-athletic female students in the case of the median value of the HEI score was only 1.1 units (Fig. 2). The Pearson correlation coefficient between the MET and HEI score values, in this case, has the value $r = 0.748$, $p = 0.028$ and it was accepted that there was a good positive correlation between the level of physical activity and food habits. Similar result has reported by Brooks-Gunn et al. (20) and Pate et al. (21).
Indices of fatness and body composition. Main study BMI index values range from 24.38 to 21.18 for boys and from 23.87 to 20.64 for girls participated in the main study. In the ancillary study, BMI index ranges from 25.12 to 22.11 for boys and from 24.55 to 20.64 for girls (Table 2).

Similar results have been reported by Johnson et al. (22) and Huang (23). A strong negative correlation between the BMI index and HEI score was observed ($r = -0.695$, $p = 0.037$). Significant negative relationships were observed between the BMI index and the IPAQ data reported for total physical activity (MET index).

We calculated the correlation coefficients between main studies and ancillary study. For physical activity (MET index), we found a very strong positive correlation $r = 0.788$, $p < 0.001 < \alpha = 0.05$.

For HEI score, main study versus ancillary study has resulted $r = 0.735$, $p < 0.001 < \alpha = 0.05$, also very strong positive correlation. For body composition (BMI index), the results obtained were in the same trend $r = 0.746$, $p < 0.001 < \alpha = 0.05$, also having a very strong positive correlation. These results validate the results obtained in the main study.

Conclusions

Both male and female athletic students involvement in PA was associated with better food habits and better BMI indexes for both gender students compared with non-athletic students. Similar results have also been observed by Pate et al. (21), Ferron et al. (24), and Middleman et al. (25).

The existing survey underlined the coexistence of positive health characteristics and the PA associated with a good diet, suggesting that it was important to get involved in various physical activities, a fact that was a necessary component of a comprehensive prevention approach among the students surveyed. Also, the future delivery of curriculum learning of various faculties during a defined training period, with clear and easy messages reinforced to support the importance of PA and healthy food habits, especially for the students. For instance, the obtained results can underline the importance of nutrition hours in the curriculum of the faculties of physical and sports education.

Conflict of interest
The authors declare that they have no competing interests.

Authors' contributions
All authors contributed equally to this manuscript.

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Supplement of liquid nutrient for fatigue recovery after tennis

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Summary. *Objective:* The objective of this study is to study the relief effect of liquid nutrient containing whey protein on fatigue of tennis players caused by plenty of exercises. *Methods:* Twenty research subjects were randomly divided into a pure water group (n = 10) and a whey protein group (n = 10), and they took tennis training after taking same dose of pure water and whey protein. The weight and fat-free weight of the players were measured before and after experiment. The red blood cells (RBC), hemoglobin (HB), blood lactic acid (BLA) and creatine kinase (CK) were detected through blood sampling. *Results:* After experiment, the fat-free weight of the whey protein group was significantly higher than that of the pure water group ($P < 0.05$), the RBC and HB of the whey protein group were higher ($P < 0.05$), and the BLA and CK of the whey protein group were lower ($P < 0.05$). *Conclusion:* The supplement of whey protein liquid nutrient is beneficial to promoting the generation of RBC and HB, reducing lactic acid accumulation, and inhibiting the increase of serum creatine phosphokinase, which is helpful to the recovery of exercise induced fatigue.

Key words: whey protein, liquid nutrient, tennis, exercise-induced fatigue, hemoglobin

Introduction

Tennis requires high on the physical ability and spirit of athletes. To maintain a high competitive level, tennis players need to do long-time and high-intensity training. Under such training, athletes usually have exercise-induced fatigue. Exercise-induced fatigue refers to a phenomenon of being unable to maintain exercise at a fixed intensity (1), which will not only affect exercise performance, but also increase the risk of excise-induced injury (2). Reasonable intake of liquid and dietary nutrients is conducive to supplementing nutrients in athletes and promoting fatigue recovery. The main research object of this study is the liquid nutrient added with whey protein. Whey protein, as a kind of high-quality protein, has been widely used in food. Through the study of 18 athletes, Hansen et al. (4) found that whey protein intake could effectively improve athletes' performance and reduce sports injury. Junior et al. (5) studied 31 old women and divided them into two groups. The two groups were given whey protein and placebo respectively. The results showed that the quality of skeletal muscle of women taking whey protein was significantly improved, and the quality of muscle was also improved. Aiming at the problem of muscle injury of football players, Philpott et al. (6) studied the effects of whey protein beverage on athletes and found that the beverage could effectively improve the muscle soreness of athletes and inhibit the increase of creatine kinase. Taking rats as the research subjects, Ren et al. (7) studied the effects of whey protein on the performance of long-term training arc in rats and found that whey protein supplement could improve the fatigue time of rats, reduce the content of propylene glycol, and relieve exercise-induced fatigue. Taking 20 tennis players as the research subjects, this study analyzed the fatigue recovery effect of whey protein liquid nutrient intake on tennis athletes after a period of tennis exercise and proved the fatigue recovery effect of whey protein by comparing blood routine and blood biochemical indicators, which is beneficial to the application and promotion of whey protein liquid nutrient in practice.

Whey protein

Whey protein, a kind of protein extracted from milk (8), contains more than 20 kinds of amino acids and can promote muscle glycogen renewal and supplement energy. Moreover it has a variety of functional active substances. In the process of sports, lack of protein will cause negative nitrogen balance, which is not conducive to the recovery of fatigue after exercise. Therefore, athletes need to timely supplement high-quality protein (9). Movement protein has characteristics of easy to absorb, rich nutrition and low cholesterol and fat. Whey protein can meet the needs of human protein supplementation and is conducive to increase muscle (10) and improve immunity. In this study, the exercise-induced fatigue recovery effect of liquid nutrients made of whey protein was studied. The formula of the liquid nutrient used is shown in Table 1.

Materials and methods

Research subjects

Twenty students were randomly selected from the athletes who were from the male tennis team of physical culture institute of Xi'an University of Architecture and Technology, China, and volunteered to attend experiment and signed informed consent. They were divided into a whey protein group and pure water group. The general information of the athletes is shown in Table 2.

Table 1. Whey protein liquid nutrient

Whey protein	3%
Saccharos and oligosaccharide	3.5%
Acidulant	2%
Carboxymethylcellulose	0.15%
Salt	1.2%
Water	90.15%

Table 2. Comparison of general information of the research subjects

	Whey protein group (n=10)	Pure water group (n=10)
Age	21.86 ± 1.27	21.55 ± 1.36
Height/m	171.24 ± 6.74	172.68 ± 5.39
Weight/kg	64.59 ± 7.12	63.27 ± 8.65
Training time/year	3.57 ± 0.52	3.61 ± 0.47

Experimental methods

The two groups of athletes received the same training task every day. Under the guidance of the coach, they were trained twice a day for tennis, 2 hours each time. The whey protein group took 400 ml of whey protein liquid nutrient half an hour before training, half an hour after training and half an hour before sleeping, while the pure water group took 400 ml of pure water at the same time. The experiment lasted for seven days, and the venous blood was collected before and after training at the first, fourth and seventh day. During the experiment, the athletes of the two groups had unified meals and did not take any extra nutrients.

Measurement indicators

Basic indicators including weight and fat-free weight were detected by research staffs before and after experiment.

Blood routine indicators including red blood cells (RBC) and hemoglobin (HB) were detected by sending blood samples to Xi'an hospital.

Serum biochemical indicators including blood lactic acid (BLA) and creatine kinase (CK) were detected by sending blood samples to Xi'an hospital.

Statistical analysis

The data were recorded in Excel 2007 and statistically analyzed using SPSS. 17.0. Different indicators were expressed in the form of mean ± standard deviation ($\overline{X} \pm S$) and processed by t test. Difference was considered as statistically significant if the value of P was smaller than 0.05.

Experimental results

Changes of basic indicators

As shown in Table 3, the weight and fat-free weight of the athletes in the two groups had no significant differences before experiment; the weight of the two groups decreased after experiment ($P < 0.05$), but the fat-free weight increased. The fat-free weight of the whey protein group was 50.84 ± 2.27 kg be-

Table 3. Comparison of basic indicators

		Pure water group	Whey protein group
Weight/kg	Before experiment	63.27 ± 8.65	64.59 ± 7.12
	After experiment	62.36 ± 6.78*	62.45 ± 6.64*
Fat-free weight/kg	Before experiment	51.27 ± 3.12	50.84 ± 2.27
	After experiment	51.42 ± 3.34	53.67 ± 3.12*#

Note: * indicated P < 0.05 compared to before experiment; # indicated P < 0.05 compared to the pure water group.

fore experiment and 53.67 ± 3.12 kg after experiment, showing a significant increase and a significant difference with the pure water group (P < 0.05). The experimental results demonstrated that the intake of whey protein liquid nutrient could effectively reduce body fat rate and promote growth of muscle.

Changes of RBC

It was found from Figure 1 that the RBC level of both groups decreased after training. At the seventh day, the RBC level of the pure water group and whey protein group was 4.94 ± 0.33 × 10^{12}/L and 4.96 ± 0.32×10^{12}/L respectively before training, and there was no significant difference. After training, the RBC level of the pure water group decreased to 4.61 ± 0.27 × 10^{12}/L, and the RBC level of the whey protein group was 4.84 ± 0.26 × 10^{12}/L, which was significantly higher (P < 0.05).

Changes of HB

It was found from Figure 2 that the HB of the two groups had no remarkable difference before training, but the HB showed a decreasing tendency in both groups after training. At the seventh day, the HB of the pure water group decreased to 131.57 ± 7.62 g/L, and the HB of the whey protein group decreased to 138.49 g/L, showing that the HB of the pure water group decreased more, but the difference had no statistical significance (P > 0.05). The change of HB demonstrated that the intake of whey protein liquid nutrient could inhibit the decrease of HB, which was beneficial to the fatigue recovery of athletes.

Changes of BLA

It was found from Figure 3 that the BLA level of the athletes in the two groups had significant increase after training (P < 0.05). At the seventh day, the BLA level of the pure water group increased to 3.62 ± 1.98 mmol/L, and that of the whey protein group increased to 1.83 ± 1.54 mmol/L after training, which was significantly lower than the pure water group (P < 0.05). It indicated that the intake of whey protein liquid nu-

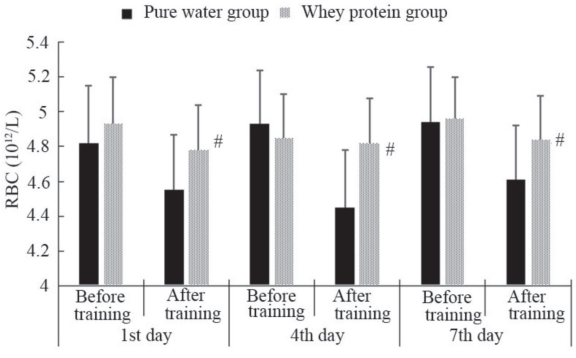

Figure 1. The changes of RBC
Note: #: P < 0.05 compared to the pure water group.

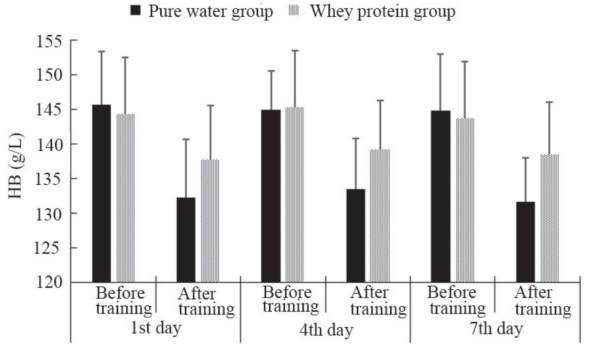

Figure 2. Changes of HB

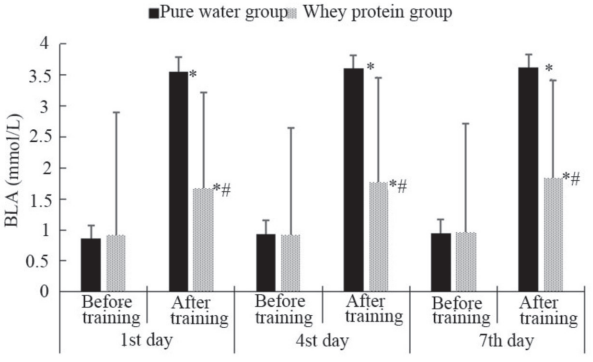

Figure 3. Changes of BLA
Note: * means P < 0.05 compared to before training; # means P < 0.05 compared to the pure water group.

trient could reduce the generation of BLA in bodies of athletes to relieve fatigue.

Changes of CK

It was found from Figure 4 that the CK level of the pure water group showed a significant difference before and after training ($P < 0.05$). At the seventh day, the CK level of the pure water group was 142.36 ± 71.64 U/L, and that of the whey protein group was 109.21 ± 52.77 U/L respectively after training, which was significantly lower than that of the pure water group ($P < 0.05$).

Discussion

As the second largest ball game in the world, tennis has developed rapidly in China. Tennis players need long-term and high-intensity tennis training in order to achieve a higher level of competition. In tennis, the physical energy consumption of athletes is very huge. Simple massage and sleep are not enough for athletes to recover. The nutrients consumed in tennis need to be supplemented in time to help tennis players recover their physical fitness. For tennis players, it is necessary to supplement some foods with high sugar, low fat and high protein.

Fat-free weight can reflect the body's adaptability to training, and its increase has a role in enhancing athletes' strength, explosive force, endurance, etc. Athletes need to maintain the best muscle mass in order to achieve better performance (11). It was found from Table 3 that the fat-free weight in the whey protein group was significantly higher than that in pure water group ($P < 0.05$), indicating that whey protein component could effectively increase the fat-free weight (12), reduce fat, and enhance the energy reserves.

In the process of human exercise, oxygen is transported through blood, mainly affected by RBC and HB. When RBC and HB decrease, the ability of oxygen transport decreases, and the human body will experience fatigue. The production of RBC and HB requires a lot of nutrients. If there is no additional supplement, RBC and HB will be reduced, so that the body's physical fitness will decline and exercise fatigue will occur. It was found from Figure 1 and 2 that the RBC and HB of the two groups showed a downward trend after experiment, and the descending range of the pure water group was significantly larger than that of the whey protein group, which indicated that whey protein component had a role in promoting the reduction of RBC and HB. Increasing the number of RBC and HB is conducive to maintaining the acid-base balance in the body, increasing the amount of exercise oxygen, and thus promoting the recovery of exercise-induced fatigue.

With the accumulation of fatigue, lactic acid accumulation will occur in the body; the more lactic acid accumulation, the more prominent the muscle soreness symptom. The content of BLA can reflect the metabolic changes and fatigue of the body (13). Whey protein component is beneficial to enhance the body's antioxidant activity and inhibit lactic acid. It was found from the results of Figure 3 that the accumulation of BLA of the whey protein group was better than that of pure water group, which proved that whey protein component could promote fatigue recovery through inhibiting BLA.

CK is an enzyme that regulates cell energy conversion (14). Its activity is closely related to muscle discomfort after exercise. High-intensity training can lead to the increase of CK. It was found from the results of Figure 4 that the CK level of both groups increased after the experiment, the CK level of the pure water group increased significantly compared with that before experiment, but the increase of the whey protein group was less ($P < 0.05$). It showed that whey protein could effectively inhibit the increase of CK after exercise, proving its role in fatigue recovery.

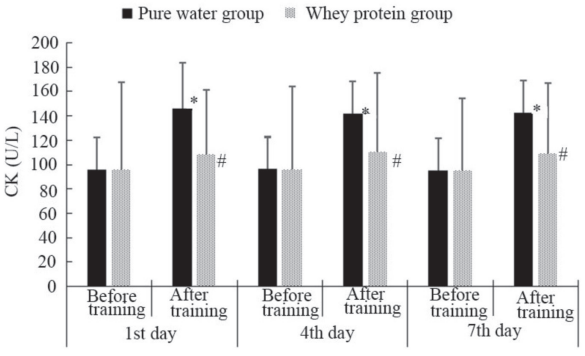

Figure 4. Changes of CK
Note: * indicated $P < 0.05$ compared to before training; # indicated $P < 0.04$ compared to the pure water group.

This study confirmed the fatigue recovery role of whey protein liquid nutrient. In the next step, we will further study liquid nutrients at different dosage and formulations.

Conclusion

In the present study, the effect of supplementation of whey protein liquid nutrients on the recovery of fatigue after tennis was studied. By comparing the indicators of the pure water group and whey protein group such as RBC and HB, it was found that:

1) after training, the weight of the athletes in both groups increased, and the whey protein group increased more;
2) the decrease of erythrocyte and hemoglobin in the whey group was lower than that in the pure water group;
3) the level of serum lactic acid produced in the whey group was significantly lower than that in the pure water group;
4) the increment of CK in the whey protein group was significantly lower than that in the pure water group.

In conclusion, whey protein liquid nutrients are beneficial to the formation of RBC and HB and can effectively inhibit the increase of BLA and CK, thereby relieving fatigue. This work makes some contributions to the study of fatigue recovery of athletes.

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Comparison of application of various anti-fatigue nutritional beverages in supplementing function of basketball players

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Summary. Basketball, as a high-intensity and high-risk sport, requires athletes to maintain a high level of physical function at all times. In recent years, with the rapid development of food manufacturing technology, a variety of anti-fatigue nutritional beverages for athletes emerge one after another. Therefore, the application of a variety of anti-fatigue nutritional beverages in supplementing the different physical function needs of basketball players has become a key topic in sports health research. In this study, basketball players were selected as experimental subjects, and the control group was set up. Three kinds of anti-fatigue nutritional beverages, namely sugar beverage, taurine beverage and tea beverage, were tested to understand the different application of different nutritional beverages in supplementing function, so that basketball players can better maintain physical strength and improve sports performance. Through the comparison of the experimental results, it is concluded that sugar beverage has the greatest application degree to blood lactic acid, taurine beverage has the greatest application degree to liver glycogen content and tea beverage has the greatest application degree to serum urea. Therefore, in practical application, attention should be paid to the coordination of various nutritional beverages and different beverages should be drunk according to the actual needs.

Keywords: anti-fatigue nutritional beverage; basketball players; physical function

Introduction

With the development of basketball, the number of basketball players is increasing day by day. However, in order to achieve good results on the court, the maintenance of physical function is the most basic bargaining chip to continue to compete. Exercise-induced fatigue is a manifestation that the human body cannot maintain the fixed exercise intensity(1), which may induce decline of exercise performance and increase risks of injuries (2). At present, basketball players mainly rely on anti-fatigue nutritional drinks to supplement the lack of physical function in a short period of time. Therefore, the functional application of different kinds of anti-fatigue food is a hot topic. Xia et al. (3) divided okra pods into seeds and skin and compared the effects of seeds (OSD) and skins (OSK) *in vivo* and *in vitro*. It was proved that the ingredients contained in okra had antioxidant and anti-fatigue functions. Di et al. (4) observed the anti-fatigue effect of small molecular oligopeptides isolated from *Panax quinquefolium L.* on mice and proved that the anti-fatigue effect of *Panax quinquefolium L.* was attributed to ginsenosides and oligopeptides. Zhao et al. (5) found that the water extract from Caulis Spatholobi had an anti-fatigue effect. The extract was further separated by alcohol precipitation method, and finally the total polysaccharide was proved to be the reason that Caulis Spatholobi could increase the level of glucose and make it have anti-fatigue effect. Li et al. (6) determined the optimal extraction conditions of Maca polysaccharide by response surface method, and discussed the anti-fatigue activity of Maca polysaccharide by using model prediction. The results of swimming parameters and biochemical parameters showed that the low dose Maca polysaccharide group had significant anti-fatigue activity. During exercise, basketball players lose a lot of water and electrolytes due to excessive sweating. Nu-

tritional beverages can provide energy for the body conveniently and quickly, and alleviate its discomfort. Functional ingredients in beverages can improve athletes' reaction level and accelerate the recovery of limb fatigue (7). The current study mostly concerns the influence of one kind of anti-fatigue food on functions of human body, but seldom concerns about the comparison of different foods. Therefore, based on physiological principle, three kinds of anti-fatigue beverages were tested in this study. The experimental data of blood sugar, hepatic glycogen and blood lactic acid in body function after drinking nutritional beverage were compared by random sampling method, and different application situations were obtained.

Causes for fatigue of basketball player

In addition to the big moves such as fast running, avoiding and leaping in the game, basketball players also need to consume a lot of energy in the daily training after the game. In this way, the alternating sport on and off the court makes the athletes easily fall into physical fatigue. The causes of fatigue of basketball players can be divided into two categories.

(1) Exercise fatigue (8). In basketball matches, there is often a continuous competition between the two sides for a certain time and several rounds. In this process, athletes need to constantly change the way of dribble and switch different technical movements. It makes the muscles of athletes constantly relax and stretch. At this time, the PH value in the body decreases, and the activity of active enzymes in the muscles is inhibited, leading to the insufficient regeneration rate of available energy of the human body and the phenomenon of fatigue. Drinking nutritious beverages can generally alleviate this situation.

(2) Injury fatigue (9). Due to the heavy training and competition intensity of basketball players, the wear and tear of each joint is more serious, so many athletes are plagued by injuries. Common injuries include Achilles tendon rupture, knee swelling and so on. The sequelae of injuries and illnesses lead to the decline of immunity, which makes it difficult for the body to recover quickly. It makes the injured parts easily fatigued after excessive exercise and unable to undertake the next intense exercise. Some anti-fatigue nutritional beverages contain certain pain-relieving ingredients, such as caffeine. But drinking nutritious beverages is only a palliative for athletes who are tired from injuries and diseases.

Experiment

Laboratory reagents

Laboratory reagents included blood sugar determination reagent, liver glycogen determination reagent and lactic acid determination reagent.

Experimental materials

To simulate the main types of anti-fatigue nutritional beverages in the market, three groups of compound beverage samples were prepared in proportion, and a control group was set up. The amount of each sample in each group was 300 ml, and the temperature, humidity and other conditions were the same. All four groups of experiments were conducted at the same time on the same day. The experimental conditions of each group were as follows:

Control group: 100% pure water.

Group 1 (Sugar beverage group): 8% carbohydrate, 0.4% taurine, 1% theanine, 5% vitamin B, 0.3% L-lysine hydrochloride, 0.015% inositol, and 0.01% nicotinamide. The rest was pure water.

Group 2 (Taurine beverage group): 4% carbohydrate, 0.8% taurine, 1% theanine, 5% vitamin B, 0.3%L-lysine hydrochloride, 0.015% inositol, and 0.01% nicotinamide. The rest was pure water.

Group 3 (Tea beverage group): 4% carbohydrate, 0.8% taurine, 2% theanine, 5% vitamin B, 0.3%L-lysine hydrochloride, 0.015% inositol, and 0.01% nicotinamide. The rest was pure water.

Experimental subjects

In a basketball team of some city, 40 male basketball players with no disease or joint injury were randomly selected, aged 20-25 years. All of them signed informed consent. All the players took part in basketball matches more than five times. The average training time was longer than 8 hours per week. At the end of the experiment, the experimental data were obtained according to the blood test results.

Experimental steps

1. Experimental preparation

The subjects were not allowed to consume any beverage containing the ingredients of this study before the start of the study. Subjects maintained good health and avoided strenuous activity. Forty subjects were randomly divided into four groups, ten in each group and drank 400 ml of water on an empty stomach one hour before the formal experiment.

2. Experiment

High-intensity continuous shooting training with 70% VO2max intensity was conducted for 1 hour. At the 0^{th}, 15^{th} and 45^{th} min, solution was supplemented to each person in the corresponding group, 100 ml each time. The physical indicators of the subjects were monitored in real time. A timer was used to measure the exhaustion time of each athlete's shot, that is, the time it took for the athlete to exhaust the total energy of the body, and the time was recorded to calculate the average time. Venous blood was collected at the 0^{th}, 30^{th} and 60^{th} min. 1 mL of venous blood was extracted using a vacuum sterile tube and then put into an anticoagulant tube for full mixing and stored at -20°C. After shooting for 30 minutes, the physical function changes of players were continued to observe and record.

3. Determination method of each index

a. Serum urea determination test

SK3003 semi-automatic biochemical analyzer (Shenzhen Sinothinker Technology Co., Ltd., China) was used for the determination. The kit used was the urea determination kit (Zhejiang Kaicheng Biotechnology Co., Ltd., China), and its principle is that urea generates NH_3 and CO_2 under urease hydrolysis and ammonia reacts with NAHD to generate glutamate and NAD + under the action of glutamate dehydrogenase.

b. Liver glycogen determination test (10)

I Serum: The blood was centrifugated for 10 minutes at the speed of 3000 rpm to collect the upper layer of serum.

II Plasma: The plasma was anticoagulated with Ethylene Diamine Tetraacetic Acid (EDTA), citrate or heparin and centrifugated for 30 minutes at the speed of 3000 rpm to collect the upper layer of serum.

III Cell supernatant: The cell supernatant was centrifugated for 10 min at the speed of 3000 rpm to remove particles and polymers.

IV Tissue homogenization: The tissue homogenization was mashed and centrifugated for 10 min at the speed of 3000 rpm to collect the upper layer of serum.

c. Blood lactic acid determination test

Beckman LX20 automatic biochemical analyzer (Beckman, USA) was used for determination. The kit used was lactate oxidase assay kit (Beijing Leadman Biochemical Technology Co., Ltd., China), and its principle is that lactate oxidase reacts with lactate to generate pyruvate and hydrogen peroxide, hydrogen peroxide reacts with 4-aminoantipyrine and p-chlorophenol to generate red quinoid dye.

Experimental results

Statistical methods were used to sort out and summarize the experimental data. Relevant calculations were carried out on the statistical analysis software statistic package for social science (SPSS). The general linear regression repeat measurement method was adopted to examine the differences in the effects of supplementing different anti-fatigue nutritional beverages on various metabolic indexes of the body.

0.05 was taken as defined value. When $P > 0.05$, there was no significant difference; when $P < 0.05$, there was a significant difference between groups; when $P < 0.01$, there was a highly significant difference between groups.

1. The comparison of the average exhaustion time between different groups of athletes is shown in Table 1.

The prolongation of exhaustion time means the improvement of exercise endurance under the same exercise intensity. The slowdown of body energy

Table 1. Effects of different anti-fatigue nutritional beverages on average exhaustion time

Group	Number of people/n	Average exhaustion time/min	P value
The control group	10	30.6	——
Sugar beverage group	10	49.8*	0.027
Taurine beverage group	10	53.7*	0.016
Tea beverage group	10	45.3*	0.047

* indicates $P < 0.05$ compared with the normal state.

consumption rate is one of the important forms to enhance the anti-fatigue ability. The results showed that the average exhaustion time of the experimental groups increased significantly. It showed that the application of these three kinds of anti-fatigue nutritional beverages could enhance sports endurance and improve athletes' anti-fatigue ability.

2. The comparison of serum urea content in each group is shown in Table 2.

Table 2 showed that the experimental group could significantly improve the basketball players' serum urea content after intense exercise, compared with the control group. Among them, the tea beverage group had the most positive effect on serum urea value of human, far more than the other two groups. It suggested that the application of tea beverages may be more effective in improving the body's serum urea levels and thus better resisting fatigue.

3. The comparison of liver glycogen content in each group is shown in Table 3.

It could be seen from Table 3 that sugar beverages and taurine beverages played a significant role in promoting the synthesis of liver glycogen. However, it could be seen from P>0.05 that the increase of liver glycogen content in tea drinks was not obvious. In this experiment, the P value of taurine beverage group was smaller than 0.01, which indicated that the application of taurine beverage in basketball players' physical function was reflected in the significant increase of liver glycogen content, thus delaying fatigue time.

4. The comparison of blood lactic acid content between different groups is shown in Table 4.

Table 4 showed that that the blood lactic acid value of all the anti-fatigue nutritional drinks in all the experimental groups decreased in different degrees

Table 2. Effects of different anti-fatigue nutritional beverages on serum urea content

Group	Number of people/n	Serum urea at 0th min /(Mmol/l)	Serum urea at 30th min/(Mmol/l)	Serum urea at 60th min/(Mmol/l)	P value
The control group	10	4.85±0.24	5.13±0.29	5.14±0.32	——
Sugar beverage group	10	5.27±0.54	5.86±0.60	6.50±0.65*	0.048
Taurine beverage group	10	4.96±0.51	5.46±0.63	6.14±0.69*	0.035
Tea beverage group	10	5.24±0.58	6.0±0.72	6.84±0.83*	0.012

* indicates P < 0.05 compared with the normal state.

Table 3. Effects of different anti-fatigue nutrition beverages on liver glycogen content

Group	Number of people/n	Liver glycogen at 0th min/g	Liver glycogen at 30th min /g	Liver glycogen at 60th min/g	P value
The control group	10	98.6±8.7	106.0±8.4	113.2±8.1	——
Sugar beverage group	10	100.2±8.3	108.3±9.2	117.8±10.6*	0.028
Taurine beverage group	10	97.4±7.9	107.6±11.3	120.5±13.9*	0.009
Tea beverage group	10	95.9±8.6	103.6±8.8	112.0±9.3	0.261

* indicates P < 0.05 compared with the normal state.

Table 4. Effects of different anti-fatigue nutritional beverages on blood lactic acid content

Group	Number of people/n	Blood lactic acid at 0th min/(Mmol/l)	Blood lactic acid at 30th min/(Mmol/l)	Blood lactic acid at 60th min/(Mmol/l)	P value
The control group	10	1.23±0.27	1.75±0.35	1.93±0.45	——
Sugar beverage group	10	1.14±0.13	1.12±0.15	1.09±0.19*	0.034
Taurine beverage group	10	1.03±0.26	1.02±0.30	0.97±0.37*	0.047
Tea beverage group	10	0.98±0.21	0.97±0.24	0.95±0.23*	0.045

compared with the normal value of the control group. Among them, the P value of sugar beverage was close to 0.01, and compared with other experimental groups, the reduction of blood lactic acid was more obvious. It suggested that the application of sugar beverage in anti-fatigue of basketball players was beneficial in slowing down the formation of lactic acid, which helped athletes maintain more sustained energy.

Discussion

Anti-fatigue nutritional beverage refers to a beverage that adds nutrient fortification ingredients on the basis of certain scientific research to supplement the special nutritional needs of some people (11). Proper consumption of nutritious beverages can help to improve the central inhibition after high-intensity exercise and achieve the ideal effect of anti-fatigue. At present, the development of anti-fatigue nutritional beverage in China still maintains the growth momentum of high sales, its application is becoming more and more diversified, and the overall industry development trend is optimistic. Nutritional beverages are gradually expanding their market share and are generally used in sports and auxiliary processes for sub-health people to improve their body functions (12). From the results of Table 1, it could be found that the exhaustion time of the experimental groups after drinking was significantly different from that of the pure water group ($P < 0.05$), indicating that the anti-fatigue nutritional beverage can effectively prolong the exhaustion time, thereby reducing the fatigue degree of the body.

Serum urea is a sensitive indicator to measure the fatigue degree, and the increase of serum urea value means that the body's exercise adaptability becomes worse (13). It could be seen from the results in Table 2 that the increase of serum urea in the tea beverage group was better than that in the control group, which proved that the components contained in tea beverage can stimulate serum urea, so as to improve the physical function.

Liver glycogen synthesis plays an important role in regulating the normal function of tissues in vivo. The more glycogen is generated, the longer the duration of exercise time that can be supported (14). As shown in Table 3, the liver glycogen content of the taurine beverage group was significantly different from that of the other three groups after the experiment ($P<0.05$), indicating that taurine beverage can effectively promote the formation of liver glycogen after exercise and proving its role in promoting fatigue recovery.

The blood lactic acid content is closely related to the acid-base balance *in vivo* . Basketball players engage in anaerobic exercise, leading to a lack of oxygen in the body, which increases blood lactic acid level. The direct result of increased lactate secretion is a decrease in the body's internal PH, which causes muscle soreness and limb weakness. As shown in Table 3, the liver glycogen content of the taurine beverage group was significantly different from that of the other three groups after the experiment ($P<0.05$), indicating that taurine beverage can effectively promote the formation of liver glycogen after exercise and proving its role in promoting fatigue recovery.

Conclusion

Starting from exploring the application of different anti-fatigue nutritional beverages, three representative anti-fatigue nutritional beverages were selected with basketball players as experimental objects. The average exhaustion time and the content of serum urea, hepatic glycogen and blood lactic acid were measured and calculated by reagents. Statistical method was used during the study. Finally, the final results were obtained by comparing the effects of three different beverages. The experiment shows that:

1) sugar drinks, taurine drinks and tea drinks can all prolong the exhaustion time of human body and improve the anti-fatigue ability of athletes;
2) anti-fatigue beverage can improve the content of urea in human serum, among which tea beverage has the most significant effect;
3) sugar drinks and taurine drinks are beneficial to the synthesis of glycogen in human liver, among which taurine drinks play the most significant role;
4) the anti-fatigue beverage can inhibit the production of blood lactate, among which sugar beverage has the most significant effect.

This experiment provides an effective attempt to study the combination of sports health and the practical application of various anti-fatigue nutritional beverages, contributes to the effective improvement of athletes' fatigue situation and the correct supplement of physical function, and provides a possibility for athletes to improve their performance to the greatest extent. It provides a scientific basis for further rational selection and application of sports beverages.

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Validation of a Turkish version of the interpersonal outcome expectancies for thinness (IOET) scale in university students

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Summary. *Aim:* The aim of this study was to determine the validity and reliability of IOET in Turkish university students. *Methods:* First, the IOET scale was translated by using the translation-back translation method for cultural equivalence. Then, IOET was carried out on 513 university students explanatory and confirmatory factor analyzes. The body shape questionnaire and the Nutritional Changes Processes Scale were used for convergent validity. Test-retest validity was performed with 117 university students 4 weeks after the scale was given. *Results and Discussion:* The IOET scale showed a one-factor structure. The one-factor structure of the scale demonstrated a good agreement with the fix index values. In addition, the internal and test-retest validity reliability of the scale was found to be good (Cronbach α= 0.933). Positive, statistically significant correlations were found between IOET, the body shape questionnaire, and the Nutritional Changes Processes Scale ($p < 0.05$). This study has shown that the Turkish version of the IOET scale is a valid and reliable instrument for assessing the extent to which thinness can affect interpersonal relationships and/or our expectations from the other people. It is thought that IOET-TR can contribute to other studies on thinness expectations by providing a different perspective.

Keywords: Body image, eating disorders, thinness expectancies, IOET, validity, reliability

Introduction

Overweight and obesity are defined as abnormal or excessive fat accumulation that may impair health. Obesity is defined as a health problem in terms of physiological, biochemical, and psychological aspects (1). In addition, it is an important health problem that needs to be addressed socially because of the prejudiced, discriminatory, and stigmatizing behaviors of other individuals in the society towards obese / overweight individuals (2).

Time at university is a period during which individuals develop into young adults (3). The individual undergoes not only physical but also psychosocial changes during this period, which has been shown to pose risks for individuals as they are prone to have both poor and unbalanced diets (4). Individuals remain away from a family setting during university education, with their free will becoming more exposed and thus they may break their habits of having a healthy and balanced diet for a variety of reasons such as peer pressure (5). It is especially during this period that individuals consume food outside home, skip their meals, ingest too much fast food that is high in carbohydrates and fat, opt for wrong diets, and eat unhealthy snacks between meals (4, 6, 7). University students have been shown to be discontent with their body image, shape, and weight, which may bring about problems in eating behavior and the emergence of eating disorder symptoms (8, 9).

Eating disorders are characterized by behaviors aimed at gaining or maintaining a slim body shape (9).

Because of the belief that " thin is beautiful" especially in the societies of Western Europe, the United States, and Asia, changes in beauty criteria and the notion of size zero in the media put pressure on adolescents and young adults, encouraging them to diet unconsciously and thus causing eating disorders (10-12). Indeed, studies have indicated that there is a positive correlation between influence of the media and body dissatisfaction, thin-ideal internalization, and eating disorder behaviors (11-14). The desire to be accepted and appreciated in social relations and the fear of exclusion and ridicule prepare the basis for starting diet and restrictive eating behaviors at an early age (15). In addition, adolescents and young women experience social pressure as a result of psychological conflicts during their life stages (identity crises, psychological separation, and a strong need for social acceptance and self-confidence), which can lead to body dissatisfaction and unhealthy eating behaviors (12, 16). There are no studies evaluating the relationship between body dissatisfaction and eating disorders and the expectations of thinness in interpersonal relations in Turkish society. Accordingly, the aim of the present study was to adapt the Interpersonal Outcome Expectations for Thinness Scale (IOET) developed by Li et al. (17) into Turkish and to perform the validity and reliability analysis of the scale.

Materials and Methods

Sample

The study was conducted with 513 students (381 females and 132 males) aged between 18–43 years (mean = 20.5 years, SD = 2.1) who were studying at Ankara Yıldırım Beyazıt University and volunteered to participate in the study. Ethics committee was granted from Ankara Yıldırım Beyazıt University Ethics Committee with research code 2019-204 (19/04/19).

Instruments

The data for the research was collected through a survey made up of three sections. Those sections are:

Interpersonal Outcome Expectancies for Thinness (IOET) Scale: Li et al. devised an 8-item scale to determine whether being thin plays a role in future relationships with others. This section explores the likely effects of being thin on interpersonal relationships. Individuals are required to provide responses to the 7-point Likert scale, like "definitely agree" and "definitely disagree". Those who "definitely disagree" receive 1 point while those who "definitely agree" receive 7 points. One can get at least 8 and at most 56 from this scale. Respondents who get a high score are of the opinion that being thin is of greater significance in interpersonal relationships (17).

Nutritional Changes Processes Scale: Prochaska et al. devised a 48-item scale with a view to determining the effect of experiences on people's eating habits and behaviors (18). This scale is made up of 12 subscales, having been prepared in the form of 5-point Likert scale. Respondents are asked to choose from the list of 5 options as "never", "rarely", "sometimes", "often", and "very often". Those who tick the "never" box receive 1 point while those who tick the "very often" box receive 5 points. Respondents receive scores ranging from 48 to 240. For all the subscales, the highest score is 20 and the lowest is 4. The assessment of the scale is by having the total score divided by the item numbers. The evaluation of reliability and validity of these scales was performed by Menekli and Fadıloglu for the Turkish population (19). The subscales of the scale are as follows:

1. *Consciousness raising:* Individuals possess awareness on the reasons and consequences of events (items 1, 13, 25, 37).
2. *Dramatic help / Emotional triggers:* Negative messages regarding unhealthy living cause individuals to act (items 3, 15, 27, 39).
3. *Re-evaluating the environment:* Individuals' assessment of the level of being affected by the social environment (items 4, 16, 28, 40).
4. *Re-evaluating own self:* Individuals' persistence in their bad habit and their assessment of themselves in the absence of this habit (items 9, 21, 33, 45).
5. *Social liberty / freedom:* A phenomenon regarding the rise of opportunities and possibilities in the immediate environment (items 10, 22, 34, 46).
6. *Compensation:* Individuals' learning a healthy behavior that can fix a bad one (items 2, 14, 26, 38).
7. *Auxiliary relationships:* Trust, openness, admission, and support that contribute to behavior change (items 5, 17, 29, 41).

8. *Fortification method:* Individuals' rewarding their positive behaviors regarding diet (items 7, 19, 31, 43).
9. *Freeing own self:* Individuals' being open to behavior change and acting toward their beliefs, purposes and decisions regarding these behaviors (items 8, 20, 32, 44).
10. *Stimulus control:* The removal of factors that lead to unhealthy behaviors by individuals and the addition of factors that help to form healthy behaviors (items 11, 23, 35, 47).
11. *Interpersonal system control:* Individuals' ability to exert control over their interpersonal relationships during the process of establishing healthy living behaviors (items 6, 18, 30, 42).
12. *Use of medicine:* Explores whether individuals resort to medicine or not to have a balanced diet (items 12, 24, 36, 48).

Body Shape Questionnaire: This questionnaire was developed by Copper et al. in order to establish individuals' opinions and concerns about their appearances in the past 4 weeks (20). This scale makes up of 34 questions. Each item was drawn up in the form of 6-point Likert scale. Respondents are asked to choose from the list of 6 options as "never", "rarely", "sometimes", "often", "very often", and "always". Those who tick the "never" box receive 1 point while those who tick the "always" box receive 5 points. The maximum score is 204 and high scores are indicative of dissatisfaction with body shape. The Turkish version of the body shape questionnaire was conducted by Akdemir et al. (21).

Translation and Linguistic Equivalence Study

Interpersonal Outcome Expectancies for Thinness scale was translated according to Brislin method (22). Scale was initially translated from English to Turkish by 3 experts who were bilingual, and the most suitable one for each item in terms of meaning and language structure was selected. The Turkish translation of the scale was re-translated into English (back translation) by two experts who were graduates of English department and the original text of the scale and the intelligibility of the translated text were checked. The Turkish form of the scale was given to 30 university students independent of the study sample and arrangements were made in line with their suggestions. After the arrangements, the items in the scale were translated back to English with the help of at least 2 different experts and the same experts finalized the scale by looking at its compatibility with the original scale. In order to determine the linguistic equivalence of the scale, after the corrections, 33 university students (who knew Turkish and English) from the Preparatory Department of Ankara Yıldırım Beyazıt University were first administered the English scale and the Turkish scale forms were applied 4 weeks later (23). Spearman correlation analysis between the mean scores of the scale obtained from the English and Turkish forms of the scale were used. According to this there was a significant positive correlation between the scores obtained from the scales in both languages ($r = 0.88$; $p < 0.01$).

Data analysis

Exploratory Factor Analysis

Factor analysis was performed for the structural validity of the scale. Exploratory factor analysis was conducted via SPSS 22.0. Before the implementation of exploratory factor analysis, the Bartlett's Test of Sphericity and the KMO Test for Sampling Adequacy were conducted in order to establish whether the data were suited to factor analysis or not. In order for the data to be suitable for factor analysis, the KMO Test for Sampling Adequacy must be over 0.60 and the significance level of the Bartlett's Test of Sphericity must be less than 0.05 (24, 25). Subsequently, the factor number of the scale was established, and the factor loadings of the items were calculated through the method of varimax rotation.

Confirmatory Factor Analysis

Confirmatory factor analysis (CFA) was conducted via AMOS 22.0. The maximum likelihood estimation method was used for this analysis. In order to evaluate the validity of the factorial structure of the scale, a large number of fit indices are used in CFA. In this study, relative chi square ($\chi 2/df$), the Comparative Fit Index (CFI), the Normed Fit Index (NFI), the Root Mean Square Residuals (RMR), the Root Mean Square Error of Approximation(RMSEA), the Standardized Root Mean Square Residual (SRMR), the Adjusted Goodness of Fit Index (AGFI), and the Goodness of Fit Index (GFI) values were used. Val-

ues with a $\chi 2$ / df ratio <5 were considered acceptable. AGFI, NFI, CFI, GFI values ≥0.90 were evaluated good fit. RMSEA and SRMR values <0.08 were considered acceptable and good fit respectively (26-28).

Reliability of the Scale

In order to determine the invariance of the scale over time, the scale was reapplied to 117 students randomly selected among 513 university students 4 weeks later. Mean, standard deviation, and intraclass reliability coefficient were used for measurement invariance testing (29).

Cronbach's alpha coefficients were used for the consistency of the scale. A Cronbach's alpha value of 0.70 was established to be acceptable and the lowest level (30).

Convergent Validity

The Nutritional Changes Processes Scale and the Body Shape Questionnaire were conducted for convergent validity. The total scores from this scale and the subscales of scales as well as the correlations between the total scores obtained from IOET were assessed. Spearman correlation analysis was performed for correlation analysis.

Results

Validity of the Scale

Exploratory Factor Analysis

When the prerequisites for the explanatory factor analysis were examined according to the Bartlett's Test of Sphericity and the Kaiser-Meyer-Olkin (KMO) Test for Sampling Adequacy, it was seen that the KMO was sufficient (0.923) and the sphericity assumption was provided (χ^2 = 3176,639; p < 0.001). When the factor analysis was applied, it was observed that the one factor of IOET-TR was the expectation of thinness and that each item had a high factor load on the thinness factor (more than 0.70) (Table 1).

Confirmatory Factor Analysis

Confirmatory factor analysis was performed for the accuracy of the one-factor structure of the scale. Standardized factor loadings and corrected correlation structures are shown in Figure 1. Items 4, 3, 6, and 2, in decreasing order of strength, have been shown to have the most significant effect on thinness expectancy. It was also found that the single-factor structure was acceptable or good fit model [($\chi 2$/ sd = 3.568, p < 0.000), RMSEA = 0.071, SRMR = 0.019, AGFI = 0.941, GFI = 0.976, RMR = 0.052, CFI = 0.988, NFI = 0.983].

According to these figures, conformity values with respect to the model were found to be within the acceptable range, with the validity of the established model confirmed.

Reliability of the Scale

The Cronbach's alpha of the IOET scale was 0.933. Furthermore, in the case that any one item is removed from the scale, Cronbach's alpha either decreases or remains the same (data not shown). Therefore the items provide scale reliability and the total Cronbach alpha value is quite high (30).

Table 1. Factor loadings, eigenvalues, and variance percentage of Interpersonal Outcome Expectancies for Thinness (IOET)

IOET items		Factor 1
1	Being thin will make me be liked by others	0.795
2	Being thin will allow me to fit in with others	0.860
3	Being thin will make me be accepted by others	0.867
4	Being thin will make me popular with others	0.871
5	Being thin will make me more desirable to others	0.744
6	Being thin will allow me to get positive treatment from others	0.848
7	Being thin will make me get compliments from others	0.793
8	Being thin will make me socially accepted by others	0.864
	Eigenvalue	5.532
	Factor's percentage for explaining the variance (%)	69.146

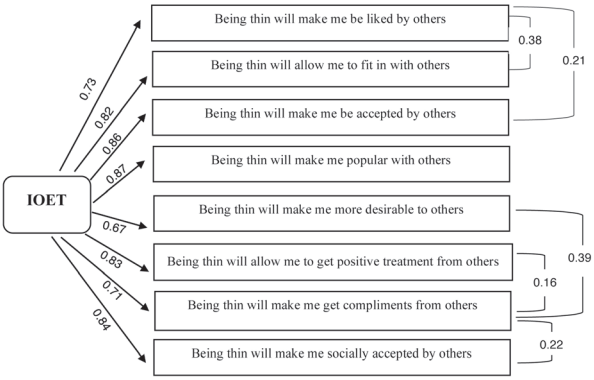

Figure 1. Standardized factor loadings of Interpersonal Outcome Expectancies for Thinness (IOET)

In order to determine the invariance of the scale over time, 2 applications were performed on 117 students with 4-week intervals. In test-retest reliability, the mean and standard deviation values of 2 applications, pre and post, were 2.53 ± 1.34 and 2.47 ± 1.46, respectively. There was no statistically significant difference between the two measurements ($t = 0.576$; $p = 0.566$). Intraclass Correlation Coefficient (ICC) values of 0.90 and above are considered excellent in the literature (30). In this study, ICC values were found to be quite high (pre = 0.926, post = 0.939).

Convergent validity

Positive and statistically significant correlations were obtained between the total scores of the Interpersonal Outcome Expectancies for Thinness and the Nutritional Changes Processes Scale and the Body Shape Questionnaire ($r = 0.410$, $p < 0.01$; $r = 0.433$, $p < 0.05$, respectively). The subscale scores of the Nutrition Change Process Questionnaire were found to vary between $r = 0.221$–0.396 ($p < 0.01$). This shows that the IOET scale has convergent validity between the IOET scale and the subscale and total scores of the scale.

Discussion

The purpose of this study was to assess the factorial structure and construct validity of the IOET questionnaire in a sample of Turkish students. The adaptation of the scale started with the translation from the source language to the target language and continued with the determination of linguistic and idiomatic equivalents and the pilot study. Finally, the Turkish form of the scale (IOET-TR) was applied to a sample group of 513 participants and analyzes were made on the data obtained. The results showed that IOET has adequate psychometric characteristics in all cases. In other words, this questionnaire can be used to determine the effectiveness of the expectations of being thin in interpersonal relationships.

The Cronbach's alpha value of the IOET scale was found to be 0.93 and it was found to be a one-factor structure. Also, Li et al. (17) found that the Cronbach's alpha value of IOET was 0.96 and that it had a one-factor structure as in our study. Item correlations ranged from 0.78 to 0.90 in the original study and between 0.74 and 0.87 in our study. In both studies, there were no items with low item correlation (r) values. Therefore, no item was removed from the scale.

In the second part of the study, the Turkish version of the scale was re-applied to test subjects after 4 weeks of the first application and the test-retest was found to be stable and did not change over time ($t = 0.57$; $p = 0.56$). In the original study of the IOET scale, IOET was found to have 0.74 test-retest reliability (6 weeks) in a subset of US female participants (n = 184) (17). These results are important in that they show the test-retest reliability of our study is good and students' opinions do not change with time between the applications. IOET scores were found to be positively correlated with the scores of the sub-scales of consciousness, dramatic help, re-evaluation of the environment and self, freedom, contradictions, auxiliary relations, empowerment, self-liberation, stimulus, interpersonal system control, and drug use in the Nutrition Change Process Questionnaire with 12 sub-scales. Similarly, a significant positive correlation was found between IOET and the total scores obtained from the Body Shape Questionnaire. Li et al. (17) showed that for construct validity, IOET scores were positively correlated with scores related to eating disorders and negative affective measures.

Therefore, it is possible to say that IOET can be used to identify individuals who are experiencing changes in their nutritional processes and who are disturbed by their body shape and make it a social pho-

bia. This issue has not been studied sufficiently since there is no valid and safe measurement tool in Turkey to measure the extent to which thinness can affect interpersonal relationships and/or our expectations from the other people. In this study, the Turkish version of the International Outcomes Expectations of Thinness Scale was found to be a valid and reliable instrument for the Turkish culture. It is thought that IOET-TR can contribute to other studies on thinness expectations by providing a different perspective.

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Osteoporosis prevention in postmenopausal female workers: beneficial effects of silicon dietary supplementation on oxidative status. A pilot study

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Summary. In the last years, the employment of ageing women is increased, and the well-being of these workers, together with the prevention of chronic disabling diseases, is an issue of great importance. Moreover, as postmenopausal ageing is associated with the loss of bone density and consequent increased fracture risk, promoting bone health in these women could be the best strategy for avoiding osteoporotic fractures. We aimed to evaluate the effects of 3-month supplementation with a commercial antioxidant product containing Silica on oxidative status and bone markers in a sample of Italian female workers. Subjects were menopausal and osteopenic women (N=29, age 59.34±6.37, mean BMI 26.19±4.01 kg/m^2). At baseline (T_0) and after three-month treatment (T_1) bone mineral density (BMD) was evaluated by phalangeal osteosonogrammetry. Haematological, serum biochemical parameters, reactive oxygen species (ROS), total antioxidant capacity (TAC), oxydated low-density lipoproteins (oxLDL) and urinary cross-links pyridinoline (PYD) and deoxypyridinoline (DPD) were assessed. Parametric or non-parametric tests were performed at T_0 and T_1. To analyse the possible association between two variables a linear correlation test was performed. At T_0, slightly high levels of ROS (86% of subjects), oxLDL (59%), Total Cholesterol (T-Chol) (90%) and LDL-Chol (59%) were observed, together with suboptimal or deficient 25-OH vitamin D (98%) concentrations. At T_1, oxLDL levels and the ratio oxLDL/LDL-Chol significantly decreased ($p<0.01$). At T_0 significant negative correlations between BMD T-score and cross-links were observed (DPD/Crea: r=-0.57, p=0.001; PYD/Crea: r=-0.45, p=0.01). At T_1, a significant reduction (p=0.03) was observed only for DPD (μg/L) but not for cross-links normalized by creatinine amounts. In conclusion 3-months Silica supplementation improves significantly oxidative status and bone resorption markers in most postmenopausal female workers, representing a complementary treatment for early phases of BMD reduction.

Keywords: osteoporosis, osteoporosis prevention, silica supplementation, female workers.

Abbreviations

Alkaline Phosphatase: ALP, creatinine: Crea, deoxypyridinoline: DPD, HDL cholesterol: HDL-Chol, LDL cholesterol: LDL-Chol, oxidized LDL: oxLDL, pyridinoline: PYD, reactive oxygen species: ROS, total antioxidant capacity: TAC, total cholesterol: T-Chol, total homocysteine: tHcy, triglycerides: TGs, 25 hydroxy vitamin D: 25-OH vitamin D.

Introduction

In many European countries, the employment of women aged 57-64 is increased, and the well-being of these workers, together with the prevention of chronic disabling diseases, has become a topic of great interest. Moreover, as postmenopausal age is associated with the loss of bone density due to a reduction in the estrogenic tone, promoting bone health in these women could be the best strategy for avoiding osteoporotic fractures.

Bone tissue undergoes constant renewal, and this process depends on the coordinated action of osteoclasts, osteoblasts and osteocytes, together with different mediators such as hormones, growth factors and cytokines (1).

Osteoblasts have oestrogen-specific membrane-receptors that act as inhibitors of interleukins and tumour necrosis factor (known to cause oxidative stress). When oestrogenic levels decrease in menopause, bone reabsorption increases, as no longer counterbalanced by bone deposition (2).

While osteoporosis is due to the imbalance between bone resorption and bone formation, osteopenia is characterized by an unbalanced metabolic-nutritional-oxidative status (3,4). The former is preceded by the latter.

Also, osteopenia and osteoporosis are related with oxidative stress, defined as imbalance between Reactive Oxygen Species (ROS) and Total Antioxidant Capacity (TAC).

As bone loss occurs insidiously and is initially asymptomatic, osteoporosis is often diagnosed after the first clinical fracture has occurred. Consequently, an early assessment of the individual risk for osteoporosis is important to prevent the first fracture, and the supplementation of calcium salts, vitamin D and antioxidants are suggested as preventive measures (5,6).

Moreover, the intake of bioavailable silicon (Si), an oligo-element positively associated with BMD in men and pre-menopausal women (7,8,9), might have a beneficial role on bone, and a protective role in atherosclerosis development, due to its effects on collagen-like molecules of blood vessels, by preserving integrity and stability of arterial walls (10,11).

The aim of the present pilot study was to evaluate the possible beneficial effects of a commercial antioxidant suspension containing Si on both BMD and oxidative status on a sample of osteopenic postmenopausal female workers. Data were evaluated at baseline (T_0) and after 3-month supplementation (T_1).

Materials and Methods

Subjects

This study was conducted on a sample of postmenopausal women attending their annual health surveillance visit as part of a workplace health promotion campaign on active ageing, organized by the Occupational Medicine Department, Occupational Unit of Clinica del Lavoro "L. Devoto", Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan (Italy) (12).

The eligibility criteria were: menopause, defined after12 months of amenorrhea following the final menstrual period, according to the Stages of Reproductive Aging Workshop (13), and osteopenia, defined according to the BMD T-scores (as defined in the Instrumental section).

Subjects with BMI>30 Kg/m^2, history of current chronic or neoplastic disease, use of anticoagulant, estroprogestinic or osteoporosis therapies, regular use of antioxidant or other dietary supplements, where considered not eligible for the study.

The selected group comes from the sample of a broader observational cross-sectional study that recruited 385 (291 females and 94 males, age range 18-69 years) consecutive participants, and enrolled them into a periodic occupational examination program in order to test, among other parameters, the seasonal variation of vitamin D status throughout the year in several occupational areas, according to official European ATECO classification (14).

Our study sample was a heterogeneous sample representative of indoor workers from several occupational areas: 52% administration, 32% trading and industry, 4% education, 10% healthcare, and 2% services area.

Among the whole sample, 29 women met the eligibility criteria and were enrolled in the present pilot study.

During the baseline clinical examination, all subjects were asked to fill in a questionnaire on general health, habitual dietary intake and lifestyle (15) (i.e.

smoking history, alcohol consumption, occupation, educational level and socioeconomic status), and anthropometric parameters (age, height, weight and body mass index, BMI) were recorded.

All participants were asked to maintain their habitual lifestyle and dietary habits for the entire period of the supplementation. Compliance with the study protocol was determined by review of patient diaries and returned remaining supplementation at the end of the 3rd month.

No adverse events were observed in any participant throughout the whole study period.

A written informed consent was signed by each participant. The study was conducted in conformity with the Declaration of Helsinki, in accordance to the Good Clinical Practice guidelines and was approved by the Human Ethic Committee of Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy (Registration number: 852).

Supplementation and intervention

Participants were provided with Cellfood® Silica Plus (NuScience Corporation, Lancaster, CA, USA), a dietary supplement (118 mL per bottle) containing a mixture of 17 amino acids, 34 enzymes and 78 trace minerals suspended in aqueous solution of deuterium sulphate (D_2SO_4) together with silicon dioxide (SiO_2) (6,6 g silicon/100g Cellfood®). The dietary supplement has been offered free of charge to the subjects by the study group.

All women were instructed to take fifteen drops of the supplement in a glass of low-mineral water two times a day (about 4 mL per day, equal to 60 mg silicon/day), at least thirty minutes before breakfast in the morning and before dinner in the evening, for the three months of the study period.

Due to the high variety of mineral waters in Italy, the study protocol recommended to use a commercial low –mineral water (fixed residue < 200 mg/L).

Subjects' self-reported compliance to the whole study period was more than 95%.

Instrumental

BMD was evaluated by phalangeal quantitative ultrasound (QUS) method at baseline (T_0) and after the three-month supplementation (T_1). As reported by several studies, QUS method (in this protocol, assessed by the portable device DBM Sonic, IGEA, Carpi, Italy) is a relatively recent non-invasive method of estimating bone mineral status in some peripheral skeletal sites (i.e. phalanges of the hand). QUS technique is safe, easy to use, radiation-free (16). The quantitative ultrasound measurement was performed at the distal meta-diaphyseal region of the proximal phalanges of fingers 2 to 5 of the hand, and the results were expressed as T-Score and Z-Score values. Osteopenia was defined for T-score value ranges from -1 to -3.2 (16-18). The device was landed for free to check its feasibility for a preventive occupational campaign.

Blood Samples

At T_0 and T_1 blood specimens from fasting subjects were collected in test tubes, either without additives for serum lipid panel, glucose, calcium (Ca), phosphate (P) and total alkaline phosphatase (ALP), vitamin D, oxidative panel and creatinine (Crea), or with EDTA to prevent coagulation for complete blood count (CBC) and plasma total homocysteine (tHcy). A specimen of whole blood was immediately centrifuged for Hcy assay. T_0 and T_1 serum and plasma samples were frozen and stored at – 80°C for batch analysis at the end of the study.

Urinary Samples

To avoid the influences of circadian rhythm, fasting 2-h morning urine samples were collected from each subject to assess urinary cross-links (PYD and DPD) concentrations.

Analytical and Biochemical Analysis

T_0 and T_1 CBC was performed as routine samples on XE 2100 analyzer (Dasit, Cornaredo, Italy) at the Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan (Italy) laboratory. Routine biochemical parameters (serum glucose, complete lipid panel, creatinine, Ca, P, ALP and plasma tHcy) were measured by commercial assays by Modular P (Roche Diagnostics International Ltd, Swiss). Serum oxLDL concentrations were measured by a commercial enzyme-linked immunoabsorbent assay (oxLDL ELISA, Mercodia, Uppsala, Sweden) on the EASIA reader (Medgenix Diagnostics, Fleurus, Belgium) (19,20).

Serum ROS levels and TAC were measured by spectrophotometric method using a commercial kit (dROMs test and OXY-Adsorbent test, respectively, Diacron International, Grosseto, Italy) on F.R.E.E. analyzer (Diacron) as previously described (21,22).

Urinary cross-links (PYD and DPD) concentrations were determined by HPLC method followed by fluorescent detection using commercially available kit (PYD and DPD, Chromsystems Instruments & Chemicals, Munich, Germany) as previously reported (23). The cross-links levels are reported both as normalized by creatinine (pmol/μmol), and as absolute concentration (μg/L). As the cut-off values in μg/L are not provided by the commercially available kit, we referred to those published by using a validated HPLC procedure using a synthesized internal standard on urine samples of 30 healthy, not menopausal women (aged 36±7.1) resulting 31.68 ±9.62 (μg/L±SD) and 190.49±49.63 (μg/L±SD) for DPD and PYD, respectively (24). Vitamin D status was evaluated measuring concentrations of the circulating form 25 hydroxy (OH) vitamin D, using DiaSorin 25-OH Vitamin D TOTAL competitive chemiluminescence immunoassay on an automated LIASON instrument (Saluggia, Vercelli, Italy). Hypovitaminosis D was defined according to the 2011 Clinical Practice Guidelines of the Endocrine Society, according to which 30 ng/ml is the minimum sufficient vitamin D level (25).

Statistical Analysis

Based on samples distribution, Student's t-test for paired data (parametric distribution) or Wilcoxon test (non-parametric distribution) were performed to compare data before and after treatment. The possible association between two variables was analysed by a linear correlation test.

Significance was set for a p-value <0.05. Data, expressed as mean ± standard deviation (SD), were analysed by using GraphPad PRISM (version 6.3) and MedCalc software (26).

Results

Of the sample of 29 subjects, mean age was 59.34 ± 6.37 years old, mean BMI 26.19 ± 4.01 kg/m^2, and mean menopausal age was 51.72± 2.53 years old. 22 women were never-smokers, 2 smokers and 5 former-smokers (who quit smoking for 15 years).

At T_0, based on the results of the standard CBC panel, all women had a normal haematological profile.

Serum 25-OH vitamin D levels (measured only at T_0), showed a hypovitaminosys D in 98% of subjects (mean 25-OH vitamin D 14.6 mcg/L, range 8.9-23,3).

Finger BMD showed a mean T-score -2.01± 0.65.

Table 1 shows the haematological and biochemical parameters of the 29 women at T_0 and T_1.

At baseline, all subjects' glycaemic profile (glucose and insulin concentrations) were within their reference range.

As far as the lipid panel is concerned, T-Chol and LDL-Chol levels were elevated in 90% and 59% of women, respectively. HDL-Chol concentrations were reduced in 38% of them, while TGs levels were within the reference range in most of the subjects. The lipoprotein ratio oxLDL/LDL-Chol was elevated in 55% of the subjects.

As regards the oxidative status, TAC was elevated in 93% of subjects; similarly, elevated ROS concentrations were observed in the majority of the participants. Ox-LDL levels exceeded the cut-off value in 59% of them. About 50% of women showed a slight hyperhomocysteinemia and in the 62% of them creatinine concentrations were slightly elevated.

At T_1, after a 3-month Cellfood® Silica Plus supplementation, as shown in Figure 1, no significant change was observed for anthropometric parameters, CBC nor BMD T-Score mean values (data not shown). Lipid profile did not show any change, except the ratio oxLDL/LDL-Chol, that decreased significantly ($p<0.01$) in most of women. Among all the other parameters, only oxLDL levels decreased significantly ($p<0.01$).

Table 2 shows the bone metabolism profile, where the cross-links values are reported both

normalized by creatinine (pmol/μmol) and as their concentration (μg/L). At T_0, the overall urinary cross-link levels were within their reference range, while PYD/Crea and DPD/Crea resulted altered in 14% and 1% of women, respectively.

A significant negative correlation between BMD T-score and cross-links was observed (DPD/Crea: r=-

Table 1. Subjects' haematological and biochemical parameters evaluated at baseline and after 3-month supplementation.

Analytes (Reference Interval or Cut-off)	T_0 Mean±SD (%)•	T_1 Mean±SD (%) •	p-value
Hb (12-16 g/dL)	13.41 ± 0.65 (0)	13.20 ± 0.72 (0)	N.S. *
HCT (%)	39.69 ± 1.88 (0)	38.98 ± 1.97 (0)	N.S. *
MCV (78-99 fL)	84.18 ± 3.70 (0)	83.77 ± 3.78 (0)	N.S. *
WBC (4-10x10^9/L)	6.25 ± 1.13 (0)	6.26 ± 1.23 (0)	N.S. *
Glucose (70-110 mg/dL)	81.87 ± 6.11 (0)	83.79 ± 7.51 (0)	N.S. *
Insulin (2.6-25 μIU/L)	8.69 ± 3.12 (0)	9.73 ± 4.50 (0)	N.S.
T-Chol (<200 mg/dL)	228.09±29.25 (90)	227.03±32.02 (79)	N.S. *
LDL-Chol (<130 mg/dL)	146.13±29.35 (59)	144.84±35.15 (62)	N.S. *
HDL-Chol (>65mg/dL)	68.25±16.22 (38)	66.13±15.20 (48)	N.S. *
TGs (<170 mg/dL)	99.62±41.09 (7)	95.28±39.32 (3)	N.S. *
TAC (>350μmolHClO/mL)	402±54.36 (7)	397.48±56.83 (17)	N.S. °
ROS (<300 U. Carr)	409.37±69.9 (90)	414.36±48.08 (97)	N.S. °
oxLDL (<70 U/L)	77.15±30.25 (59)	65.19±22.88 (28)	0.01 °
oxLDL/LDL-Chol (<0.50)	0.54±0.20 (55)	0.45±0.10 (16)	0.01 °
tHcy <10.5μmol/L	11.65±1.90 (51)	12.33±1.96 (49)	N.S. °
Creatinine (0.5-1.0 mg/dL	1.08±0.55 (62)	0.83±0.41 (24)	0.03°

In brackets: % of subjects with values out of reference interval or cut off. (* t-test, ° Wilcoxon test).
Abbreviations: WBC: white blood cells, RBC: red blood cells, Hb: hemoglobin, MCV: mean corpuscolar volume, tHCy: total homocysteine.

0.57, p=0.001; PYD/Crea: r=- 0.45, p=0.01). However, when PYD and DPD values were reported as concentrations (μg/L), both PYD and DPD levels exceeded the cut-off. Moreover, both Calcium and ALP concentrations were elevated in 14% of the subjects.
Only DPD concentration (μg/L) showed a significant reduction (p<003), whereas no changes were observed for cross-links normalized by creatinine amounts. At T_1, phosphorous level was lightly increased (p=0.05), whereas no change was observed for both Ca and ALP concentrations.

Discussion

The present pilot study evaluated the potential beneficial effect of a commercially available antioxidant nutritional supplement on bone metabolism and nutritional-oxidative status in 29 Italian osteopenic menopausal female workers, who attended a periodic occupational examination program.

The findings of our study show that the supplementation, enriched with organic Silicon in colloidal form (more bioavailable and easily assimilable), can influence the oxidative stress condition, represented by

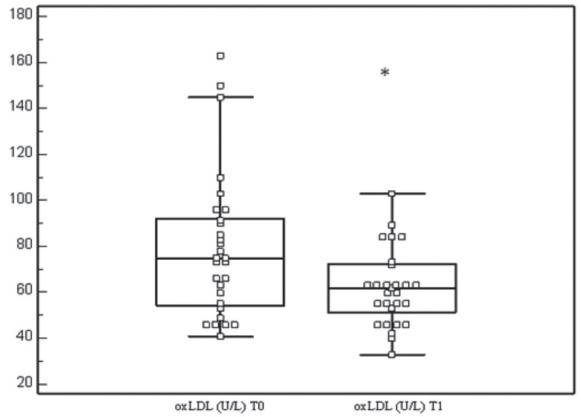

Figure 1. The box plot describes the reduction (p=0.01) in ox-LDL levels (U/L) after treatment, compared to baseline.

a significant reduction of both serum oxLDL levels, but also bone metabolism, as showed by urinary DPD reduction in most of the women.

These preliminary results point out the possible utility of the Silica supplement (containing silicon and several trace minerals as iron, copper and zinc), in the prevention of female workers osteopenia and in buffering their oxidative status condition, a risk factor for various non-communicable chronic diseases.

In the course of the whole life, bone continuously undergoes a process of remodeling, characterized by a fine balance between bone reabsorption by osteoclasts, and deposition by osteoblasts. When a state of imbalance between the two activities occurs, the resulting bone loss may lead to the pathological condition known as osteoporosis. This condition is characterized by reduced bone mass and density and increased fracture risk, and represents the most prevalent metabolic bone disease.

In the last few years, the importance of workers' well-being and the prevention of chronic disabling diseases as Public Health strategy issues, is increasing. In the European countries, the workforce is aging rapidly in all sectors and physically demanding jobs becomes increasingly difficult, especially for female postmenopausal workers (27). Workers' ageing will be accompanied by a progressive reduction in their aerobic fitness, muscle strength and bone density, which means a reduction of life quality (28). In the setting of Occupational Medicine, even a simple preventive intervention (i.e. a nutrient supplementation) in postmenopausal female workers can led to beneficial occupational health perspectives.

Bone matrix consists of an inorganic component (mostly hydroxyapatite), which provides stiffness, and an organic component, the collagen secreted by osteoblast, which supplies tensile strength, ductility and toughness. The collagen structural unit is stabilized by the formation of intra- and inter-molecular pyridinium collagen cross-links such as pyridinoline (PYD) and deoxypyridinoline (DPD), thus allowing aggregation in fibres (29,30).

Table 2. Women' urinary and serum markers of bone metabolism evaluated at T_0 and T_1.

Analytes (Reference Interval or Cut-off)	**T_0 Mean±SD (%)•**	**T1 Mean±SD (%)•**	**p-value**
PYD/Crea (25-83 pmol/μmol)	54.64±25.08 (14)	55.10±16.32 (7)	N.S. °
DPD/Crea (6-23 pmol/μmol)	11,71±5,23 (1)	11,71±3,64 (0)	N.S. °
PYD (190.49±49.63 μg/L)	229.18±129.04 (23)	179.10±128.81 (18)	N.S. °
DPD (31.68 ±9.62 μg/L)	46.21±26.73 (22)	35.61±24.38 (12)	0.03 °
Ca (8.40-10.20 mg/dL)	9.65±0.36 (14)	9.66±0.39 (10)	N.S. *
P (2.7-4.5 mg/dL)	3.62±0.43 (0)	3.76±0.57 (3)	0.05 *
ALP(35-104 U/L)	77.90±25.81 (14)	76.28±21.00 (14)	N.S. °

In brackets: % of subjects with values out of reference interval or out of cut off. (* t-test, ° Wilcoxon test).
Abbreviations: Crea: creatinine, PYD: pyridinoline, DPD: deoxypyridinoline, Ca.calcium, P: phosphate, ALP: bone alkaline phosphatase.

Although bone mineral density is the routine indicator of bone strength in clinical practice, many others biochemical markers of bone turnover could better reflect the status of bone metabolism (31,32). The pyridinium cross-links PYD and DPD are considered good markers to identify an increase in bone resorption during metabolic bone disorders. In particular, PYD is the major cross-link in all connective tissues, whereas DPD is found in high amounts in mineralized tissues. For this reason, DPD is considered more specific for bone collagen degradation than PYD (31-34).

PYD and DPD levels are usually expressed as the ratio of pyridinium crosslinks to urinary creatinine excretion (pmol/μmol). However, as creatinine is produced from the catabolism of muscle proteins, its levels can be affected by various factors (i.e. lean body mass, derangement in muscle metabolism, high or reduced dietary proteins intake, physical activities) (35). Thus, in the present study the urinary excreted cross-links were evaluated both as normalized by creatinine and as their concentrations (μg/L) (as reported in Table 2).

Notably, after Silicon supplementation, a significant decrease in the excreted DPD levels was observed, whereas no significant differences were observed when the crosslinks were related to creatinine. This finding confirms the positive effect of the supplementation on bone. We did not observe a reduction in urinary PYD excretion; this is possibly due to the poor specificity of this crosslink for bone tissue, as it is distributed also in cartilage and synovium (31-33).

The improvement in urinary markers can be justified by the reported action of silicon on collagen synthesis and reduction of bone resorption. *In vitro* studies have shown that physiological concentration of silicon stimulated collagen type I synthesis, and osteoblastic differentiation in human osteoblast-like cells (10,11).

The present findings show that the supplementation with Cellfood® Silica Plus could act on bone metabolism reducing bone resorption.

The significant association between BMD T-score and urinary cross-links observed at T_0, but lost after treatment, might be due to the faster changes in the levels of bone resorption markers than to the T-score modifications (probably occurring after a longer treatment).

In addition, it is possible that the supplementation period was too short. It appears, however, that there was a partial reduction in some of the damaging effects of osteoporosis related to the significant improvement in oxidative alterations, thus suggesting the potential use of nutraceutical treatment to reduce working-related complications.

As expected, the slight increase in ROS remained approximately unchanged. In physiological conditions, ROS are produced in our body (approximately 90% at mitochondrial level) as the result of cellular metabolism and are counterbalanced by a system of antioxidant enzymes and scavenger molecules, able to prevent and stop the chain propagation of radical reactions. The balance between the ROS endogenously produced and their neutralisation by antioxidant defence mechanisms is known as "oxidative status". When ROS concentration is higher than the physiological amount, and TAC is insufficient to neutralise them, a condition known as "oxidative stress" is produced. This condition gives rise to cellular functional and structural alterations that are potentially responsible for various diseases. Oxidative stress can be counteracted by exogenous antioxidant compounds able to reduce the osteopenic and/or osteoporosis risk (19,36).

Also, oxidative stress is considered to be closely associated with osteoporosis. Under physiological conditions, ROS production by osteoclasts is involved in bone remodeling. Post-menopausal reduction in skeletal mass seems to be associated with excessive osteoclastic activity together with decreased osteoblastic action; this is partially due to lower stimulatory effect of estrogens (molecules with antioxidant properties) or to an unbalanced oxidative status (ROS/TAC imbalance).

Moreover, Maziere C. et al. (37) reported a role for oxLDL in bone remodelling by impairing the Receptor Activator of Nuclear factor k B Ligand (RANKL), a cytokine involved in osteoclasts differentiation by preventing the effect of the inorganic phosphate (P) released by bone resorption (38).

Brodeur *et al.* (39) reported that low oxLDL concentrations induced proliferation of osteoblasts whereas high levels were cytotoxic. Thus, the noteworthy findings of the present pilot study suggest that a supplementation with antioxidant could help reduce oxidative stress and subsequently prevent bone loss in postmenopausal women.

Our previous studies in different clinical settings, highlighted that elevated oxLDL concentrations can be a consequence of oxidative stress (19,40). Oxidized LDL are formed by the reaction among LDL with the terminal compounds deriving from the free oxygen radical attach on poly-unsaturated fatty acids.

Another noteworthy point, after the three-month Silicon supplementation, was that the lipid panel remained unaltered, except for the significant decrease in the oxLDL concentrations and in the oxLDL/LDL-Chol ratio.

Oxidized Low Density Lipoprotein cholesterol (oxLDL), is able to generate ROS. As well known, oxLDL play a major role in the formation and progression of the atherosclerotic plaque, and atherosclerosis is often accompanied by osteoporosis (41).

The decrease in oxLDL concentration (Table 1, Figure 1) might be explained by different hypotheses due to the silicon presence in the supplementation, as reported by some authors: i.e. a direct scavenger effect of silicon on free oxygen radicals or its collaboration with enzymes and/or minerals or its inhibiting action on the lipoxygenase activity (42), or its interaction on superoxide dismutase activity (43,44), but this was not confirmed by other authors.

According to Pawlak *et al.* (45), the lipoprotein ratio confirms the beneficial effects of antioxidant in improving the risk of cardiovascular diseases. The oxLDL/LDL-Chol ratio can help predict the degree of clinical benefit in lowering such risk (12,40). Moreover, these markers can be associated with good and balanced antioxidant conditions (TAC).

The oxidative stress condition affects both bone and skeletal muscle structure, thus the beneficial antioxidant effect of the silicon dietary supplementation could explain the significant creatinine decrease (Table 1) (36).

As Silicon and Carbon have the same chemical affinity, we might hypothesize for SiO_2 a reaction between SiO_2 and ROS, as that reported for carbon dioxide (CO_2) (35,42), which contrasts the formation of superoxyde anion-radicals ($O_2^{\cdot-}$) by inhibiting the activity of NADPH-oxydase, the enzyme producing $O_2^{\cdot-}$ (46).

The several trace minerals, essential cofactors for enzymes involved in the synthesis of bone matrix constituents, and present in Cellfood® Silica Plus, could explain the significant increase in phosphorus (P) levels observed after supplementation (as seen in Table 2). The intake of bioavailable Silicon, an oligoelement, is positively associated with bone mineral density in men and pre-menopausal women, even after adjustment for confounding factors (7-9), and the role of silicon in bone formation, mainly due to its promotion of collagen synthesis contributing to prolyl-hydroxylase activity, has been reported elsewhere (10,11).

Therefore, Silicon supplementation could be useful as a preventive or therapeutic agent against osteopenia and/or osteoporosis and, in addition, might have a protective role in the atherosclerosis development, due to its effects on collagen-like molecules of the vasculature by preserving the integrity and stability of arterial walls (17).

In order to better define the women's bone metabolism status, vitamin D levels were measured only at baseline because the supplementation does not contain this vitamin. The fat-soluble Vitamin D is needed for Calcium absorption and bone health. In 98% of the population sample, serum 25-OH vitamin D levels were low and at the end of the study, the subjects diagnosed with low vitamin D levels were supplemented with individual dose of vitamin according to EFSA protocol.

Several findings reported that vitamin D insufficiency is more prevalent than previously thought, particularly among the elderly, among people living in northern latitudes and individuals with poor nutrition. At least a half of the women of the general population present vitamin D deficiency in their midlife, and our findings totally agree with these figures. Consequently, vitamin D deficiency could contribute to the increased risk of osteoporosis, accelerating bone loss.

In conclusion, the present study highlighted that three-months' Cellfood® Silica Plus supplementation improves the oxidative status. These promising results are confirmed by the significant decrease in oxLDL levels and oxLDL/LDL-Chol ratio (a new and more powerful biomarker than the standard lipid assessment). Notably, according to our findings, most of the women who took the supplement, showed a significant decrease in the bone resorption markers and in oxidative parameters of lipid peroxidation over the

supplement period, even if it was for a limited time. Moreover, considering its rapid clearance, silicon may be a potential benefit of long-term intake without excessive retention and accumulation in the body. Therefore, a longer silicon supplementation period could be suggested to obtain a greater effect and could represent a complementary treatment for the early phases of BMD reduction.

The present study has some limitations to be considered. First, it has to be mentioned the reduced number of participants. Indeed, only 29 women met the T-Score criteria of osteopenia and were enrolled. It must be stressed, however, that this is a pilot study, and the promising findings should be confirmed on a larger population. Second, the evaluation of BMD by routine measurement of phalangeal osteosonogrammetry (QUS). In fact, belonging this study to the wider frame of a nutritional promotion program, it was necessary to identify efficient, precise, reliable, cheap and simple indexes applicable in clinical practice and public economic management. Dual energy x-ray absorptiometry (DXA) is the most commonly used technique for bone mineral assessment worldwide but the subject is exposed to ionized radiation. However, taking measurement by DXA at femur and lumbar column as "gold standard", Omodei et al.'s study (47) reported that DXA and QUS showed an agreement in 90% of cases. This means that the agreement between the two methodologies is very high. Moreover, Albanese et al.'s contribution (48) suggested that QUS at the phalanges may represent an index of bone tissue condition usable to evaluate the degree of bone mineralization after estrogen decreases in early postmenopause.

Thus, allowing Occupational and Preventive Medicine and Governance stakeholders to plan strategic preventive measures in order to promote female workers' health and to prevent disabling chronic diseases.

Achieving the goal of extended health-span in the setting of occupational medicine will depend on elucidating and exploiting successful interactions among biological, psychosocial and environmental factors.

Promoting bone health in postmenopausal female workers can be considered part of Public Health primary and secondary life-style preventive strategies, as it can lead to beneficial working health perspective, thus preventing morbidity and improving survival by avoiding osteoporotic fractures.

The observed beneficial effects of intervention with nutraceutical formulations could encourage further investigations.

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Maternal and fetal outcomes of pregnant females after a nutritional health education program. An interventional study

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Summary. *Background:* Nutrition during pregnancy is an important element for the pregnant women and their fetus, they must take enough calories and nutrients to provide the essential requirements for both themselves and their fetus and to prevent complications of abnormal weight gain in pregnancy. *Objectives:* To determine the effect of the nutritional health education on changing knowledge, attitude and practice towards healthy pregnancy, obtaining optimal weight and its effect on obstetric outcome. *Subjects and Methods;* An interventional study (pre-posttest) was conducted in Zagazig university antenatal care clinic and included 135 pregnant female. *Methods:* Data collection was done through a semi-structured questionnaire about females' socio-demographic characteristics, obstetric, family and clinical history. Health education sessions were applied on the pregnant females and their knowledge, attitude and practice about healthy nutrition were assessed before and after the intervention. Ultrasound was performed, obstetric outcomes were detected. *Results:* This study was conducted on 135 pregnant female. After the nutritional education program, the proportion of adequate knowledge, attitude and practice was increased from (28.2% to 77.3%-8.2% to 75.5% and 32.7% to 77.3%) respectively (p-value <0.001). There was statistically significant higher cesarean section, intra and post-partum complications (85.7% vs 42.9%p<0.001), (71.4% vs17.2% p<0.001) and (25.7%vs7.1% p=0.008) (Odds (C.I 95%); 8(2.7-23.1), 12.1(4.6-31.6) and 4.5(1.4-14.7)), higher neonatal weight and blood glucose (p<0.001& 0.009) in over-weight versus optimal weight gain groups respectively. *Conclusion:* The intervention was effective on improving pregnant females' knowledge, attitude and practice towards healthy nutrition during pregnancy, getting optimal weight gain and consequently maternal and fetal outcome.

Key words: Gestational weight gain, nutritional education, obstetric outcome.

Introduction

The nutrition in pregnancy has a great influence on subsequent maternal and offspring health, inadequate intake increase the risk of preterm delivery and low birth weight (1) while excess intake is associated with having larger babies and postpartum weight retention (2). Moreover, adequate nutrition during pregnancy is important for the development of the placenta, for a healthy delivery and for future lactation (3). The recommended weight gain for obese women during pregnancy is up to 6.8 kilograms; for overweight women, gain would be from 6.8 to 11.2 kilos and for non-obese women, between 11.2 to 15.9 kilos (3). Maternal obe-

sity during pregnancy is a wide spread problem and related to several comorbidities for both mother and child (4). Excessive gestational weight gain (GWG) using the Institute of Medicine, Washington, criteria increases maternal risks for preeclampsia, gestational diabetes, Caesarean section and weight retention postpartum with associated long-term health consequences (1) and fourfold increased risk of large-for-gestational-age (LGA) infants (2). Although the great role of nutritional health education intervention on improving maternal and fetal outcomes through improving knowledge, attitude and practice, there little reports on this issues were done before.

So the objectives of this study were to improve maternal and neonatal outcomes through raising knowledge attitude and practice of pregnant females towards healthy lifestyle and obtaining optimal weight gain using nutritional health education program.

Materials and methods:

Study design and settings: an interventional study (pre-posttest) was conducted in the Antenatal Care Clinic of Obstetrics and Gynecology Department at Zagazig University Hospitals in the period from May 2017 to June 2019.

Target group: pregnant females in the first trimester attended the Antenatal Care Clinic at Zagazig University Hospitals, aged 18-35 years, Nonsmokers, take no medication and didn't have any chronic medical disorder with Body mass index (BMI) between 18.5 and 24.9 kg/m^2.

Sample size: Calculated through Open-EPI (version 3.01), according to the following collected data: Assuming that the knowledge of pregnant female about healthy nutrition during pregnancy was changed from 9% before intervention to 31% after intervention, the power of precision was 80%, and the confidence interval was 95%, so the sample size was 112 pregnant women. Twenty percent (23 women) were added to overcome the drop-out rate so the total number was 135 pregnant female.

Sample technique: systematic random selection of the sample population from all pregnant women was carried out. Total number of registered women was 570 and the needed sample size was 135 so we took every 4th women (K-interval) beginning by the 5th one chosen by lottery. Nearly sample collection persisted for seven months.

Data collection: All study participants took part in individual face-to-face interviews and completed the questionnaires to collect socio-demographic data about age, marital status, level of education, income, residence, sources of health information and service delivery (5). Family history of obesity and clinical data were detected. Females' knowledge about healthy food, supplements, iron, calcium, effect of anemia and obesity on pregnancy, their attitude and practice about healthy nutrition were detected before and after the intervention using (pretest) and (posttest) through face to face interview, the differences in body weight, fasting blood glucose, blood pressure, urine analysis were assessed before and after intervention.

After intervention, Ultrasonography was done at time of birth to detect any cause of cesarean section. Mode of delivery, intrapartum complications (prolonged, obstructed and precipitate labor, shoulder dystocia, labor and anesthetic complications) were assessed, neonatal weight and blood glucose were measured and follow up for 24 hours postpartum to detect atonic complications in the form of postpartum haemorrhage and traumatic complications in the form of traumatic injury to the genital tract.

Field work;

A) *First session*, the nutritional education session persisted for 20 minutes for each women individually, after collection of data and filling questionnaires, using (Health education message) in a face to face interview using posters and booklet which covered;
   1. Knowledge about healthy foods, importance of calcium intake, importance of iron intake and the foods decrease absorption of iron, anemia during pregnancy, importance of folic acid intake, importance of supplementation intake, knowledge about ideal gestational weight gain during pregnancy and consequences of abnormal weight gain.
   2. Attitude: positive attitude about importance of getting enough calcium, iron, folic acid and supplementation during pregnancy.

3. Definition of obesity in pregnancy, its risk factors (modifiable and non-modifiable) and its complications.

B) Follow up monthly visits along the pregnancy period ranged in time from 15 to 20 minutes for each female one session per month at antenatal care unit done by the researcher using posters and booklet, the females were taught about the healthy balanced diet, habits and their weight gain was followed up.

C) Activities during last visit before delivery: Ultrasonongraphy to assess the fetal conditions and to exclude any indication for cesarean section (malpresentation and fetal abnormalities), body weight, blood pressure, fasting blood glucose, urine analysis, convulsion and deciding mode of delivery were assessed.

D) The pregnant women were followed till 24 hours after delivery to detect any intra or post-partum complications (atonic complications in the form of post-partum Hge and traumatic complications in the form of traumatic injury to the genital tract), detect neonatal weight and blood glucose.

Data management: The collected data were entered, checked and statistically analyzed using SPSS program (Statistical Package for Social Science) version 22.0 (SPSS, Chicago, IL, USA). For the statistical calculations, data coding was done, and qualitative data were represented as frequencies and percentages, Chi-square test (χ 2), Fischer exact test and McNemar test were carried out for testing the association between the qualitative data. Quantitative data were presented as mean, SD and median and compared using independent t-test, paired t-test and Wilcoxon signed rank test. Binary regression analysis was conducted to identify the predictors of maternal outcome. The test results were considered significant when p-value ≤ 0.05.

Scoring of socio-economic status:

- Socio-economic level was classified into low, moderate, high level depending on the score calculated (39).
  - score less than 50% (low) ≤19.5
  - score 50%- less than 75% (moderate)= 19.6-29.25
  - score 75% and more (high) ≥ 29.25
- This is the updated scale for assessing the socio-economic status (5)

Scoring of knowledge, attitude and practice:

Total knowledge score about healthy foods types was (23), Importance of calcium and vitamin D intake was (18), Importance of iron intake was (21), Anemia was (34), Folic acid was (16), Supplementations intake was (18) and knowledge about weight gain in pregnancy was (16). So the grand total score of all items of knowledge about healthy nutrition during pregnancy was (146), total attitude toward importance of healthy foods was (12), while total practice towards health habits was (8).

Adequacy level of Knowledge, attitude and practice:

Cut off point (70%) (6) Whereas: >70% was considered as satisfactory, while ≤ 70 % was considered as unsatisfactory knowledge attitude and practice levels.

Weight gain classification:

According to Institute of Medicine ((IOM), 2009) (7), Gestational weight gain in pre-pregnancy normal weight women (with BMI from (18.5 to 24.9) was classified as follow; women with gestational weight gain <11.2 kg (subnormal weight gain),women with gestational weight gain from 11.2 to 15.9 kg (optimal weight gain) and women with increased gestational weight gain >15.9 kg (overweight gain) (Fig. 1).

Results

Socio-demographic characteristics of the studied group;

The age of the studied group was 27.1±4.4 (ranged from 20-37 years old), about half of them (48.2%) were of low social class and (40.9%) had positive family history of obesity (mainly first degree relatives). After the

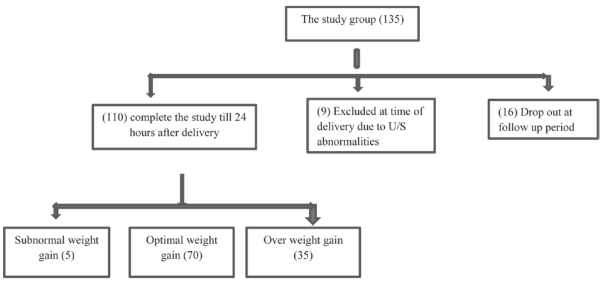

Figure 1. Flow chart of the studied group

intervention, most of the study group (63.6%) had optimal weight gain and (31.8%) had over weight gain, while only (4.5%) had subnormal weight gain.

Knowledge, attitude and practice improvement after the nutritional health education program;

There was statistically significant improvement in total knowledge, attitude and practice scores of pregnant females about healthy lifestyle during pregnancy from (53(33-125), 3(0.0-11) & 4(2-7)) before the health education sessions to (127(44-146), 11(1-12) & 6(3-8)) respectively after the intervention. With statistically significant higher adequacy levels of knowledge, attitude and practice after than before the intervention (77.3%, 75.5% and 77.3%), (28.2%, 8.2% and 32.7%) respectively (p-value <0.001) Table 1.

Characteristics of the subnormal weight gain group;

In regard to the five subnormal weight gain females, weight gain was (9±1.6) ranged from (7-11) kg, the adequate levels of knowledge, positive attitude and healthy practice were (60.0%, 20.0% and (0.00%) respectively. All of them (100.0%) had normal fasting blood glucose and blood pressure levels with no one (0.0%) had neither convulsion nor proteinuria. Twenty percent of them had cesarean sections and majority of them (80.0%) had intra-partum complications mainly traumatic injury to the genital tract (60.0%) followed by precipitate labor (50.0%) then prolonged labor and shoulder dystocia (20.0%) with no post-partum complications between them.

Binary logistic regression for prediction of the role of knowledge, attitude and practice improvement on gestational weight gain;

For detection of the effective change in knowledge, attitude and practice as explanatory (independent) factors for the gestational weight gain as a dependent factor in the studied group, Binary logistic regression proved that practice followed by attitude then knowledge were statistically significant explanatory variables for gaining optimal weight after the intervention (Odds (C.I 95%); 21.1(5.5-80.0), 10.7(3.4-29.3) and 4.5(1.7-11.9)) respectively (P-value <0.001). Table 2.

Maternal and neonatal outcome after the intervention;

Concerning maternal outcome, there was statistically significant difference between optimal and overweight gain groups regarding maternal weight gain, BMI, maternal fasting blood glucose and presence of proteinuria (14.3±0.9 VS 21.3±2.8), (23.1±2.3 VS 27.1±1.4), (1.4% VS 14.3%) and (0.00% VS 5.7%) (P-value <0.001, 0.001, 0.007 and 0.04) respectively. But regarding blood pressure and convulsions, there was

Table 1. total knowledge, attitude and practice of the studied group about health nutrition during pregnancy before and after the intervention.

Variables	Pre intervention	Post intervention	p-value
-***Total Knowledge score*** Median (interquartile range)	53 (33-125)	127 (44-146)	**0.001**^^
-***Total attitude score*** Median (interquartile range)	3 (0.0-11)	11 (1-12)	**0.001**^^
-***Total practice score*** Median (interquartile range)	4 (2-7)	6 (3-8)	**<0.001**^^
Total Knowledge adequacy *NO (%)*	31 (28.2)	85 (77.3)	**<0.001**^^^^
Total adequacy of positive attitude *NO (%)*	9 (8.2)	83 (75.5)	**<0.001**^^^^
Total practice and healthy habits well done *NO (%)*	36 (32.7)	85 (77.3)	**<0.001**^^^^

^= Wilcoxon signed rank test, ^^= Mc Nemar test and **statistically highly significant difference (P ≤ 0.001).

Table 2. Binary logistic regression for the effect of knowledge, attitude and practice as explanatory (independent) factors for the weight gain as a dependent factor in the studied group.

Variable	Regression coefficient	S.E	Wald test	p-value	Odds (C.I 95%)
Knowledge	*2.2*	*0.65*	*11.5*	***0.001****	*4.5(1.7-11.9)*
Attitude	*0.05*	*0.01*	*14.6*	***<0.001****	*10.7(3.4-29.3)*
Practice	*2.3*	*0.6*	*15.2*	***<0.001****	*21.1(5.5-80.0)*

Table 3. Maternal and neonatal outcomes between females with optimal and over weight gain after the intervention

Items	**Optimal weight gain (N=70)**	**Over weight gain (N=35)**	**p-value^**	
Weight gain (kg) mean ± SD (Range)	14.3±0.9 (11.5-15.6)	21.3±2.8 (16.7-25)	**0.001****	
-BMI (kg/m2) mean ± SD (Range)	22.3±0.3 (18.8-24.9)	27.1±1.2 (25-29.8)	**<0.0001****	
-Neonatal weight (grams) mean ± SD (Range)	3611±400 (2150-4050)	4102±501 (2200-4950)	**<0.0001****	
-Neonatal blood glucose level at birth	121±7 (110-130)	128±13 (105-140)	**0.009***	
	NO (%)	**NO (%)**	**p-value^^**	**OR (C.I 95%**
-Fasting blood glucose *(mg/dl)*				
Normal	69 (98.6)	30 (85.7)	**0.007***	11.5
Abnormal	1 (1.4)	5 (14.3)		(1.3-102.7)
-Blood pressure				
Normal	69 (98.6)	32 (91.4)	0.07	6.4
Abnormal	1 (1.4)	3 (8.6)		(0.6-172.5)
-Convulsions				
Normal	70 (100.0)	34 (97.1)	0.15	NA
Abnormal	0.0(0.00)	1(2.9)		
- Proteinuria				
Normal	70 (100.0)	33 (94.3)	**0.04***	NA
Abnormal	0.0 (0.00)	2 (5.7)		

^=independent t-test, ^^=Fischer Exact test, OR = Odds ratio (C.I 95%), NA= not applicable, *= significant p-value and **= highly significant p-value.

no statistically significant difference (p-value 0.07 and 0.15) respectively. Table 3.

In regard fetal outcome, there was statistically significant increased neonatal birth weight and blood glucose level at birth (4102±501 VS 3611±400)gm and (128±13 VS 121±7) (p-value <0.001 and 0.009) respectively in over-weight gain group than optimal weight gain group. Table 3.

Cesarean section, intra-partum complications and post-partum hemorrhage were statistically significant higher in over-weight gain group than optimal one (85.7% VS 42.9%), (71.4% VS 17.2%), and (25.7% VS 7.1%) (Odds (C.I 95%); 8(2.7-23.1), 12.1(4.6-31.6) and 4.5(1.4-14.7)) (P-value <0.001, <0.001and 0.008) respectively. Figure 2.

Discussion

Nutrition counseling is a cornerstone of prenatal care for all women during pregnancy as woman's nutritional status not only influences her health, but also pregnancy outcomes and the health of her fetus and neonate (8).

Abnormal GWG, whether excessive or inadequate, is associated with a series of maternal and neonatal complications and even life-threatening diseases (9). Excessive GWG is associated with postpartum weight retention, obesity, abnormalities in maternal prenatal blood glucose level, hypertension during pregnancy, cardio-metabolic problems in women, and macrosomia and later childhood obesity in children (10). While poor maternal weight gain during pregnancy is associated with SGA infants if mothers didn't

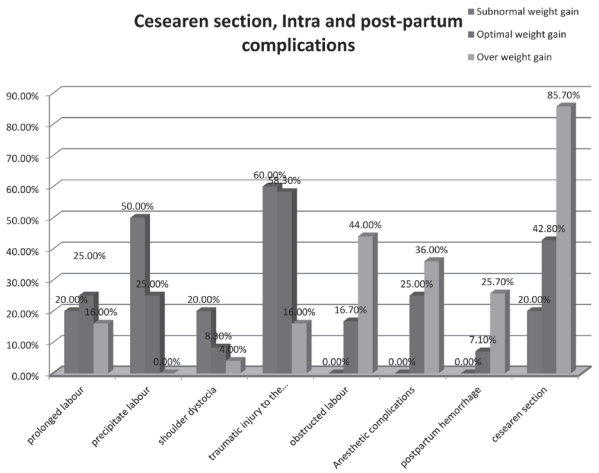

Figure 2. Bar chart for intra-partum complications, postpartum hemorrhage and cesarean section between subnormal, optimal and over weight gain groups.

gain more than 20 pounds, miscarriage, preterm births, LBW infants and gastroschisis (11).

This study included 135 pregnant women in the 1st trimester of pregnancy attending antenatal care clinic at Zagazig University Hospitals. Body weights, FBG, blood pressure, urine analysis were assessed. Nutrition education was applied and the pregnant females were followed up till 24 hours after delivery. At the time of delivery, Ultrasonography was done for all women to exclude any fetal abnormalities (mal-presentation was detected in 5 cases, three women had macrocosmic infants and one woman had cephalo-pelvic disproportion so these nine women were excluded from the final statistical analysis).

The present study showed that there was statistically significant improvement in total knowledge score of pregnant women about healthy nutrition during pregnancy, this attributed to the health education intervention, and this finding was in consistent with Girard & Olude (12) who conducted meta-analyses for the effect of NEC on maternal, neonatal and infant health outcomes including gestational weight gain, maternal anaemia, birthweight, low birthweight and preterm delivery and reported that nutrition education resulted in an increase in the level of nutritional knowledge also, similar results by Fallah et al. (6), found significantly increase in the awareness level of pregnant women about healthy nutrition from 3% before intervention to 31% after the nutritional education intervention ($P < 0.001$). However, the present results were in contrast with some Turkian studies which found that NE did not provide women with adequate knowledge about nutrition during the pregnancy period (13). The difference between the current study and studies which didn't have effect might be due to the fact that more positive outcomes could be gained if nutrition education was given by a dietitian (nutritional health care practitioner) not by midwives, this justification was supported by studies carried out in USA which stated that as a result of nutrition education provided by a dietitian, unhealthy food was decreased, and healthy food consumption were increased during the pregnancy period (14).

Regarding attitude and practice of pregnant women towards health nutrition during pregnancy, the current study demonstrated statistically significant improvement. This finding was in concomitant with Aşcı and Rathfisch (15), who found that the lifestyle interventions had a significant effect on improving attitude in the form of increased calcium, magnesium, iron, zinc, and vegetable intakes. Also a NE programs conducted by Dunneram and Jeewon (16), had been effective in positive behavior modification measured in terms of eating pattern and health quality and the pregnancy specific healthy dietary practice of the pregnant women increased from 46.8% to 83.7% after the nutritional education sessions applied by Zelalem et al. (14).

Regarding the increased adequacy levels of on knowledge, attitude and practice, the significant improvement in this study concise with Zelalem et al. (14), where the proportion of pregnant women with proper knowledge, attitude and practice towards nutrition during pregnancy increased from 53.9 to 97% and another study revealed that the percent of the pregnant women who had correct information after the intervention was (78.6%) (17).

After the intervention, most of the study group (63.6%) had optimal weight gain (31.8%), had over weight gain, while only (4.5%) had subnormal weight gain. this good outcome regarding gestational weight gain was statistically significant explained by the improvement in practice, attitude and knowledge after the NHEP which was supported by Walker et al. (18), who found that traditional face to face delivery of weight management interventions during pregnancy could be successful in obtaining optimal weight gain.

Considering weight gain and maternal complications, the present study was in agreement with Fallah et al. (6), whose study resulted in (41%, 28%, 26% and 5%) were normal, overweight, obese and underweight depending on BMI. Only (1%) had an abnormal blood pressure, diabetes, nephropathy, also the present study was coincided with Whitaker et al. (19), whose study was ended by 79 % within the normal range, 9 % below and 11 % above.

The statistically significant decrease in C.S, intra and post-partum complications and neonatal birth weight and blood glucose level in this study was similar to the results of Stang and Huffman (20) who concluded that lifestyle interventions that moderate gestational weight gain may reduce the risk of poor pregnancy outcomes, such as gestational diabetes, gestational hy-

pertension, large for gestational age, and macrosomia, as well as lower the risk for significant postpartum retention. Consistently, Denison et al. (21), noted that obese pregnant women were at increased risk of complications, including shoulder dystocia (OR 2.9, 95% CI 1.4–5.8), shoulder dystocia (OR 2.9, 95% CI 1.4–5.8), gestational diabetes, pre-eclampsia, venous thromboembolism (VTE), dysfunctional or prolonged labour and anaesthetic complications. Also Fukami et al. (22), reported that risk factors for postpartum hemorrhage among the deliveries were fetal macrosomia (over 4000 g), pregnancy-induced hypertension, severe vaginal or perineal lacerations and all these factors can be caused if weight gains over 15 kg during pregnancy. Such high weight gain significantly increased the incidence of PPH compared with women showing less than 10 kg weight gain during pregnancy. Finally Butwick et al. (23), had an increased risk of postpartum hemorrhage which was atonic especially as a consequence of less postoperative movement and more congested vessels among excess weight gain pregnant women.

Conclusion

Nutritional education program to pregnant women can improve their knowledge, attitude, habits and practice towards healthy nutrition during pregnancy and optimize their weight gain, which in role, succeeded to improve maternal and fetal outcome.

Ethical Considerations and Consent to Participate

An official permission was taken from Zagazig University, Faculty of Medicine, obstetrics and gynecology department. The title and objectives of this study were explained to the participants to ensure their cooperation and informed consent was obtained. Institutional Review Board (IRB) of the Faculty of Medicine, Zagazig University approved the study protocol (No. 3168).

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Availability of data

Data are available with coauthor at any time of request.

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