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# Index

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Review

# Osteoporosis and mineral nutrition. A literature review

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**Summary.** Osteoporosis is a disease affecting millions of people in the world. The work consists in a review of data on the main nutrition-related minerals. The following minerals have been analyzed: calcium, phosphorus, potassium, magnesium fluoride, sodium, iron, silicon, zinc, copper, manganese and strontium.

**Key words:** osteoporosis, mineral, nutrition

## Introduction

Osteoporosis is a progressive disease which leads to the depletion in the bone structure with loss of bone mineral density (BMD) increasing the risk of fractures over the years. In the United States, adults in these conditions over 50, are more than 12 million and 40 million adults are also at high risk of developing osteoporosis because of a low BMD.

In Italy are estimated 3.5 million osteoporotic women and 1 million men. In addition, there are 250.000 fractures due to osteoporosis each year, of which 80.000 hips and 70.000 femurs. It is important to note that patients with fracture of the proximal femur show, within a year, a mortality rate of 15-30% (1).

Osteoporosis is characterized by low BMD and shows a deterioration of the microarchitecture with trabeculae smallness, reduced mineralization and is associated with an increase in cortical porosity (2). The BMD is the result of a balance between bone resorption due to osteoclasts and bone formation due to osteoblasts, during an ongoing remodelling process. During the growth of children bone formation requires a balance in favour of bone growth and the achievement of peak bone mass until they reach the adult state where the BMD tends to remain relatively stable. With aging the activity of osteoclasts increases compared to that of osteoblasts and this leads to a loss of bone mass (3).

## Measurement of bone mineral density

The most widely used method is the DXA (dual energy absorbed x-ray absorptiometry) based on the different X-ray absorption of soft tissues and bone. DXA provides the measurement of BMD in specific locations such as the hip and spine and the bone mineral density is expressed as g/cm². These measures are compared with an healthy population (normally healthy Caucasian women at their bone mass peak) considered as a standard; a score (T-score) lower than -2,5 SD is defined as osteoporosis, between -2,5 and -1 is considered as "low bone mass" and a T-score higher than -1 is considered normal.

## BMD and risk of fracture

The measurement of BMD to define the state of osteoporosis is important because there is an inverse relationship in adulthood between BMD and fracture risk. A meta-analysis of 12 cohorts in different pop-

ulations show that, using DXA in the femoral neck, BMD is a strong predictor of subsequent fractures in both men and women (4).Vertebral compression fractures may lead to curvature of the spine that can cause chronic pain and disability and are more common in women than men (5). Age-related reduction in bone density, associated with falls due to decreased muscle strength, loss of balance, arthropathies, decreased vision, use of drugs, increases the risk of fractures (6).

As shown in the Framingham Osteoporosis Study (FOS), in the elderly, in women there are many important risk factors associated with bone loss such as age, low weight, a weight loss, while the use of estrogen appears to be a protective factor. In men bone loss appears to be associated with smoking. Surprisingly both in men and in women physical activity, intake of caffeine and calcium or serum concentration of 25-OH Vit.D aren't associated the loss of bone mass (7)

On the contrary the Rotterdam study with older adults, bone loss is associated with low weight and smoking in both men and women while the calcium intake is protective in men but not in women (8). In another study done with 9516 older female patients it was found that the risk of fracture associated with previous fractures, is associated with high weight, poor health care, hyperthyroidism, treatment with benzodiazepines, caffeine intake and sitting for more than 4 hours per day (9).

## Nutritional factors in BMD and fracture risk

Bone is a living tissue with a constant remodelling and appears dependent on a wide variety of nutrients. The nutrients in foods as principal minerals, clearly associated with bone status are listed in Table 1.

### *Minerals*

The bone matrix is composed of calcium, phosphorus, protein, magnesium and other minerals contained n traces. In the past calcium was thought to be the only nutritional factor for bone health; nowadays others diet components allows to understand bone health (10).

*Calcium*

Calcium is the largest mineral of bone tissue and about 99% of calcium in an adult is contained in the bone in the form of hydroxyapatite. Although growing children are thought need more calcium intake than adults, studies on children supplemented with calcium provided conflicting data. A review by Wosije et al. concluded that calcium contributes to an higher BMD primarily on the cortical bone and was more effective in low calcium consuming people and in pubertal rather than pre-pubertal children (11). In another review on 2859 children who were supple-

**Table 1**

| Mineral | Daily value° | Foods |
|---|---|---|
| Calcium | 1000-1200 mg | Milk,yogurt and cheese.<br>Small or canned fish edible bones (sardines, salmon)<br>Calcium set tofu<br>Fortified soy milk |
| Magnesium | 240 mg | Whole grains and whole grain cereals (wheat bran, wheat germ, brown rice, quinoa, otmeal, raisin bran, shredded wheat) |
| Potassium | 3900 mg | Baked potato, sweet potato, tomato paste,tomato sauce<br>Mature beans (kidney beans, white beans, soy beans, lima beans, lentils)<br>Yogurt milk<br>Fish (halibut, rockfish, cod, trout)<br>Winter squash<br>Orange juice<br>Banana |

° Italian Society Of Human Nuitrition SINU 2014 in adults

mented with calcium it was found that supplementation has a small effect on upper limb BMD but no effect on the femoral neck or lumbar spine. Furthermore, there is no evidence that sex, calcium, puberty, ethnicity and physical activity may affect bone mass and calcium supplementation in children does not reduce the risk of fracture (12). Another review demonstrates that calcium supplementation in adults leads to an improvement in bone condition, ameliorates bone growth and reduces fractures due to bone loss during ageing (13).

A follow up study focused on calcium and vitamin D supplementation for 3 years period in older men and women showed that BMD benefits were lost 2 years after the end of the supplementation (14).These studies seem to demonstrate that calcium supplementation does not influence the final state of the bone mass and does not reduce the risk of fracture except in those cases where the basal calcium level was low. It may be that calcium intake with the diet may be more effective than calcium supplementation. A follow-up analysis demonstrates that low intake of milk during childhood and adolescence is associated with a significantly lower BMD and doubles the risk of fractures in women over the age of 50 (15). Studies with calcium-enriched foods have shown beneficial effects on the bone. In one study, spinal bone loss was significantly lower in premenopausal women who used food that increased calcium intake from 900 to 1500 mg per day compared to a control group (16). In another study, 3 portions of yoghurt per day were provided and significant reductions were found in urinary excretion of bone turnover markers in older women (17). A recent study on 6-month effects of kefir treatment in 40 osteoporotic patients showed that BMD increases, the serum beta c-terminal telopeptide of type 1 collagen decreases, serum osteocalcin increases, PTH increases after treatment, but decreases in control group: Authors concluded that kefir therapy is associated with bone turnover and increases bone BMD in osteoporotic patients at 6 months (18).The calcium contained in foods like milk and yoghurt seems to be more effective than supplementation because it comes along with other important nutrients including vitamin D, protein, potassium and magnesium

*Phosphorus*

Phosphorus is essential for the bone but taking too much phosphorus in combination with low calcium can lead to reduced calcium bioavailability and boost bone loss. The phosphorus intake deficiency in older adults seems to be due to malnutrition, intestinal malabsorption or prolonged use of phosphorus-binding medicines including antacids (19). In general, the population tends to exceed the amount of phosphorus intake. In a study in the United States, average phosphorus intake was 1123 mg/day for women and 1550 mg/day for men, with a recommended intake of 700 mg/day, while calcium intake was 883 mg/day in women and 1038 m /day for adult men with a recommended intake of 1200 mg for both women and men (20).

Excess phosphorus forms chemical complexes with calcium and interfere with calcium intake. This leads to lowering of serum calcium level, increasing PTH production , lowering production of 1.25 $(OH)_2D$ and calcium absorption in the intestinal tract and consequently releasing calcium from the bone (21). One of the main sources of phosphorus intake is the cola drinks. A study in teenage girls has shown that cola consumption leads to an increased risk of fracture (22). Women who consume daily cola have a significantly lower hip BMD than those consuming less than once a week (23). From these studies, it seems likely that prolonged consumption of large amounts of phosphoric acids directly affect BMD causing small/moderate BMD loss.

*Potassium*

Potassium promotes calcium retention by the kidney, neutralizes the load of dietary acids and may therefore protect calcium loss from the bones. Potassium administration increases the serum concentration of osteocalcin and decreases the excretion of urinary hydroxyproline (24). Several studies have shown positive and protective association between potassium intake and bone health. In premenopausal women, a difference of 8% in femoral BMD was observed between the highest and the lowest quartile of potassium intake (25). In the elderly, potassium and, in general, alkaline-producing dietary contribute to maintenance of BMD (26). In another study with older women, higher baseline urinary potassium concentration is as-

sociated with a total BMD greater than 4% and trabecular BMD greater than 11% at 5 years (27). Some authors have pointed out that the modern diet is very deficient in potassium (on average 2500 mg versus 3900 mg recommended daily) and contains excess of sodium (about 4000 mg to 1200-1500 mg daily recommended) (28). This combination seems to have a particularly negative effect on the bone.

*Magnesium*

Magnesium plays an active role in crystallization because it is important in the formation of hydroxyapatite and can promote bone hardness (29). The magnesium concentration in the bone is significantly lower in women with osteoporosis than in normal ones (30). In observational studies, magnesium intake is significantly and positively associated with BMD and protects against bone loss (31). In a US study, the median magnesium intake ranged from 177 mg/die in African American women to 326 mg/die among non-Hispanic white men (32). In some studies has been shown the benefit of magnesium intake in bone mass growing in adolescent girls (33), in suppression of turnover markers in young men (34) and in preventing bone loss in osteoporotic women (35). For these reasons this mineral element, often underestimated, is important in maintaining and promoting bone health.

*Sodium*

Sodium intake is generally higher than the recommended dose of 1500 mg per day against an average intake of 4000 mg for men and 2800 in the United States (36) although the situation is similar in Italy. This leads to greater elimination of calcium from the kidneys. Some studies have shown that every 1000 mg of sodium over the recommended value leads to an increase in calcium loss with urine (37) and consequently to a lower BMD. Balanced optimum intake to protect the bone mass is between 1000 mg and 2000 mg of sodium per day. The effect of sodium may also depend on the potassium intake. A metabolic study found that in postmenopausal women giving 5175 mg of sodium per day increased urinary calcium and N-telopeptide, whereas in those that additionally sodium was given potassium citrate had a decrease in urinary calcium and no increase in N–telopeptide (38). Dietary Approaches to Stop Hypertension (DASH), a diet rich in fruits, vegetables, low-fat dairy products and therefore a potassium-rich diet, reduces serum markers of bone turnover reducing serum osteocalcin and PTH, in the control group (39). In another study with postmenopausal women whose sodium was reduced to less than 2000 mg/ ay for 6 months, calcium excretion of calcium and turnover markers decreased (40). However, another study shows no adverse event on BMD of 3000 mg/day of sodium compared to 1500 mg/day when participants were given adequate calcium and vitamin D intake (41). In another study involving 69.735 postmenopausal women studied over an average of 11,4 years, there is no association between sodium consumption and BMD at hip or lumbar spine, as well as with fracture risk, and concludes that sodium intake recommendations are unlikely for a significant development of osteoporosis(42).

*Fluoride*

Fluoride has long been known to prevent dental caries and has been added for long time to many water supplies. Fluoride replaces the hydroxyl group in the hydroxyapatite by forming fluorapatite. It has been shown that fluoride appears in the bone in the form of large crystals and increases BMD but decrease elasticity (43). In a randomized sodium fluoride study in postmenopausal women with osteoporosis, BMD spine increase but also increases the risk of vertebral fractures (44). In a meta-analysis of 25 studies, fluoride treatment increases BMD of the hip and spine, but there is no effect on the risk of fracture. The protective effect was seen at low doses (≤20 mg/day) (45). In another study comparing the bone structure of the common individuals in municipalities with or without fluoride water, it is shown that there is no difference in the physical characteristics of the bone in both groups (46).

*Iron*

Iron is an important cofactor for hydroxylases in the formation of collagen. The lack of iron intake and, conversely, an iron overload, are negatively associated with BMD. An iron overload in patients with genetic hemochromatosis and African hemosiderosis is associated with low BMD (47). Rats with a poor iron diet, shows impairment in bone morphology, strength and

density and decreases serum osteocalcin (48). Studies in postmenopausal women show that higher iron intake is associated with a higher BMD (49, 50). In contrast, other studies show that there is no association between iron status and BMD in women (51)

*Silicon*

Silicon is important for the formation of collagen and glycosaminoglycan in the bone and cartilage by influencing the formation of the organic matrix. Silicon is also one of the major ions in osteogenetic cells. Orthosilicic acid is the form that is absorbed by the diet and appears to be associated with bone formation by increasing the synthesis of type I collagen and stimulation of osteoblasts (52). Chicks fed with a silicon-free diet have abnormal bone formations (53), while silicon addition to the impoverished rats diet, causes less osteoclast production, increases bone formation, decreases bone turnover, and increases BMD (54). Few studies have been made in men but silicon seems to have shown a protective action. In a study the silicon added diet showed a positive association at hip sites in men and premenopausal but not in postmenopausal women (55). French patients with osteoporosis show an increase in trabecular bone volume with silicon treatment (56) and femoral BMD increases in women with osteoporosis by silicon intramuscular injection over fluoride, magnesium and control (57). All these findings show that high silicon intake may have a protective effect on BMD even if further studies are needed.

*Zinc*

Zinc influences the bone for its role in nucleic acids and protein metabolism (58). Low zinc concentrations in serum and bone have been observed in patients with osteoporosis (59). In animals zinc increases alkaline phosphatase and DNA synthesis that stimulates bone formation (60). Although study debate still exist, intake of calcium, copper and zinc, seems to show a benefit in BMD preservation in post-menopausal women (61).

*Copper*

Copper is a co-factor of the lysil oxidase catalyzing the cross-linking of lysine and hydroxyproline in collagen.

Animals to which copper has been removed from diet show a reduction in bone strength (62) and a greater bone loss with aging (63). In women, copper plasmatic concentration is correlated to BMD in lumbar spine (64). In a controlled study in men, an increase in activity of bone resorption markers has been shown, ranging from a diet rich in copper (6 mg/day) to a poor (0.7 mg/day) and this is reversible by returning to a copper-rich diet (65)

*Boron*

Boron intake can protect the bone by decreasing calcium, phosphorus and magnesium loss and increasing the serum concentration of estradiol (66). In rats, the lack of boron alters trabecular bone and reduces the strength demonstrating the importance of boron in the cortical force and microstructure of the bone (67). However, there are currently no randomized studies on humans.

*Manganese*

Manganese can contribute to the good bone mineral state. In rats a manganese supplement leads to an increase BMD in the lumbar vertebrae and increases serum osteocalcin suggesting that manganese contributes to bone formation (68). In a study with postmenopausal women who received daily copper and calcium zinc supplements for 2 years, there was evidence that there was less bone mass loss and BMD increase compared to a control group (69).

*Strontium*

Strontium has similar characteristics to calcium. Strontium ranelate doses of 1 to 2 grams per day for 2 years increase BMD in postmenopausal women by 2-3% compared to placebo (70) and reduce the risk of vertebral and non-vertebral fractures (71).A meta-analysis of two clinical trials shows that strontium ranelate is associated with 31% reduction in osteoporosis femoral neck and with reduction of 40% of vertebral fractures as well. (72). Biopsies of the bone show that strontium is predominantly organized in new bone deposits and cross-link collagen and bone quality is preserved (73). Strontium ranelate is approved for the treatment and prevention of osteoporosis in Europe but in 2014 the European Medicines Agency's Pharma-

covigilance Risk Assessment Committee (PRAC) has recommended that strontium ranelate should no longer be used to treat osteoporosis due to severe cardiovascular side effects (extensive vascular calcifications).

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Review

# Alcohol consumption and risk of Barrett's Esophagus. Mini-review of recent literature

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**Summary.** Alcohol consumption has a substantial importance in the causation of cancer of the oral cavity, pharynx, liver, colon, rectum; and in women, breast. It is also recognized as an independent risk factor for esophageal squamous cell carcinoma (ESCC). Nevertheless, the association with esophagus adenocarcinoma (EAC) is still not completely defined; as well as the association between alcohol intake and Barrett's Esophagus (BE). The aim of this mini-review is to summarize recent findings from population studies focused on the association between alcohol consumption and risk of BE. The research was carried out in PubMed, filtering for studies conducted in the period 2009-2015. Our mini-review has shown no association between the consumption of alcohol and BE. Some type of alcoholic beverages has shown an inverse association. Direct public health applications of these findings are limited, considering the causal link between moderate-to-heavy alcohol consumption with increased risks of several cancers. Given the rising incidence of BE and EAC, it is important to understand the interplay of dietary and lifestyle factors that influence the development of these conditions.

**Key words**: Barrett's Esophagus/Oesophagus, alcohol, alcohol consumption, risk factor, wine, beer, liquor, spirits, ethanol, alcoholic beverages

## Abbreviations

**BE**: Barrett's Esophagus; **EAC**: esophagus adenocarcinoma; **ESCC**: esophageal squamous cell carcinoma; **GERD**: Gastroesophageal reflux disease; **IARC**: International Agency for Research on Cancer; **OR**: Odds Ratio; **RR**: Relative Risk; **LES**: Low Esophageal Sphincter; **CI**: Confidence Interval; **HR**: Hazard Ratio

## Introduction

Esophageal cancer is the 8th and 19th most common cancer worldwide and in Europe, respectively.

In 2012, the estimated incidence of esophageal cancer worldwide was 456,000 new cases (3% of all cancers), representing the sixth most common cause of death from cancer with an estimated 400,000 deaths (5 of total deaths) (1, 2). The disease is three to four times more common among men and it could account in two different histotypes: adenocarcinoma (EAC) and squamous cell carcinoma (ESCC). Cases of EAC have risen dramatically in the last decades: a fivefold incidence increase from the figures given in 1970s have been reported; and it has become the main esophageal malignancy in many Western countries (3-5). Barrett's esophagus (BE) is considered the premalignant precursor lesion and the strongest risk factor for EAC (6). The incidence of BE can be comparable with the increase of EAC as well (7-9).

The American Gastroenterological Association defines BE as a condition in which any extent of metaplastic columnar epithelium replaces the stratified squamous epithelium that normally lines the distal esophagus (10, 11). BE was initially categorized as long segment (currently define as >3 cm) and short segment (currently define as ≤3 cm) (12).

BE affect 1-2% of general population (13) and, currently, only about 5% of patients with esophageal adenocarcinoma have a pre-cancer diagnosis of Barrett's esophagus (8, 14).

The most recognized and strong risk factor for BE is gastroesophageal reflux disease (GERD). Other important risk factors for BE include: abdominal obesity, tobacco use, and male gender (15) while, alcohol consumption is one of the most debated risk factor in Barrett's Esophagus (BE) onset.

Identifying, understanding and intervening on potentially modifiable risk factors for BE onset could have a major impact on the rate of esophageal adenocarcinoma.

Based on a systematic review of the available evidence and robust scientific consensus, alcohol was classified by the International Agency for Research on Cancer (IARC) as group 1, "carcinogenic to humans". Especially, alcohol is shown to have a dose dependent risk association and is related to the duration of the habit. Alcohol consumption has a substantial importance in the causation of cancer of the oral cavity, pharynx, liver, colon, rectum; and in women, breast (16-18). In particular, it is also recognized as an independent risk factor for esophageal squamous cell carcinoma (ESCC) (17, 19-23). Nevertheless, the association with EAC is still not complete defined, as well as the association between alcohol intake and Barrett's esophagus.

The aim of this mini-review is to summarized recent findings from population studies focused on the association between alcohol consumption and risk of BE. The research was carried out in PubMed, filtering for studies conducted in the period 2009-2015, with keywords: Barrett's Esophagus/Oesophagus and Alcohol, alcohol consumption, risk factor, wine, beer, liquor, spirits, ethanol, alcoholic beverages; combined with the Boolean operator: OR/AND.

## Total Alcohol consumption and risk of Barrett's Esophagus

Several studies (Table 1) evaluating the association between alcohol intake and risk of BE have shown that alcohol intake is not consistently associated with the risk of Barrett's esophagus (24-30).

Thrift et al. (25) have shown a significant inverse association between lifetime total alcohol consumption and the risk of nondysplastic or dysplastic BE. The duration of drinking median in subjects with nondysplastic BE was 35 years (Q1: 27; Q3: 43) in males and 29 years (Q1: 21; Q3: 39) in females; in dysplastic BE duration of drinking median was 41 years (Q1: 35; Q3: 48) in males and 34 years (Q1: 25;Q3: 45) in females. The inverse association between Barrett's esophagus and alcohol consumption was found in subjects with an alcohol intake which ranged between 7-20 drinks/week (intermediate consumption) (nondysplastic BE: OR = 0.53, 95 % CI: 0.31 –0.91; dysplastic BE: OR=0.52, 95 % CI: 019-1.43) and 21-41 drinks/week (high consumption) (nondysplastic BE: OR = 0.37, 95 % CI: 0.19 – 0.73; dysplastic BE: OR=0.22 95% CI= 0.007-0.73), compared with nondrinkers and consumption of less than 1 drink/week. Steevens et al. demonstrated that subjects with an intake ≥30g of ethanol per day had an RR of 0.82 when compared with abstainers (26). Furthermore in subjects aged 20 and 30 years old, alcohol consumption was not associated with an increased risk of BE (25, 27, 28).

Moderate drinking (7 - 13 drinks/week or 1 - <3 drinks/day) has a borderline or no statistically significant inverse association (24, 29, 30), whilst subjects who reported to typically consume between three and five alcoholic drinks per day (intermediate intake) had statistically significant lower risk of Barrett's esophagus compared with non-drinkers (29).

The association between alcohol and BE do not appear to be modified by other factors such as Body Mass Index (BMI), Waist-to-Hip Ratio (WHR), Gastroesophageal reflux disease (GERD), or the duration of GERD symptoms, smoking and gender (25, 29, 30).

Although there are trends for a lower risk among subjects with moderate intakes and a higher risk for those with heavier intakes, there is no conclusive evidence.

## Type of alcohol and risk of Barrett' Esophagus

*Beer*

Most studies show that drinking beer is not associated with BE (24, 27, 29,). One case-control study conducted by Trifth et al. has shown a lower risk, which was not statistically significant, among subjects consuming 14 to 28 drinks/week of beer, compared to life-long non-drinkers (30). These results were also confirmed by another study founding a significant inverse linear trend between beer consumption and non-dysplastic BE (P = 0.04) (25). Inverse association between high beer consumption (>21 pints per week) and BE was also seen for consumption at age 21 years (27).

*Liquor*

No significant relationships between drinking liquor and BE was found (24, 27, 29). However, an Australian population-based case-control study and a recent meta-analysis has provided evidence of increased BE risk with increased liquor consumption (11, 25).

*Wine*

Wine consumption has been reported to be inversely associated with BE risk (26, 27). Thrift et al. have shown a statistically significant inverse association with any level (drinks/day) of wine consumption (any vs. non-drinkers, summary OR=0.71, 95% CI 0.52–0.98, I2=0%); these figures were also adjusted for total alcohol consumption (29). The authors also retrospectively evaluated wine consumption at the age of 21 years old, and no significant association with an increased risk of BE was found (27). Kubo et al., showed that subjects consuming a glass of wine a day on average (≥7 glasses of wine a week), had less than half the risk of Barrett's esophagus compared with non-alcohol drinkers (OR, 0.44; 95% CI, 0.20–0.99) (24). Finally, in the study of Yates et al., no association was seen between wine and BE, which suggests there may be any protective effect of wine that can prevent the malignant transformation of metaplastic epithelium (28).

Comparing non-drinkers to drinkers, no associations were seen for wine consumption (HR 0.90, 95 % CI 0.48–1.69), beer (HR 1.55, 95 % CI 0.73–3.29) or spirits (HR 0.68, 95 % CI 0.38–1.22) (28).

## Discussion and Conclusion

Findings from this mini-review suggest that total alcohol consumption is not a risk factor for BE. In actual fact, when comparing population controls and BE patients, there is no statistical significant association between alcohol consumption and BE, nor studies have shown an inverse association between the two (11, 24-30). When analyzing in-depth the beverage type assumption, liquor was associated with an increased risk of BE (11, 24-26) whereas, wine and beer do not appear to increase the risk for BE (11, 24-30).

These differences between the various alcohol types could be due to diverse drinking patterns. Wine drinkers usually consume their alcoholic beverage with meals, whereas liquor drinkers are less inclined to do so (31). Consuming alcohol with food can reduce potential damage to esophageal epithelium and, consequently, the risk of carcinogenic process (31). Liquor with its high alcohol concentration may cause a direct esophageal tissue irritation, which may already have been injured by frequent reflux. (24)

Several studies had provided a number of plausible mechanisms to explain these findings. For example, the amount of antioxidants provided by wine intake (32, 33), and to a lesser extent in beer (34), may confer benefits (35). Moreover, the protective effects of chronic low ethanol consumption may improve insulin resistance and decrease advanced glycation end-products (AGEs) (33). Another possibility is that the seemingly protective effects of lifetime alcohol consumption may simply be an aversion effect, as BE patients may refrain from alcohol consumption over time after enduring prolonged reflux discomfort. Despite this, in a large population-based case-control study, Thrift et al. observed similar reduced risks in BE patients compared with inflammation controls (patients with GERD) (25). These results seems to suggest that alcohol avoidance by BE patients are unlikely to explain in full the observed effects.

In conclusion, our mini-review has shown that alcohol consumption seems to be not associated with BE. The review has shown also an inverse association for some types of alcoholic beverages and BE onset. Nevertheless, these findings have to be interpreted with caution due to multiple sources of possible bias.

It is possible that reported results may be affected by recall bias and selection bias. The main and common recall bias is referred to misclassification of overreporting or underreporting of alcohol intake by the individuals. A possible selection bias is represented by the fact that BE is usually diagnosed in patients with GERD, but we cannot exclude BE in asymptomatic individuals, who are usually undiagnosed and this may have influenced our results.

Moreover, alcohol has shown to reduce LES pressure and promote GERD symptoms. Hence, the protective effect demonstrated by various forms of alcoholic beverages may be due to an aversion effect.

Nevertheless, the direct public health applications of these findings are limited, considering the causal link between moderate-to-heavy alcohol consumption with increased risks of several cancers (16). On the other hand, given the rising incidence of BE and EAC, it is important to understand the interplay of dietary and lifestyle factors that influence the development of these conditions.

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Original article

# Determination of monthly changes in mineral content of Spiny Atraphaxis (*Atraphaxis spinosa* L.) as an alternative fodder crop

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**Summary.** This study was conducted in 2015 with the aim of determining monthly changes in mineral content of Spiny Atraphaxis (*Atraphaxis spinosa* L.) as to growth phases. Wild plants grown spontaneously on the wind erosion site in Aralık district of Iğdır province were the subject of the study. Plant samples were collected and changes in N (Nitrogen), P (Phosphorus), K (Potassium), Ca (Calcium), Mg (Magnesium), Na (Sodium), Cu (Copper), Fe (Iron), Zn (Zinc) and Mn (Manganese) contents were determined according to months. Highest values for N, P, K, Ca, Mg, Na, Cu, Fe, Zn and Mn contents of Spiny Atraphaxis during the 7-month growth period within which this study was conducted were measured as, 1.27%, 0.98%, 1.46%, 1.17%, 0.46%, 0.19%, 2.66 ppm, 344.76 ppm, 42.46 ppm and 64.90 ppm, respectively.

**Key words:** *Atraphaxis spinosa* L., mineral content, growth phase

## Introduction

Meadows and pastures are important sources of fodder crops in Turkey as well as in other parts of the world. There is an increasing demand for coarse fodder in Turkey in recent years due to an increasing animal population. However, meadows and pastures, which are the most important coarse fodder resources in Turkey, do not meet with this increasing demand because of decreasing productivity resulting from irregular grazing regimes, use of agricultural lands for non-agricultural purposes, mechanization of agriculture and ecological factors (1-4). Stockbreeding is an important source of income in the rural areas of Turkey. Thus, solving the problem of providing a regular supply of quality, cheap and abundant coarse fodder crops is important for a more economic and profitable conduct of stockbreeding in Turkey (5, 2).

Plants grown on pasture lands do not always have the same amount of mineral content during growth periods. High mineral contents at the beginning of the growth period of some plants decrease as the growth period progresses (6). It is reported that mineral contents of grass obtained from ravaged pasture lands which have low-yielding soils are much lower than the levels needed by ruminants (7). Species such as bushes and trees which are grown naturally in arid and semi-arid regions are important feed sources for ruminant during periods in which pasture plants grow pale and their mineral contents decline (8-10). Since quality losses occur lesser and more slowly in these species in comparison to herbaceous species, they can produce feed which has high energy and nutrient content and which is rich in vitamins and minerals (11, 12) .

During periods in which mineral contents of pasture plants are decreasing, deficiencies of nutritional elements occur in animals that are fed with these plants (13). These elements which are very important for living organisms and expressed in mg/kg and µg/kg terms are called trace elements (14). Such elements from these group as N, P, K, Ca, Mg, Na are called macro elements while Cu, Fe, Zn, Mn are called micro elements. Mineral elements have a quite important role in increasing rumen activity and enabling more effective fodder utilization in ruminants (15). Mineral substances have an important place in metabolic activities of animals, how-

ever, they cannot be synthesized within the animal body (16) and animals intake these needed minerals mostly from plants (13). Reproductive, growth, yield and immunity systems are adversely affected in case of an absence or excess of mineral substances (17, 18).

*Atraphaxis spinosa* (Spiny atraphaxis) is a member of *Polygonaceae* family. Approximately 30 species related to Atraphaxis genus of *Atraphaxis spinosa* are identified in Southern Europe, Southwest and Central Asia, South Siberia, Mongolia and China (19-21). *Atraphaxis spinosa* is found in the nature as scrubs; it is deciduous in winters and usually has thorns in its branches. Its fruits are either shaped trigonal or flat. It grows on sunny, arid, sandy-gravel soils and known as plants of step or desert climates (22).

The aim of this study is to determine mineral contents of Spiny Atraphaxis at different growth phases. Thus, it will be revealed whether Spiny Atraphaxis is an important feed source in the nutrition of animals, which grows well under relatively more microclimatic conditions of Iğdır province, in comparison to other provinces of Eastern Anatolia Region where continental climate is the dominant climatic system.

## Material and Methods

This study was conducted to determine monthly changes in mineral content of Spiny Atraphaxis *(Atraphaxis spinosa)*. For this aim, sample materials were collected from the wind erosion site in Aralık district of Iğdır province between April 2015 and October 2015. Looking at climatic data of Iğdır province in 2015; total amount of annual precipitation is 302,4 mm, with the lowest temperature of -9,8°C recorded in January and the highest temperature of 41,4°C recorded in August 41,4 (23).

In order to determine mineral content of the plant, samples were taken totally 1 kg from the stems + leaf of the plant, imitating grazing habits of the animals. The research was established in 3 blocks according to randomized blocks trial design. Samples were taken from 5 plants in each block and a total of 15 samples were collected. Sample collection times were included as a factor of determining mineral contents of plants. In this respect, P, N, K, Ca, Mg, Na, Cu, Fe, Zn and Mn values were measured for 7 months between April and October and monthly changes in these values were recorded. Collected samples were analyzed for macro (P, K, N, Ca, Mg, Na,) micro (Cu, Fe, Zn, Mn) elements.

P, K, Ca, Mg, Na, Fe, Cu, Zn and Mn contents of the plant samples were determined by reading in P, K, Ca, Mg, Fe, Mn, Zn, Cu, Na ICP OES spectrophotometer (Inductively Couple Plasma spectrophotometer) (Perkin-Elmer, Optima 2100 DV, ICP/OES, Shelton, CT 06484-4794, USA) (24), after samples were treated with nitric acid and hydrogen peroxide (2:3) in three steps (1st Step; 5 minutes at 75% microwave power at 145, 2nd Step; 10 minutes at 90% microwave power at 180' and 3rd Step; 10 minutes at 40% microwave power at 100) and processed at pressure tight microwave wet decomposition unit resistant to 40 bars of pressure (25).

Total nitrogen contents of the samples were determined by micro Kjheldahl method after subjecting to wet decomposition salicylic-sulphuric acid (26).

## Results and Discussion

Mineral content of stem + leaf sample of *Atraphaxis spinosa* has shown differences according to sample collection times, defined in months, in the research. Changes in N, P, Na, Cu, Fe and Zn ratios were found to be very significant ($p<0.01$) while changes in K and Mn ratios were found to be significant ($p<0.05$) and changes in Ca and Mg ratios were found to be insignificant (Table 1 and Table 2). Although changes in Ca and Mg ratios were insignificant, it was observed that Ca and Mg contents of the plant were still over the level (0.1mg %) (27) required by ruminants.

*Macro minerals*

In the study, the effects of different growth periods on Ca and Mg content of *Atraphaxis spinosa* have been insignificant. Nitrogen (N) content was observed to change between 0.98-1.27%. The highest Nitrogen content of 1.27% was obtained in October and the lowest nitrogen content of 0.98% was recorded in August.

Phosphorus (P) content was observed to change between 0.43-0.98%. The highest P value of 0.98% was recorded in April and the lowest P value of 0.43%

was recorded in July. P content of plants varies according to phase of growth and sample collection time (12; 28). All mineral content, including that of phosphorus, of fodder crops decrease with as plants mature (29). P level must be between 0.12% and 0.48% in fodder in order to meet nutritional requirements of ruminants (30). The P levels obtained in the present study are above these values (Table 1). However, there is no possibility of occurrence of adverse effects of excess phosphorus since fodder crops in the region do not solely comprised of Spiny Atraphaxis.

Potassium (K) value varied between 1.08 and 1.46%. The highest K value was observed in May as 1.46% while the lowest K value was observed in October as 1.08%. K value is at the highest level between the months of April and August (13). It was also reported in another study that K ratio drops as fodder crops mature and reaches the lowest levels during winters (15). K level in ruminant rations must be between 0.5% and 1.0% (30). In our study K levels were observed to be over these values. However, K toxicity is not possible according to the obtained results because all values are determined below the 3% limit (31).

Sodium (Na) concentration of the samples collected in the study varied between 0.12% and 0.99%. The highest Na value was recorded in April as 0.99% and the lowest value was recorded in July as 0.12%. Recommended Na concentration in fodders must be in the range of 0.06-0.18% (30). Previous studies confirm that the most common mineral deficiency for the ruminants over the world is Na deficiency (32). Na deficiency was also reported for many different regions around the world (33). The results obtained from this study suggest that using *Atraphaxis spinosa* in feed rations may be a solution to overcome this deficiency.

*Micro minerals*

Copper (Cu) content of the samples varied between 0.86 and 2.66 ppm. the highest Cu content of 2.66 ppm was observed in June while the lowest content of 0.86 ppm was observed in August. Cu content of *Atraphaxis spinosa* varied significantly according to sample collection times. According to data reported by (30), Cu content of the ruminant feed must be in the range of 6-12 ppm. Cu content was found to be lower than these values in our current study. This means that Cu deficiencies may occur in case animals are grazed only with Spiny Atraphaxis. However, this is a very low possibility for the region since feed rations contain other plants.

Iron (Fe) content of the samples varied between 112.03 and 344.76 ppm. The highest Fe content was recorded in August as 344.76 ppm and the lowest value was recorded in April as 112.03 ppm. Fe content of the ruminant feed must be in the range of 30-60 ppm (30). Fe values observed in this study are much higher than the recommended limits. An abundance of Fe causes iron toxicity and this situation results in disfigurement in the bone and teeth structures of ruminants (17). Grazing of animals solely with Spiny Atraphaxis may cause iron toxicity and the ratio of this plant in the rations must be adjusted carefully.

**Table.1.** Macro mineral contents and F values of Spiny Atraphaxis harvested at different growth periods

| Months | N (%) | P (%) | K (%) | Ca (%) | Mg (%) | Na (%) |
|---|---|---|---|---|---|---|
| **April** | 1.02 c | 0.98 a | 1.42 a | 0.90 | 0.33 | 0.99 cd |
| **May** | 1.21 ab | 0.66 b | 1.46 a | 0.86 | 0.32 | 0.80 d |
| **June** | 1.02 c | 0.46 c | 1.44 a | 1.06 | 0.27 | 0.13 b |
| **July** | 1.00 c | 0.43 c | 1.13 b | 1.03 | 0.41 | 0.12 bc |
| **August** | 0.98 c | 0.51 c | 1.13 b | 1.07 | 0.41 | 0.18 a |
| **September** | 1.10 bc | 0.50 c | 1.15 b | 1.17 | 0.46 | 0.19 a |
| **October** | 1.27 a | 0.51 c | 1.08 b | 1.06 | 0.35 | 0.13 bc |
| **F values and significance** | 6.588** | 25.675** | 3.798* | 2.308in | 2.288in | 2.371** |

** denotes very significant F values at 1% confidence level; * denotes very significant F values at 5% confidence level; in: denotes insignificant F values.

a, b, c, and d Different letters in the same column indicate significant differences in the mineral contents between the growing periods.

**Table.2** Micro mineral contents and F values of Spiny Atraphaxis harvested at different growth periods

| Months | Cu (ppm) | Fe (ppm) | Zn (ppm) | Mn (ppm) |
|---|---|---|---|---|
| **April** | 1.46 b | 112.03 d | 42.46 a | 44.13 b |
| **May** | 1.40 b | 160.80 cd | 33.00 bc | 54.76 ab |
| **June** | 2.66 a | 208.73 bcd | 35.76 b | 47.23 b |
| **July** | 2.53 a | 236.53 bc | 25.30 d | 62.00 a |
| **August** | 0.86 b | 344.76 a | 28.03 cd | 59.43 a |
| **September** | 0.93 b | 280.66 ab | 33.70 bc | 64.90 a |
| **October** | 1.53 b | 193.13 bcd | 32.73 bc | 58.86 a |
| **F values and significance** | 9.261** | 5.472** | 10.102** | 4.653* |

** denotes very significant F values at 1% confidence level; * denotes very significant F values at 5% confidence level; in: denotes insignificant F values.

a, b, c, and d Different letters in the same column indicate significant differences in the mineral contents between the growing periods.

Zinc (Zn) content of the samples collected in the study varied between 25.30 and 42.46 ppm. The highest Zn content was recorded in April as 42.46 ppm and the lowest Zn content was recorded in July as 25.30 ppm. Zn content required in feeds of ruminants varies between 7.0 and 100.0 ppm (30). Results obtained in the presents study for Zn content fall within this range.

Manganese (Mn) content varied between 44.13 and 64.90 ppm. The highest Mn content for *Atraphaxis spinosa* was recorded in September as 64.90 ppm and the lowest content was recorded in April as 44.13 ppm. Mn ratio recommended by (30) for all ruminant classes is between 18 and 36 ppm. Mn content obtained in the research was higher than the recommended values.

## Conclusions

In this study conducted on Spiny Atraphaxis (*Atraphaxis spinosa*), samples were taken at different growth phases in order to determine changes in the mineral content of the plant. It was observed that sample collection times (growth phases) have affected the mineral content. The highest Nitrogen content was obtained in October and the lowest nitrogen content was recorded in August. While Phosphorus, Potassium, Sodium and Zinc contents were high at the beginning of the growth, they observed to decrease gradually through the end of the growth period. However, these contents were still over the levels required by ruminants despite this decline. Mn contents were observed to increase through the end of the growth period. Changes in Ca and Mg contents were found to be insignificant, while Na content was observed to be compatible with levels recommended in the literature. While the highest copper content was obtained in June and July, the copper content of the plant was similar in the initial stages (April and May) of development and in the late stages of development (August, September and October). Fe contents of the plant increased in comparison with the start of the growth period as the growth phases progressed, and then decreased through the end of the growth period. Cu content was found to be lower than recommended while Fe and Mn content were observed to be higher than the required level. According to the obtained results, the mineral content decreased to an extent as the plant matured. Nevertheless, Spiny Atraphaxis may be a good source of alternative feedstuff in the areas with extreme climatic and soil conditions during times when feedstuff cannot be supplied in desired amounts and quality due to paling of pasture plants and the decline in their mineral content.

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Original article

# Effects of longitudinal abuse of anabolic steroids on liver enzymes activity and lipid profiles of male bodybuilders

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**Summary.** The purpose of this study was to investigate the effects of anabolic steroids (AS) abuse on liver enzymes activity and lipid profiles in male bodybuilders. 40 well-trained bodybuilders, with 20 self-reporting regular AS use and 20 self-reporting never taking AS (NAS) were recruited for this study. Participants reported to the laboratory for blood sampling to assess liver enzymes activity (Aspartate transaminase [AST], Alanine aminotransferase [ALT] and Alkaline phosphatase [AP]), lipid profiles and fasting blood sugar (FBS). Moreover, maximal strength and muscle volume were measured. The results indicated that AS users had higher strength in the bench press (113±11.8 vs. 93.7±13.3 kg) and leg press (329.5±40.4 vs. 248.5±41.0 kg), muscle volume (arm, 41.2±3.5 vs. 35.1±4.2 cm and thigh, 60.6±6.4 vs. 53.7±5.6 cm), LDL (179.2±34.1 vs. 155.8±37.7 mg/dL), TG (166.5±74.4 vs. 126.9±48.2 mg/dL), TC (253.2±59.6 vs. 143.5±48.0 mg/dL), AST (53.2±14.3 vs. 34.5±11.11 IU/L) and ALT (53.5±15.1 vs. 33.3±7.8 IU/L) ($p < 0.05$). However, NAS users indicated higher HDL (43.5±15.2 vs. 30.7±10.0 mg/dL) and AP (82.7±30.6 vs. 75.6±30.1 IU/L) ($p < 0.05$) in comparison to AS users. In conclusion, AS abuse is associated with alterations in liver enzymes function and lipid profiles that, represent an increased risk profile in athletes who used AS.

**Key words**: anabolic steroids, liver, strength, lipid, bodybuilding.

## Introduction

Anabolic steroids (AS) are one of the most commonly used drugs among athletes, especially in strength-trained men, to improve muscular performance, muscle size and increase strength. (1,2). Outside of some physiological advantage of AS, abuse of this drug has become a serious problem in the United States, United Kingdom as well as other parts of the world (2), and during past 2 decades, the number of AS users increased more than 2000% in the world (3). There is an adverse effect of AS in some organs such as hepatic (4), endocrine, and cardiovascular systems (5). For example, it has been shown that AS may induce pathological left ventricular hypertrophy (6) with disproportional extracellular collagen accumulation and/or interstitial fibrosis (7).

Liver is a key organ actively involved in numerous metabolic and detoxifying functions during exercise. During exercise training liver play an important role to release ATP or glucose. Abuse of AS has an adverse effects on liver function. The liver adverse effects are among the most common and serious associated with AS abuse and are virtually always associated with the oral active 17-α alkylated androgens such as methyltestosterone, methandrostenolone, oxandrolone, and stanozol. In fact, AS allows increased oral absorption and slower hepatic degradation and clearance, so resulting in greater hepatic toxicity (8). Welder et al. (9) showed that AS are directly toxic to rat hepatocytes with increase of liver enzymes levels. Animal studies clearly shown liver alterations induced by AS. Gragera et al. (10) observed ultrastructural alterations of hepatocytes, the most prominent changes being swelling of

mitochondria and marked increase in the number of lysosomes. Saborido et al. (11) and Molano et al. (12) observed that treatment with stanozolol, either with or without concurrent exercise training, affects lysosomal hydrolases and mitochondrial respiratory chain electron transport in rat liver, without modifying classical serum indicators of hepatic function. Acute adaptative changes on the liver tissue (slight to moderate multifocal lobular inflammation with acidophilic degeneration and evident Kuppfer cells reactivity) were observed by Boada et al. (13) in rats administered with stanozol for a short time in association with minimal to mild variability in the size of cell nuclei and increased mitosis and binucleation. In the majority of the livers from long-term treatment, the researchers observed cytoplasmic vacuolation, and lipidic degeneration; in addition, as in the case of acute AS-treated animals, they found increased mitosis and binucleation and variability in the size of cell nuclei.

Although there are several reports concerning the physiological abnormalities induced by AS abuse, the liver enzymes activity after using this drug in human subjects is unclear. Since previous studies used Rats to identify the effects of AS on liver enzymes activity and lipid profile, the information about the effects of longitudinal AS abuse on changes in liver enzymes and lipid profiles in human subjects especially in strength-trained men is scarce. Therefore, the purpose of this investigation was to determine the influence of longitudinal abuse of AS on liver enzymes activity and lipid profiles of men bodybuilders.

## Methods

*Subjects*

The subjects of this study were 40 weight trained men, with 20 self-reported regular AS use and 20 self-reported never taking AS (NAS) (Table 1). Inclusion criteria were resistance training history of minimum of 5 yr with four to five training sessions per week. The specific inclusion criterion for the AS group was a documented self-reported history of AS abuse for 1 to 3 years and inclusion criterion for the NAS group was self-reported history of never taking AS. Before taking part in the study, the participants were notified about the potential risks involved and gave their written consent. This study was approved by the Guilan university human research ethics committee.

*Design*

A cross-sectional cohort design was used for the study, with participants required to make a single visit to laboratory. Initially, subjects completed self-report questionnaires related to general health, training status, and history as well as detailed accounts of AS abuse. This was followed by assessment of body composition, arm and tight circumferences, strength test and a venous blood sample. All tests were conducted on the participants after an overnight fast, as well as a 24-h abstention from resistance training.

*History of AS use*

The participants in the AS group had experience of AS abuse at least 1 to 3 years. The types of AS currently being used by some of the AS participants included trenbolone (number of use (N) = 4), testosterone (N = 2), sustanon (N = 3), boldenone (N = 1), nandrolone (N = 3), oxandrolone (N = 3), and stanozolol (N = 4). Of those in the AS group who provided sufficient information to perform an analysis of their daily usage (n = 20), we found that the mean AS dose was 220 $mg.d^{-1}$ with a SD of 152 mg.

*Body composition*

Height was measured using a wall-mounted stadiometer (Seca 0123, Germany) to the nearest centimeter. Body mass was measured to the nearest 0.1 kg using a medical scale (Seca 760102, Germany). Percentage of body fat was measured using 3-site skin fold thickness (chest, abdominal, and thigh). The measurement was used according to the method by Jackson and Pollock (14). All skin fold measurements

**Table 1**. Subjects characteristics (mean±sd).

| | AS (n=20) | NAS (n=20) | P value |
|---|---|---|---|
| Age (y) | 25±2.9 | 24.2±3.1 | 0.12 |
| Height (cm) | 172.7±5.9 | 174.1±5.4 | 0.31 |
| Body mass (kg) | 81.1±10.3 | 74.6±6.7 | 0.04 |
| Body fat (%) | 18.1±4.5 | 15.7±3.9 | 0.08 |

were taken using Lafayette caliper (Skin Fold Caliper, Model 01127-INDIANA). Skinfold thickness was based on the average of the two trials. If the two skinfold measurements at the same site differed by more than 0.5 mm, a third measurement was obtained and the mean value used.

*Muscle circumference*

The circumferences of mid thigh and mid arm of the right side were assessed during full muscle contraction using tape measure with nearest to 0.1 cm (15).

*Strength assessment*

Strength was measured using the one repetition maximum (1RM) bench press and leg press exercises. The 1RM testing was performed according to method previously described by Kraemer and Fry (15). The participants performed a warm-up set of 8 to 10 repetitions at a light weight. A second warm-up consisted a set of three to five repetitions with a moderate weight, and third warm-up included one to three repetitions with a heavy weight. After the warm-up, each subject was tested for the 1RM by increasing the load during consecutive trials until the participants were unable to perform a proper lift, complete range of motion and correct technique. The 1RM test was determined by ~5 sets of one repetition, with 3–5 minutes of rest among attempts. Spotters and investigators were present to provide verbal encouragement and safety for the subjects.

*Blood sampling and analysis*

Blood samples were drawn (10 cc) from the antecubital vein into plain evacuated test tubes. All the blood samples were drawn after 12 h of fasting and 8 h of sleeping. The blood was allowed to clot at room temperature for 30-min and centrifuged at 1500×*g* for 10 min. The serum layer was removed and frozen at –20°C in multiple aliquots for further analyses. Assessment of fasting blood sugar (FBS), total cholesterol (TC), HDL, LDL, and triglycerides (TG) were performed using the Daytona RS blood analysis machine (Randox, Co., Antrim, N. Ireland). Liver enzymes (i.e., AST, ALT and AP) were analyzed using conventional spectrophotometric methodology using a DxC 600 autoanalzyer (instrument and reagents from Beckman Coulter, Fullerton, CA).

*Statistical analysis*

All data were subjected to tests of normality. Differences between AS and NAS participants were analyzed using paired t-tests. The level of significant was set at $p \leq 0.05$. Statistical analysis of data was performed using statistical software package SPSS Version 16 (SPSS, Inc., Chicago, IL).

## Results

Although height did not differ between groups, participants in the AS group were significantly heavier than NAS group ($p < 0.05$). However, body fat percentage was not significantly different between groups.

The participants in AS group indicated greater strength in the bench and leg press exercises than NAS group ($p < 0.05$), and there were also differences in the arm and thigh circumferences between groups ($p < 0.05$) (Table 2).

LDL and HDL were significantly elevated and reduced, respectively, in the AS group compared with NAS group ($p < 0.05$). FBS, TG and TC were higher in the AS group in comparison to NAS group ($p < 0.05$). There was little difference in partial thromboplastin time (PTT) between groups (33.4±5.7 vs. 32.9±5.3 sec) (Table 2).

AST and ALT levels were significantly elevated in the AS group than NAS group, whereas the AP level was higher for the NAS group ($p < 0.05$) (Table 2).

## Discussion

The aim of this study was to compare the effects of longitudinal abuse of AS on liver enzymes activity, lipid profiles, strength and muscle volume in men bodybuilders. The results indicated that body mass, strength and muscle volume were greater for the athletes who used AS. However, the liver enzymes activity and lipid profiles were higher in the AS users than NAS group.

The findings of this study indicated that the AS users were heavier and the arm and thigh circumferences were greater in the AS users compared to NAS group. Moreover, the AS users were stronger than NAS users in the 1RM of bench press and leg press

**Table 2.** Data for the strength, muscle volume, lipid profiles and liver enzymes activity in AS and NAS groups (mean±SD).

| | AS (n=20) | NAS (n=20) | P value |
|---|---|---|---|
| 1RM bench press (kg) | 113±11.8* | 93.7±13.3 | 0.02 |
| 1RM leg press (kg) | 329±40.8* | 248.5±41 | 0.003 |
| Arm circumference (cm) | 41.2±3.5* | 35.1±4.2 | 0.04 |
| Thigh circumference (cm) | 60.6±6.4* | 53.7±5.6 | 0.03 |
| PTT (sec) | 33.4±5.8 | 32.9±5.3 | 0.13 |
| FBS (mg/dL) | 87±11.9* | 81.3±9.8 | 0.05 |
| TG (mg/dL) | 166.5±74.4* | 126.9±48.2 | 0.04 |
| TC (mg/dL) | 253.2±59.6* | 143.5±48 | 0.01 |
| HDL (mg/dL) | 30.7±10* | 43.5±15.2 | 0.02 |
| LDL (mg/dL) | 179.2±34.1* | 155.8±37.7 | 0.04 |
| AST (IU/L) | 53.2±14.3* | 34.5±11.1 | 0.02 |
| ALT (IU/L) | 53.5±15.1* | 33.3±7.8 | 0.02 |
| AP (IU/L) | 75.6±30.1 | 82.7±30.6† | 0.05 |

*1RM: 1 repetition maximum, PTT: partial thromboplastin time, FBS: fasting blood sugar, TG: triglyceride, TC: total cholesterol, HDL: high density lipoprotein, LDL: low density lipoprotein, AST: Aspartate transaminase, ALT: Alanine aminotransferase, AP: Alkaline phosphatase. *Significant differences compared with NAS group (p≤0.05): †Significant differences compared with AS group (p≤0.05).*

exercises. These findings are in line with previous studies which reported larger gains in body mass, muscle size and strength performance after AS abuse (17-22). A large number of studies reported that use of AS can increase the body mass (2-5 kg) (17,18). Alen and Hakkinen (23) reported that 6 months AS use induced 5 kg gains in body mass. In relation to muscle size or circumference, some studies reported no alterations of circumferences after AS use (24,25). In contrast, other researchers addressed that use of AS could induce increases in muscle size (20,21,22). The largest gains of muscle circumferences were seen at the thorax, shoulders and upper arm (19). Although, AS have been demonstrated to stimulate protein synthesis (21), the effects on muscle size and circumference could not be established. It has only been in the last decade that clear evidence for the muscle building properties of AS in males and athletes became available (20-23). It seems that these mechanisms could be a reason to greater body mass and muscle size in the AS users.

The most prevalent results for AS abuse is to promote muscle mass and strength. Bhasin et al (26) examined the effects of AS abuse and strength training on muscle size and found that 10 weeks AS use + strength training increased arm and thigh muscle circumferences and these changes were greater than strength training only. Moreover, higher strength for the AS user have been supported in previous studies (21,23,25). It can be concluded that AS administration may increase muscle mass and circumference and whether type I or type II muscle fibers are more profoundly affected is not clear yet. It appears that increase in muscle mass can be attributed to muscle hypertrophy and also the formation of new muscle fibers (20). The key roles seem to be played by satellite cells (i.e., they are enhanced by AS administration) and androgen receptors. Androgen receptors are expressed in myonuclei of muscle fibers and in capillaries and are more present in upper limb than in lower limb. AS administration induced an increase in androgen receptor-containing myonuclei in the muscles and also increase the myonuclear number per fiber in the muscle (19,20). Sinhahikim et al. (22) observed that muscle hypertrophy induced by exogenous testosterone administration was associated with an increase in satellite cell number, changes in satellite cell ultra-structure and a proportionate increase in myonuclear number (21,22,23). These observations may explain the regional differences in body mass, muscle fiber adaptation, muscle circumferences and strength development between AS and NAS users.

In addition, we observed an altered lipid profile in AS users. The TC was higher in AS users, the difference between groups was statistically significant, which supports data from Baldo-Enzi et al. (27), but contradicts Sader et al. (28). It can be explained that lipid profiles and overall cholesterol are important when determining cardiovascular and atherosclerotic risk (29). The decrease in HDL in AS users in the present study agrees with past research (30,31). Likewise, an increase in LDL in the current study also supports previous data (31). Supraphysiological doses of AS lead to high hepatic androgen exposure, and high androgen levels can alter levels of lipoprotein, which directly affects the formation of HDL (32) and these changes could increase cardiovascular disease in athletes who use AS.

In accordance with the findings of this study, number of studies reported elevation of liver enzymes activity after AS abuse (10-13). The results indicated that long-term abuse of AS increased basal levels of ASP and ALP in athletes, whereas this drug induced decreases in the AP levels in comparison to NAS athletes. Even though the numbers of subjects were small, the results indicated that in the general collective consciousness of the medical community, use of AS closely associated with liver disease. Previous reports on athletes who use AS have suggested that AS may cause serious hepatic dysfunction using ASP and ALP (12,33). It is presumed that AS are responsible for liver damage (33). Also, these lesions are reversible, at least partially, as has been reported in several case reports, and in some series of long-term follow-up (34,35); however, progression to hepatic insufficiency has been published (36). It would be conclude that long-term abuse of AS induced elevation of liver enzymes activity resulting hepatic toxicity. AS treatment is known to induce hepatic structural and ultrasturactural changes (33) that may cause modifications in the liver subcellular fractionation pattern consequently enhances of liver enzymes activity.

In conclusion, the results from this study indicate that longitudinal abuse of AS coupled with strength training in athletes is associated with greater muscle mass, strength and muscle size than NAS user athletes. However, these greater enhancements are in accordance with elevation of lipid profiles and liver enzymes activity resulting liver damage and toxicity.

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Original Article

# The acute effects of combined supplementation of beta-alanine, carbohydrate and whey protein on biochemical parameters of athletes after exhaustive exercise

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**Summary.** *Background:* The beneficial effect of popular supplements and use of combined supplementation in athletes which purpose to increase sports performance. *Objective:* This study aimed to review biochemical responses that the athletes gave to combined supplementation received after exercise and some changes in hematological values. *Material and Method:* 16 volunteers, in shape, male athletes with ages between 18-25 participated into the study. Athletes were divided into two groups as experiment (supplement) (n=8) and control (placebo) (n=8). After the exercise made until exhaustion (shuttle run test), beta-alanine/vitargo(carbohydrate-electrolyte)/whey protein supplement was given to the experimental group while the control group received placebo (water). Blood was taken from the athletes three times as basal, post-exercise (PE) and 2 hours after ingestion supplement (PS); Urea, Creatinine, Cholesterol, Triglyceride, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), Gamma Glutamyl Transferase (GGT) and Alkaline Phosphatase (ALP) values have been analyzed. *Results:* Statistically significant differences in many biochemical parameters were found when comparisons of in-group basal, PE and PS time courses of supplement and control groups were reviewed ($p<0.05$). When inter-group comparison of triglyceride and cholesterol levels were reviewed, a significant difference was seen in basal levels ($p<0.05$) no difference was detected in values other than that ($p>0.05$). *Conclusions:* It is possible to say that acute combined supplementation used after exercise does not create a negative effect on biochemical parameters of athletes, on the contrary when the research result data were compared with control group, by looking at the basal, exercise and after supplementation values such as creatinine, cholesterol, triglycerides, HDL and LDL, the combined supplement intake showed positive results in terms of health.

**Key words:** sports nutrition, nutritional health, ergogenic aids, dietary supplements, carbohydrate, electrolyte, protein, amino acids, biochemical changes, exercise

## Introduction

In recent years, the beneficial effects of nutrition on exercise performance were clearly documented with researches (1). The first and most important nutritional needs of athletes is adequate amounts of food and a balanced diet to supply their energy consumption. Then for training, competition and a successful recovery, nutrition strategy should be determined (2). Besides when looking at the reasons of nutrition consumption of athletes; the most important ones are to be healthy, optimum body weight and composition, fast recovery after exercise and, of course, to provide fuel for energy throughout the exercise together with high exercise performance (1). But of many athletes throughout the world even in elite level; level of nutrition knowledge is very low not being very different from the general population. Ergogenic aids are being used to eliminate inefficiencies and needs of nutrition or to maintain adequate intake of certain nutrients (3).

Nutrition is an important element in the training program of athletes. It also holds an important place in the training period of many athletes. Pre and post training proper nutrition intake can supply the nutritional needs of athletes to a large extent. However, it may not be possible to supply the increased nutritional need with natural foods at challenging and intense match traffic. Though it is thought that exercise and sports training increase the nutritional needs in some of the athletes, appropriate calories with balanced diet can substantially meet the nutrient requirements. Despite, for a variety of reasons it is not possible for all athletes to meet the increased nutritional needs with the natural diets, and so the natural supplements referred to overcome the deficiencies will improve the performance (4). Exercise capacity of people, as a result of involved regular physical activity combined with the advancement of age, incurs individual losses and regresses. But the diet supplements increase the capacity of exercise, protect physical compliance and improves general health (5). Athletes who want to succeed, go down the path of performance increasing by using physiological, nutritional and pharmacological factors. All these factors that help the athletes in developing the performance are referred to as ergogenic aid. Ergogenic aid includes drugs as well as normal nutrients consumed by people (6). It is thought that ergogenic aid was to use to provide help to the level of the athlete's performance. It is seen that some ergogenic aids safely increase the performance (7). As a result; optimum processing of human muscle energy systems depends on a variety of diet nutrients (8).

A wide range of dietary supplements are produced for the use of athletes to improve their performance (9). In the studies, it was determined that the use of supplement starts at college and university level and athletes over age of 18 use supplement (4). According to the statistics, many national, international and Olympic level elite athletes often prefer the use of supplement. When looking at reasons to use supplement; it is emerging that it is used for upgrading the percentage of sports performance impact, enhancing energy, improving performance, preventing nutrition deficiencies, preventing diseases, increasing muscle mass and improving recovery (4). When looking at the most popular dietary supplement that athletes use, the most common intake is aminoacids. This distribution is seen as; protein/aminoacid, electrolyte and carbohydrate (10). When looking at the causes of use of supplement of athletes; reasons such as energy requirement and providing fuel during exercise, fluid and electrolyte balance, adaptation to special environmental conditions, physical activity, athletic performance, recovery after exercise, general health, body weight and composition come in the first place. It is very important that these products are safe in terms of production, effective, potential/strong and legal (1).

In the study beta-alanine, vitargo (carbohydrate-electrolyte) and whey protein supplements were used as nutritional support. Beta-alanine that some athletes use to improve performance and increase exhaustion threshold at the same time, was qualified by experts as a nutritional support that would be useful and needs raising awareness about (11). Carbohydrate that is important in terms of providing sufficient energy and repletion of muscle glycogen stores, is also an important nutrient support for general health (1). Whey protein that athletes usually use for muscle development, and increasing strength and performance is known to improve general health as well (12).

In the study presented; it was aimed to investigate the effects of acute combined supplementation on physiological responses and some hematological values of athletes.

## Materials and Methods

*Research group*

In this study, 16 volunteers, in shape, male athletes between the ages of 18-25, participated into this study. Athletes were divided into two groups as experiment (supplement) (n=8) and control (placebo) (n=8). In athletes, requirements of being healthy, not having chronic or acute disease and not having any movement limitation depending on disability occurred for any reason were looked for. For this study, by Sinop University Human Research Ethics Board it was decided that there was no inconvenience ethically and it was found appropriate (Number: 57452775-604.01.02-E.).

*Study design*

In the study firstly some biochemical blood parameters were analyzed by taking basal blood sam-

ples prior to shuttle run and supplementation. Later 20-metre shuttle run was applied to athletes and right after the exercise done until exhaustion venous blood was again taken from the athletes for tests and then drink products were given to the supplement and control groups. After athletes consumed the drinks given to them under the supervision of researchers and in allotted time venous blood was taken for the last time for biochemical blood analysis two hours after consumption and some hematological parameters of athletes were analyzed after acute supplementation. Measurements and tests were made at the same physical conditions in both the supplement and control groups.

*Blood measurements*

Venous blood was taken from the athletes by expert nurses for hematological tests and analysed by biochemistry specialists. Biochemistry analysis was studied from the serum samples acquired by 3500 cycle/minute and 15 minute centrifuge speed of venous bloods at Abbott Architect c16000 biochemistry autoanalyzer. The upper phases were transferred to eppendorf tubes and kept at –80 °C until the use. Blood was taken from the athletes three times as basal, post-exercise (PE) and 2 hours after supplement ingestion (PS); Urea, Creatinine, Cholesterol, Triglyceride, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), Gamma Glutamyl Transferase (GGT) and Alkaline Phosphatase (ALP) values were analyzed.

*Supplementation*

Immediately after 20-metre shuttle run test 16 athletes were randomly divided into two groups as supplement (n=8) and control (n=8) group. The study was conducted as a single blind application. Beta-alanine/vitargo(carbohydrate-electrolyte)/whey protein supplements were given to the experiment group in accordance with administration and daily dosage (with 1000ml water), an equal amount of placebo (water) to the given nutritional supplement was given to the control group. The supplementation was prepared beforehand and as a single dose that includes beta-alanine 3 g, vitargo (carbohydrate-electrolyte) 75 g and whey protein 30 g. The athletes were not informed about the substance given to them. So the psychological effects that may occur in athletes were removed and the study was conducted in more reliable conditions. In addition, the athletes were warned about not consuming any alcohol and stimulants one day before the test, caring the nutrition and resting.

*Shuttle run test*

In the study, 20-metre shuttle run test was applied to increase level of fatigue of athletes. This test that is used to measure aerobic capacity test is frequently preferred since it is a method that can be applied easily. In addition, 20-metre shuttle run test is a test that its validity and reliability to measure aerobic capacity is proven (13). They do not need to warm up before starting to the test. Because the 20-metre shuttle run test is a multistage test, first stages are in warming tempo (14). This test; is a test starting with 8.5 km/h and in every 1 minute running speed increases by 0.5 km/h, 20-metre distance is run as round trips (15). Running speed is controlled with a tape that beeps at regular intervals. The subject begins to run from the signal heard first and has to reach the other line until the second beep. And when the second beep is heard, returns to the starting line and these running signals continue. When the subject hears the signal, he sets the tempo himself to be on the other end of the runway at the second signal. If the subject misses a signal and catches the second, he continues to the test. The test is over when the subjects cannot catch the line 3 times before the beep or they quit running due to exhaustion (14, 16).

*Statistical analyses*

The research data obtained were given in the form of the standard error of the mean (M±SEM). We assessed the distribution of the analyzed variables using a Shapiro-Wilk test. The results showed that the distributions deviated from normal distribution. The Mann–Whitney U test was used to compare basal, PE, PS values between the two groups. A Friedman rank test was undertaken to evaluate the statistical differences in time for each parameter. When a significant F-value in Friedmans' analysis was found, a post-hoc test with a Bonferroni correction was used to determine the between-means differences. Statistical significance was accepted as $p<0.05$. In making of statistical analysis derived from the study and comparing the results SPSS v.22 package program was used.

## Results

A statistically significant difference was found when serum urea level after exercise (PE) was compared to urea level after supplement (PS) in the supplement group ($p<0.05$). There was no statistically significant difference at any phase when serum urea levels were checked in the control group ($p>0.05$). And when inter-group comparisons of urea levels were considered, there was no significance at any phase ($p>0.05$) (Figure 1a). Statistical significance was found when creatinine values of supplement group were compared in all processes (basal with PE, basal and PS, PE and PS) ($p<0.05$). And in the control group while there was a significant difference between basal creatinine level and PE and PE and PS ($p<0.05$), there was no between basal and PS ($p>0.05$). When inter-group comparisons of creatinine levels were considered, no statistically significant difference was found at any phase ($p>0.05$) (Figure 1b).

A statistically significant difference was found when basal cholesterol was compared to PS cholesterol level and PE level to PS level in the supplement group ($p<0.05$). There was statistical difference between basal and PE and PE and PS phases when cholesterol levels of the control group was considered ($p<0.05$). When inter-group comparisons of cholesterol levels were considered, there was significance at basal level ($p<0.05$) (Figure 2a). Statistical significance was found when PE triglyceride levels of supplement group was compared to PS level ($p<0.05$). When looking at the control group also there was a significant difference between the PE and PS levels ($p<0.05$). When inter-group comparisons of triglyceride levels were considered, there was significance difference at basal level ($p<0.05$) (Figure 2b).

There was statistical difference in both groups between basal and PE and PE and PS phases when HDL levels of the supplement and control group were considered ($p<0.05$). When inter-group comparisons of HDL levels were considered, there was no significance at any phase ($p>0.05$) (Figure 3a). A significant difference was detected only between PE and PS when levels of LDL of the supplement and control groups were compared at all phases ($p<0.05$) In inter-group comparisons of LDL levels, there was no statistical significance ($p>0.05$) (Figure 3b).

A statistically significant difference was found between basal and PE and PE and PS phases when GGT levels of supplement group were examined ($p<0.05$). There was no statistically significant difference at any

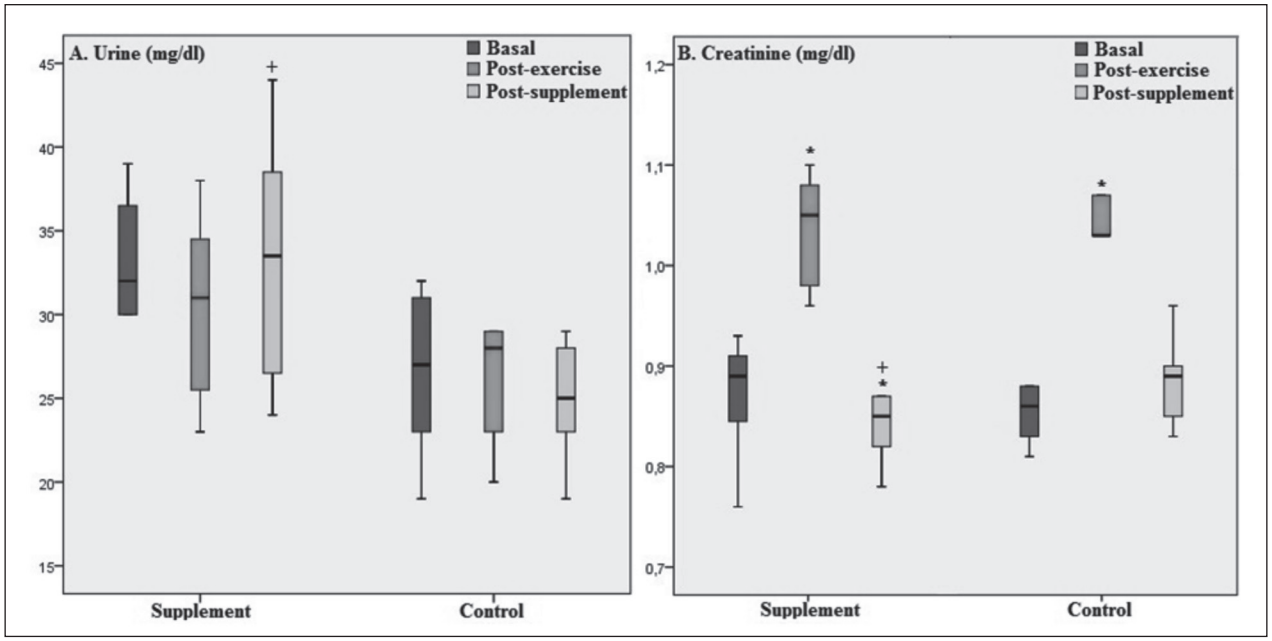


**Figure 1.** Changes in the serum urine (mg/dl) and creatinine (mg/dl). *Significant difference compared with basal ($p<0.05$). + Significant difference compared with post-exercise ($p<0.05$). #Significantly different between supplement and control groups ($p<0.05$) (M±SEM).

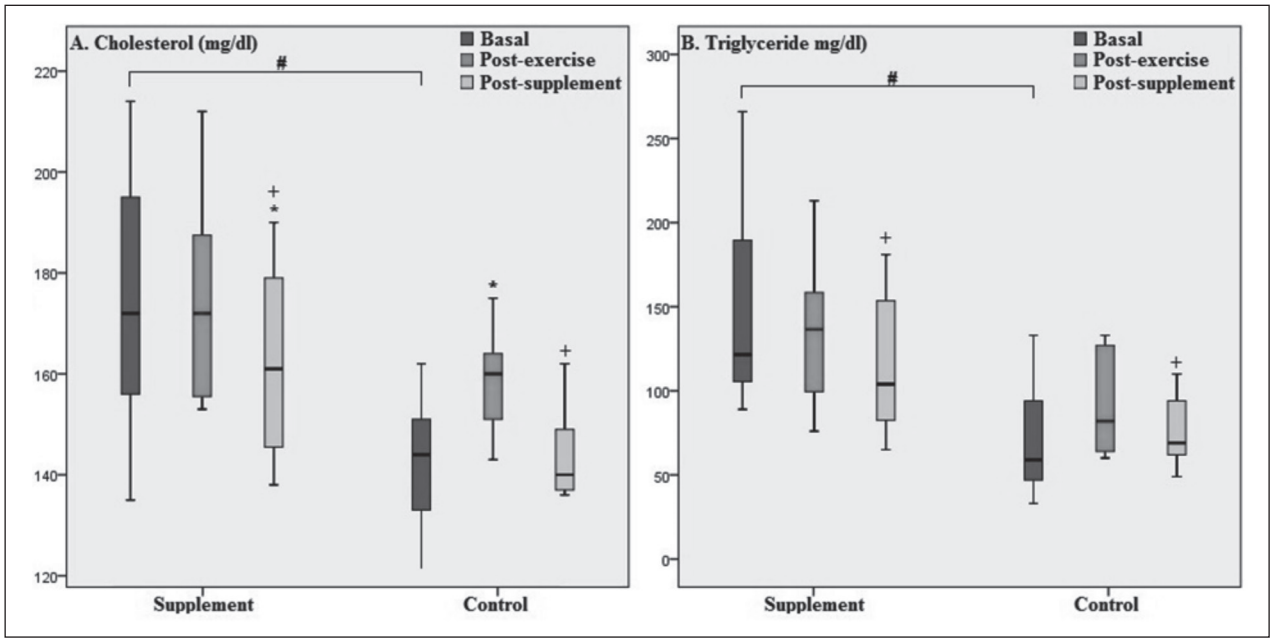


**Figure 2.** Changes in the serum cholesterol (mg/dl) and triglyceride (mg/dl). *Significant difference compared with basal (p<0.05). + Significant difference compared with post-exercise (p<0.05). #Significantly different between supplement and control groups (p<0.05) (M±SEM).

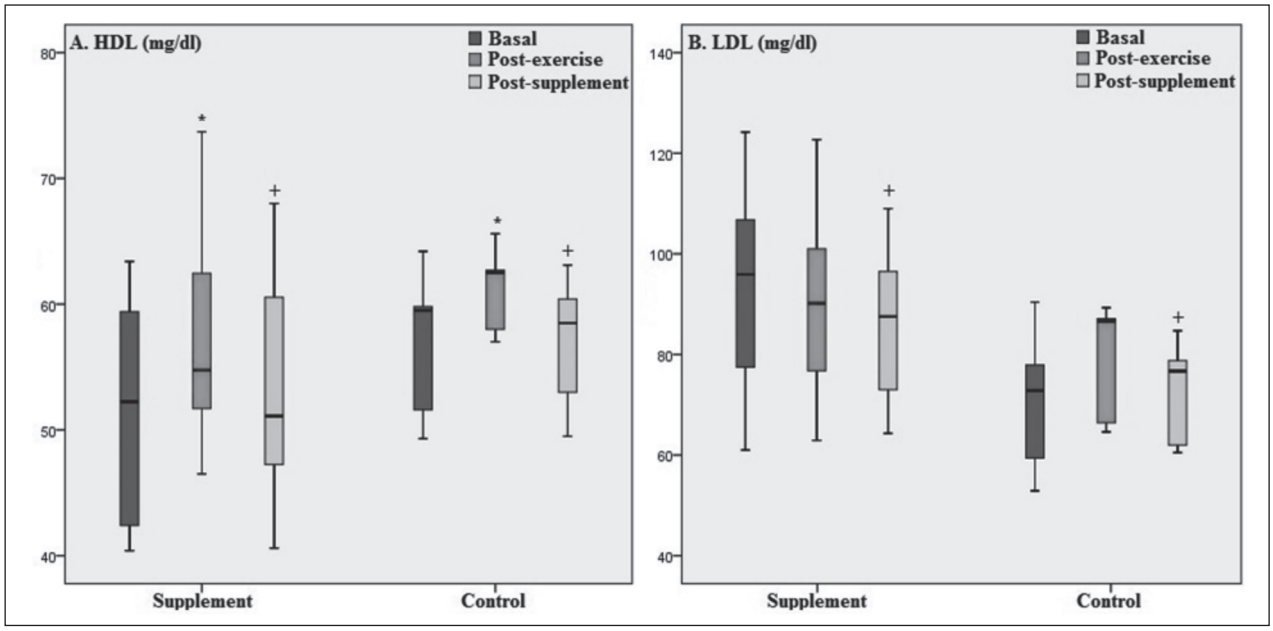


**Figure 3.** Changes in the serum HDL (mg/dl) and LDL (mg/dl). *Significant difference compared with basal (p<0.05). + Significant difference compared with post-exercise (p<0.05). #Significantly different between supplement and control groups (p<0.05) (M±SEM).

phase when serum GGT levels of control group were considered (p>0.05)(Figure 4a). In addition, when ALP values of both the supplement and control group were compared in groups statistical significance was found in all phases (basal with PE, basal with PS, PE and PS) (p<0.05). When inter-group comparisons were considered, no statistically significant difference was found at any phase (p>0.05) (Figure 4b).

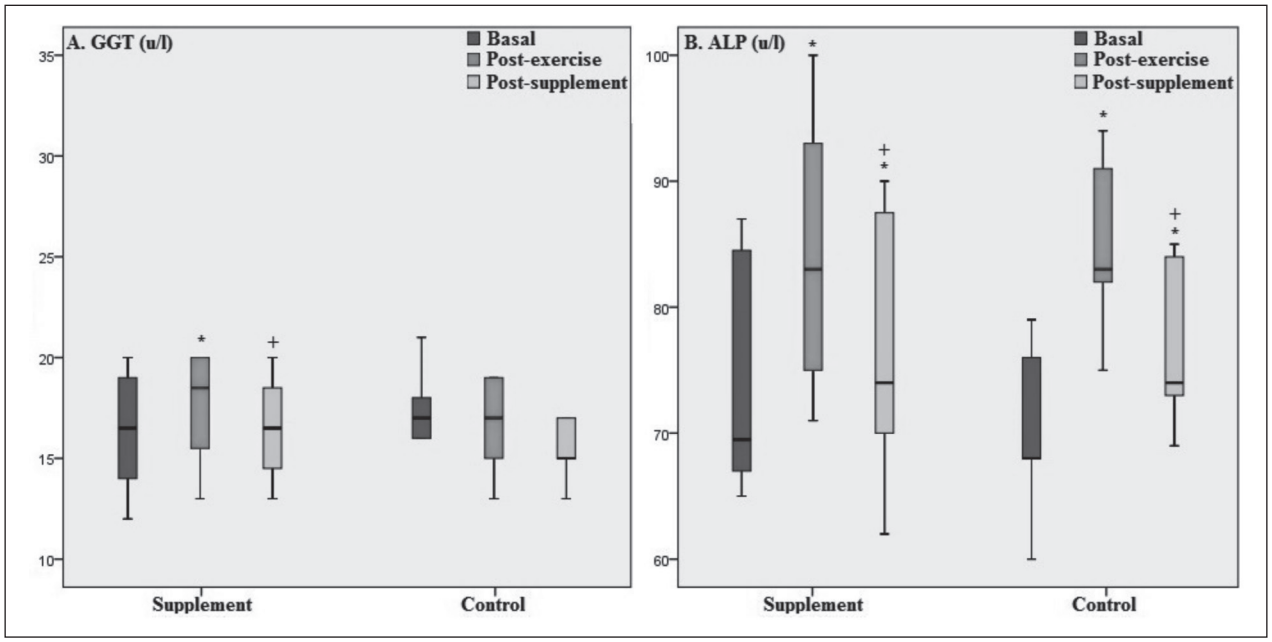


**Figure 4.** Changes in the serum GGT (u/l) and ALP (u/l). *Significant difference compared with basal (p<0.05). + Significant difference compared with post-exercise (p<0.05). #Significantly different between supplement and control groups (p<0.05) (M±SEM).

## Discussion and Conclusions

All athletes need body fuel, liquid and nutrition to bring their performance levels to the best. Roles of sports nutritionist here are, choosing products that would protect general health and make it fine while recommending proper supplements needed before, during and after the training (1). Because health has a place like 16% when popular reasons of supplement usage are examined (17). Also, there are many conditions that nutritional supplements play very important role for health and performance (18). While this is the case, supplements athletes use to improve their performance should create no threat in terms of health. On the contrary, the expectations of athletes from supplements consumed to enhance performance are to also upgrade general health.

In the study presented; acute combined beta-alanine, vitargo (carbohydrate-electrolyte) and whey protein supplementation was made to the athletes after the exercise made until exhaustion. When physiological responses and hematological values received after supplementation were considered; biochemical changes were within the body's normal level ranges after intake of the supplement, while creating no risk in terms of metabolic balance, positive results in terms of overall health have emerged in some biochemical values in the group taking nutritional supplement.

In a study, it was established that 88% of college athletes in America are using at least one natural supplement, 58% of them are using 2 or more supplements (4). The contents and the effects of consumed supplements on metabolism are usually evident. However the use of 2 or more supplements which is quite common in athletes, brings the question of what impact would they do on metabolism when supplement are used in combination.

Many combined supplements of which their effects are proved in a scientific way are offered to consumers. Competition, effort to be superior and taking action for maximizing personal potential of athletes together with increased awareness about nutrition preferences to effect athletic performance caused an increase in combination of supplements that boost sportive performance. And manufacturers produce these products usually thinking that combined products would benefit more than a single product. Besides, the efficiencies of very few combined products given to athletes are proven scientifically (19).

In the results obtained from the study, it was seen that similar results were present when the basal, after exercise and after supplement hematological values of the supplement and control groups were compared. This circumstance shows that the acute supplementation does not cause a noxious change in biochemical values of athletes. Besides, the results suggest that rise and fall in hematological values are physiological effects of the workout. In addition, it was shown that supplementation could lead to positive results in terms of health when basal, training and post supplement creatinine, cholesterol, triglyceride, HDL and LDL values were compared between the two groups. These positive changes revealed that the combined supplements consumed with a good timing after the training brought the metabolism from the catabolic state to the anabolic state. If rise in sportive performance and a rapid recovery after exercise are added to besides these results, the net effect of conscious use of supplement on the athletes' life will be clearly seen.

It is already known that intake of important nutrients before, during and after exercise improves health, performance and recovery (20, 21). From a literary perspective; while some studies have found that the use of combined supplements has a positive effect on athlete's health and performance (22, 23, 24), the results of some studies suggest that combined supplementation does not benefit performance or metabolism (25, 26). When looking at other acute and chronic supplementation studies in literature; in the study Flakoll et al. (2004) made with U.S. Marine recruits, they gave protein/carbohydrate/fat to the experiment group, carbohydrate/fat to the control group and none to the placebo group for 54 days right after exercise. According to the emerging results, when three groups were compared, in the experimental group consuming the combination supplement a 33% reduction in referring to health facilities for general reasons, a 28% decrease in referring for bacterial / viral infections, a 37% decrease in referring for muscle and joint problems and a 83% reduction in referring for heat exhaustion. Flakoll et al. (2004) reported that combined supplementation improved overall health, reduced muscle pain, and had positive results on tissue hydration according to the emerging results.(27). Jialal and Grundy (1993) gave combined α-tocopherol, ascorbate, and beta-carotene supplements to 12 male subjects in their study and examined LDL changes in metabolism. In the obtained data, it was found that supplement intake had a blocking role on LDL. In the study done with rats, Maxwell et al. (2001) supplemented rats with L-arginine with drinking water for 4-8 weeks and measured their cholesterol levels. The researchers found that the supplements of L-arginine had no effect on the cholesterol levels of rats (29). In the study done with rats, Suzuki (2009) exercised the rats and performed L-arginine / L-ornithine loading. As a result of the study, supplementation facilitated the development of blood vessels in the feet of the rats, which is an additional effect in exercise; led to the development of capillary vessels in the sole and soleus muscles of both feet (30). In the study Jang et al. (2011) made with 9 training wrestlers, they divided wrestlers into three groups as placebo, carbohydrate and carbohydrate+BCAA+arginine and looked at some biochemical parameters. In the obtained data, they found an increase in glucose and insulin levels, decrease in glycerol, and non-esterified fatty acid concentration in carbohydrate and carbohydrate+BCAA+arginine groups according to the placebo group (31). In the study El-Kirsh et al. (2011) did with rats, they investigated the effect of combined L-arginine or L-citrulline supplements on biochemical parameters taken with high-fat and cholesterol diets. In the study subjects were divided into 6 groups. Those groups were determined as; group 1-control, group 2-basal diet+L-arginine, group 3-basal diet+L-citrulline, group 4-high fat and cholesterol diet (HFC), group 5-HFC diet+L-arginine, group 6-HFC diet+L-citrulline. They found that the combined supplementation caused a significant decrease in ALT and AST enzymes and stated that the supplementation had a protective effect for metabolism. In the study, there was significant increase in urea levels of group 3 and group 4 compared to that of group 1 and group 2, significant decline in urea levels of group 5 and group 6 compared to group 3 and group 4. Researchers explained this situation as that the supplement does not generate any damage in the kidney. In addition, the study shows that high-fat and cholesterol diet resulted in a significant decrease in HDL cholesterol, but caused the increase of all the other lipid parameters when compared with a control group. In

the study, biochemical parameters such as total cholesterol, creatinine, LDL cholesterol and HDL cholesterol of the subjects were maintained and in this results cholesterol, creatinine, LDL cholesterol values of subjects using supplement in high fat and cholesterol diet were found low and HDL cholesterol was found high. These results showed that supplementation caused in reduction of serum lipid profile and brought about the rise in HDL cholesterol. In addition, it showed the supplements prevented the damage that high fat and cholesterol diets give to the body (32).

As a result, it is possible to say that acute combined supplementation used after the exercise does not create a negative effect on biochemical parameters of athletes, on the contrary when the research result data were compared with control group, by looking at the basal, exercise and after supplementation values such as creatinine, cholesterol, triglycerides, HDL and LDL, the combined supplement intake showed positive results in terms of health. In addition, it showed that the combined supplements consumed consciously and with a good timing after the training could bring the metabolism from the catabolic state to the anabolic state and these generated biochemical responses would have positive effects on athletic performance and recovery. In the future works on the subject; a controlled lifestyle that can be created on athletes, chronic supplementation with a regular nutritional program and watching the changes in different biochemical mechanisms may reveal more clear information in terms of the result of the study.

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Original article

# The evaluation of body composition and anthropometric measurements of males aged 18-25 years, based on the regularity of physical exercise

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**Summary.** There were 200 participants in total, on a voluntary basis in the study. Of these participants, 100 were male students aged 18-25 years who exercised regularly and vigorously, and 100 were male aged 18-25 years who did not participate in any physical activity. The measurements of BMI, body fat composition, hip-to-waist ratio, and skinfolds measured at nine different anatomical areas of their bodies (pectoral, biceps, triceps, subscapular, abdominal, suprailiac, thigh, midaxillary and the medial part of the leg) using skinfold caliper were recorded in the Excel format. It was determined that there was a significant difference ($p<0.05$) between the BMI of those who exercised regularly and of those who did not, and that there were significant differences ($p<0.05$) between muscle weights, hip-to-waist ratio, body fat composition, total body water, and basal metabolic rates of the participants who exercised regularly and of those who did not. It was determined that the total body water was increased in the subjects who exercised regularly due to increased muscle weight and mass, their hip-to-waist and body fat ratios were lower, and basal metabolic rates were higher when compared to the non-exercisers. It was also determined that there were significant differences ($p<0.05$) between the two groups regarding of skinfold caliper measurements at nine different areas. When the data from the study, which is the first to include nine different anatomical regions, was evaluated, it was concluded that the sedentary lifestyle influences the onset of obesity and it can be partially treated with physical activity.

**Key words:** anthropometric measurement, regular exercise, athlete, physical activity, obesity

## 1. Introduction

Exercising can be defined as making a habit of performing physical activity on a regular basis, which is important for overall health (1). With the recognition of the relationship between a sedentary lifestyle and chronic disorders, the tendency to exercising is continuously increasing.

The World Health Organization defines sports or exercises as any physical movement produced by the skeletal muscles, requiring energy consumption (2). The increased prevalence of obesity, which is due to the improvement of socioeconomic conditions in developing and developed countries and the subsequent withdrawal from an active lifestyle, brings about many chronic disorders with it (3). It is known that exercising has positive impacts on obesity, coronary heart disease, hypertension, diabetes, arthritis, osteoporosis, dyslipidemia, depression, cancer, muscles, bones, and joints (4-6).

The most commonly used measure for overweight and obesity is the Body Mass Index (BMI), a simple index to classify overweight and obesity in adults. It is defined as the weight in kilograms divided by the square of the height in meters (kg/m2). Body mass index (BMI) is a measure of body fat based on height and weight that applies to adult men and women (6).

Body composition is defined as the relative weight ratio of fatty and fat-free tissues of the body (2), whereas anthropometry is measurement of physical parameters

**Table 1.** Classification of Overweight and Obesity by Body Mass Index (BMI) (7)

| Body Weight | BMI (Kg/M²) | Morbidity Risk |
|---|---|---|
| Underweight | <18.5 | Low (Clinical Problems High) |
| Healthy Weight | 18.5-19.9 | Middle |
| Recommended | 20.0-24.9 | Mid |
| Overweight | ≥25:00 | Lightweight |
| Pre-Obese | 25.0-29.9 | High Mid-Lightweight |
| Obese | ≥30.00 | High |
| Obese Class I | 30.0-34.9 | Very High |
| Obese Class III | 35.0-39.9 | Very High |
| Obese Class III | ≥40 | Extremely High |

(height, weight, circumferences, etc.) of individuals with different ages, genders, and nutritional status and determination of the body composition (fat and muscle tissues) (8,9). Anthropometric measurements are important for the evaluation of development and growth, the determination of the amount of lean tissue (muscle tissue) and fatty tissue, and also because it is an indicator of fat distribution in the body. The measurement of body weight, height, mid-upper arm circumference (MUAC), head circumference (HC), waist circumference, and hip circumference are commonly used anthropometric methods (9). Anthropometric data from adults is a good indicator for the evaluation and recognition of health status and diet, disease risk, and the comparison of different body types by comparing measurements from various regions of the body (6, 10). The purpose of our study was to investigate the presence and tendency to obesity together with the wellness status of 18-25-year old male individuals by comparing the body composition and anthropometric measurements to the reference values according to their status of regular exercise and to determine reference values for mildly overweight, overweight, skinny or underweight individuals.

## 2. Material and Method

There were 200 participants in total, on a voluntary basis in the study. Of these participants, 100 were male students aged 18-25 years from Police Vocational School of Higher Education who exercised regularly and vigorously, and 100 were male university students aged 18-25 years who do not participate in any physical activity. The study was conducted after the obtainment of the permission dated 06/11/2014 and No.: E.1459 from the Bitlis Eren University Ethical Committee. At least one day prior to the measurements, the participants were informed about the measurement process, and the measurement devices were introduced. The participants signed a "Voluntary Consent Form." For both groups, the BMI, body fat ratio, hip-to-waist ratio, and skinfolds from nine different anatomical areas of their bodies (the pectoral, biceps, triceps, subscapular, abdominal, suprailiac, thigh, midaxillary and the medial part of the leg) using skinfold caliper were measured and recorded in Excel format.

### *2.1. Anthropometric measurements*

#### *2.1.1. Measurement of height*

Measurements were made by using a Harpenden stadiometer (ADE/Hamburg MZ10020) ultrasonic height measurement unit with 0.1 cm accuracy (11) parallel to the floor at the level of the crown of the head, and without any clothes or accessories that might affect the measurements.

#### *2.1.2. Measurement of Body Mass Index (BMI), Body Fat Composition, Hip-to-Waist Ratio, Total Body Water, Basal Metabolic Rate (BMR)*

Measurements were performed with InBody230 (MW160) Bio-Impedance Body Analysis Machine with the participants not wearing anyclothing except for shorts, at least 12 hours after the last meal, and at a state of rest.

#### *2.1.3. Skinfold Thickness (SFT) measurement*

The thickness of skin was measured using special callipers named Holtain Skinfold Calipers (Holtain, Crymch, Dyfed, UK) on nine different anatomical regions of the body (12) (pectoral, biceps, triceps, subscapular, abdominal, suprailiac, thigh, midaxillary and the medial part of the leg) on the dominant side of the body.

The measurement of skinfold thickness was done by holding the skin and subcutaneous fat with the

thumb and index finger (13), pulling it in the direction of the fold and away from the muscle tissue. The value on the skinfold indicator was recorded in millimeters. This process was done in all subjects by a single person to minimize the chance of error.

*2.1.4. Bi-iliac, bitrochanteric, and biacromial diameter measurements*

Measurements were done using Holtain Harpenden Anthropometer measurement device (14), subjects wearing nothing but shorts.

*2.2. The Physical Activity Status*

*2.2.1. Moderate-level physical activities*

These activities are accompanied by small increases in heart and respiratory rates. Paced walking, dancing, gardening, low-intensity swimming and biking are considered moderate-level physical activities (15). To be able to consider this type of activity as regular, it must be done at least 5 times a week and 30 minutes a day.

*2.2.2. Moderate-severity activities*

This is the type of activity that requires moderate physical exertion and causes a small increase in respiration, performed for at least 10 minutes at a time (15).

*2.2.3. Vigorous physical activities*

This level of physical activity requires immense physical effort and produces prominently increased respiration. These activities are performed for more than 10 minutes at a time (15). The participants of this study performed vigorous exercise on a regular basis.

*2.3. Statistical analysis*

All data were statistically analysed using STATGRAPHICS Centurion XVI (Version 16.2.04), STATGRAPHICS plus 5.1 (Statpoint Technologies, Warrenton, VA, USA), or SPSS v21 (IBM Corp., Chicago, IL, USA) and means ± SD were calculated for every parameter measured.

## 3. Results

*3.1. Demographic characteristics*

The BMI distributions and means of BMI, age, and height of the exercising and non-exercising participants of the study were given in Table 2.

*3.2. Body Mass Index, Body Fat Ratio, Hip-to-Waist Ratio, Muscle Mass, Total Body Water, Basal Metabolic Rate (BMR) Analysis Results*

In the study, it was determined that there was a significant difference ($p<0.05$) between the BMI of exercising subjects and non-exercising subjects and the mean BMI value of exercising subjects was lower.

It was determined that there was a significant difference ($p<0.05$) between the exercising and non-exercising subjects regarding muscle mass, hip-to-waist ratio, body fat ratio, total body water, and basal metabolic rate. It was determined that, due to increased

**Table 2.** The exercising and non-exercising participants of the study; means of BMI, age, and height avarage

| **Avarage** | **Regular Exercise** | | **Not Regularly Exercising** | |
|---|---|---|---|---|
| BMI | 22.66±1.90 | (n=100) | 23,57±1.77 | (n=100) |
| Age | 21.04±1.58 | (n=100) | 20.90±1.52 | (n=100) |
| Height | 178.86±5.74 | (n=100) | 177.64±5.23 | (n=100) |

| **BMI (kg/m²)** | **Regular Exercise participants (n)** | **Not Regularly Exercising participants (n)** | **Total** |
|---|---|---|---|
| 18.5-19.9 | 10 | - | 10 |
| 20.0-24.9 | 80 | 82 | 162 |
| 25.0-29.9 | 10 | 18 | 28 |
| General Total | 100 | 100 | 200 |

**Table 3.** Regular exercise status of groups; body fat ratio, hip-to-waist ratio, muscle mass, total body water, basal metabolic rate (BMR) analysis values (*n:200*)

| Measurement Parameters | Exercise Situation (F-value) |
|---|---|
| Body Mass Index (BMI) | 0.2394 * |
| Percentage Body Fat (PBF) | 0.5937 *** |
| Waist-Hip Ratio (WHR) | 0.5183 *** |
| Muscle Weight | 0.4338 *** |
| Total Body Water | 0.3656 *** |
| Basal Metabolic Rate (BMR) | 0.2394 *** |

** 0.01<P<0.05 *** P<0.001*

muscle weight and mass, the total amount of water in the body was higher, the hip-to-waist and body fat ratios were lower, and basal metabolic rates were higher in the subjects who regularly exercised when compared to the non-exercisers.

*3.3. Skinfold thickness analysis results*

It was determined from measurements of the nine different anatomical areas using skinfold caliper that there was a significant difference (p<0.05) between the exercising and non-exercising subjects.

*3.3. Bi-iliac (Navel), Bitrochateric (Hip), Biacrominal (Shoulder) Diameter Analysis Values*

It was also determined that the differences between the bi-iliac, bitrochanteric and biacromial diameters of the exercising subjects and non-exercising subjects were not significant (p>0.05).

**Table 4.** Regular exercise status of groups; skinfold thickness measurements analysis values (*n:200*)

| Measurement Parameters | Exercise Situation (F-value) |
|---|---|
| Pectoral | 0.4070 *** |
| Subscapula | 0.4209 *** |
| Biceps | 0.4919 *** |
| Triceps | 0.5033 *** |
| Suprailiac | 0.4932 *** |
| Abdomen | 0.5292 *** |
| Middle Axillary | 0.4132 *** |
| Femur | 0.2578 *** |
| Leg Medial | 0.5159 *** |

**** P<0.001*

**Table 5.** Regular exercise status of groups; diameter measurements analysis values (*n:200*)

| Measurement Parameters | Exercise Situation (F-value) |
|---|---|
| Bi-iliac (Navel) Diameter | 0.0619** |
| Bitrochateric (Hip) Diameter | 0.1743** |
| Biacrominal (Shoulder) Diameter | 0.1690** |

*** P>0.05*

## 4. Discussion

Regularly performed physical exercise ensures that the muscles, bones, joints, and cardiovascular system function optimally (17). With exercise, the respiratory, digestive, excretory, and skeletal systems can be maintained in the ideal condition (18). Individuals who remain sedentary for too long can lose their mobility and may suffer from poor health. Systematic sports activities have a definite positive effect on the body composition (19). The body is mainly composed of fat tissue, bones, muscle tissue, other organic material and extracellular fluids. The body composition can be divided into fatty and fat-free masses (20). The fat-free mass involves muscles, bones, water, nerves, blood vessels and other organic material. The fatty mass can be classified as subcutaneous fats, stored fats, and essential fats (21).

The results of the study indicate that in the exercising individuals, BMI, the body fat and the hip-to-waist ratios are lower, and therefore, exercise reduces fatty mass in the body, the BMI, and the obesity risk. It is also shown that muscle mass and total body water is higher in exercising subjects when compared to non-exercising ones; therefore, it can be suggested that exercise increases muscle mass, strength, speed, and endurance (22). While the body fat mass decreases, the muscle mass and endurance improve in individuals who exercise regularly. Besides, a sedentary lifestyle can lead to energy input and output imbalance, and thus, may lead to increased prevalence of obesity.

It was determined that the BMR of the regularly exercising individuals were higher than those who did

not (3) and the exercise-related increase in muscle mass elevated the BMR. Another study has found that the aerobic metabolic capacity increased in subjects who participated in exercises such as walking, running, gymnastics, swimming, and ball games (22).

Significant differences ($p<0.05$) were found between the exercising and non-exercising individuals regarding skinfold thicknesses measured with a skinfold caliper from nine different anatomical regions (pectoral, biceps, triceps, subscapular, abdominal, suprailiac, thigh, midaxillary and the medial part of the leg). It was shown in conducted studies that exercise reduces fatty mass, particularly leading to a reduction of the subcutaneous fat thickness in the abdominal region (10, 13, 23-24,).

Similar to this study, Kayıhan et al. (25) have found significant differences between exercising and non-exercising individuals. However, in contrast with this study, the studies performed by Twisk et al. (26) and Düzgün (27) have indicated that the differences between their exercising and non-exercising groups regarding subcutaneous fat thickness were insignificant. This may be a result of differences in the intensity and frequency of the workouts, and the number of participants in the studies.

Even though no significant differences were discovered between the bi-iliac, bitrochanteric, and biacromial diameters of the exercising and non-exercising groups measured by Holtain Harpenden Anthropometer, this might be associated with the low number of participants and the similarity of BMI in the two groups. A study conducted with a higher number of participants with wider ranges of BMI and age groups can better address this issue.

**To conclude:** The research done until now have proven that exercise improves the cardiovascular and respiratory systems, the musculoskeletal system, hypertension, diabetes, being overweight or obese, cholesterol, mental clarity, psychological balance, stress, and cancer (10).

- Today, obesity is a disorder leading to a great financial loss all over the world. This problem can be taken under control by providing better living conditions, healthy nutrition, and step-by-step improved lifestyle changes.
- Although the regularly exercising group in this study included male students from Police Vocational School of Higher Education, exercise is not limited to certain age and vocational groups, only. People from every age group, background, and occupation should be informed with the importance of regular exercise and be provided with regular courses if necessary. It should not be forgotten that exercise can prevent many health problems and exercise should not be considered as treatment, but a preventive measure for obesity and related disorders.
- In this study, we have once again proven that, in the treatment of obesity, it should be aimed to increase the energy expenditure through regularly performed exercise, activity, and sports, in addition to the restriction of energy input.

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Original article

# "Mediterranean Diet 'reflections' ". Estimating adherence to the Mediterranean diet through secondary data

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**Summary.** *Purpose:* to compare several countries against many Mediterranean adherence indices, calculated by looking at 19 European Member States. The value of a population-level Mediterranean Diet Index, the Mediterranean Adequacy Index (P-MAI) is at the core of the analysis. *Design/methodology/approach:* the EFSA's Concise European Food Consumption Database (mean g/day/per capita) and the FAO-FBS dataset (grams and calories/day/per capita values) were used as the unique sources currently available, in order to derive a simple yet harmonised secondary-data framework, which could serve for policy analysis and policy making therein of. *Findings:* The adherence to a Mediterranean-like dietary pattern outlines a general rank correlation among countries, and a broader north-south divide within Europe. Scores remain relatively stable across time. Although there has been a decrease in Mediterranean adherence in southern Europe, some central and northern European regions have seen gains. *Research limitations/implications:* Several data gaps do not allow a full comparison across all the indices used (i.e., lack of foodstuff detail of key-foods of the Med diet). A further problem of Med-adherence indices is that it does not consider the overall caloric intake. *Practical implications:* The relatively low discriminatory power of the emerging clusters of countries, reflecting the national diets- limits their usefulness in terms of policy-making recommendations. Furthermore the indices used were originally built on first-hand data (i.e., cohort studies relying on real persons), and not on aggregated mean-median values at population level (secondary data). Social implications In a period in which the interest for the health outcomes of the Mediterranean diet is on the rise in terms of preventive medicine, the P-MAI is an interesting indicator due to its user-friendliness, which allows the classification of European countries' diets using food intake data. *Originality/value:* Mediterranean adherence indices may be useful as synthetic indicators for monitoring the evolution of diets and for identifying sub-regions with similar dietary patterns or changes. The P-MAI index in particular, due to its simplicity, may help to monitor the overall healthiness of national diets, and could help to inform subsequent nutrition policies, including emerging labelling provisions both at National and European level, in order to achieve public health targets (i.e., reduction of NCDs).

**Keywords:** Mediterranean Diet, National Food Patterns, monitoring

## Introduction

The "Mediterranean Diet" is a dietary pattern relying for the most part on fresh fruits&vegetables (including seeds, nuts and legumes), whole grains and olive oil, and on relatively low consumption (2-3 times per week) of animal (by)products such as meat and dairy products (but with high intake of fish), and a sparing ethanol intake, generally during meals (1,2).

The Mediterranean Diet is globally recognised as one of the healthiest dietary patterns, and one of the most studied as well. In spite of existing only one model, it is apparent that there are different regimes which may fit to the "Mediterranean Diet" definition, also outside the key-countries of the Mediterranean basin, and which proved to be healthful as well (3). The health and social benefits of the Mediterranean diet have been extensively documented (4-8) and although

a causal link between Mediterranean Adequacy and mortality (prevention) has been suggested (9), it has not been definitely demonstrated (10). Since 2010 UNESCO awarded the Med Diet with the Intangible Cultural inheritage status.

However, is still unclear if Mediterranean countries maintained along time adherence to the Med diet, or -due to increasing trade in processed foods, long food chains and globalisation- transited to other dietary patterns. Another concern regards the real "existence" of something like the Mediterranean diet outside abstractions. Criticisms have been raised about the delimitation and definition of "what is" eventually a Mediterranean dietary pattern.

This paper tries to answer to both questions. Even if there are several metrics and underneath biological rationales endorsing Mediterranean-style diets (linking food items to health outcomes), there is a core of food which eventally cannot be missed inside a balanced Med Diet. Also, as authors are going to demonstrate, the resilience of dietary patterns-including the Med diet in southern EU countries is apparent, in spite of a general loss of adherence to the original model, and gains from Northern countries due to specific policy making interventions along the last decades.

## The challenge of comprehensive database to monitor food patterns

National dietary assessment and its consequent monitoring represent a key aspect in public health management. While cross-country comparisons are complex to study, they have received support from the European Commission (EC) (11,12). Furthermore, complicated, resource-intensive, nationwide food consumption surveys, which aim to estimate dietary patterns, are not currently carried out on an annual basis. This was examined in the European Food Consumption Survey Methods project (EFCOSUM project) (13) and subsequently taken on by the European Food Safety Authority (EFSA), which then went on to establish the Network of Food Consumption Data (former Expert Group on Food Consumption Data) and set up the Concise Database (14,15) and the Comprehensive Database (16), in order to collect EU- harmonized data. The increasing attention paid to comparable, compatible and reliable individual food consumption data –with in mind a wide EU- risk assessment standardization- led EFSA to launch the EU-MENU programme to support Members State in collecting harmonized data (17).

This seems particularly relevant considering preliminary cues for the worsening of national dietary patterns in recent decades, at least with respect to a number of EU countries. According to a set of 88 health indicators collected in the European Community Health Indicators Monitoring (ECHIM, www.echim.org/) database, fruit and vegetable consumption ranks 49$^{th}$ and 50$^{th}$ respectively (www.echim.org/indicators.html) reflecting a minor importance to the health-status - in front of other, more prominent indicators.

Furthermore, the number of EU countries that failed to comply with WHO guidelines for sugar consumption has increased from 1961 to 2003 from 8 to 10 and in the last 40 years, sugar energy shares converged with all countries at around 11% level (18, 19). Although- according to identical sources during the same period-, countries with an adequate intake of fruits and vegetables more than doubled. The Food Balance Sheets (FBS) published by the Food and Agriculture Organisation (FAO) of the United Nations[1] showed an increase in the consumption of animal protein and saturated fat in the same last 40 years timeframe, particularly within Mediterranean countries, such as Greece, Italy, Spain, and Portugal (20). However, there remain remarkable differences across countries, especially in terms of saturated fat intake (Fig. 1), in spite of an apparent convergence phenomenon: countries with high consumption levels of saturated fatty acids (15%) like Finland or Ireland reduced them to close to the recommended maximum, (18,19). Given the difficulties associated with analysing individual dietary patterns (16,21), and hence in inferring how dietary patterns converge or diverge with the Mediterranean diet across countries and across time, it would be useful to examine existing aggregated population-level food consumption datasets. This is an "a priori approach" of food consumption patterns – as

[1] FAO – Food and Agriculture Organisation of the United Nations-Food Balance Sheet in FAOSTAT (http://faostat3.fao.org/faostat-gateway/go/to/home/E).

defined by Efsa- and "is based on prevailing knowledge concerning favourable or adverse effects of various dietary constituents. Diets are assessed for the presence or absence of certain food or nutrient characteristics, and the resulting score is then operationalised as a dietary exposure variable" (22)- but without an empirical, *a-posteriori* assessment of the health outcomes as integral part of the research. The Mediterranean Adequacy Index (MAI) is one of the most predictive indicators of a Mediterranean diet (9, 23, 24). The MAI inversely correlates with 25 years of figures for deaths from coronary heart disease (6).

This study aims to analyse the possibility of building a Mediterranean diet adequacy index-, the Population level Mediterranean Adequacy Index, P-MAI- to allow for monitoring dietary trends using figures from national Food Balance Sheets (FBS), which are published annually on the FAO website (2011).While the index *per se* is not new (MAI), it has never been applied before to aggregated data at population-level (P-MAI). At the same time, more precise *food-intake* data collected at EU Member State level (EFSA Concise Food Consumption Database, hereinafter "CONCISE") are available, but under a more limited timeframe (*i.e., differently from FAO FBS,* only in selected years of surveys, not harmonised at the EU level and with each Member State having different surveys years).

These two sources of data –even if different by nature-enable us to draw some kind of comparison on specific periods of time (i.e. *the years* during which the dietary surveys were carried out in the Member States based on individual national dietary surveys (14).

Therefore, the index proposed in this study is the P-MAI (Population-level Mediterranean Adequacy Index), which provides geographical and temporal insights into food consumption patterns across the EU Member States. In this way, it creates a *user-friendly* tool for public health policy-making, at a time when there is increasing focus on food-related diseases and costs.

## Methods

The P-MAI, as previously stressed, is an extension of the original (and well-established) concept (MAI) proposed by Alberti and Fidanza (2004) to measure the adequacy of *national diets* against the Mediterranean diet. Both MAI and P-MAI are calculated as the *ratio* between the summed weight (or the summed energy value) of food items from the core Mediterranean Diet (vegetables, fruit, cereals, red wine, vegetable oils, potatoes and fish) and of non-core foods (meat, dairy products, animal fat, eggs and sugar) (9 ,25).

The P-MAI was computed using the average intake of 15 food groups and 21 sub-groups from the EFSA CONCISE European Food Consumption Database (g/day/per capita intakes), hereafter referred to as the CONCISE database/data, and from the FAO Food Balance Sheets (FBS database/data) (g or kcal/day/per *capita* intakes 1961-2007). The CONCISE database comprises mean food consumption data for adults (aged 16-64 years) departing from different food categories. FBS data were calculated by dividing the total amount of food available for consumption by the aggregate population of a given country[2].

It is important to point out that some foods were not included in the CONCISE database, for example, vegetable oils and red wine. Red wine was also not available in the FBS database. Instead, 'wine' (FBS database) and 'wine and substitutes' (CONCISE database) were used. Furthermore, as vegetable oils were not recorded separately from animal fats, the broader category 'fats' was excluded when using the CONCISE database.

Countries were selected based on national surveys availability inside the CONCISE and consequently, more available FBS data were in turn included for comparison. This means that all FBS data were aligned to those in the CONCISE database according to survey year. However, in a separate analysis relying on longitudinal FBS data only -Spain and Greece were added, as examples of Mediterranean countries. Whilst data for Estonia in 1961 was not available in the FBS database. In fact, in order to assess historical trends, P-MAI scores were estimated in both 2007 and 1961 using FBS data (the only dataset allowing for this diachronic assessment).

[2] The total amount is obtained by examining production and *import* figures, less *export* and *re-use* figures (supply fed to livestock or used for seed, and losses during storage and transportation). divided by the national population level for the given year. FBS are inherently advantageous as they take into account both *domestic and non-domestic* food consumption (catering, restaurants, etc.).

Energy intake was derived from the FBS (grams instead were used for CONCISE).

All data was entered into Excel spreadsheets versions (2010 and 2013) and Scott's choice analysis, was used to identify the number of classes with internal and external consistency (26). The Scott's choice test is a simple rule for describing an optimal grouping for the identification of clusters of countries (see Figure 1 below).

Concordance between the different elaborations of P-MAIs (CONCISE vs. FBS, FBS grams and FBS calories, and FBS time series) was measured using Spearman's rank (ρ) and the Kendall's Tau (τ) correlational analysis. Other correlations were included once added other diet-focused indices, in order to compare the resulting classification with that determined by P-MAI.

The indices are:

a) A simplified form of the Diet Quality Index for the Mediterranean Region (Med-DQI) developed by Gerber (2006)(27). The Med-DQI is a screening tool which gives scores (from 0 to 2) for the intake of the following food items, meats, olives, fish, cereals, fruit and vegetables, as based on Table 1.
   With regard to the analysis of the Med-DQI, neither cholesterol nor SFAs were included –in spite of being present in the original DQI- due to a lack of European population-level data. Instead, to make directly comparable the DQI with the other Indexes, the complement to 10 of the Gerber's Index was calculated, such that the higher value obtained, the better the diet.
b) The Global Nutrition Index (GNI), (28), accounting for three indicators of nutritional status: deficits, excess, and food security.
c) The Mediterranean Score (29). This score seizes the adherence to the Med-diet and relies on specific cut-off points for healthy vs unhealthy foods (i.e. 1 point for healthy foods such as cereals, fruit, vegetables and legumes, fish and moderate ethanol amounts; 0 points for 'unhealthy', non-Mediterranean foods such as meat and dairy products). Unfortunately, the ratio between monounsaturated/saturated fats, as originally indicated in Trichopoulou (2003)(29) could not be provided. Therefore, olive oil consumption versus animal fat consumption was used instead as a proxy for the monounsaturated fats-saturated fats ratio. Nor was it possible to analytically separate legumes from vegetables using the CONCISE database.

$$h = \frac{3{,}5 \cdot S}{\sqrt{N}}$$

**Figure 1.** The Scott's choice test for the optimal number of classes/members of a class

Other indices were also initially considered within this analysis, namely, the Mediterranean-Style Dietary Pattern Score (MSDPS) proposed by Sanchez-Villegas *et al*. (2002) (30); the Mediterranean Dietary Pattern (MDP) by Rumawas *et al*. (2009) (31); and the Mediterranean diet score by Panagiotakos *et al*. (2006) (32). However, these were *subsequently excluded* either due to a lack of available data (i.e. on *trans* fatty acids), or due to other classification difficulties.

A one-way analysis of variance (ANOVA) was performed to measure the impact of the aforementioned data sources (CONCISE or FBS) as a major contributor to changes in the P-MAI value, in order to depurate results from *dataset effects*. The rank correlation coefficients (the Spearman's Rho ρ and the Kendall Tau τ) were used in the analysis to measure correlations among GNI and Med-DQI with the other indices.

**Table 1**. Scoring system derived as simplification of the Diet Quality Index for Mediterranean Region

| Scores | Meats (g) | Olive oil (ml) | Fish (g) | Cereals (g) | Vegetables+fruit (g) |
|---|---|---|---|---|---|
| 0 | <25 | >15 | >60 | >300 | >700 |
| 1 | 25-125 | 15-mag | 60-30 | 300-100 | 700-400 |
| 2 | >125 | <5 | <30 | <100 | <400 |

## Results and Discussion

Results showed that, the estimated Population-level Mediterranean Adequacy Index (P-MAI) scores in European countries when estimated from average food intake from the CONCISE (Table 2) varied from 0.86 to 2.34, whereby higher scores indicated increased adherence to a Med-Dietary pattern.

FBS data were aligned to those in the CONCISE database according to survey year[3].

From the results obtained, a geographical gradient can be seen in Table 3, for example, Italy was among the highest in terms of P-MAI scores according to all three calculations (P-MAI, MDQI, and MSC). In general, a North-South trend can be observed with northern countries in the cluster of lower P-MAI scores (i.e. lower adherence to the Mediterranean diet). At the lowest levels, Scandinavian countries maintain their ranking in the first two (lower adherence) clusters, despite differences between the CONCISE database and FBS database computations. The Netherlands, Iceland and Finland remain in the first cluster, whereas Norway moves from the first cluster to the

[3] Where figures in the CONCISE dataset referred to multiple years, the mean of the corresponding years in the FBS was used (e.g. if CONCISE(country i)1986-87 was the reference period of the survey, then a mean of FBS(i)1986 and FBS(i)1987 was calculated for country i).

**Table 2**. P-MAI (Population-level Mediterranean Adequacy Index) scores for the aggregated average national diets, estimated by food weight (g) from the FAO Food Balance Sheets ("FBS") and the EFSA CONCISE database ("CONCISE"). For comparability reasons

| | FBS g* | CONCISE g* (mean values) | Delta (% difference between the 2 values) | Delta (absolute g) | Years of reference (EFSA) |
|---|---|---|---|---|---|
| AUT | 1.18 | 2.20 | 86.42 | 1.02 | Average 2005-2006 |
| BEL | 1.14 | 2.12 | 85.94 | 0.98 | 2004 |
| BGR | 1.35 | 1.88 | 39.35 | 0.53 | 2004 |
| CZE | 1.13 | 1.59 | 40.30 | 0.46 | Average 2003-2004 |
| DEU | 1.05 | 1.67 | 59.29 | 0.62 | 1988 |
| DNK | 1.09 | 1.31 | 20.63 | 0.22 | Average 2000-2001-2002 |
| EST | 1.39 | 1.49 | 7.23 | 0.10 | 1997 |
| FIN | 0.81 | 0.86 | 6.34 | 0.05 | 2002 |
| FRA | 1.1 | 1.70 | 54.25 | 0.60 | 1999 |
| GBR | 1.31 | 1.53 | 16.97 | 0.22 | Average 2000-2001 |
| HUN | 1.33 | 1.48 | 11.01 | 0.15 | Average 2003-2004 |
| IRL | 1 | 1.67 | 66.80 | 0.67 | Average 1997-1998-1999 |
| ISL | 0.98 | 1.01 | 3.48 | 0.03 | 2002 |
| ITA | 1.5 | 2.34 | 56.30 | 0.84 | Average 1994-1995-1996 |
| NLD | 0.79 | 1.17 | 48.20 | 0.38 | Average 1997-1998 |
| NOR | 1.07 | 0.95 | 11.11 | 0.12 | Average 1993-1997 |
| POL | 1.47 | 2.30 | 56.43 | 0.83 | 2000 |
| SVK | 1.42 | 2.26 | 59.26 | 0.84 | 2006 |
| SWE | 0.78 | 1.28 | 63.78 | 0.50 | Average 1997-1998-1999 |

**EFSA, European Food Safety Authority; FBS, FAO-Food Balance Sheets. P-MAI calculated as the ration between Med foods (vegetables, fruit, cereals, red wine, vegetable oils, potatoes and fish) and of non Med foods (meat, dairy products, animal fat, eggs and sugar).*
*Austria (AUT); Belgium (BEL); Bulgaria (BGR); Czech Republic (CZE); Germany (DEU); Denmark (DNK); Estonia (EST); Finland (FIN); France (FRA); United Kingdom (GBR); Hungary (HUN); Ireland (IRL); Iceland (ISL); Italy (ITA); the Netherlands (NLD); Norway (NOR); Poland (POL); Slovakia (SVK).*

second from the CONCISE database to the FBS database. Sweden moves from the second to the first and Denmark remains in the second cluster in both the CONCISE and FBS calculations. Interestingly, when adopting the synth-MDQI, Norway scores in the first cluster of Mediterranean Diet adherence. However, this may be biased, as cholesterol and SFAs were not included in this synth MDQI, and historically Nordic countries have a high intake of these, as observable when considering food matrices of departure. In both the CONCISE and FBS databases, Poland, Slovakia and Italy showed/had the best Mediterranean adherence scores.

Italy shows relatively good fruit and vegetable consumption (respectively 203g/day and 249g/day, about 4 to 5 portions, against a virtual recommendation of at least overall 4 portions-or 400 g/day- from WHO in 1991) (33), low meat intake (137g/day- against the standard Med Diet advice of a moderate consumption of 2-3 servings per week- no WHO recommendations here) and low sugar intake (19g/day.- well below the 10% of total energy intake as suggested by WHO). In general, Italy has a more homogeneous ranking along the different datasets used with only minor variations in the ranking in response to the use of different Med Diet indicators or datasets used.

The high P-MAI value (i.e. adherence to the Mediterranean diet) for Italy is not surprising. However, other figures require insight into the data in order to be explained further. For example, taking into account the CONCISE database result for Austria, the relatively high P-MAI (2.20) was due to its relatively high fruit and vegetable consumption (202g/day and 211g/day respectively, and 59g/day of potatoes), as well as its low intake of dairy products (171g/day) and sugar (23g/day). As regards Poland, which has a P-MAI value of 2.30 in the CONCISE database, we note a high consumption of vegetables (292g/day), potatoes (304g/day) and fruit (282g/day). Although consumption levels for meat (259g/day) and dairy products (181g/day) are high, the overall P-MAI remains relatively good and potatoes play a key role here, as they are considered as 'vegetables' inside the traditional P-MAI score (even if this is questionable from a public health perspective: in the UK potatoes are not valid for the "5 a day" F&V purposes- see the Discussion section later on). We should however take into account here the fact that the FBS P-MAI based on grams is slightly lower than he FBS P-MAI based on kilocalories (1.47 and 1.49). This could mean that allegedly calorie-dense, healthy foods play some minimal role in meliorating the score (again, potatoes, or alcohol).

As for Germany, according to the CONCISE database, vegetable consumption is relatively high (252g/day, and 125g/day for potatoes) as is fruit consumption (190g/day), while dairy product consumption stands at 313g/day. Beer consumption, which covers 184g/day of the 231g of alcoholic beverages consumed on a daily basis, is not taken into account in this computation because of the potential bias of the indicator used (excessive consumption, which is unhealthy, equally enhances the score a higher value since - to determine the "right amount" of alcohol to be consumed in order to have health benefits- no thresholds are in place for the traditional P-MAI- as on the contrary, other indicators do, such as the MDS).

In fact, FBS-based computations for Germany show lower P-MAI values (0.97 based on kilocalories and 1.05 based on grams), most likely due to the FAO's more detailed food categories (in particular, the vegetable oil/animal fats ratio and wine).

Despite this, there is a noticeable divergence *versus* the same indicator (P-MAI) when relying on the CONCISE dataset (with a value of 1.67) as illustrated via a comparison of the CONCISE database vs. FBS (Z-scores) (see Figure 2).

Results from the historical trend observing the P-MAI score both in 2007 and 1961 using FBS dataset, showed that during this time period, P-MAI scores decreased in most countries. Across all countries (i.e. Mediterranean and northern European countries), average P-MAI decreased from 1.83 to 1.37 (from 1961 to 2007) and the standard deviation (SD) decreased from 1.27 to 0.34 (Table 3). This may be interpreted on the one hand as result of more globalised lifestyles and dietary patterns; on the other one, as public health policies in charge to Nordic countries governments to meliorate dietary behaviours since the '70es. In the same period, all core-Mediterranean countries experienced decreases in P-MAI (Italy: -1.32; Portugal: -2.03; Spain: -2.25; and Greece: -2.59). On a relative scale, southern European countries (Greece, Italy,

**Table 3**. Clusters determined by Scott's choice applied to -P-MAI calculated from FBS - calories and grams and CONCISE database (g). by CONCISE database countries (*) and according FBS years. The MDQI and MSC scores and rankings ("Cluster") are added for comparison.

| Country | P-MAI CONCISE (g) | Cluster | Country | P-MAI FAO FBS (g) | Clusters | Country | P-MAI FAO FBS (cal) | Clusters | Country | MDQI CONCISE (g) | Clusters | Country | MDQI FAO FBS (g) | Clusters | Country | MDS CONCISE (g) | Clusters | Country | MDS FAO FBS (g) | Clusters |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| FIN | 0.86 | 1 | SWE | 0.78 | 1 | ISL | 0.68 | 1 | CZE | 3 | 1 | SVK | 2 | 1 | NLD | 1 | 1 | ISL | 2 | 1 |
| NOR | 0.95 | | NLD | 0.79 | | DNK | 0.82 | | DNK | 3 | | AUT | 3 | 2 | NOR | 2 | 2 | DEU | 3 | 2 |
| ISL | 1.01 | | FIN | 0.81 | | NLD | 0.93 | | EST | 3 | | POL | 3 | | GBR | 2 | | NOR | 3 | |
| NLD | 1.17 | | ISL | 0.98 | 2 | DEU | 0.97 | 2 | IRL | 3 | | DEU | 3 | | IRL | 2 | | NLD | 3 | |
| SWE | 1.28 | 2 | IRL | 1 | | FIN | 1.03 | | HUN | 3 | | EST | 3 | | ISL | 2 | | FIN | 3 | |
| DNK | 1.31 | | DEU | 1.05 | | SWE | 1.04 | | NLD | 3 | | IRL | 3 | | FIN | 2 | | CZE | 4 | 3 |
| HUN | 1.48 | | NOR | 1.07 | | FRA | 1.05 | | BEL | 4 | 2 | HUN | 3 | | EST | 2 | | SWE | 4 | |
| EST | 1.49 | | DNK | 1.09 | | NOR | 1.15 | | BGR | 4 | | CZE | 3 | | BEL | 3 | 3 | DNK | 5 | 4 |
| GBR | 1.53 | | FRA | 1.1 | | BEL | 1.16 | | DEU | 4 | | GBR | 3 | | SWE | 3 | | AUT | 5 | |
| CZE | 1.59 | 3 | CZE | 1.13 | | HUN | 1.18 | | SVK | 4 | | NLD | 3 | | CZE | 3 | | BGR | 5 | |
| IRL | 1.67 | | BEL | 1.14 | | IRL | 1.22 | 3 | SWE | 4 | | FRA | 4 | 3 | DNK | 3 | | EST | 5 | |
| DEU | 1.67 | | AUT | 1.18 | 3 | AUT | 1.23 | | GBR | 4 | | BGR | 4 | | HUN | 3 | | POL | 5 | |
| FRA | 1.70 | | GBR | 1.31 | | CZE | 1.32 | | ISL | 5 | 3 | DNK | 4 | | SVK | 3 | | GBR | 5 | |
| BGR | 1.88 | | HUN | 1.33 | 4 | GBR | 1.35 | | AUT | 5 | | FIN | 4 | | BGR | 3 | | FRA | 5 | |
| BEL | 2.12 | 4 | BGR | 1.35 | | SVK | 1.47 | 4 | FIN | 5 | | ISL | 4 | | POL | 4 | 4 | IRL | 5 | |
| AUT | 2.20 | | EST | 1.39 | | POL | 1.49 | | FRA | 5 | | SWE | 4 | | FRA | 4 | | ITA | 5 | |
| SVK | 2.26 | | SVK | 1.42 | | EST | 1.52 | | ITA | 5 | | BEL | 5 | 4 | AUT | 4 | | BEL | 6 | 5 |
| POL | 2.30 | | POL | 1.47 | | BGR | 1.89 | 5 | POL | 5 | | NOR | 5 | | DEU | 5 | 5 | HUN | 6 | |
| ITA | 2.34 | 5 | ITA | 1.5 | | ITA | 1.95 | | NOR | 5 | | ITA | 7 | 5 | ITA | 6 | 6 | SVK | 6 | |

*(*) Country indicated by the three digits standard*

*P-MAI CONCISE (g): Population-level Mediterranean Adequacy Index calculated on EFSA's Concise database (grams-based calculation); P-MAI FAO FBS (g): Population-level Mediterranean Adequacy Index calculated on FAO Food Balance Sheets database (grams-based calculation); P-MAI FAO FBS (cal): Population-level Mediterranean Adequacy Index calculated on FAO Food Balance Sheets database (calories-based calculation); MDQI CONCISE (g): Mediterranean Diet Quality Index (synth version based on available aggregated data) calculated on EFSA's Concise database (grams-based calculation); MDQI FAO FBS (g): Mediterranean Diet Quality Index (synth version based on available aggregated data) calculated on FAO Food Balance Sheets database (grams-based calculation); MDS CONCISE (g): Mediterranean Diet Score calculated on EFSA's Concise database (grams-based calculation); MDS FAO FBS (g): Mediterranean Diet Score calculated on FAO Food Balance Sheets Database (grams-based calculation).*

*Austria (AUT); Belgium (BEL); Bulgaria (BGR); Czech Republic (CZE); Germany (DEU); Denmark (DNK); Estonia (EST); Finland (FIN); France (FRA); United Kingdom (GBR); Hungary (HUN); Ireland (IRL); Iceland (ISL); Italy (ITA); the Netherlands (NLD); Norway (NOR); Poland (POL); Slovakia (SVK).*

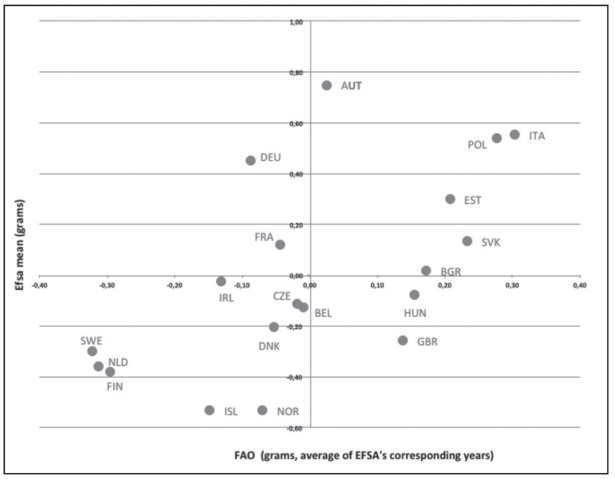


**Figure 2.** Biplot of P-MAI (Population-level Mediterranean Adequacy Index) Z- scores for the Efsa mean national diet, estimated from the EFSA concise database, by food weight versus the FAO FBS data. Scores are standardized and compared with the average of the variable. Data collection period as per Table 1. Norway (NOR); United Kingdom (GBR); Belgium (BEL); Ireland (IRL); Iceland (ISL); Finland (FIN); Sweden (SWE); Czech Republic (CZE); Denmark (DNK); Netherlands (NLD); Poland (POL); Germany (DEU); Hungary (HUN); France (FRA); Austria (AUT); Slovakia (SVK); Italy (ITA); Bulgaria (BGR); Estonia (EST).

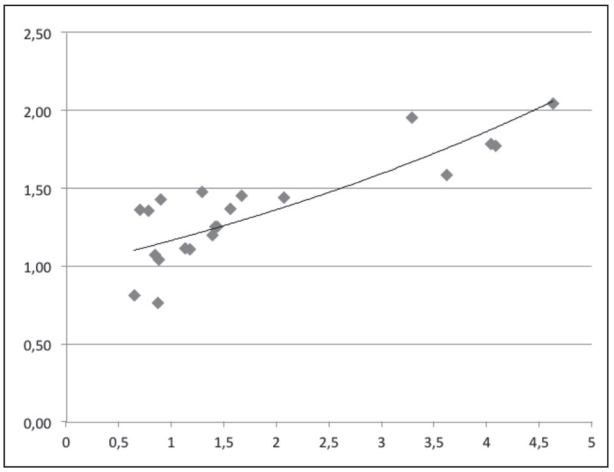


**Figure 3.** P -MAI (Population-level Mediterranean Adequacy Index) scores for the aggregated average national diets from FAO FBS in 2007 – X axis- and 1961 Y axis-, and change in selected EU Member States (calories-based computation) (Pearson correlation: 0,84 p-value not significant 1.58).

Spain and Portugal) halved their P-MAI, whereas northern European countries (Norway and the UK, but also Finland and Sweden) increased their adherence to the Mediterranean Diet.

For these FBS-based historical trends, as expected, g-based values and kcal-based values were positively correlated (Pearson = 0.77), as was the FBS database correlated with the CONCISE database (0.71).

However, a substantial stability of patterns for the interested countries emerges (Figure 3)-, P -MAI scores for the aggregated average national diets from FAO FBS in 2007 – X axis- and 1961 Y axis-, in selected EU Member States (calories-based computation) showed a Pearson correlation of 0,84 and a R-squared of 0,617.

Finally, P-MAI values did not appear to be associated with total energy intake, regardless of the dataset used for the P-MAI estimate (CONCISE, FBS(g), or FBS(kcal)), with r varying from -0.16 to 0.25 and 0.07, respectively, in 2007 in the FBS data.

For the sake of comparison we provide detailed P-MAI values for the indicator used, whether it be the CONCISE datatabase, FBS database (g), or FBS database (kcal) (see Tables 2 and 3).

Given that one of the goals of this study was to assess the P-MAI's discriminatory power, it was tested against rank correlation both internally (CONCISE P-MAI and FBS P-MAI) and externally, taking into consideration other food quality indices capable of providing further insight. In particular, the simplified form of the Diet Quality Index for the Mediterranean Region (synth Med-DQI) developed by Gerber (2006) (27) was added for comparative purposes (limited to food items with and without cholesterol and saturated fatty acids), as the Global Nutrition Index (GNI) of Rosenbloom *et al.* (2008) (28).

Spearman's rank ($\rho_s$) and Kendall's tau ($\tau$) correlations were calculated to compare the list of countries as classified by the P-MAI ranks (derived from the CONCISE and FBS(g)), obtaining $\rho = 0.72$ (p-value 0.002) and $\tau = 0.54$ (p-value 0.001). When comparing P-MAI ranks derived from the CONCISE and FBS(kcal), $\rho = 0.67$ (p-value 0.004) and $\tau = 0.50$ (0.003) were found. All coefficients showed a high level of concordance (value$^3$ 0.5).

An overview on how such indicators performed in the clustering analysis is shown in Table 3, whereas a correlation matrix across the indicators used can be seen in Table 4.

Comparison with the Rosenbloom Global Nutritional Index (GNI), which sums up both food deprivation and overweight burden (the index combines deficits, excesses and food security), failed to show any correlation with P-MAI. Examining the CONCISE P-MAI ranks versus the GNI ranks for the same countries results in no correlation found for any of the rank-correlation indices and for any of the datasets used. The Diet Quality Index for Mediterranean Region (Med-DQI) developed by Gerber (2006) (27), provided clustering with some differences from the P-MAI. This may be explained by the different items taken into account (i.e. mostly nutrients instead of real foods), but also by the cut-off based system (assigning values ranging from 0 to 2 according to consumption thresholds). This implies an underestimation of specific food consumption not reaching the threshold level of the specific nutrient.

## Discussion

The health status of persons and even more of population is linked to a number of factors, only a part of which refer to a healthy diet (Waxman, 2005/34). A healthy diet is in fact only part of broader healthy life-

**Table 4**. A comparison across the indicators, either considering absolute values or ranks

| **Spearman rank correlation** | **nCLUS-PMAI-CONCISE-mean** | **nCLUS-PMAI-FBS-calories** | **nCLUS-PMAI-FBS-grams** | **Rank MDQI-CONCISE** | **Rank MD QI-FBS** | **Rank MDS -CONCISE** | **Rank MDS-FBS** |
|---|---|---|---|---|---|---|---|
| nCLUS-PMAI-CONCISE-mean | 1 | 1 | 1 | 0 | 0 | 1 | 0 |
| nCLUS-PMAI-FBS-calories | | 1 | 1 | 0 | 0 | 0 | 1 |
| nCLUS-PMAI-FBS-grams | | | 1 | 0 | 0 | 0 | 1 |
| Rank MDQI-CONCISE | | | | 1 | 0 | 0 | 0 |
| Rank-MDQI-FBS | | | | | 1 | 0 | 0 |
| Rank MDS CONCISE | | | | | | 1 | 0 |
| Rank MDS-FBS | | | | | | | 1 |
| nCLUS-PMAI-CONCISE-MEAN sign + | | | sign + | sign + | sign + | sign - | sign + |
| nCLUS-PMAI-FBS-CALORIES sign + | | | | sign + | sign + | sign + | sign + |
| nCLUS-PMAI-FBS-GRAMS sign + | | | | | sign + | sign - | sign + |
| Rank-MDQI-CONCISE sign - | | | | | | sign + | sign + |
| Rank-MDQI-FBS | | | | | | sign + | sign - |
| Rank-MDS-CONCISE sign + | | | | | | | |
| Rank-MDS-FBS | | | | | | | |

style. However, to measure the adherence to a healthy diet is of the upmost interest since it is one of the most controllable variable either individually or inside public policy initiatives. Furthermore, the focus on diets instead than on food items is increasingly prevalent, as a full risk- assessment cycle requires also *exposure data* (consider the Trans Fatty Acids-TFAs debate at the EU level, if provide for labelling on food or not, due to the unquestionable risk characterization, but also with a low exposure of the population) (35EC, 2005)) and also a risk-benefit assessment -(i.e., for eating oily fish- with benefits from omega 3 fatty acids and risks from PCBs, heavy metals and dioxins- (EFSA,2015/36)). Clear trade-offs hence refer more and more to the balanced consumption of foods more than on the mere intake (or avoidance) of a food matrix..

Once cleared this aspect, it can be disclosed that to a certain extent, P-MAI appears to discriminate between different diets across Europe and to detect changes over time. Across databases, eastern European countries seem to have a higher adherence to a Mediterranean-like dietary pattern than northern and central European countries. This is perhaps due to a lower meat consumption, which can most probably be attributed to reduced access to meat and to higher potato consumption levels. The apparent phenomenon of converging diets in Europe is interesting and has been investigated elsewhere (18,37).

As potatoes and starchy foods are a controversial category as regards their place in the Mediterranean diet, they have alternatively been included either in the list of Mediterranean foods or on the contrary, in the list of non-Mediterranean foods (Tables 5 and 6).

For this reason, P-MAI scores were higher when potato values were calculated in grams rather than in calories. This is due to the relatively low energy density of potatoes compared to other food categories. On average, the difference between the P-MAI with potatoes and the P-MAI without potatoes equals a value of 47.5% for FBS g/day computations, but "only" 20.9% for calories/day computations (Table 5). The inclusion of potatoes in Med Foods seems to increase discrimination between MS dietary patterns -resulting in higher standard deviation values-, but the scientific rationale of such inclusion is questionable from a public health perspective.

When ranking and clustering was carried out using the Scott's choice test (Tab. 3) for the FBS(g) P-MAI, most countries maintained their position or at least maintained their original cluster from the CONCISE (Tab.6). The only major changes were seen with Estonia (from 0.71 to 1.39 and from cluster 2 to 4), Poland (from 0.76 to 1.47 and from cluster 2 to 4) and the UK (from 0.72 to 1.31 and from cluster 2 to 3). Italy and Slovakia remained at the top of the ranking of countries for Mediterranean Adequacy in both cases, other countries worsened passing onto calories-based computation and others ameliorated. However, non-Med foods (meat, dairies, fats) are generally denser from a caloric perspective than Med- foods (cereals, F&V, legumes..,), i.e., *a lower intake of Non-Med foods gives better scores than a proportionally increased intake of Med foods- even if hardly there is a general rule (olive oil is more caloric than butter; cereals have the same 4 calories per g than meat)*. Many of the Nordic countries maintained both their position and clusters. Despite their relatively high potato consumption, scoring positively-adherence to the Mediterranean diet was low and no significant effect was seen by including starchy foods in the list of Mediterranean foods.

When examining calories instead of grams, the effects of *ranking variations* were subtle, yet present-the SD –reasonably- increased. The UK and Ireland each gained a rank in the clusters partition (from 1.02 to 1.35 and 0.94 to 1.22 respectively), as does Estonia (from 1.08 to 1.52).

It is possible to draw similar conclusions from the EFSA database. Here, it is important to bear in mind that starchy foods only play a minor role in the diet, regardless of whether calories or grams are used as indicator units.

It is possible to advance similar considerations to those pinpointed for potatoes about other food items that do not yet have a clear nutritional status, such as *beer* or *fruit juices* with low fruit content[4]. When including them in the P-MAI (CONCISE g)-by food weight-, with the assumption of beer at the numerator (ethanol was positively considered within the MDS) and soft drinks at the denominator, the indicator

[4] Soft drinks are considered drinks with a fruit content lower than 'nectar', as defined by the European Commission (EC) Directive 2001/112, typically containing 25-50% fruit, but with added sugars.

**Table 5**. PMAI on the datasets FBS (grams and calories) and CONCISE (mean and median values), with potatoes/starchy foods alternatively included in Med foods (NUMERATOR) or in non Med foods (DENOMINATOR)

| | FBS Grams | | | FBS Calories | | | CONCISE MEAN | | |
|---|---|---|---|---|---|---|---|---|---|
| | Potatoes in "non-med foods" | potatoes in "med-foods" | delta(%) | potatoes in "non-med" foods | potatoes in "med" foods | delta(%) | potatoes in "non med foods" | potatoes in "med foods" | % Delta |
| AUT | 0,9 | 1,18 | 31,1 | 1,08 | 1,23 | 13,9 | 1,78 | 2,20 | 23,9 |
| BEL | 0,81 | 1,14 | 40,2 | 0,99 | 1,16 | 17,2 | 1,48 | 2,12 | 43,6 |
| BGR | 1,07 | 1,35 | 26,2 | 1,69 | 1,89 | 11,8 | 1,32 | 1,88 | 42,5 |
| CZE | 0,75 | 1,13 | 50,7 | 1,1 | 1,32 | 20 | 1,09 | 1,59 | 45,8 |
| DEU | 0,68 | 1,05 | 54,4 | 0,78 | 0,97 | 24,4 | 1,18 | 1,67 | 42,1 |
| DNK | 0,77 | 1,09 | 41,6 | 0,68 | 0,82 | 20,6 | 0,94 | 1,31 | 39,8 |
| EST | 0,71 | 1,39 | 95,8 | 1,08 | 1,52 | 40,7 | 0,79 | 1,49 | 88,6 |
| FIN | 0,58 | 0,81 | 39,7 | 0,85 | 1,03 | 21,2 | 0,61 | 0,86 | 40,5 |
| FRA | 0,82 | 1,1 | 34,1 | 0,91 | 1,05 | 15,4 | 1,39 | 1,70 | 22,2 |
| GBR | 0,72 | 1,31 | 81,8 | 1,02 | 1,35 | 32,7 | 1,04 | 1,53 | 48 |
| HUN | 0,94 | 1,33 | 41,5 | 1,01 | 1,18 | 16,8 | 1,04 | 1,48 | 41,6 |
| IRL | 0,55 | 1 | 81,8 | 0,94 | 1,22 | 29,8 | 0,78 | 1,67 | 114,2 |
| ISL | 0,75 | 0,98 | 30,7 | 0,6 | 0,68 | 13,3 | 0,85 | 1,01 | 19,8 |
| ITA | 1,26 | 1,5 | 19 | 1,78 | 1,95 | 9,6 | 1,97 | 2,34 | 18,7 |
| NLD | 0,54 | 0,79 | 46,3 | 0,77 | 0,93 | 20,8 | 0,79 | 1,17 | 48,6 |
| NOR | 0,74 | 1,07 | 44,2 | 0,96 | 1,15 | 19,5 | 0,64 | 0,95 | 48,9 |
| POL | 0,76 | 1,47 | 93,4 | 1,1 | 1,49 | 35,5 | 1,09 | 2,30 | 110,1 |
| SVK | 1,2 | 1,42 | 18,3 | 1,23 | 1,47 | 19,5 | 1,52 | 2,26 | 48,3 |
| SWE | 0,59 | 0,78 | 32,2 | 0,91 | 1,04 | 14,3 | 0,84 | 1,28 | 52,2 |
| Mean | 0,8 | 1,15 | 47,53 | 1,03 | 1,23 | 20,89 | 1,11 | 1,62 | 50,96 |
| SD | 0,2 | 0,22 | | 0,29 | 0,32 | | 0,37 | 0,45 | |

*(*) Country indicated by the three digits standard; Austria (AUT). Belgium (BEL); Bulgaria (BGR); Czech Republic (CZE); Germany (DEU); Denmark (DNK); Estonia (EST); Finland (FIN); France (FRA); United Kingdom (GBR); Hungary (HUN); Ireland (IRL); Iceland (ISL); Italy (ITA); the Netherlands (NLD); Norway (NOR); Poland (POL); Slovakia (SVK).Mediterranean Foods as by original Mediterranean Adequacy Index: vegetables, fruit, cereals, red wine, vegetable oils, potatoes and fish. Non Mediterranean Foods as by original Mediterranean Adequacy Index: meat, dairy products, animal fat, eggs and sugar*

shows substantial variations as compared to the baseline model. The mean P-MAI value without including beer and soft drinks is 1.37 (SD 0.43), but changes to 1.28 (SD 0.40) with both foods included in the above-mentioned positions.

Although the inclusion of beer inside Med-foods is questionable, as several countries have a high average beer consumption (i.e. the Czech Republic 373g/day, Ireland 299g/day, and the UK 257g/day), fruit juices with added sugar and a low fruit content require also proper examination from a public health perspective. In fact, there are also quite high consumption levels for these fruit juices across the EU (Norway 330g, Iceland 339g, Belgium 275.2g, and the UK 219g).

With regard to scores other than the P-MAI, after taking into account the basic Mediterranean Score index (MDS), the scope was enlarged to include non-Mediterranean foods, as well as potatoes and eggs,

**Table 6**. PMAI on the datasets FBS (grams and calories) and CONCISE (mean and median values), with potatoes/starchy foods ("POT") alternatively included in Med foods or in non-Med foods. Clusters provided depending on the rankings.

| Pot "Non-Med" | | | Pot "Med" | | | Pot "Non-Med" | | | Pot "Med" | | | Pot "Non-Med" | | | Pot "Med" | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | FBS g | Cluster | | FBS g | Cluster | | FBS cal | Cluster | | FBS cal | Cluster | | CONCISE mean | Cluster | | CONCISE mean | Cluster |
| NLD | 0,54 | 1 | SWE | 0,78 | 1 | ISL | 0,6 | 1 | ISL | 0,68 | 1 | FIN | 0,61 | 1 | FIN | 0,86 | 1 |
| IRL | 0,55 | | NLD | 0,79 | | DNK | 0,68 | | DNK | 0,82 | | NOR | 0,64 | | NOR | 0,95 | |
| FIN | 0,58 | | FIN | 0,81 | | NLD | 0,77 | | NLD | 0,93 | | IRL | 0,78 | | ISL | 1,01 | |
| SWE | 0,59 | | ISL | 0,98 | 2 | DEU | 0,78 | | DEU | 0,97 | 2 | NLD | 0,79 | | NLD | 1,17 | |
| DEU | 0,68 | | IRL | 1 | | FIN | 0,85 | 2 | FIN | 1,03 | | EST | 0,79 | | SWE | 1,28 | 2 |
| EST | 0,71 | 2 | DEU | 1,05 | | FRA | 0,91 | | SWE | 1,04 | | SWE | 0,84 | | DNK | 1,31 | |
| GBR | 0,72 | | NOR | 1,07 | | SWE | 0,91 | | FRA | 1,05 | | ISL | 0,85 | | HUN | 1,48 | |
| NOR | 0,74 | | DNK | 1,09 | | IRL | 0,94 | | NOR | 1,15 | | DNK | 0,94 | 2 | EST | 1,49 | |
| CZE | 0,75 | | FRA | 1,1 | | NOR | 0,96 | | BEL | 1,16 | | GBR | 1,04 | | GBR | 1,53 | |
| ISL | 0,75 | | CZE | 1,13 | | BEL | 0,99 | | HUN | 1,18 | | HUN | 1,04 | | CZE | 1,59 | 3 |
| POL | 0,76 | | BEL | 1,14 | | HUN | 1,01 | | IRL | 1,22 | 3 | CZE | 1,09 | | IRL | 1,67 | |
| DNK | 0,77 | | AUT | 1,18 | 3 | GBR | 1,02 | | AUT | 1,23 | | POL | 1,09 | | DEU | 1,67 | |
| BEL | 0,81 | | GBR | 1,31 | | AUT | 1,08 | | CZE | 1,32 | | DEU | 1,18 | | FRA | 1,70 | |
| FRA | 0,82 | | HUN | 1,33 | 4 | EST | 1,08 | | GBR | 1,35 | | BGR | 1,32 | 3 | BGR | 1,88 | |
| AUT | 0,9 | 3 | BGR | 1,35 | | CZE | 1,1 | 3 | SVK | 1,47 | 4 | FRA | 1,39 | | BEL | 2,12 | 4 |
| HUN | 0,94 | | EST | 1,39 | | POL | 1,1 | | POL | 1,49 | | BEL | 1,48 | | AUT | 2,20 | |
| BGR | 1,07 | 4 | SVK | 1,42 | | SVK | 1,23 | | EST | 1,52 | | SVK | 1,52 | 4 | SVK | 2,26 | |
| SVK | 1,2 | 5 | POL | 1,47 | | BGR | 1,69 | 4 | BGR | 1,89 | 5 | AUT | 1,78 | | POL | 2,30 | |
| ITA | 1,26 | | ITA | 1,5 | | ITA | 1,78 | | ITA | 1,95 | | ITA | 1,97 | 5 | ITA | 2,34 | 5 |

*(*) Country indicated by the three digits standard. Austria (AUT); Belgium (BEL); Bulgaria (BGR); Czech Republic (CZE); Germany (DEU); Denmark (DNK); Estonia (EST); Finland (FIN); France (FRA); United Kingdom (GBR); Hungary (HUN); Ireland (IRL); Iceland (ISL); Italy (ITA); the Netherlands (NLD); Norway (NOR); Poland (POL); Slovakia (SVK). Mediterranean Foods as by original Mediterranean Adequacy Index: vegetables, fruit, cereals, red wine, vegetable oils, potatoes and fish. Non Mediterranean Foods as by original Mediterranean Adequacy Index: meat, dairy products, animal fat, eggs and sugar.*

which within Trichopoulou's highly relevant results showed an increase in the mortality rate of 1.07 (0.95-1.21 and 0.98-1.17 respectively). For comparative purposes, in this study there was an increased hazard of 1.05 for saturated fats, 1.06 for meat and 1.11 for dairy products. Results suggest that when eggs and potatoes were considered part of the Mediterranean diet entries, as expected they gave rise to a higher apparent adherence to the Mediterranean diet, with a number of countries benefitting from same (including Italy, Austria and Estonia). This loophole, due to the inability of the traditional Med Diet to reflect on either new foods nor on foods traditionally outside the Med pattern, requires for sure additional research and modelling (Table 7).

Also, some considerations can be drawn about the different data sources used. Previous international comparisons (38, 39[5]-"Dafne") relied exclusively on other datasets, such as the FAO Food Balance Sheets, a food supply database, or Dafne, a household food availability database. Both of these datasets provided data obtained via the food balance method (FBS) or from household food purchases (Dafne) whereas on the contrary the EFSA dataset collected data from national food consumption surveys. While the known limitations of the EFSA dataset include its lack of harmonisation in collection and survey methods, the different timeframes of the national surveys and underreporting, it may still provide more accurate information than previously used datasets. As for the P-MAI, the apparent discrepancies between the CONCISE and FBS average values could be due to waste along the food consumption chain (from distribution to consumption). In fact, FBS are corrected for food reused for other production purposes, but not for retail/kitchen waste or table leftovers.

For future research, it would be worth exploring whether Mediterranean diet adherence depends on socio-economic or cultural factors, i.e. the degree of inequality in income distribution inside a given country (using the Gini Index; (40)). We also used wine and wine substitutes as well as beer as a proxy of moderate alcohol consumption (an element of the Mediterranean diet). This may be misleading, as alcohol is known to damage health. Moreover, starchy foods (refined, as opposed to whole foods) contribute to a higher P-MAI. However, if they are not consumed as a substitute animal protein, they do not lead to a healthier diet, which can lead to an increased BMI (9, 41).

In this study, we estimated the P-MAI with weight or energy content, depending on the available data. Where data was missing, it was recommended to use the number of grams consumed daily (25, 9). In countries where EFSA data was available, the P-MAI (estimated in 1961 and 2007) demonstrated a decline in all southern European countries surveyed, confirming changes in the consumption of the Mediterranean diet.

**Table 7**. The MDS with potatoes and eggs regarded as Mediterranean entries or not (original model of Trichopoulou)- EFSA mean data considered, grams.

| | MDS (pot + eggs as MED) | | MDS Original |
|---|---|---|---|
| GBR | 2 | NLD | 1 |
| NOR | 2 | NOR | 2 |
| NLD | 2 | GBR | 2 |
| IRL | 2 | IRL | 2 |
| EST | 3 | ISL | 2 |
| HUN | 3 | FIN | 2 |
| FIN | 3 | EST | 2 |
| BEL | 3 | BEL | 3 |
| DNK | 4 | SWE | 3 |
| SWE | 4 | CZE | 3 |
| ISL | 4 | DNK | 3 |
| BGR | 5 | HUN | 3 |
| SVK | 5 | SVK | 3 |
| FRA | 5 | BGR | 3 |
| DEU | 5 | POL | 4 |
| POL | 5 | FRA | 4 |
| CZE | 5 | AUT | 4 |
| AUT | 6 | DEU | 5 |
| ITA | 7 | ITA | 6 |

*(*) Country indicated by the three digits standard. Austria (AUT); Belgium (BEL); Bulgaria (BGR); Czech Republic (CZE); Germany (DEU); Denmark (DNK); Estonia (EST); Finland (FIN); France (FRA); United Kingdom (GBR); Hungary (HUN); Ireland (IRL); Iceland (ISL); Italy (ITA); the Netherlands (NLD); Norway (NOR); Poland (POL); Slovakia (SVK).*

[5] Dafne stands for DAta Food NEtworking, and aims at the creation of a pan-European food data bank

According to FBS data, the reason for this lies in increased meat consumption, rather than in the reduced consumption of fruit and vegetables or vegetable oils. Reductions have been observed in the consumption of beans and wine only. Furthermore, southern European diets have also increased their total food and energy intake, which is not reflected in the P-MAI. Several countries in northern and central Europe had higher levels of Mediterranean diets in 2007 than in 1961, as measured by the P-MAI. However, this change coincided with a general increase in energy intake (kcal). More generally, most national diets tend to display some sort of 'inertia' in terms of the P-MAI, which can easily be attributed to dietary cultures and recipes.

When measured with the FBS, more food in terms of both weight and energy was consumed in 2007 than in 1961 in all countries. National diets, which were below average P-MAI in 1961, were generally still below average in 2007. The 2007 P-MAI scores are correlated with those in 1961 (Pearson's r = 0.84), Table 8.

For comparative purposes for each food category in the EFSA database, Confidence Intervals (CI) have been derived in order to assess whether related FAO data fall inside them. The results obtained were informative and lead to the following consideration. Categories that were too broad in scope did not allow values to overlap in the 2 datasets (i.e. values inside the confidence intervals), even if there appeared to be a strong correlation (i.e. countries with a high consumption of dairy products were the same across databases, although the intake varied significantly depending on the source used, which in turn may depend upon a different level of aggregation of single food items).

Other categories, even if they were more restricted, showed that despite the same direction being seen in the CONCISE and FBS (expressed by the correlation); the magnitude was quite different to the well-known variation expected between FBS and real consumption (fish and starchy products). Hence, it generally did not allow FBS values to fall inside the confidence intervals of the CONCISE DB. As for wine, there was a degree of correlation and, in some national cases, an overlapping between FBS and survey data is apparent. This similarity between data from the CONCISE and FBS, which on the contrary food availability record at home, could easily be explained by the fact that wine is not processed at home meaning that waste can be reduced. Hence, wine intake and availability are similar, unlike other food categories for which waste within the home environment is to be expected.

Such comparisons may also drive reflections on the impact that adult consumption levels have on the overall population. Although, as confidence intervals from the CONCISE data in most cases did not include corresponding FBS values, it could be deduced that this is not in fact the case.

**Table 8**. P-MAI (Population-level Mediterranean Adequacy Index) scores for the aggregated average national diets from FAO FBS in 2007 and 1961, and change in selected EU Member States (calories-based computation).

| | 1961 | 2007 | Change | Change % |
|---|---|---|---|---|
| Norway | 0.7 | 1.36 | 0.66 | 94.7 |
| Great Britain | 0.78 | 1.36 | 0.58 | 74.3 |
| Belgium | 1.56 | 1.37 | -0.19 | -12.2 |
| Ireland | 0.89 | 1.43 | 0.54 | 60.4 |
| Iceland | 0.64 | 0.82 | 0.18 | 27.5 |
| Finland | 0.88 | 1.04 | 0.16 | 18.3 |
| Sweden | 0.84 | 1.07 | 0.23 | 27.6 |
| Czech Republic | 1.29 | 1.48 | 0.19 | 14.4 |
| Denmark | 0.87 | 0.77 | -0.10 | -11.8 |
| The Netherlands | 1.17 | 1.11 | -0.06 | -5.3 |
| Poland | 1.66 | 1.45 | -0.21 | -12.5 |
| Germany | 1.13 | 1.11 | -0.02 | -1.3 |
| Hungary | 1.39 | 1.20 | -0.19 | -13.8 |
| France | 1.41 | 1.25 | -0.16 | -11.1 |
| Austria | 1.43 | 1.26 | -0.17 | -12.1 |
| Slovakia | 2.07 | 1.44 | -0.63 | -30.2 |
| Italy | 3.28 | 1.96 | -1.32 | -40.3 |
| Portugal | 3.62 | 1.59 | -2.03 | -56.1 |
| Bulgaria | 4.08 | 1.77 | -2.31 | -56.5 |
| Spain | 4.04 | 1.79 | -2.25 | -55.7 |
| Greece | 4.63 | 2.04 | -2.59 | -55.9 |
| Mean | 1.83 | 1.37 | | |
| St.Dev | 1.27 | 0.34 | | |
| Pearson correlation | 0.75 | | | |
| Pearson correlation (calories/PMAI 2007) | 0.07 | | | |

**Table 9**. Correlation and overlapping between FAO and EFSA DB (grams) for specific food categories

| Food Category | Pearson's correlation r CONCISE - FBS (g) | Countries presenting overlapping Confidence Intervals (i.e., comparable values) |
|---|---|---|
| Fish | 0.59 | Austria (P <0.01) |
| Cereals | 0.25 | France (P <0.01 and P< 0.05), Germany (P< 0.01) |
| Fruit | -0.3 | none |
| Vegetables | 0.34 | none |
| Dairy products | 0.72 | none |
| Meat | 0.08 | Slovakia (P <0.01 and P< 0.05) |
| Sugars and sweeteners | 0.41 | none |
| Starchy products | 0.62 | Austria (P <0.01 and P< 0.05) |
| Wine products | 0.81 | Austria (P <0.01 and P <0.05), Czech Rep. (P <0.01 and P <0.05), Slovakia (P <0.01 and P <0.05), Sweden (P <0.01). |
| Eggs | 0.22 | Poland P < (0.01) |

However, we believe that the P-MAI, for all its simplicity, may be worth exploring as an initial summary population-level measure of the level. Such an index is currently available in several forms (42, 9, 41).

Another outcome of this study was the comparison between different databases, which can provide problematic results from a public health perspective. When political and administrative resources are limited and there is a need to address emerging issues (such as diet) at population level, the use of different indicators and databases may result in different policy indications. Although we were already aware (43) that variations in percentiles of national populations exist, whereby there are higher variations between countries. This contribution once again underlines the difficulties encountered when managing the need for country-specific policies and the need for specific relief for specified target groups (percentiles) of the populations, at the time of adopting aggregated data.

## Limitations

A first limitation is that some foods were not included in the CONCISE database- for example, vegetable oils and red wine-distorting the overall value of the indices.

Another limitation depends on the different years of surveys in the CONCISE, which make inherently difficult to compare Mediterranean Diet adherence during diverse temporal windows- as well as the different survey methodologies (i.e., 24 hours recall, 48 hours recall, etc)

Also, with regard to the FAO FBS, they are not deflated for domestic food waste (i.e., apparent consumptions may be exaggerated).

In addition, the use of dietary indices, which, in this case, rely on average or median data do not account for the variability in the population's dietary habits.

Furthermore, many indices used relying on population-level data discount the lack of all the information: i.e, for the Med Diet Score the ratio between monounsaturated/saturated fats, as originally indicated in Trichopoulou (2003) (28) was absent, nor was it possible to analytically separate legumes from vegetables departing from the CONCISE database; neither the Med- DQI was able to capture TFAs or cholesterol.

Another major limitation of this study is that the indices used were originally built on first-hand data (i.e., cohort studies relying on real persons), and not intended for aggregated mean-median values analysis at population level (secondary data). Eventually, a further problem of Med-adherence indices is that *it does not consider the overall caloric intake* and also, specific nutrients or other indicators of nutritional status such as BMI. Further considerations reflect the uncertain status inside the Mediterranean diet of food items, i.e. soft drinks, potatoes or beer/ethanol. This will likely also present a challenge in terms of the scientific background and overall conceptual framework.

Nonetheless such indicators fitted the clustering purposes only and obviously, the paper did not intend to infer health-based outcomes, such as hazard ratios presented in the original works.

In conclusion, it is apparent that there is something like a real "Mediterranean Diet" and Mediterranean dietary lifestyle. However, internationalisation of food consumption and trade, global lifestyles and attention to preventive medicine-(starting from healthier diets)- result in an increase in Med diet patterns in Nordic EU countries and a relative loss in traditional Southern ones.

In the end, different dietary patterns seem to continue to exist across Europe, which is consistent with the findings of other studies. Future refinements require a better definition of the Mediterranean diet (42). Moreover, proper consideration should be paid to food categories with still uncertain Mediterranean-taxonomy (i.e. potatoes, beer or fruit juices with a low fruit content). Equally, a future refinement could intend to model FAO FBS data in order to mimic real survey data, should there be a lack of such data. Consequently, correction factors for Mediterranean countries could be extrapolated (i.e., *food waste- in order to better describe real at home intake of F&V, for which the difference between intake surveys data and FBS seems relevant*).

The P-MAI is an interesting indicator due to its user-friendliness, lack of a-aprioristic assumptions or modelling with a minimal recourse to hypothesis - and allows the classification of European countries' diets using food intake data. In fact, exploring variations with a one-way ANOVA and considering the 3 comparable data sources used (FAO FBS calories, FAO FBS grams, and EFSA mean values in grams), we cannot reject the null hypothesis that mean values are not the same. Therefore, using different data can lead to not completely different results (F 4.73, F critical 3,16). The residual variance/overall variance ratio, is in fact 0.88 (values closer to 1 imply that -regardless the dataset used- the results can still be compared, suggesting *that different dietary patterns count more than diverging collections methods/dataset).*

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Original article

# Association between intakes of macro- and micro- nutrients and serum lipid profiles among Jordanian adults: a preliminary study

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**Summary.** Dyslipidemia is one of the most important risk factors for cardiovascular disease. Diet is considered as a major contributor for developing dyslipidemia. This study aimed to assess the intakes of macro- and micro-nutrients with serum lipid profile in disease-free adults. A convenient sample of 167 adults was recruited in this cross-sectional study. Serum lipid profile including total cholesterol, triglycerides (TG), low-density lipoprotein cholesterol LDL-C and high-density lipoprotein cholesterol HDL-C were measured. Nutrients and food groups' consumption was assessed using a validated quantitative Food Frequency Questionnaire. The findings revealed different significant differences between the levels of total energy intake and macronutrients' contribution to energy with serum lipid profiles. Cholesterol serum level was significantly higher among individuals with the highest energy intake ($P$=0.03), and was significantly lower with highest energy intake from trans-fatty acids ($P$=0.02). HDL-C tertiles were significantly associated with of percentage of energy from protein ($P$=0.04). Serum total cholesterol and LDL-C levels were significantly different with vitamin K intake levels ($P$=0.03). The intake of meat, fish, eggs, and beans was significantly different among HDL-C tertiles. ($P$=0.04). Many possible associations between Jordanian diet components and serum lipids were reported which indicates that diet is an important factor that should be considered when preventive or treatment strategies are implemented.

**Key words:** macronutrients, micronutrients, cholesterol, triglycerides (TG), low-density lipoprotein cholesterol LDL-C, high-density lipoprotein cholesterol HDL-C

## Introduction

Dyslipidemia is defined as an abnormal lipid metabolism including increased levels of total cholesterol, triglycerides (TG), and low density lipoprotein cholesterol (LDL-C) and/or decreased levels of high-density lipoprotein cholesterol (HDL-C) (1). Dyslipidemia is a major risk factor for developing cardiovascular disease -the first cause of mortality worldwide (2, 3). An estimated 17.7 million people died from cardiovascular diseases (CVDs) in 2015, representing 31% of all global deaths (4). The prevalence of dyslipidemia among Jordanian adults in 2010 was high with 48.8% had high cholesterol, 40.7% had high LDL-C, 40.1% had low HDL-C, while 43.6% had high TG concentrations (5).

The underlying risk factors suggested for dyslipidemia are divided as non-modifiable (e.g., age, gender and genetics), and modifiable (e.g., diet, smoking, physical activity, and stress) (6). Diet plays a key role in the prevention and management of dyslipidemia.1 Previous studies focused on the relationship between dietary fat and cholesterol intake and serum lipids (7-9). Dietary cholesterol is a highly controversial nutrient since it increases serum cholesterol, especially among individuals who are not able to maintain plasma cholesterol homeostasis by reducing its absorption in the small intestine or by suppressing its endogenous synthesis (10). In contrast, studies conducted in young adults (11, 12) and elderly individuals (13), who were assigned to consume additional 640 mg of cholesterol for (4) weeks, found that hyper-responders raise both LDL-C and HDL-C thus the LDL-C/HDL-C ratio is maintained. In addition, those individuals who were not hyper-responders, the LDL-C/HDL-C is maintained since no significant raises in plasma cholesterol were shown (13).

Several epidemiological studies revealed the effectiveness of dietary modifications in preventing and reducing the risk of cardiovascular diseases. For example, low level of protein rich diet in saturated fat has been suggested to reduce the levels of cholesterol, TG, and LDL-C in comparison to a diet that is rich in carbohydrate and unsaturated fat (14). In addition, high intake of polyunsaturated fatty acids was negatively associated with cholesterol and LDL-C concentrations (15), but positively associated with HDL-C concentrations (16). While, excessive intake of total carbohydrates exerts a lowering effect on HDL-C concentration (14, 17), it has been reported to increase TG concentration in adults (18). Conversely, diets enriched in monounsaturated fatty acids (19-21) or both monounsaturated fatty acids and polyunsaturated fatty acids (22) showed no significant difference in serum cholesterol and LDL-C concentrations. However, consumption of fruits and vegetables was found to be associated with low LDL-C levels (23). Also, increased consumption of milk and other dairy products showed a protective effect against the increase in serum TG and decrease in HDL-C (24).

The equivocal results regarding the association between dietary factors and serum lipids may be due to the influence of several confounding factors which could have been inconsistently taken into account. Genetic differences arise among the factors that may cause variation in serum lipid levels through various gene loci in which a direct or indirect involvement in lipid metabolism and/or transport may take place (25, 26). Gender-specific differences may influence serum lipids; women have higher levels of cholesterol and HDL-C, while men have higher LDL-C levels. HDL-C and LDL-C are also influenced by age (27) and body mass index (BMI) (28). The physical activity level mostly increases HDL-C, but findings for cholesterol and LDL-C are less consistent (29). Although the examination of the dietary factors associated with lipid profiles is essential to prevent dyslipidemia, cardiovascular diseases, type 2 diabetes and another chronic disease; the effects of diet on lipid profiles are not yet fully understood. Therefore, the aim of the present study was to investigate the differences in macro and micro-nutrient intake and serum lipids in apparently healthy Jordanian adults.

## Methods

*Study design and participants*

A total number of 167 (83 males and 84 females) disease-free Jordanian volunteers (students and employees), aged 18-51 years were recruited conveniently from the King Hussein Medical Center (KHMC) during the period of October 2014 to July 2015. Based on an alpha probability of 0.05 and power of 0.8 the sample size was calculated. Eligibility criteria to be enrolled in the study were: being Jordanian and above 18 years old. Pregnant and lactating women and individuals with eating disorders, major surgeries or any chronic diseases were excluded from this study. A consent form was obtained from each participant. The study protocol was approved by the Jordanian Royal Medical Services (JRMS) ethics committee.

*Measurement of lipid profiles*

Blood samples were drawn from participants after overnight fasting by a specialized medical laboratory technician. Serum samples were centrifuged and separated from the whole blood and stored at −80°C until

further analysis. Fasting blood lipid profiles, including total cholesterol, LDL-C, HDL-C, and TG levels were measured by Jenway 6305 UV/Visible Spectrophotometer, USA using commercially available Enzymatic kits (TECO DIAGNOSTICS, USA).

*Assessment of nutrient intake*

A validated quantitative Food Frequency Questionnaire (FFQ) was used for dietary assessment (30). The FFQ was completed by a trained dietitian through face-to-face interviews. Participants were asked how frequently, on average, during the past year they had consumed one standard serving of specific food items in nine categories (<1/month, 2-3/month, 1-2/week, 3-4/week, 5-6/week, 1/day, 2-3/day, 4-5/day, or 6/day). Food lists in the modified FFQ questions were classified based on types of food: 8 items of cereals; 9 items of milk and dairy products; 21 items of fruits and juices; 21 items of vegetables; 16 items of meat such as red meat (lamb and beef), chicken, fish, cold meat, and others; 4 items of beans; 4 items of soups and sauces; 5 items of drinks; 9 items of snacks and sweets; and 14 items of herbs and spices (30). Portion size estimation was carried out using food models and standard measuring tools. Dietary intakes were analyzed using dietary analysis software (ESHA Food Processor SQL version 10.1.1; ESHA, Salem, OR, USA) with additional data on foods consumed in Jordan (31).

*Physical activity level*

7-Day physical activity recall (PAR) is a questionnaire that focuses on a participant's recall of time spent doing the physical activity over a seven-days period (32). The validated PAR questionnaire was used to measure physical activity level. Our study participants were asked to respond to the PAR questions with an emphasis on their personal exercise pattern and behavior during the noted time period. Physical activity level was calculated according to the Sallis et al., (1985) protocol (32).

*Statistical analysis*

The data were analyzed using SPSS statistical package version 20. Energy, macronutrients and micronutrients were presented as a mean ± standard error. Cholesterol, TG, HDL-C and LDL-C were grouped into tertiles. Post-hoc Analysis of Variance (ANOVA) was used to assess the impact of energy, macronutrients and micronutrients intake on serum lipid profiles after adjustment for age, sex, BMI, energy intake, physical activity and smoking. Different letters denote significant differences among the tertiles. *P*-values<0.05 were considered statistically significant.

## Results

Participants' demographic and anthropometric data, the concentration of serum cholesterol, TG, HDL-C, and LDL-C were published previously in another publication (33). They revealed that serum levels of IL-6, cholesterol, LDL and HDL were significantly ($P<0.05$) greater in obese participants than those reported for overweight and normal body weight participants. TG serum concentration was the only biochemical variable which was significantly higher ($P<0.05$) in overweight and obese participants when compared with normal body weight participants. However, the values of the all variables were within the normal levels as shown in table 1.

Table 2 shows the possible associations between participants' daily total energy intake and macronutrients' contribution to energy intake with serum lipid profiles. Cholesterol serum level was the only lipid profile which was significantly higher among individuals with the highest total energy intake ($P=0.03$), and was significantly lower among individuals with highest energy intake from trans-fatty acids ($P=0.02$). Similarly, HDL-C serum concentration was the only lipid profile that was significantly different with energy intake from protein ($P=0.04$). There was no significant relationship between energy intake from carbohydrate, fat and saturated fat across all tertiles for serum cholesterol, TG, HDL-C and LDL-C.

Table 3 shows participants' daily intake of different macronutrients through different tertiles of serum cholesterol, TG, HDL-C, and LDL-C. Individuals with the lowest level of serum cholesterol reported increased consumption of trans fatty acids as compared to the middle and highest level of serum cholesterol ($P=0.02$).

There was no statistically significant difference between fat soluble and water soluble vitamins as well

**Table 1.** Anthropometric and biochemical parameters of the study sample based on BMI[ſ].

| Parameter | Normal | | | Overweight | | | Obese | | | *p-value |
|---|---|---|---|---|---|---|---|---|---|---|
| | N | Mean | SEM | N | Mean | SEM | N | Mean | SEM | |
| Total Cholesterol (mg/ml) | 68 | 149.5[a] | 3.5 | 40 | 158.8[a] | 5.1 | 42 | 171.5[b] | 5.9 | **0.005** |
| TG (mg/ml) | 68 | 84.2[a] | 5.1 | 40 | 109.7[b] | 11.4 | 42 | 125.9[b] | 10.2 | **0.008** |
| HDL (mg/ml) | 68 | 44.5[a] | 1.3 | 40 | 40.5[a] | 1.2 | 42 | 38.8[b] | 1.3 | **0.022** |
| LDL (mg/ml) | 68 | 101.0[a] | 2.9 | 40 | 109.9[a] | 3.7 | 42 | 123.1[b] | 5.5 | **0.001** |

[ſ] *Al-Radaideh et al, 2016*

*Abbreviations: SEM - standard error of mean; BMI - body mass index; TG - triglycerides; HDL- high-density lipoprotein cholesterol; LDL - low-density lipoprotein cholesterol.*

** p<0.05: those were significantly different based on LSD analysis are labeled with different letters.*

**Table 2 -** Percentages of energy intakes and distribution across different tertiles of cholesterol, TG, HDL-C and LDL-C

| Nutrients | | Serum cholesterol | | TG | | HDL-C | | LDL-C | |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean±SEM | *P*-value | Mean±SEM | *P*-value | Mean±SEM | *P*-value | Mean±SEM | *P*-value |
| Energy (kcal) | $T_1$ | 2985.0±183.6[ab] | | 2838.5±159.4 | | 2997.9±156.2 | | 2930.8±180.5 | |
| | $T_2$ | 2615.1±151.9[a] | **0.03** | 2853.2±172.9 | 0.36 | 3019.5±201.2 | .67 | 2806.6±156.2 | 0.47 |
| | $T_3$ | 3248.1±175.1[b] | | 3151.9±188.2 | | 2815.2±167.1 | | 3106.6±184.3 | |
| Percent Energy from Carbohydrates (%) | $T_1$ | 61.2±1.2 | | 61.5±1.1 | | 62.1±1.2 | | 61.8±1.2 | |
| | $T_2$ | 63.4±1.3 | 0.29 | 62.6±1.4 | 0.29 | 63.2±1.5 | 0.78 | 62.1±1.4 | 0.24 |
| | $T_3$ | 63.9±1.4 | | 64.4±1.4 | | 63.3±1.3 | | 64.7±1.3 | |
| Percent Energy from Fats (%) | $T_1$ | 29.7±1.1 | | 29.8±0.9 | | 28.8±0.9 | | 29.37±1.06 | |
| | $T_2$ | 28.5±1.1 | 0.30 | 28.91±1.2 | 0.11 | 28.9±1.2 | 0.76 | 29.16±1.05 | 0.21 |
| | $T_3$ | 27.4±1.1 | | 26.7±1.0 | | 27.8±1.1 | | 26.97±1.08 | |
| Percent Energy from Protein (%) | $T_1$ | 12.2±0.4 | 0.76 | 12.1±0.3 | 0.71 | 12.6±0.4[b] | **0.04** | 11.9±0.4 | 0.37 |
| | $T_2$ | 11.9±0.3 | | 11.9±0.4 | | 11.3±0.4[a] | | 12.5±0.4 | |
| | $T_3$ | 12.2±0.4 | | 12.3±0.4 | | 12.3±0.3[b] | | 11.9±0.3 | |
| Saturated Fatty Acids. Kcal | $T_1$ | 292.5±14.9 | 0.90 | 300.3±12.8 | 0.87 | 286.1±12.6 | 0.12 | 291.9±14.4 | 0.91 |
| | $T_2$ | 304.4±14.5 | | 303.7±14.5 | | 328.9±25.8 | | 300.7±14.8 | |
| | $T_3$ | 297.6±23.1 | | 290.7±24.4 | | 281.6±12.6 | | 302.1±23.2 | |
| Trans-Fatty Acids. kcal | $T_1$ | 37.1±4.8[b] | **0.02** | 30.7±3.1 | 0.82 | 31.9±4.4 | 0.54 | 35.0±4.9 | 0.12 |
| | $T_2$ | 23.5±2.3[a] | | 27.5±3.4 | | 27.9±3.3 | | 25.1±2.5 | |
| | $T_3$ | 26.6±3.0[a] | | 28.7±4.3 | | 26.6±2.7 | | 27.0±3.0 | |

*- Statistical significant difference (P< 0.050)*

*- Different letters to denote significant differences among the tertiles.*

*T stands for Tertile.*

as calcium, copper, iron and zinc intake through all tertiles of the analyzed serum lipid profiles, except for total cholesterol and LDL-C levels, which were positively affected by vitamin K intake (*P*=0.03) (Table 4).

Table 5 presents the differences in food groups' intake according to serum lipid levels. The highest intake of meat, fish, eggs, and beans was inversely affected by HDL-C (*P*=0.04). No significant differences

**Table 3** - Mean±SEM for adjusted macronutrients intake in different tertiles of cholesterol, TG, HDL-C and LDL-C

| Nutrients | | Serum cholesterol | | TG | | HDL-C | | LDL-C | |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean±SEM | *P*-value | Mean±SEM | *P*-value | Mean±SEM | *P*-value | Mean±SEM | *P*-value |
| Total Carbohydrate (g) | $T_1$ | 511.4±12.2 | | 510.1±9.9 | | 518.4±10.5 | | 515.4±12.1 | |
| | $T_2$ | 518.6±12.4 | 0.34 | 518.9±13.4 | 0.26 | 525.2±16.1 | 0.91 | 512.8±12.8 | 0.27 |
| | $T_3$ | 538.1±14.7 | | 539.6±15.7 | | 525.2±13.4 | | 540.1±14.4 | |
| Fiber (g) | $T_1$ | 56.9±2.4 | | 58.2±1.9 | | 59.19±2.07 | | 57.2±2.2 | |
| | $T_2$ | 57.4±1.8 | 0.43 | 56.3±2.2 | 0.42 | 55.2±2.3 | 0.21 | 57.9±2.0 | 0.69 |
| | $T_3$ | 60.6±2.1 | | 60.3±2.2 | | 60.3±1.9 | | 59.4±2.2 | |
| Soluble Fiber (g) | $T_1$ | 6.9±0.6 | | 7.1±0.6 | | 7.6±0.6 | | 6.7±0.6 | |
| | $T_2$ | 7.5±0.56 | 0.53 | 7.0±0.7 | 0.59 | 6.8±0.7 | 0.51 | 8.1±0.6 | 0.32 |
| | $T_3$ | 7.9±0.7 | | 7.9±0.6 | | 7.8±0.7 | | 7.5±0.7 | |
| Insoluble Fiber (g) | $T_1$ | 12.0±1.0 | | 12.7±0.9 | | 13.4±1.0 | | 11.9±1.0 | |
| | $T_2$ | 12.4±0.9 | 0.40 | 11.8±1.1 | 0.45 | 11.8±1.1 | 0.55 | 12.9±0.9 | 0.62 |
| | $T_3$ | 13.8±1.0 | | 13.6±1.0 | | 12.9±0.9 | | 13.3±1.1 | |
| Protein (g) | $T_1$ | 98.9±2.9 | | 98.2±2.3 | | 101.2±2.6 | | 97.2±2.8 | |
| | $T_2$ | 95.5±2.2 | 0.49 | 95.5±2.6 | 0.43 | 93.6±3.0 | 0.12 | 100.5±2.8 | 0.52 |
| | $T_3$ | 99.9±3.0 | | 100.5±3.1 | | 98.9±2.4 | | 96.4±2.5 | |
| Fat (g) | $T_1$ | 116.1±4.7 | | 117.1±3.9 | | 113.0±4.0 | | 115.0±4.7 | |
| | $T_2$ | 115.6±4.5 | 0.21 | 114.9±5.2 | 0.18 | 113.5±5.5 | 0.91 | 116.0±4.6 | 0.28 |
| | $T_3$ | 105.5±5.1 | | 105.1±5.2 | | 110.7±5.1 | | 106.2±5.1 | |
| Saturated Fat (g) | $T_1$ | 32.5±1.7 | | 33.4±1.4 | | 31.8±1.4 | | 32.4±1.6 | |
| | $T_2$ | 33.8±1.6 | 0.89 | 33.8±1.6 | 0.87 | 36.6±2.9 | 0.12 | 33.4±1.7 | 0.91 |
| | $T_3$ | 33.1±2.6 | | 32.3±2.7 | | 31.3±1.4 | | 33.6±2.6 | |
| Monounsaturated Fat (g) | $T_1$ | 28.9±1.8 | | 28.9±1.6 | | 28.3±1.5 | | 28.2±1.7 | |
| | $T_2$ | 26.9±1.4 | 0.11 | 27.2±1.7 | 0.07 | 23.6±1.7 | 0.09 | 27.8±1.7 | 0.14 |
| | $T_3$ | 24.1±1.7 | | 23.7±1.6 | | 27.8±1.6 | | 24.0±1.6 | |
| Polyunsaturated Fat (g) | $T_1$ | 21.8±1.8 | | 23.0±1.7 | | 21.9± 1.6 | | 21.9±1.8 | |
| | $T_2$ | 23.3±1.7 | 0.24 | 22.8±1.9 | 0.12 | 19.3± 1.8 | 0.29 | 23.6±1.7 | 0.13 |
| | $T_3$ | 19.2±1.7 | | 18.6±1.6 | | 23.2±1.8 | | 18.8±1.6 | |
| Trans-Fatty Acids (g) | $T_1$ | 4.2±0.5[b] | | 3.4±0.3 | | 3.6±0.5 | | 3.9±0.5 | |
| | $T_2$ | 2.7±0.3[a] | **0.02** | 3.1±0.4 | 0.82 | 3.1±0.4 | 0.55 | 2.8±0.3 | 0.12 |
| | $T_3$ | 2.9±0.3[a] | | 3.2±0.5 | | 3.0±0.3 | | 3.0±0.3 | |
| **Cholesterol (mg)** | $T_1$ | 228.4±26.5 | | 201.9±14.3 | | 226.6±22.9 | | 226.7±25.9 | |
| | $T_2$ | 205.7±18.4 | 0.67 | 200.4±20.1 | 0.36 | 217.7±23.3 | 0.50 | 216.4±20.5 | 0.58 |
| | $T_3$ | 205.7±16.0 | | 237.5±26.3 | | 193.1±13.1 | | 196.5±14.1 | |
| Omega 6 Fatty Acids (g) | $T_1$ | 18.9±1.8 | | 20.2±1.7 | | 19.2±1.6 | | 18.8±1.8 | |
| | $T_2$ | 20.8±1.6 | 0.19 | 20.2±1.9 | 0.96 | 15.9±2.1 | 0.19 | 20.9±1.7 | 0.17 |
| | $T_3$ | 16.1±2.0 | | 15.4±1.9 | | 20.7±1.9 | | 16.1±1.9 | |
| Omega 3 Fatty Acids (g) | $T_1$ | 1.4±0.2 | | 1.5±0.1 | | 1.4±0.1 | | 1.4±0.2 | |
| | $T_2$ | 1.7±0.1 | 0.08 | 1.5±0.2 | 0.15 | 1.2±0.2 | 0.29 | 1.6±0.1 | 0.23 |
| | $T_3$ | 1.2±0.2 | | 1.2±0.2 | | 1.6±0.2 | | 1.2±0.2 | |

*- Statistical significant difference (P<0.05)*

*- Different letters denotes significant differences among the tertiles.*

*- T stands for Tertile.*

**Table 4** - Mean±SEM for adjusted micro-nutrients intake for different of cholesterol, TG, HDL-C and LDL-C

| Nutrients | | Serum cholesterol | | TG | | HDL-C | | LDL-C | |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean±SEM | *P*-value | Mean±SEM | *P*-value | Mean±SEM | *P*-value | Mean±SEM | *P*-value |
| Vitamin A. IU | $T_1$ | 17773.9±2117.9 | | 17768.4±1642.7 | | 20193.9±1916.6 | | 17522.2±1993.4 | |
| | $T_2$ | 19501.7±2425.7 | 0.38 | 18165.7±2352.2 | 0.12 | 21035.8±2744.1 | 0.62 | 19288.8±2454.3 | 0.26 |
| | $T_3$ | 22065.8±1995.1 | | 23454.7±2477.0 | | 18037.9±1905.4 | | 22535.2±2065.1 | |
| β-carotene (µg) | $T_1$ | 7566.4±1084.1 | 0.41 | 7519.9±827.5 | 0.17 | 9154.0±1012.2 | 0.43 | 7315.3±1040.9 | 0.24 |
| | $T_2$ | 8578.5±1353.2 | | 8001.1±1420.0 | | 9422.9±1613.4 | | 8481.5±1337.1 | |
| | $T_3$ | 9854.9±1161.9 | | 10512.9±1306.3 | | 7341.5±937.2 | | 10205.2±1204.5 | |
| Vitamin D (µg) | $T_1$ | 1.0±0.3 | | 0.7±0.2 | | 0.7±0.1 | | 1.1±0.3 | |
| | $T_2$ | 0.9±0.2 | 0.78 | 0.8±0.2 | 0.44 | 1.1±0.3 | 0.43 | 0.8±0.2 | 0.57 |
| | $T_3$ | 0.7±0.2 | | 1.1±0.3 | | 0.8±0.3 | | 0.7±0.2 | |
| Vitamin E (mg) | $T_1$ | 16.5±4.1 | | 15.4±2.6 | | 11.8±0.9 | | 17.8±4.2 | |
| | $T_2$ | 16.8±2.8 | 0.82 | 16.3±2.8 | 0.98 | 18.1±3.6 | 0.25 | 15.0±2.7 | 0.75 |
| | $T_3$ | 14.2±2.4 | | 15.90±4.1 | | 18.3±4.4 | | 14.7±2.4 | |
| Vitamin K (µg) | $T_1$a | 143.8±19.7 | | 179.8±22.7 | | 221.2±37.1 | | 141.2±18.9[a] | |
| | $T_2$b | 173.1±27.9 | **0.04** | 241.4±73.1 | 0.67 | 227.4±71.5 | 0.72 | 171. 7±28.9[a] | **0.03** |
| | $T_3$c | 309.7±75.5 | | 206.6±39.3 | | 175.2±30.6 | | 313.7±75.1[b] | |
| Folate (µg) | $T_1$ | 507.8±125.3 | | 463.2±72.8 | | 369.5±26.0 | | 548.2±128.2 | |
| | $T_2$ | 510.9±78.9 | 0.90 | 511.2±81.2 | 0.90 | 576.1±101.9 | 0.20 | 461.4±73.9 | 0.80 |
| | $T_3$ | 474.3±71.1 | | 520.7±123.1 | | 568.6±133.8 | | 484.5±70.9 | |
| Vitamin B12 (µg) | $T_1$ | 7.6±1.8 | | 6.4±1.1 | | 4.9±0.5 | | 8.13±1.9 | |
| | $T_2$ | 7.1±1.3 | 0.73 | 7.1±1.2 | 0.90 | 7.9±1.6 | 0.18 | 6.31±1.2 | 0.57 |
| | $T_3$ | 5.9±1.1 | | 7.2±1.8 | | 8.2±1.9 | | 6.21±1.1 | |
| Vitamin C (mg) | $T_1$ | 281.6±46.7 | | 268.2±39.5 | | 277.1±36.7 | | 285.8±45.8 | |
| | $T_2$ | 262.3±40.3 | 0.37 | 287.4±73.1 | 0.54 | 309.9±48.4 | 0.84 | 265.5±43.5 | 0.48 |
| | $T_3$ | 344.7±41.3 | | 333.4±46.3 | | 303.9±45.1 | | 337.2±39.1 | |
| Calcium (mg) | $T_1$ | 1468.9±333.4 | | 1386.0±199.5 | | 1095.6±88.7 | | 1582.2±335.0 | |
| | $T_2$ | 1441.5±202.5 | 0.99 | 1423.9±235.1 | 0.93 | 1680.0±286.2 | 0.20 | 1325.5±198.8 | 0.77 |
| | $T_3$ | 1417.5±216.3 | | 1520.0±323.0 | | 1608.8±349.7 | | 1422.7±215.7 | |
| Copper (mg) | $T_1$ | 1.5±0.1 | | 1.5±0.1 | | 1.5±0.1 | | 1.4±0.1 | |
| | $T_2$ | 1.3±0.1 | 0.09 | 1.4±0.1 | 0.80 | 1.3±0.1 | 0.34 | 1.4±0.1 | 0.74 |
| | $T_3$ | 1.6±0.1 | | 1.5±0.1 | | 1.4±0.1 | | 1.5±0.1 | |
| Iron (mg) | $T_1$ | 32.3±5.7 | | 29.7±3.3 | | 26.1±1.2 | | 34.1±5.8 | |
| | $T_2$ | 32.3±3.5 | 0.85 | 30.8±3.5 | 0.81 | 34.1±4.6 | 0.26 | 30.3±3.3 | 0.72 |
| | $T_3$ | 29.3±3.1 | | 33.4±5.6 | | 34.6±6.0 | | 29.5±3.0 | |
| Zinc (mg) | $T_1$ | 19.8±4.5 | | 17.8±2.9 | | 14.4±0.9 | | 20.9±4.7 | |
| | $T_2$ | 20.0±3.0 | 0.72 | 19.4±2.9 | 0.94 | 20.8±3.9 | 0.24 | 18.6±2.9 | 0.71 |
| | $T_3$ | 16.5±2.6 | | 19.2±4.5 | | 21.9±4.8 | | 16.8±2.6 | |

*- Statistical significant difference (P<0.05)*
*- Different letters to denote significant differences among the tertiles.*
*- Abbreviations: Retinol Equivalent, RE; International Unit, IU; Tertiles, T*

**Table 5** - Food groups distribution across different tertiles of cholesterol, TG, HDL-C and LDL-C

| Nutrients | | Serum cholesterol | | TG | | HDL-C | | LDL-C | |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean±SEM | *P*-value | Mean±SEM | *P*-value | Mean±SEM | *P*-value | Mean±SEM | *P*-value |
| Grain group (oz) | $T_1$ | 13.1±1.1 | | 13.1±0.9 | | 13.0±0.9 | | 13.1±1.1 | |
| | $T_2$ | 12.6±0.9 | 0.63 | 12.4±0.9 | 0.50 | 13.3±1.1 | 0.98 | 13.5±0.9 | 0.92 |
| | $T_3$ | 13.8±0.8 | | 14.0±1.0 | | 13.1±0.9 | | 12.9±0.8 | |
| Vegetables group (cup) | $T_1$ | 4.9±0.6 | | 4.3±0.5 | | 5.0±0.5 | | 4.9±0.6 | |
| | $T_2$ | 3.8±0.4 | 0.12 | 4.2±0.4 | 0.27 | 4.1±0.4 | 0.39 | 3.9±0.4 | 0.21 |
| | $T_3$ | 5.1±0.5 | | 5.2±0.6 | | 4.6±0.6 | | 5.0±0.5 | |
| Fruit group (cup) | $T_1$ | 3.7±0.8 | | 3.9±0.8 | | 4.3±0.7 | | 3.6±0.7 | |
| | $T_2$ | 3.8±0.9 | 0.20 | 4.0±1.0 | 0.49 | 4.9±1.2 | 0.77 | 4.1±1.1 | 0.31 |
| | $T_3$ | 5.8±1.0 | | 5.3±0.9 | | 4.0±0.8 | | 5.5±0.9 | |
| Dairy group (cup) | $T_1$ | 0.6±0.1 | | 0.6±0.1 | | 0.6±0.1 | | 0.5±0.1 | |
| | $T_2$ | 0.5±0.1 | 0.60 | 0.6±0.1 | 0.97 | 0.7±0.1 | 0.26 | 0.6±0.1 | 0.57 |
| | $T_3$ | 0.7±0.1 | | 0.6±0.1 | | 0.5±0.1 | | 0.7±0.1 | |
| Meat group (meat, fish, eggs, and beans) (oz) | T1 | 4.6±0.5 | | 4.0±0.4 | | 4.7±0.4[b] | | 4.1±0.5 | |
| | T2 | 3.3±0.3 | 0.06 | 3.8±0.4 | 0.72 | 3.3±0.3[a] | **0.04** | 3.9±0.4 | 0.91 |
| | T3 | 4.1±0.4 | | 4.2±0.4 | | 3.9±0.4[ab] | | 4.0±0.3 | |
| Fats and oils (tsp) | $T_1$ | 10.4±0.9 | | 10.0±0.8 | | 10.2±0.9 | | 9.9±0.8 | |
| | $T_2$ | 8.9±0.8 | 0.23 | 10.4±1.1 | 0.92 | 10.8±1.1 | 0.56 | 9.9±0.9 | 0.88 |
| | $T_3$ | 11.1±1.1 | | 9.9±1.0 | | 9.4±0.8 | | 10.5±1.1 | |

*- Statistical significant difference (P<0.050)*
*- Different letters to denote significant differences among the tertiles.*
*- T stands for Tertile; C strands for Cup; oz strands for ounces; tsp stands for teaspoon.*

between grains, vegetables, fruits, milk, fats and oils intake and cholesterol, TG, HDL-C and LDL-C concentrations were observed.

## Discussion

Dyslipidemia is the abnormal lipid metabolism which is regarded as a strong predictor for the development of cardiovascular diseases. Little is known about the associations between serum lipid profiles and dietary factors. In this study, we examined the associations between macronutrient and micronutrient intake and serum lipid profile among healthy Jordanian adults.

The present study showed that total energy intake was associated with serum total cholesterol. Extra caloric intake is not recommended, whether the extra calories are from fat, protein, or carbohydrate (34). In contrast to our finding, Song et al. (2016) did not find an association between total energy intake and TC and LDL-C pattern (35). This could be attributed to the highest energy intake among our study tertiles (around 3250 calories) compared to what was found by other researchers. Total energy intake among our study group when stratified according to the BMI was significantly higher among obese participants as compared to overweight and normal body weight (3369.2±173.9, 2806.6±142.6, 2801.8±195.6 kcal for obese, overweight and normal body weight, respectively; p<0.03). These results are not presented in the tables. Excessive energy intake leads to obesity which associated with states of hyperinsulinemia and insulin resistance (36). Insulin resistance was linked to the increase in cholesterol synthesis. Insulin stimulates lipid synthesis, in particular, cholesterol synthesis, thus raising the total cholesterol level (37, 38).

Additionally, serum cholesterol was significantly lower among Jordanian adults who consumed the highest amount of trans-fatty acid. This can be interpreted as the highest amount of trans-fatty acid that can be found in hydrogenated vegetable oils, which were considered free of cholesterol (39). As a consequence, serum cholesterol could be lower for those who consumed a considerable amount of hydrogenated fats and oils. On the other hand, it is well known that trans-fatty acid intake is highly associated with elevated level of serum LDL-C and reduced level of HDL-C (39). However, the present study did not report any significant differences between trans-fatty acid intake levels and levels of serum LDL-C and that was consistent with a reported randomized crossover study in healthy young Japanese which also showed no significant effects of 0.6% energy trans-fatty acid intake on total serum cholesterol concentrations (40). Therefore, it could be concluded that the intake of trans-fatty acids among this study sample is lower than the level which may affect serum lipids.

The 3 tertiles of HDL-C were significantly associated with percentage of energy from protein, irrespective to protein sources: plants or animals. However, no specific trend could be detected in the 3 tertiles. In a study conducted in Tehran, Bahadoran et al. (2013) reported that the higher dietary intake of protein as % of energy and g/kg body weight in men was inversely related with 3-year changes in HDL-C levels (41). However, Song et al. (2016) did not find any association between % of energy from protein with TG and HDL-C pattern or even with TC and LDL-C pattern (35). The mechanism by which protein is associated with upregulated HDL cholesterol production and the extent to which body weight status modulates this response requires further study. However, because consuming a high protein diet regularly was associated with higher HDL cholesterol (and lower adiposity) regardless of total dietary energy, carbohydrate, and fat intake, the intrinsic properties of protein appear to be partially responsible for these effects (42). In addition, a higher ratio of dietary protein to carbohydrates is related to more efficient glycemic control and satiety during weight loss which may enhance HDL (43).

Regarding the results of calcium, copper, iron and zinc intake and their associations with serum lipid profile, no significant associations have been detected. In a Québec Family Study, Jacqmain et al. (2003), found that the plasma lipoprotein-lipid profile in both women and men is apparently affected by a low dietary calcium intake (<600 mg) compared to a high dietary calcium intake (>1000 mg) (44). Inconsistent with our findings, Song et al. (2016) observed an inverse association between calcium intake and TG and HDL-C pattern (35). In a cross-sectional study on healthy females, Zaribaf et al. (2014) reported that there was no significant correlation between total amount of iron, heme iron, and non-heme dietary iron with serum lipid profile (45). On the other hand, an inverse association between iron intake and total cholesterol and LDL-C pattern was observed by other (35). Additionally, a negative association between dietary zinc intake and total cholesterol and triglycerides was noticed in a cross-sectional study (41). Regarding dietary copper intake, limited cross-sectional studies tend to suggest that dietary copper is associated with a better lipoprotein profile (46).

Both water and fat soluble vitamins, except vitamin K, were not significantly associated with serum lipid profile. In this study, the differences between vitamin K intake levels were significant with serum cholesterol and LDL-C values. The effect of vitamin k intake and blood lipid profile in the literature is equivocal and depends on its sources if they are plant (vitamin K1: phylloquinone) or animal (vitamin K2: menaquinones) sources. Indeed, vitamin K1 was found to be inversely associated with a fatal heart attack (47), while vitamin K2 was not associated with increased incidence of heart disease and increased fatal heart attack (48). In a prospective cohort study of Dutch men and women, neither vitamin K1 nor vitamin K2 was found to be associated with stroke risk (49). In our study, total vitamin K is estimated irrespective of its source, so further studies are warranted to assess the association between vitamin K from the two sources and its effect on serum lipids.

It was observed that number of servings consumed from the following groups: grain, vegetables, fruits, milk and dairy products and fats and oils were not associated with serum lipid profile. Similar results were obtained in another study, except for vegetable group, which was inversely associated with total cholesterol

and LDL-C pattern and milk as well as dairy products, which was inversely associated with TG and HDL-C pattern (35). In the present study, food groups that were inversely associated with HDL-C included meat, fish, eggs and beans. In consistent with our findings, Daoud et al. (2014) reported that high protein diets resulted in a reduction in HDL-C and LDL-C (50).

## Limitations

Our study has several limitations including the cross-sectional design that did not determine a causal relationship between dietary factors and dyslipidemia. Furthermore, the one year dietary recall period, which may be affected by changes in memory and bias, is one of the major limitations of the present study. However, we believe that because food selection and the taste are mostly based on availability and habits that influence deliberate choices, including endemic cultural biases, we accept that the recall period of one year is very likely to be reflective of the previous years. In addition, our sample size is small due to the limited financial support for the biochemical analyses but within the recommended size as calculated using the sample size power calculations. Additionally, the subjects' lipid profile is generally within the normal levels which showed few associations with some nutrients. Therefore, another study on a large-scale is warranted to generalize these findings.

## Conclusion

In conclusion, there are many possible associations between Jordanian diet and its components and serum lipids, even after adjustment for potential confounding factors. The consumption of meat, fish, eggs and beans should be within the recommendations.

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Original article

# The relationship between BMI and blood pressure in school-age children in Izmir, Turkey

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**Summary.** *Objective:* Prevalence of hypertension and obesity are increasing in children. This study aimed to determine the relationship between hypertension and obesity among school-age children in Izmir (western part of Turkey), which is the third largest city in Turkey. *Design:* Cross-sectional descriptive study. *Participants:* The sample consisted of 1289 students aged between 6 and 10 attending two primary schools in Izmir, a province in Turkey. *Measurements:* The students' weights, heights and blood pressures were measured. *Data Analysis:* The independent sample t-test, anova test and multiple regression analysis were used for statistical analyses. *Results:* The prevalence of hypertension and obesity was 20.2% and 14.7% respectively. Gender was not associated with hypertension and obesity in children. The body mass index was statistically significant as an explanatory variable of hypertension for both genders. *Conclusion:* Overweight and obese children are at a significantly higher risk for hypertension than are normal weight children.

**Key words:** hypertension, obesity, school children, Turkish

## Introduction

There is evidence that hypertension in adults' starts in childhood (1, 2). Children whose blood pressure and body weight are high for their age are more likely to develop hypertension in the future (3). In recent years, the prevalence of hypertension in children and adolescents is increasing. The increase in childhood hypertension leads not only to an increase in the prevalence of hypertension in adulthood but also to an increase in cardiovascular mortality and morbidity. Therefore, early diagnosis of childhood hypertension is an important public health strategy for the prevention and control of cardiovascular diseases (4).

Many chronic diseases such as diabetes, hypertension and cardiovascular diseases are known to be associated with obesity. Childhood obesity is also known to be associated with high blood pressure. Increase in the prevalence of hypertension in parallel with obesity epidemic in children has become a major problem (5). Studies conducted with large samples in several countries have demonstrated that high blood pressure is an important determinant for obesity in children (4, 6). However, in Turkey, data on the relationship between hypertension and obesity, and disease burden in children at the national level are limited. To overcome these diseases and to develop effective prevention strategies, knowing the prevalence of the disease is a priority. This present study was aimed to determine the prevalence and the relationship between obesity and hypertension in Turkish school-age children aged 6-10 years, living in the province of Izmir, Turkey. İzmir is the third largest city in Turkey, with a city population of more than four million.

## Materials and methods

According to Turkey Population Statistics, 522.390 children, aged 5-14 years were living in Izmir

in 2015. Using this information and the probable prevalence of overweight and obesity was accepted as 15 % with a margin of error of 0.05, and the minimum sample size was calculated 194 students for this study with a 95% confidence interval. Because of this study aims (statistical test selection, subgroup size, etc.) sample size was raised to 1000.

This cross-sectional and descriptive study was conducted among all grades (from grade 1 to grade 4) of two primary schools in Balçova district of Izmir city center, Turkey, between January 2014 and January 2015. Balçova was inhabited by people of different socio-economic levels and had a dynamic population structure. There were six primary schools and 3240 students were attending primary schools in the Balçova district. Two of these six primary schools were selected with a simple randomize method. For all of the schools, the classes were mixed sex and school attendance was imperative (primary school is enforced by the government in Turkey). All students enrolled in all grades in these two schools (n=1452) were asked to participate. The response rate was 88.7% (n=1289).

After data collection days were determined, data collectors were trained by researcher. The students were visited in schools and data collection was completed in classrooms. All students were invited for data collection but some students were excluded because they were absent on data collection days. The students' age and gender information was taken before height, weight and resting blood pressure (BP) measurements were obtained.

*Weight and height measurements:* Children's body weights were measured with a digital scale (Bosch) (±0.1 kg). While their weights were measured, the students took off their shoes and clothes except for school uniforms. For the height measurements, a digital tape measure (Bosch) (±0.1 cm) was used. Height measurements were made with shoes off, shoulders relaxed and arms at the sides. Body Mass Index (BMI) was calculated by dividing weight (kg) by the square of the body height ($m^2$), which is expressed in units of $kg/m^2$. After the children's BMIs were calculated, percentile values based on their age and gender were determined. Those in the less than the 5 th percentile were considered as underweight, from the 5 th percentile to less than the 85 th percentile as normal weight, from the 85 th to less than the 95 th percentile as overweight and equal to or greater than the 95 th percentile as obese (7).

*Resting blood pressure:* Resting blood pressure measurements were performed manually using a mercury sphygmomanometer (ERKA) with a cuff appropriate to their age. Measurements were performed in the sitting position after at least a 10-minute rest. The assessments were performed in accordance with the criteria defined in National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents (8). According to age and height, systolic or diastolic blood pressures in the ≥95 th percentile were considered as hypertension, between the ≥90 th percentile and the <95 th percentile as prehypertension, and lower than the 90 th percentile as normal.

Data on the children's age, gender, BMI and blood pressure were assessed with the descriptive statistics (numbers, percentages, means). While the comparison of the BMI and blood pressure values of boys and girls for age and gender were performed with the anova test, inter-gender comparisons were performed with the Independent Samples t test. To identify the variables affecting blood pressure in girls and boys, the multiple regression analysis was used. Data were analyzed using Statistical Package for the Social Sciences version 15.0 (SPSS Inc., Chicago, IL, USA) and the statistical significance was defined as $P<0$.

To carry out the study, the approval was obtained from the non-interventional clinical research ethics committee of Dokuz Eylul University.

## Results

In this present study, data of 1289 students aged between 6 and 10 years were evaluated. Of the students, 51.4% (n=663) were male. The mean age of the girls and boys were 7.71±1.21 and 7.70±1.23, respectively. The students' mean BMI value was 17.93±3.51, mean systolic blood pressure value was 108.24±10.95 mmHg and mean diastolic blood pressure value was 66.62±8.28 mmHg. Of them, 61.8% (n=796) were normal weight, 12.7% (n=164) were overweight and 14.7% (n=190) were obese. The prevalence of hypertension and prehypertension was 20.2% (n=261) and

10.0% (n=129), respectively. Of the students, 4.8% (n=62) were both obese and hypertensive (Table 1).

According to age groups, the highest prevalence of obesity was in 8-year olds (21.5%), and the highest prevalence of hypertension was in the 7-year olds (26.1%) (Table 2). The distribution of BMI and blood pressure values of the participants for age and gender is shown in Table 3. An increase was determined in the BMI and diastolic blood pressure mean values with age in both genders (p<0.000). For the 6-year-old boy students, the BMI and diastolic blood pressure mean values were 17.02±3.01 kg/m$^2$ and 66.36 ± 9.17 mmHg respectively. These values increased to 19.68±4.68 kg/m$^2$ and 68.67±6.99 mmHg for the 10-year-old boy students. The values were 16.66±2.40 kg/m$^2$ and 65.02±7.12 mmHg for the 6-year-old girl students, and 18.98±3.34 kg/m$^2$ and 68.91±7.68 mmHg for the 10-year-old girl students. The comparison of girls' and boys' BMI (17.78±3.32 vs 18.07±3.67), systolic (107.94±11.19 vs 108.52±10.71) and diastolic blood pressure (66.52±7.86 vs 66.72±8.65) mean values revealed no significant difference (p>0.05).

**Table 1.** Obesity and hypertension prevalence (N=1289)

| | n | (%) |
|---|---|---|
| Weight status | | |
| Underweight | 139 | (10.8) |
| Normal weight | 796 | (61.8) |
| Overweight | 164 | (12.7) |
| Obese | 190 | (14.7) |
| Blood pressure status | | |
| Normal | 899 | (69.8) |
| Prehypertension | 129 | (10.0) |
| Hypertension | 261 | (20.2) |

Comparison of the blood pressure values of normal-weight students with those of the overweight and obese students revealed significant differences between the two groups in terms of both the systolic blood pressure values (106.71±10.34 and 112.12±11.42, respectively) and diastolic blood pressure values (65.69±8.01) and 68.50±8.28, respectively) (p<0.000). Blood pressure levels of the overweight and obese students were higher than were those of the normal weight students.

The results of regression analysis indicating the factors affecting the blood pressure values of the boys and girls are shown in Table 4. In the girls, BMI and age accounted for 10% and 9.5% of the variance for the systolic blood pressure and diastolic blood pressure respectively. These rates were 11.3% and 7.3% for the boys. BMI was identified as the variable directly affecting both the systolic blood pressure and the diastolic blood pressure for the two genders (p<0.000).

## Discussion

In this current study, the prevalence of hypertension and obesity among school-age children (aged 6 to 10 years) in Balçova District of Izmir and the relationship between hypertension and obesity were assessed. The study findings showed that the prevalence of obesity and hypertension was high, and that diastolic blood pressure and BMI increased with age. Childhood obesity was determined to be associated with hypertension.

In this current study, anthropometric characteristics of the 1289 children were assessed and the prevalence of hypertension was found as 20.2%. In previous studies conducted in Turkey, the prevalence

**Table 2.** Hypertension and obesity prevalence according to age (N=1289)

| Age | 6 | | 7 | | 8 | | 9 | | 10 | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | n | % | n | % | n | % | n | % | n | % | n | % |
| Hypertension | 70 | (24.3) | 74 | (26.1)* | 38 | (13.2) | 73 | (19.6) | 6 | (10.5) | 261 | (20.2) |
| Pre-hypertension | 32 | (11.1) | 41 | (14.4) | 26 | (9.0) | 27 | (7.3) | 3 | (5.3) | 129 | (10.0) |
| Obesity | 57 | (19.8) | 34 | (12.0) | 62 | (21.5)* | 30 | (8.1) | 7 | (12.3) | 190 | (14.7) |

** The highest prevalence in the age group*

**Table 3.** Body mass index (BMI (kg/m$^2$) and systolic and diastolic blood pressures according to age and gender

| | Age | n | BMI (kg/m$^2$) | | Systolic BP (mmHg) | | Diastolic BP (mmHg) | |
|---|---|---|---|---|---|---|---|---|
| | | | Mean | SD | Mean | SD | Mean | SD |
| Boys | 6 | 148 | 17.02 | 3.01 | 108.18 | 11.56 | 66.36 | 9.17 |
| | 7 | 150 | 17.01 | 2.76 | 107.70 | 11.10 | 64.32 | 8.66 |
| | 8 | 148 | 17.76 | 2.92 | 108.54 | 8.83 | 66.52 | 8.09 |
| | 9 | 183 | 19.74 | 4.41 | 109.39 | 11.09 | 68.77 | 8.46 |
| | 10 | 34 | 19.68 | 4.68 | 108.82 | 10.73 | 68.67 | 6.99 |
| Total | | 663 | 18.07 | 3.67 | 108.52 | 10.71 | 66.72 | 8.65 |
| | | | F=19.392 $p$<0.001 | | F=0.569 $p$=0.686 | | F=6.131 $p$<0.001 | |
| Girls | 6 | 140 | 16.66 | 2.40 | 106.36 | 10.47 | 65.02 | 7.12 |
| | 7 | 134 | 16.61 | 2.34 | 107.53 | 11.61 | 65.07 | 8.04 |
| | 8 | 140 | 18.17 | 3.53 | 107.30 | 10.01 | 66.00 | 7.70 |
| | 9 | 189 | 18.98 | 3.79 | 109.49 | 12.14 | 68.75 | 7.91 |
| | 10 | 23 | 18.98 | 3.34 | 111.08 | 10.43 | 68.91 | 7.68 |
| Total | | 626 | 17.78 | 3.32 | 107.94 | 11.19 | 66.52 | 7.86 |
| | | | F=17.109 $p$<0.001 | | F=2.234 $p$=0.064 | | F=7.160 $p$<0.001 | |

**Table 4.** Multiple regression analysis of blood pressure results

| Dependent variable | | | $R^2$ | β | t | p value |
|---|---|---|---|---|---|---|
| Systolic BP | Girls | BMI | - | 0.311 | 7.811 | <0.001 |
| | | Age | - | 0.017 | 0.440 | 0.660 |
| | | BMI and Age | 0.100 | - | 28.073 | <0.001 |
| | Boys | BMI | - | 0.348 | 9.062 | <0.001 |
| | | Age | - | -0.056 | -1.471 | 0.142 |
| | | BMI and Age | 0.113 | - | 33.681 | <0.001 |
| Diastolic BP | Girls | BMI | - | 0.252 | 6.320 | <0.001 |
| | | Age | - | 0.116 | 2.916 | 0.004 |
| | | BMI and Age | 0.095 | - | 22.647 | <0.001 |
| | Boys | BMI | - | 0.244 | 6.208 | <0.001 |
| | | Age | - | 0.066 | 1.681 | 0.093 |
| | | BMI and Age | 0.073 | - | 22.896 | <0.001 |

of hypertension in school-age children ranged between 7.9 and 15.1% (9-11). In studies conducted in various countries, the prevalence of hypertension was 6.2% in Italy (12), 5.9% in India (13) and 20.6% in the United States (14). In this present study, the obesity prevalence in school-age children was determined as 14.7%. In a study conducted in Bursa, a province in Turkey, the prevalence of obesity in school-age children was reported as 11.2% (9). In another large-scale study conducted with students from fifty-three provinces in Turkey, the prevalence of obesity in school-age children was 10% and 6.6% for boy and girl students re-

spectively (15). In two studies conducted in Greece and China, the prevalence of obesity in school-age children was similar to the prevalence of obesity determined in this present study 13.1% (16) and 15.7% (17). It is difficult to determine the exact prevalence of hypertension in children, because results vary from one study to another due to the differences in participants' age groups, populations of the studies (schoolchildren or general population), blood pressure measurement methods, the number of blood pressure measurements, the place of residence and ethnicity. Another reason may be due to the differences between the prevalence of obesity (10).

The results showed that BMI and diastolic blood pressure increased with age in both genders. Polat et al. carried out a study with schoolchildren in Ankara, the capital of Turkey and found similar results indicating that BMI, and systolic and diastolic blood pressure increased with age in both gender (11). The results showed that blood pressure and BMI did not differ by gender. The relationship between gender, and blood pressure and BMI is controversial. Previous studies showed that in Iranian and Turkish children, BMI and blood pressure values of boys were higher than were those of girls (11, 18). On the other hand, Gündogdu reported that girl students' BMI was higher than that of boy students, but that blood pressure values did not vary for gender (19). Although it is not clear, the possible causes of the gender-related differences are considered to be associated with the effects of sex hormones in the control of blood pressure (18).

The results of the regression analysis showed that high BMI is an important predictor for the risk of hypertension in children. In both sexes, both diastolic and systolic blood pressure increased as BMI increased. A study conducted in Turkey indicated that obese children had higher systolic and diastolic blood pressure values (20). Similarly, a study of Chinese schoolchildren showed that obesity was a risk factor for hypertension (4). In another study conducted in the United States, the risk of hypertension in overweight and obese schoolchildren was found to be 3 times more than that in other children (15).

The results obtained from this present study are applicable only to the students surveyed and thus they cannot be generalized to other schoolchildren in Turkey.

## Conclusion

The results of the present study confirm that the prevalence of hypertension and obesity in school-age children is increasing, and that childhood obesity is a significant risk factor for hypertension. To protect children against cardiovascular disease risks in adulthood, it is important to diagnose their problems at an early stage and to implement preventive interventions. Conducting yearly body weight and blood pressure measurements in children is the first step in protecting them against hypertension and obesity. Routine follow-ups and screenings carried out in schools, and primary and secondary healthcare facilities offer significant opportunities for health professionals to diagnose childhood hypertension and obesity. In order to reduce cardiovascular morbidity and mortality, it is recommended that regular screening programs should be conducted in schools, children at risk should be followed, appropriate treatment programs should be organized, education programs on the prevention of obesity and hypertension should be prepared for children, families and teachers, and environmental and political measures to promote a healthy lifestyle should be taken.

## Ethics statement

This study was reviewed and approved by the Dokuz Eylül University Ethical Committee.

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# Major Dietary patterns among female adolescents with eating disorders: A factor analysis approach

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**Summary** *Aim:* Present study was conducted to determine major dietary patterns among female students as major factors in preventing eating disorders. *Methods and Material:* In this cross-sectional study, 515 samples were detected through 2766 female students who were selected by a multistage random sampling from 5 distinct of Tehran and assessed for having eating disorders. A two-stage procedure including screening using Eating Attitude Test-26 questionnaire and a semi structured questionnaire based on Diagnostic & Statistical Manual of Mental disorders, 4th ed. diagnostic criteria was used to diagnose eating disorders. All participants fulfilled a modified version of the Body Shape Satisfaction Scale and a qualitative food frequency questionnaire. Dietary patterns were defined using factor analysis and three interpretable factors were obtained. Then, people were categorized based on their dietary patterns' scores quartiles. To identify the association between dietary patterns and eating disorders, logistic regression was used in three models. *Findings:* Using factor analysis, three major dietary patterns were obtained: High carbohydrate- High fat pattern, High Proteins- High fat pattern and High Fibre-Low fat pattern. Findings showed that the Odds ratio of eating disorder reduced in high protein- high fat pattern and after adjustment of confounding factors, this reduction is still remained significant. Odds ratio of eating disorder is increased in high fibre- low fat dietary pattern significantly in all models. *Conclusions:* It seems that odds of eating disorders is higher in adolescents who follow a high fibre – low fat (vegetarian) dietary pattern and lower in adherence to "high protein-High fat" dietary pattern.

**Key words:** eating disorders, major dietary pattern, factor analysis, female, adolescent

## Introduction

Eating disorders are a group of food and nutrition related diseases in which dramatic changes occur in eating behaviours and predominantly during adolescence for the first time, mostly in girls. The symptoms of eating disorders are mainly due to pathological fear of being overweight or obese and their diagnostic criteria are based on psychological, behavioural and physiological characteristics (1-4). Epidemiological studies reported the prevalence of partial syndrome of eating disorders among adolescent from 0.8-14% (5). Prevalence of bulimia nervosa and partial syndrome among Iranian female adolescents has been reported 2.1% and 6.5%, respectively(6).

In these patients, weight preoccupation cause a range of misbehaviours includes severe restriction of energy intake on voluntary, self starving and unusual eating patterns. This may result in inappropriate intake of nutrients and malnutrition that could affect adolescents' health status via different mechanism (7, 8). The results of the studies have shown menstrual disorders, decreased bone mineral density, reduced height growth, delayed puberty in addition to Calcium, Iron,

Zinc, Copper, Magnesium, and C , A, D, E, $B_1$, $B_2$, $B_6$, $B_9$ and $B_{12}$ Vitamins deficiencies in these patients resulting from limiting food and calorie intake(2, 5, 9).

Only a few data is available about the intake pattern of patients with eating disorders and limited conducted studies just assessed their nutrient intake. This traditional approach won't be able to identify the relation between food intake and disease, because of ignoring the interactions of foods and nutrients and confounding effect eating habits. So this approach is substituted nowadays by "dietary pattern analysis" method that was provided in 1986 by Jacobson and et .al (10). In this method, food items are put in a "factor" based on the degree of their correlation while factors are totally independent to each other; then, using leaner combination of variables (food items) for each factor, each people is given a "food score" which can be used in logistic regression analysis to examine the relation of disease with dietary patterns. Actually, dietary patterns make it possible to identify the whole diet and not its ingredients. So, it will be easier to design and implement nutritional intervention in the form of changes in dietary patterns and will be more successful (11-12).

Studies on "dietary pattern" around the world as well as Iran have been focused mainly on adults and a few studies assessed dietary pattern of children and adolescents(13). Up to date, no study has been investigated the dietary patterns among patients with eating disorders, so the present study was conducted to determine the major dietary pattern of adolescent girls with eating disorders in Tehran, Iran as a basic step to design appropriate nutritional intervention to prevent eating disorders.

## Material and Methods

In this descriptive cross-sectional study, 2766 female high school students were selected with a stratified random sampling from 5 distinct of Tehran regarding to pupils population in each distinct. Then, a two-stage approach were used to diagnose eating disorders among them and 515 individuals, all diagnosed eating disorders(n=231) and 284 healthy, were entered study.

In this study 25 food groups were considered for factor analysis and while it requires at least 10 samples for each variable (food groups), at least 250 samples was needed. According to the 515 samples analyzed in this study, sample size was adequate for factor analysis.

This study was approved by "The ethics committee of National Nutrition and Food Technology Research Institute".

### Data collection tools

In present study, a demographic questionnaire were used to gathering data about number of family members, parents' job, education and their marital status and students' date of birth, parity and their age at menarche.

In addition, for screening and identification of suspected samples, Farsi translation of EAT-26 questionnaire was used that its reliability and validity have been verified by Dezhkam and colleagues (14). Responses of EAT question are classified based on the Likert scale and for each statement, always, usually and often, gets 3, 2 and 1 point respectively. Three remaining options were "sometimes", "rarely" and "never" are zero-rated. Thus, EAT-26 scores can be from zero to 78 and score of 20 or higher indicates a possible eating disorder.

Also a semi structured questionnaire that was prepared and validated by Dezhkam and colleagues based on DSM-IV diagnostic criteria was used to diagnose eating disorders(14). On the basis of DSM-IV criteria, anorexia nervosa is defined by «Refusal to maintain body weight at or above a minimally normal weight for age and height, intense fear of gaining weight or becoming fat, disturbance in the way one›s body weight or shape is experienced, in postmenarcheal females, amenorrhea, i.e., the absence of at least 3 consecutive menstrual cycles»(15). Bulimia nervosa is defined by «recurrent episodes of binge eating characterized by eating, in a discrete period of time (e.g., within any 2-hour period), an amount of food that is definitely larger than most people would eat during a similar period of time and under similar circumstances with a sense of lack of control over eating during the episode, in addition to recurrent inappropriate compensatory

behaviour to prevent weight gain, such as self-induced vomiting, misuse of laxatives, diuretics, enemas, or other medications, fasting, or excessive exercise. The binge eating and inappropriate compensatory behaviour both occur, on average, at least twice a week for 3 months»(15). Eating disorder not otherwise specified includes disorders of eating that do not meet the criteria for the above two eating disorder diagnoses. Examples include: «For female patients, all of the criteria for Anorexia Nervosa are met except that the patient has regular menses or despite significant weight loss, the patient›s current weight is in the normal range or all of the criteria for Bulimia Nervosa are met except that the binge eating and inappropriate compensatory mechanisms occur less than twice a week or for less than 3 months or the patient has normal body weight and regularly uses inappropriate compensatory behaviour after eating small amounts of food»(15).

To determine body satisfaction, a modified version of the Body Shape Satisfaction Scale was used in which satisfaction with ten different body parts (height, weight, body shape, waist, hips, thighs, stomach, face, body build, shoulders) were administered and scored with a five Likert scale, ranging from one point for "very dissatisfied" to five points for "very satisfied" and classified as weak, medium and good based on tertiles of obtained scores (16-17). To investigate physical activity level, a valid questionnaire for adolescent that includes four questions was used(18). Responses are classified based on the Likert scale as the most active choice scored 4 and less active scored 1 in each question. The tertiles of total score were considered as high, medium and low physical activity. To investigate usual dietary intake, a valid (19), semi-quantitative FFQ included 168 food items was used but because of time limitation for fulfilling questionnaires at school and special characteristics of eating disorders, according to other studies on food consumption in this age group, mentioned questionnaire merged and modified to a qualitative FFQ questionnaire including 70 items. Content validity of this new questionnaire confirmed by group of experts and Cronbach's Alpha was used to determine its reliability (r=0.68).

**Study procedure**

To implement study, all the students completed Demographic and EAT 26 questionnares in first phase. Then screening questionnaire were rated and samples with high risk of eating disorders were defiened (n=578). In the second phase, all these high risk samples fullfiled the diagnostic questionnaire of eating disorders which was supplemented with a short interview by a trained expert to ensure the correction of answers. Thus, no case of anorexia nervousa, 59 cases of bulimia nervousa and 178 cases of eating disorders not otherwise specified (EDNOS), totally 237 preson were diagnosed with eating disorder which Food Frequency Questionnaire (FFQ) questionnaire was completed for 231 of them with interview by an expert. 293 students with screening score less than 15 selected randomly and after the completion of diagnostic questionnaire to ensure lack of eating disorders, were selected and 284 of them fullfiled FFQ questionnaire in same method. The Procedure is shown in Figure 1.

**Anthropometric measurments**

Weight and height of selected samples were measured using standard methods by a trained expert. Weight was measured with a portable digital scale (Seca), with an accuracy of 10 g and height was meas-

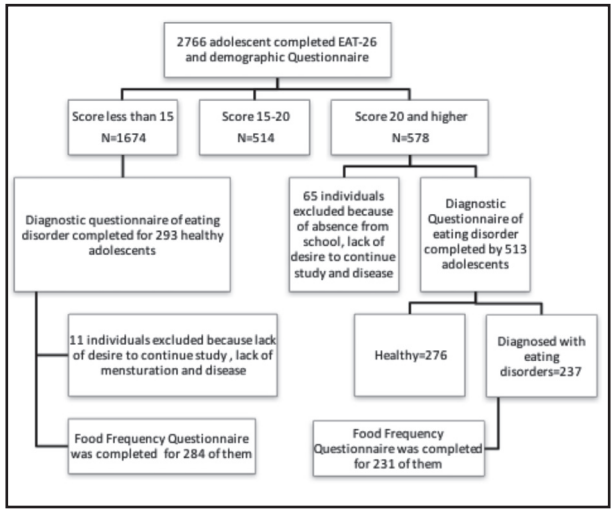


**Figure 1**. Procedure of screening and diagnosis of eating disorders among female students of Tehran, Iran

ured with a non-elastic tape measure with a precision of 0.1 cm. Then BMI was calculated and underweight (≥5$^{th}$), overweight (85$^{th}$-95$^{th}$) and obesity (≥95$^{th}$) were defined compared to BMI for age of CDC2000 for adolescents (20-21).

*Data analysis:*

Data were analyzed using SPSS (Version 18) and dietary patterns were defined using factor analysis. In order to perform factor analysis, due to the large number of food items in the food frequency questionnaire, food items were categorized to 25 "Food Groups" (Table 1) based on the similarity of their nutrients content or their culinary usage and according to previous studies(11). If a food item had a unique nutrient profile or its consumption indicated a distinct food pattern, considered individually as a food group. Principal Component Analysis (PCA) with Varimax rotation was used to extract independent dietary patterns and based on Eigen value more than 1.6 and using Scree test, three interpretable factors were retained. Loading factor equal or more than 0.3 was considered to determine items of each dietary pattern according to previous studies and scores of each people were calculated based on loading factors and food items in each factor (12, 22). Then, people were categorized based on their dietary patterns' scores quartiles. To identify the association between dietary patterns and eating disorders, the data of both groups (with and without eating disorders) were analyzed together and logistic regression was used in three models: in first model, effect of age, age at menarche and weight status, in second model previous variables in addition to body satisfaction and physical activity and in third model, effect of all variables including age, age at menarche and weight status, body satisfaction, Physical activity, parity, parents marital status, parents job and education were modified. First quartile of dietary patterns' score was determined as reference in all models (11, 23). Demographic characteristics were defined as mean and standard deviation for quantitative variables and percent for qualitative ones. One way ANOVA were used to compare means between groups of quantitative variables and Chi square and fisher exact test were used to compare the frequency of qualitative variables. Data normality was checked using Kolmogorov–Smirnov test before analyzing. Anthropometric data were analyzed using Epi-info.

## Results

*General charactersistics*

Result showed that Mean±SD of age was 15.63±0.91 and age at menarch was 12.61±1.21 years among studied population. Also the Mean±SD of their weight, height and body mass index was 60.54±12.02 (Kg), 160.92±6.07 (Cm) and 23.37±4.42 (Kg/m$^2$), respectively. No significant difference was observed between these groups (Bulimia, EDNOS and Normal) regarding these variables.

**Body mass index status of samples has been shown in chart 1, and no significant difference observed between groups in this regard.**

Chart1- Frequency of Body mass index distribution among 515 female students

The most frequent parity was "first" (45.5%), and most of them (48.8%) were belonged to "four members" families. Majority of their parents were living together (80.1%). Assessing their parents hob showed that the most frequency of their father job was self-employee and Majority of mothers was housewives. The most frequent level of parents' education was high school Diploma, 51.1% and 39.23% in fathers and mothers, respectively. Using Qi-square, no statistical significance was observed between eating disorders and these variables.

Assessing their body satisfaction revealed that 12.8% of them had low satisfaction, 53% medium satisfaction, and 34.2% were well satisfied with their bodies.

*Major Dietary Patterns*

*Using factor analysis among studied population, three major dietary patterns were identified and named*

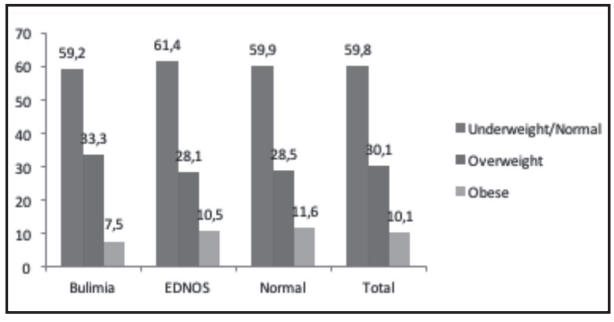


**Figure 2**.

*based on their dominant macro nutrients as:*

- High carbohydrate-high fat pattern including high consumption of rice, corn, French fries, salty snacks, confectionary products, mayonnaise, fast food.
- High protein-high fat pattern including high consumption of Other cereals, red meat, fish, egg, organ meat, dairy products, nuts, olive and olive oil, saturated fat.
- High fiber-low fat pattern including avoidance of rice and high consumption of vegetables, sour snacks, fruits, nuts and dried fruits.

Table 1 is showing the "factor loading" of food items in each of patterns. These three patterns totally represent %26.4 of the variance explained. Indeed, some other dietary patterns were identified too, but due to the low variance explained by these models were not considered in other analyzes. As the dietary patterns defined by factor analysis are formed on the basis of association between

**Table 1.** Loading factor of food groups in extracted dietary patterns

| **Food Groups** | **High carbohydrate, High fat** | **High Protein, High fat** | **High fibre, Low fat** |
|---|---|---|---|
| Salted snacks | 0.683 | -0.042 | 0.090 |
| Fast foods | 0.646 | -0.063 | -0.038 |
| Mayonnaise | 0.620 | 0.157 | 0.001 |
| French fries | 0.509 | 0.112 | -0.050 |
| Confectionary Products | 0.447 | 0.008 | 0.035 |
| Boiled corn | 0.353 | 0.287 | 0.080 |
| Hydrogenated fat | 0.353 | 0.458 | -0.148 |
| Rice | 0.303 | 0.200 | -0.373 |
| Dairy products(Regular and low-fat) | -0.153 | 0.580 | 0.087 |
| Red meats | 0.025 | 0.517 | 0.006 |
| Cereals | 0.258 | 0.457 | -0.157 |
| Olive and olive oil | -0.216 | 0.386 | 0.297 |
| Fish | 0.098 | 0.385 | 0.176 |
| Egg | 0.001 | 0.370 | 0.004 |
| Organ meats | 0.198 | 0.359 | 0.009 |
| Nuts | 0.242 | 0.315 | 0.419 |
| Dried fruits | 0.173 | -0.064 | 0.705 |
| Sour snacks | 0.249 | -0.166 | 0.624 |
| Vegetables | -0.079 | 0.230 | 0.521 |
| Fruit and fruit juice | 0.091 | 0.258 | 0.382 |
| Potato(boiled or grilled) | -0.037 | 0.252 | 0.212 |
| Legumes | 0.003 | 0.273 | 0.218 |
| High fat dairy products | 0.227 | 0.030 | 0.041 |
| Poultry | 0.157 | 0.055 | 0.124 |
| Tea | 0.206 | 0.043 | 0.073 |
| % Variance | 12.06 | 7.89 | 6.48 |

*Factor loading values greater than 0.3 is considered*

food items (rather than similarity between dietary patterns of individuals), so all the subjects are present in all patterns with different scores.

As it is shown in Table 2, odds ratio of eating disorders is different in quartiles of High carbohydrate- high fat pattern, as it is decreased in the second quartile to the first. In 3rd and 4th quartiles, although there is a slight increase in odds ratio compares to the second quartile, odds ratios are still lower than first quartile. The same pattern is observed in odds ratio values in all models and after adjusting the effect of confounding variables.

Odds ratio of eating disorders is decreased in high protein- high fat pattern. Although after adjusting for confounding variables, odds ratio in second quartile was not significant, but a significant decreasing trend is observed in all other quartiles of all models.

Odds ratio of eating disorders is increased significantly in quartiles of high fibre- low fat pattern and although the odds ratio is not significant in second quartile of any of these models, but its trend of increasing is significant.

## Discussion

In the present study, using factor analysis, three major dietary patterns were identified among the population: "**high carbohydrate and high fat**" pattern include salty snacks, fast food, mayonnaise, French-fries, confectionary products, corn and rice, "**high protein, high fat**" pattern includes saturated fat, other grains, low-fat or regular dairy products, red meat, olives and olive oil, fish, eggs, organ meats and nuts and "**high fibre and low fat** " pattern which includes rice (negative relationship), nuts, dried fruits, sour snacks, vegetables and fruits. All three models showed a significant relationship with eating disorders after adjusting for confounding variables.

In the most of studies which have examined dietary patterns, food items are classified in patterns under titles like «unhealthy or western» versus «healthy or traditional» patterns which include items like olives, nuts, and fish (24-27). Dietary patterns obtained in present study do not exactly follow this classification,

**Table 2.** Linear regression analysis of the association between dietary patterns and eating disorders

| **Quartiles of Dietary Patterns** | | | | | **P-Value Trend** |
|---|---|---|---|---|---|
| | **First** | **Second** | **Third** | **Forth** | |
| **High carbohydrate, High fat** | | | | | |
| Crude | 1 | 0.43(0.26-0.73) | 0.50 (0.3-0.85) | 0.59(0.35-0.98) | 0.007 |
| Model 1 | 1 | 0.44(0.25-0.77) | 0.53(0.31-0.92) | 0.62(0.36-1.07) | 0.009 |
| Model 2 | 1 | 0.46(0.26-0.81) | 0.53(0.30-0.93) | 0.67(0.38-1.18) | 0.039 |
| Model 3 | 1 | 0.39(0.21-0.71) | 0.50(0.28-0.91) | 0.60(0.33-1.08) | 0.017 |
| **High Protein, High fat** | | | | | |
| Crude | 1 | 0.61(0.36-1.01) | 0.40(0.24-0.68) | 0.34(0.20-0.57) | <0.0001 |
| Model 1 | 1 | 0.54(0.31-0.94) | 0.38(0.22-0.66) | 0.32(0.19-0.57) | <0.0001 |
| Model 2 | 1 | 0.60(0.34-1.04) | 0.37(0.20-0.66) | 0.35(0.19-0.62) | 0.001 |
| Model 3 | 1 | 0.59(0.33-1.06) | 0.38(0.21-0.70) | 0.35(0.19-0.64) | 0.002 |
| **High Fibre, Low fat** | | | | | |
| Crude | 1 | 1.67(0.98-2.85) | 2.48(1.45-4.24) | 3.18(1.86-5.44) | <0.0001 |
| Model 1 | 1 | 1.61(0.91-2.84) | 2.38(1.35-4.17) | 3.19 (1.8-5.66) | <0.0001 |
| Model 2 | 1 | 1.70(0.94-3.06) | 2.24(1.25-4.01) | 2.78(1.53-5.05) | 0.005 |
| Model 3 | 1 | 1.72(0.93-3.19) | 2.53(1.37-4.65) | 2.74 (1.47-5.1) | 0.005 |

*Model 1: Justified for weight status, age and age at monarch; Model 2: Justified for weight status, age, age at monarch, body satisfaction and Physical activity; Model 3: Justified for weight status, age, age at monarch, body satisfaction, physical activity, family size, parity, parents' marital status, parents' job and education*

as it›s «high-carbohydrate, high-fat» patterns is some how similar to «unhealthy» pattern because of food items such as mayonnaise, French-fries, confectionery products, corn and rice in the form of refined grains. But «high protein-high fat» pattern is a mixture of both «unhealthy» and «healthy» patterns because of including food items such as red meat, eggs, organ meats along with olives, cheese, milk, nuts, and fish; and in the third pattern, «high fibre, low fat», although it is some how similar to «healthy» pattern in other studies because of including fruits, vegetables and nuts, but it lacks some basic food items mentioned in these models, such as dairy, poultry or legumes.

In fact, «high fibre, low fat» resemble to «vegetarian» pattern because of lacking animal foods or could called «snack» pattern as it›s food items (fruit, dried fruit, nuts) are mainly consuming as snacks.

While using factor analysis, numbers of variables enter analysis and the number and name of factors are determined by researchers, so dietary patterns are not iterative and comparison between studies are difficult, especially among communities with different diets.

This difference between patterns also may be due to the different nature of eating disorders. This means that people with eating disorders have a two-dimensional attitude regarding foods and as they have fear of obesity, according to their own perception of «fattening», categorize foods to «good or bad» and «permitted or prohibited» and select their food in this way rather than noticing the effects of food on their health(28-29). In other words, they think about content of macronutrients in their diet rather than fibre content or using suitable oils which result in formation of different dietary patterns.

In the present study, «high-carbohydrate, high-fat» pattern showed an inverse relationship with eating disorders. «Food avoidance» pattern in female with eating disorders was first described in 1965 as «carbohydrate Phobia». Studies showed that restrictive anorectic female have symptoms of «carbohydrate starvation» and their carbohydrate consumption is low or non existent. These patients expressed strong hatred of all fattening foods; especially avoided starch, sweets and desserts(28). However, strict avoidance of sugar and starch is not the constant characteristics of eating disorders. In being eating episodes, anorectic and bulimic peoples consume different types of snacks and desserts(28). This may be the reason that not any specific «trend» was observed in quartiles of «high carbohydrate» food pattern in present study. Similar to these findings, the attitude of «getting fat by consuming carbohydrates» in Greek society has also been reported by Yannakoulia and colleagues, as with increasing the risk of eating disorders, less energy was supplied by carbohydrates(30).

In present study, an inverse relationship was observed between eating disorders and «high protein-high fat» pattern. Findings of other studies indicate fat aversion among people with eating disorders (28, 31-34). Vaz and colleagues compared food aversions of people with eating disorder with a control group and showed that aversion of foods with high quality protein content (meat, fish, milk and eggs) was characteristic of patients with eating disorders(35). In another study, da Costa and colleagues reported lower intake of protein and calcium among adolescent with eating disorder in compare to control group(36).

A significant relationship was observed in present study between «high fibre-low fat» pattern which was free of confectionery products, fats and starchy carbohydrates and eating disorder. Specially, low consumption of rice in this pattern is considerable. Although studies that have conducted in recent years, mention that the total calorie intake and not macronutrient composition is effective in weight control (37-40), but the Iranian society widely accepted the old belief that restricting carbohydrate intake, especially rice is an effective way to lose weight(41) and it is clearly visible in this pattern.

«High fibre-Low fat» dietary pattern in present study is also similar to vegetarian diet. In a study that was conducted on female adolescents, Chang and colleagues reported lower intake of energy, protein, carbohydrate, zinc, vitamin B6 and vitamin B12 and higher intake of raw and dietary fiber in adolescent with disordered eating patterns(42). Micali and colleagues assessed dietary intake, food frequency and dietary pattern of pregnant women with life time eating disorder in a longitudinal study. Their results showed that in compare with control group, women with eating disorder had higher score in «vegetarian» dietary pattern. They consumed less amount of meat that was compensated by soy products(43). So far there is no proof for the causal relationship that why people with eating disorders often eliminate meat from their diet (32, 44-45).

Present study had some limitations: first of all, samples were restricted to females. Second, in this study, dietary patterns were evaluated only on the basis of dietary intake; whereas some researchers believe that nutritional behaviour, such as pattern, time and number of snacks and meals should also be considered. In addition, in assessing dietary intake using the food frequency questionnaire, errors such as measurement error on the total number of food items or some of them may be exist. Therefore, it is recommended that the relationship between food consumption and eating disorders evaluate in prospective studies with larger sample size including both male and females and in different subtypes of disorder.

In conclusion, it seems that following high fibre and low fat dietary pattern (with avoidance of rice and high consumption of vegetables) increase the odds of eating disorders among adolescents and they are more likely to involve unhealthy weight control behaviours. In contrast, adherence to dietary pattern high in protein (e.g. dairy, meat, fish products) and fat (e.g. nuts, olive and olive oil) might be associated with reduced odds eating disorders among this group. Further studies are required to confirm our findings.

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Original Article

# The analysis of the collective diet of preschool children in Niš, Serbia and potential health risks

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**Summary.** Preschool age is an important period for acquiring the eating habits and evaluation of the children's collective nutrition in kindergartens is especially important for prevention of potential health risks. The aim of the paper was to assess the kindergarten servings in Niš (Serbia), in respect to probable health risks. The study was done in the period from 2011 to 2014. In daily portion, macronutrient shares were determined by using proximate analysis in the accredited laboratory and micronutrient shares were calculated by food composition tables. The mean energy value of the examined servings was (range: 657.7-1,1363 kcal). The share of macronutrients in the total energy intake were in accordance with the national recommendations: protein (13.54%), fat (32.32%) and carbohydrates (54.16%). An average daily serving contained 6.22 mg of Fe, 107 mg of Mg, 2411 mg of Na, and 351.4 mg of Ca. The results of our study indicate that children in kindergartens in Nis, Serbia consumed servings with no risk of developing obesity. The total quality of servings should be improved, because of the observed inadequacies of minerals and vitamins and possible health risks.

**Key words:** risk management, food quality, preschool children

## Introduction

The analysis process of the health risks regarding the food consists of risk assessment of food products, risk management and interactive information exchange regarding the risk. Identifying hazards, i.e. anything that may cause harm is the first step when carrying out health risk assessment (1).

In the preschool period, children learnt about appropriate and balanced diet and acquire good eating habits in later life. It should provide sufficient nutrient and protective substances for proper growth, development and physical activity. During the first year of life, growth and development are extremely rapid, especially in the first six months when a child needs to double its birth weight. At the end of the first year of life, a threefold increase in birth weight is expected, as well as an increase in body length by about 50%. In the early childhood, the stages of intense growth interchange with the stages of a slightly slower growth, and puberty is the next intense growth stage.

Energy needs depend on the age and in infants they are up to 150 kcal/kg of the body weight. The needs of children in their early childhood are about 1000-2000 kcal, in the preschool age of about 1700 kcal and about 2500 kcal in the school age. During adolescence, energy needs differ significantly depending on gender and range from 2500-3600 kcal (2, 3).

Protein intake in children should be significantly higher than in adults. Requirements are greatest in the

youngest children (2-3 g/kg of body weight) and they gradually decrease with the age. Proteins that children intake should be of high quality, in order to satisfy the intake of essential amino acids. Carbohydrates should satisfy 55-60% of a daily intake, where, as in adults, preference is given to complex carbohydrates. Unlike adults, high intake of dietary fibers is not recommended for younger children due to the insufficient development of their digestive tract. Recommended fat intake is slightly higher in children than in adults. By the age of five, the fat intake ranges up to 40% of the total daily intake (due to energy security, the development of the nervous system and the intake of liposoluble vitamins), and after the fifth year of life it should be reduced to 30% (4, 5).

Regarding the protective substances, the requirements for calcium, iron, zinc, vitamin A and vitamin C are increased in children than in adults and deficiencies may be due to inadequate diet. Iron deficiency is common in young children aged 6-36 month, even in Europe (6).

Pre-school nutrition-related behaviours influence diet and development of lifelong eating habits (7). In addition to genetic predisposition and insufficient physical activity, nutrition has the greatest effect on the appearance of obesity in children, which today is one of the biggest problems in the world (8).

The aim of the paper was to assess chemical analysis of the servings in kindergarten in Niš (Serbia), including the different type of food, in respect to a probable health risks.

## Method

*Study design*

The study had been conducted in the period from 2011 - 2014 in 30 kindergartens in Niš, Serbia. The material for analysis (the entire daily serving) was collected seasonally during five random days (20 annually) and the sample of each serving was collected from the serving on the dining table in front of a child. Chemical analysis was carried out after taking samples in sterile packaging by the method of random selection, after serving a meal, directly from the dishes.

*Chemical analysis*

In daily portion, macronutrient shares and serving weight were determined by using proximate analysis in the accredited laboratory of the Public Health Institute, Niš (9).

Measured serving ingredients were first measured in grams (meat separated from food, husked bananas, etc.), and then the total weight of the daily serving was measured. (10). the homogenization of the shares in servings was done by using mixers for fine homogenization. The weight of the serving was determined via the water share which was determined on the basis of the weight difference in the sample weight before and after drying in a drying oven at a temperature of 103±2°C to a constant weight:

$$\text{Water quantity (\%)} = \frac{\text{weight difference}}{\text{measured amount}} \cdot 100$$

From the obtained percentage of proteins, fats and carbohydrates, by the mathematical proportion from the total mass of the serving sample grams were calculated, and then calculated into kcal (kJ) (11, 12).

The share of the protein in the sample was defined by using the method of determining the share of total nitrogen in food according to the SRPS ISO 1871: 1992 guidelines by assessing the nitrogen share (13). Factor 6.25 was used in the calculation because the short-chain amino acids have higher nitrogen share, and longer-chain amino acids have lower nitrogen share. The process involves the mineralization by sulfuric acid in the presence of a catalyst, the alkalization of the reaction product, distillation of released ammonia and titration with sulfuric acid (14, 15). The result is presented as a percentage of the protein in a whole-day serving sample and it is calculated according to the following formula:

$$\text{Total nitrogen (\%)} = \frac{(b-a) \cdot N \cdot 14 \cdot V \cdot 100}{V_1 \cdot m}$$

*a* - the volume of the sulfuric acid solution used to titrate the blinded experiment (ml)

*b* - the volume of the sulfuric acid solution used for the sample titration (ml)

$N$ - concentration of sulfuric acid solution

$V$ - volume of the solution obtained after the mineralization of the sample portion for testing (ml)

$V_1$ - volume of the stock solution taken for distillation (ml)

$m$ - mass of the sample portion for testing (mg)

The fat share was determined by the Soxhlet total fat determination method which, after the hydrolysis of the hydrochloric acid sample, involves a multiple fat-extraction with an organic solvent in the Soxhlet apparatus. (16) The amount of total fat is expressed in percentages and calculated according to the following formula:

$$\text{Total fat (\%)} = \frac{a}{c} \cdot 100$$

$a$ - mass of extracted fat (g)
$c$ - mass of the sample taken for analysis (g)

The carbohydrate share was calculated by the following formula:

Carbohydrates (%) = 100% - (warer %) + (protein % + fat % + 2.2*)
** 2.2 applies to salt and non-digestible carbohydrates*

*Calculation and interpretation*

Based on the consumption of food determined by kitchen warehouse lists and the number of presented children in kindergartens, the average daily intake of certain vitamins (vitamin B1, PP and C) and minerals (K, Na, Mg, Ca, P, Cu and Fe) is calculated by using a specially designed software tool, based on the chemical composition of different foods.

The statistics was done by using the Microsoft Excel software.

The obtained results were interpreted in relation to the rulebook on the norm of social nutrition of children in institutions for children. Accordingly to the Serbian Book of Regulations, the kindergarten servings must provide at least 75% of the daily energy requirements and 90% of the daily requirements in animal proteins and vitamins. As children in the "Pčelica" institution are mostly aged 3-5, the results are commented in relation to the Book of regulation Value for that age (17).

## Results

*Chemical analysis of the pre-school children's servings*

The mean weight of daily servings in the investigated period was 1142 g, ranging: 712 g - 1552 g (Fig. 1).

No trend was observed in the average daily weight in the studied period. The energy value of the examined daily servings ranged from 657.7 kcal to 1,1363 kcal and the average energy value of a daily serving was 897.0 kcal. As the daily energy needs of children aged 3-5 years are 1600 kcal per day, the examined servings meet 56.1% of the daily energy needs of this population group.

Table 1 shows the macronutrients shares of servings in the investigated period. The average amount of protein in the energy value of a serving during the investigated four-year period was 34.58 g. The average amount of fat was 36.34g (ranged: 28.50 g - 42.30

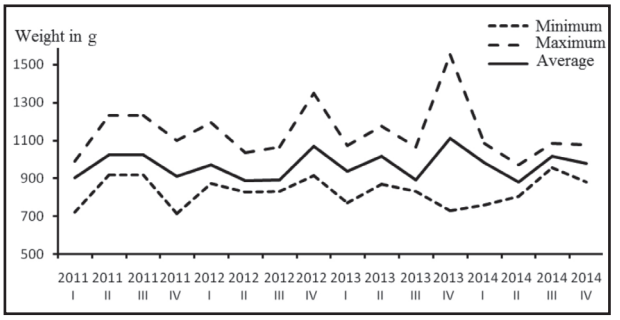


**Figure 1.** Trends of the mean weight of kindergarten servings in Niš in 2011-2014

**Table 1.** The macronutrients shares of kindergarten servings in Nis in the 2011-2014 period

| Nutrient substances (g) | n | X | Min. | Max. | SD | % | Norm (g) | Norm (%) |
|---|---|---|---|---|---|---|---|---|
| Protein | 80 | 34.58 | 28.30 | 46.40 | 4.87 | 13.54 | 29 | 10-15 |
| Fat | 80 | 36.34 | 28.50 | 42.30 | 3.98 | 32.32 | 39 | 25-30 |
| Carbohydrates | 80 | 138.21 | 118.50 | 166.20 | 15.75 | 54.16 | 176 | 55-60 |

g), whereas the average amount of carbohydrates was 138.21 g. Proteins were present in a full-day serving in the value that was above the normative (29 g), while the fat (32.32%) and carbohydrates share (54.16%) was a little below the normative standards.

Figures 2-4 present the trend of the share of proteins, fats and carbohydrates in a daily serving. Proteins are increased, and fats and carbohydrates are below the recommended values.

*Micronutrient shares in the daily servings of pre-school children*

Table 2 presents the share of minerals in daily servings in the investigated period.

The share of calcium in the examined servings was lower than the recommended value (720 mg) and amounted to 351.41 mg. The phosphorus share in the servings ranged from 451.80 mg to 828.00 mg and is also lower than the value prescribed by the norm. The average potassium share (1697.87 mg) is optimal, and copper share (0.50 mg) is lower. An average daily serving contained 1528.50 mg of sodium. The average magnesium share was 107.95 mg (60.08%), which was lower than the recommended value. The average iron share was 6.22 mg (69.14%), which was also below the value prescribed by the norm (9 mg) (Figures 5-11) present the trend of mineral shares in daily servings.

The vitamins share in servings is presented in Table 3.

In the average children's serving, the share of vitamin B1 (34.25%) ranged from 0.44 mg to 0.90 mg, which was insufficient compared to the values prescribed by the norm. The share of vitamin PP (67.07%) ranged from 5.06 mg to 8.80 mg, which was also insufficient compared to the norm. The share of vitamin C (173.65%) was above the recommendation. Vitamins

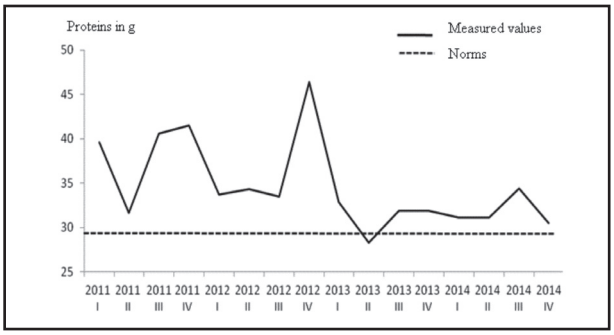


**Figure 2.** Trends of protein share in the energy value of a serving compared to the norm

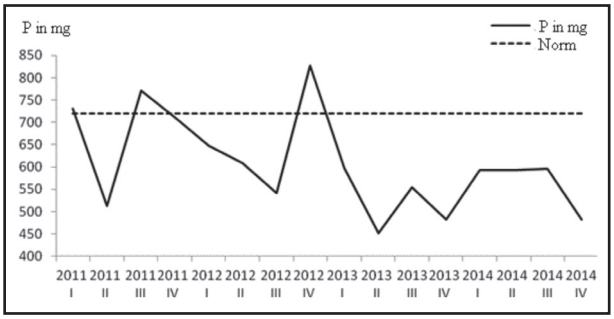


**Figure 3.** Trends of fat share in the energy value of a serving compared to the norm

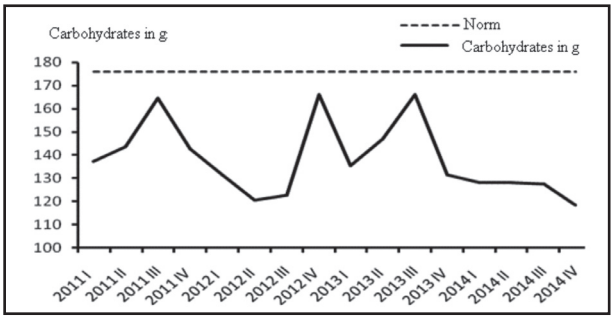


**Figure 4.** Presents trends of carbohydrate share in the energy value of a serving compared to the norm

**Table 2.** Minerals share in daily servings

| Minerals (mg) | N | Min. | Max. | X | SD | % | Norm (mg) |
|---|---|---|---|---|---|---|---|
| Ca | 80 | 165.00 | 512.70 | 351.41 | 104.59 | 48.78 | 720 |
| P | 80 | 451.80 | 828.00 | 606.36 | 108.19 | 85.51 | 720 |
| Mg | 80 | 75.20 | 158.60 | 107.95 | 26.88 | 60.08 | 180 |
| Fe | 80 | 4.60 | 9.00 | 6.22 | 1.27 | 69.14 | 9 |
| Cu | 80 | 0.36 | 0.63 | 0.50 | 0.09 | lower | 0.7-1.8 |
| Na | 80 | 1103.00 | 2411.20 | 1528.50 | 360.39 | higher | 405-215 |
| K | 80 | 1033.60 | 2191.40 | 1697.87 | 314.82 | prescribed | 697-2092 |

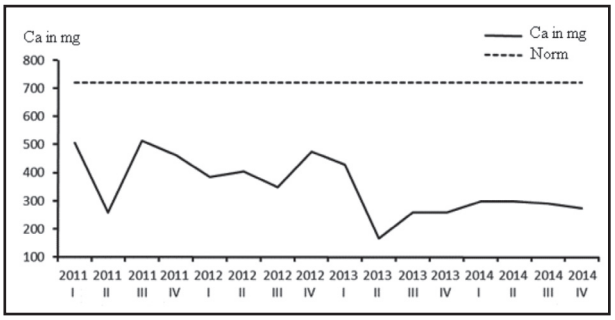


**Figure 5.** Calcium share trends in daily servings compared to the norm

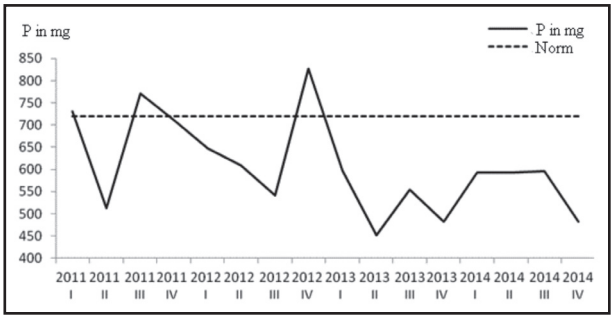


**Figure 6.** Trends in phosphorus share in daily servings compared to the norm

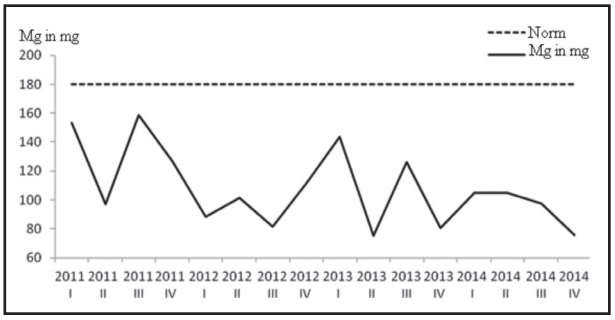


**Figure 7.** Trends of magnesium share in daily servings compared to the norm

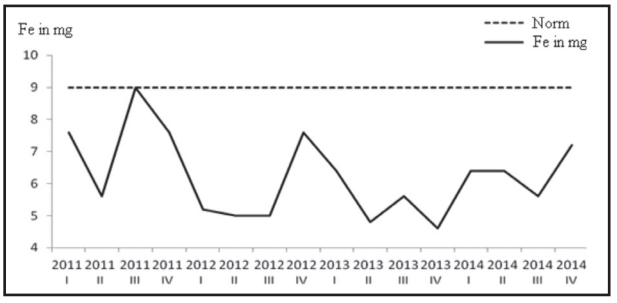


**Figure 8.** Trends of iron share in daily servings compared to the norm

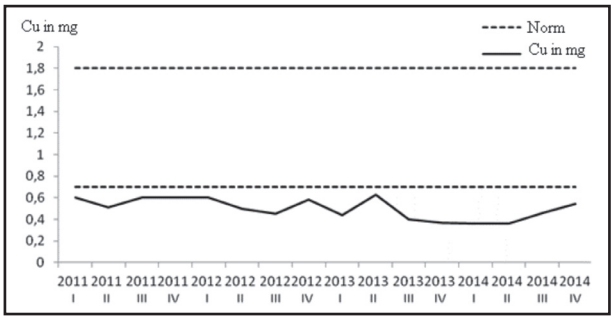


**Figure 9.** Trends of copper share in the daily servings compared to the norm

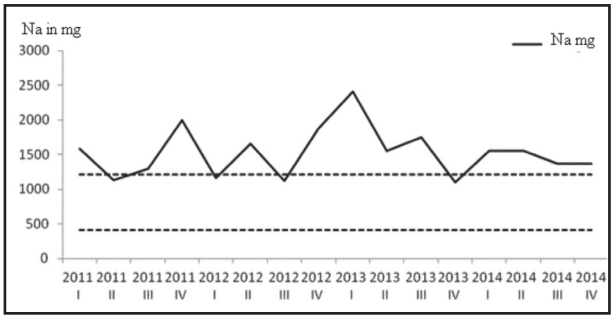


**Figure 10.** Trends in sodium share in daily servings compared to the norm

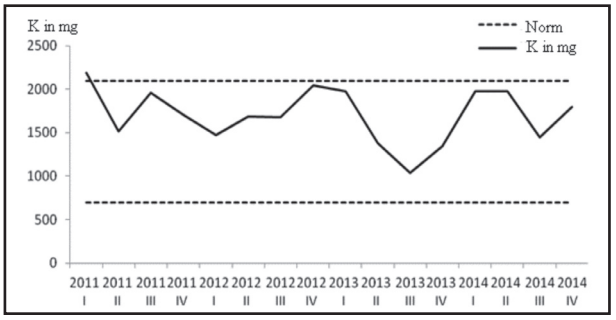


**Figure 11.** Trends of potassium share in daily servings compared to the norm

share in the examined period in relation to the normative is presented in Figures 12-14.

## Discussion

Pre-school servings make significant contributions to a healthy dietary behaviour, at a time when eating habits and food preferences are being formed. Prevention of the unhealthy dietary habits in preschool

**Table 3.** Vitamins share in daily servings

| Vitamin (mg) | N | Min. | Max. | X | SD | % | Norm (mg) |
|---|---|---|---|---|---|---|---|
| B1 | 80 | 0.44 | 0.90 | 0.62 | 0.14 | 34.25 | 1,81 |
| PP | 80 | 5.06 | 8.80 | 6.62 | 1.16 | 67.07 | 9,90 |
| C | 80 | 21.20 | 108.40 | 70.14 | 26.71 | 173.65 | 40,50 |

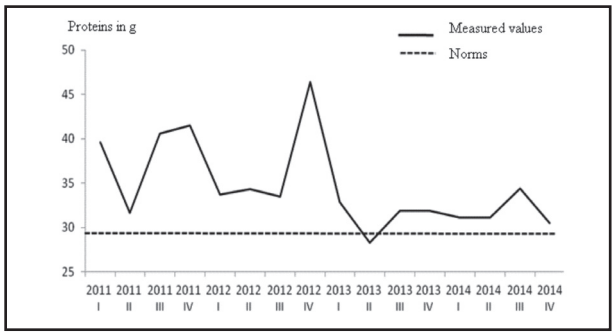


**Figure 12.** Trends of B1 vitamin share in daily servings compared to the nor

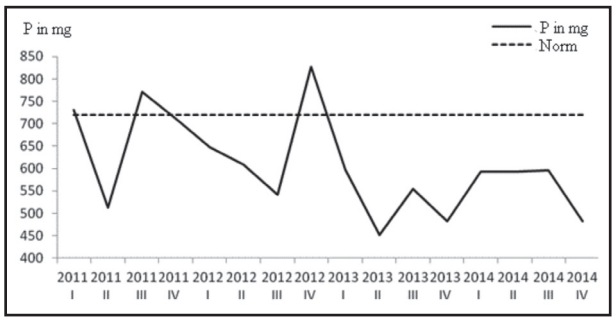


**Figure 13.** Trends of PP vitamin share in daily servings compared to the norm

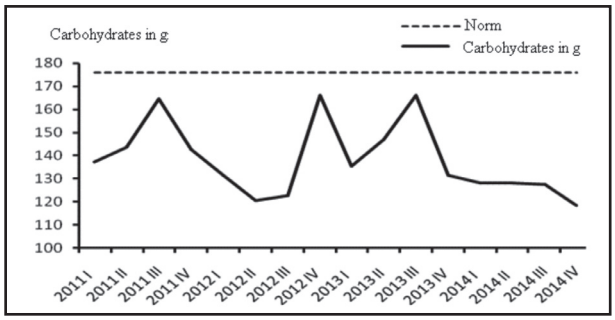


**Figure 14.** Trends of vitamin C share in daily servings compared to the norm

children is very important for their future health and permanent concern about children's food intake in kindergarten is serious social task (18, 19).

The results of our study indicate that the energy values of preschool servings consumed in kindergarten in Nis did not pose a risk of developing obesity. The energy value of servings did not exceed the level recommended by the national regulative and were adequate to the time spent by children in kindergartens, usually shorter than 10 hours. Compared to our results, kindergarten children in Brazil consumed servings of lower energy value than required (13). On the other hand, on average, the children consumed 1.280 kcal per day, in Hong Kong or 92% of the Chinese Nutrition Society's energy recommendation (20).

The result of the study indicated that the share of macronutrients in the total energy intake were in accordance with the national recommendations: protein (13.54%), fat (32.32%) and carbohydrates (54.16%). On the other hand, shares of fat were higher than suggested in the children servings in kindergartens of six cities in China had the higher energy value higher due to fat (21).

The share of calcium, magnesium, iron, cooper and phosphorus in the analyzed servings were inadequate. The obtained results indicate a significant deficiency in mineral share in the collective diet of the preschool population in Nis. According to the chemical analysis, Djermanovic M. et al. reported similar data for the intakes of calcium and iron through the collective diet of the preschool children aged up to 7 in Republika Srpska (22).

Minerals have significant roles in the human organism and are very important in the process of children's growth and development. According to the WHO, there are approximately 2 billion people worldwide with a mineral deficiency and children belong to the particularly sensitive to the lack of minerals (23).

Determined low calcium intake in our study may predispose the studied preschoolers to osteoporosis in later life (24). According to Ekbote V. et al., the mean calcium intake was 57% of the RDA in 2–16-year-old

Urban Western Indian children. The modifications of servings in kindergarten, with the choice of calcium-rich foods, the estimated calcium share of the diet may be increased. Preschool children are consuming more nutrient-dense foods and a more servings of fruit and vegetables at childcare during lunch than at home during dinner. Childcare and parents should work together to provide early and consistent exposure to nutrient-rich foods to ensure optimal nutrition for developing children (25).

This study also indicates inadequate magnesium intake through the servings in kindergartens. Magnesium deficiency is not usual in this period of life, but a comparison between the whole wheat bread versus the refined wheat bread in Serbian Food composition database reveals higher amounts of magnesium (86 vs 15 mg/100 g) and one of the basic change could be using more of whole grains in children's servings.

Iron deficiency is considered to be one of the most widespread micronutrient deficiencies in the world. Due to the increased need for iron during the period of accelerated growth and development, children belong to the group that is exposed to the highest risk of this deficiency. Several studies indicated the connection between sideropenic anemia in infants and their slower cognitive development.

Copper deficiency manifests as hypochromic anemia, and neutropenia, and in children has been associated with bone abnormalities, including osteoporosis, fractures of the long bones and ribs, and stunting. This metal is an essential nutrient, also very important for iron metabolism.

This study demonstrated a too excessive intake of sodium in the preschool servings in Nis, which is one of the factors for hypertension development. Investigations have brought evidence that salt intake is positively related to systolic blood pressure and that children with higher blood pressure are more susceptible to hypertension in adulthood. Much further effort is required to reduce salt share of consumed food. Our findings of a high sodium intake in children are similar to data from recent research studies in Serbia and other countries.

Our examination of vitamin shares in an average children's serving showed that vitamin B1 was lower in relation to the norm, as well as the share of vitamin PP. Vitamin C was just above the prescribed values. As well as in our study, investigators from Poland found many inadequacies for vitamin intakes (26).

If the nutritional share of preschool menus was not balanced, it is not surprising that the result is preschoolers' inadequate intake. To prevent health effects resulting from inadequate and excessive intakes of nutrients, it is necessary to plan balanced preschool menus and for this purpose, a dietician has been employed in the kindergarten of the city of Nis.

In modern science, both in our country and in the world, the quality of products and its safety are closely linked and they represent a good basis for further research. Nutrition is a significant factor in the prevention of various diseases and the improvement of the health of the pre-school children. It is necessary for growth, development and body function, and nutrition planning is based on the physiological needs of each and every individual.

The issues of management of the risk of preschool children's nutrition as well as the methods of the quality control and safety standards are increasing in their importance in the modern world because the consumers are the most sensitive population – children. Risk management is significant in terms of comprehensively addressing problems that include the safety of technological systems, types of risks and risk assessment methods, with a special emphasis on the health risk caused by food. Our analyses show that compliance with norms is of great importance for the quality of the final product.

## Conclusion

To conclude, preschool diets in kindergarten need continuous improvement to prevent diet-related diseases in the preschoolers. Even though the mean energy value of servings did not exceed the recommendation and the share of proteins, fats and carbohydrates was optimal, the total quality of investigated servings should be improved by introducing food with high-nutrient density. Better planning of the children's nutrition in kindergartens with constant laboratory control of servings may be the future strategy, any deviation from the prescribed intake of nutrients negatively af-

fects the growth, development and health of children. Multidisciplinary consideration of nutrition issues is significant because of its complexity and actuality.

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Original Article

# An examination of the relationship between hypertension and healthy lifestyle behaviors in adults

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**Summary.** *Objectives:* The modern lifestyle has led to an increase in the risk of hypertension and cardiovascular diseases. *Design:* This analytical cross-sectional study aimed to examine the relationship between hypertension and healthy lifestyle behaviors in adults. *Participants:* The research was carried out universe consisted of individuals aged 30+ years who applied to the family health center and the study sample included 400 individuals. *Measurements:* The participants' weights, heights and blood pressures were measured, the data of the study were collected by using " Questionnaire Form" and "Healthy Lifestyle Behaviors Scale". *Results:* 32.4% of the women and 31.0% of the men had blood pressures of 140/90 mmHg and above. Average BMI of the participants was 26.69±4.39. The total point average of Healthy Lifestyle Behaviors Scale obtained by the of the participants was 121.86±24.35. The participants on the subscales of self-realization, nutrition and interpersonal support were high, while the lowest score was obtained on the subscale of exercising. Further, those with lower blood pressure measurements were determined to have higher levels of self-realization, exercise, interpersonal support, stress management and healthy lifestyle behaviors compared to the levels of those with high blood pressure ($p<0.05$). *Conclusion:* It has been determined that the male, who ate meals more frequently, with higher levels of physical activity, without chronic diseases, and the participants with normal blood pressure values had higher healthy lifestyle behaviors.

**Key words:** adult, hypertension, lifestyle, nurses

## Introduction

Globally, cardiovascular disease (CVD) is responsible for 17 million annual deaths, which translates to nearly one-third of total deaths across the world (1-3). Hypertension (HT) accounts for at least 45% of deaths from cardiovascular diseases (1). HT is a major risk factor for the development of CVD and as a worldwide public health concern, it requires intensive prevention and treatment management programs to address it, on account of its prevalence, costs, and health effects (3,4).

The modern lifestyle has led to an increase in the risk of HT and CVD (5). The principal modifiable behavioral risk factors for CVD include unhealthy diets, physical inactivity, alcohol abuse, and smoking. Long-term exposure to these factors has also been linked to obesity, hypertension, diabetes mellitus, and dyslipidemia. Lifestyle modifications have proved to be essential and effective in the prevention and treatment of HT (6).

The prevalence of hypertension among in the 35–64 age group is about 30% in the US population and about 44% in European countries (7). The estimated HT prevalence for the population aged 20+ years was 26.4% globally in 2000 (26.6% for men and 26.1% for women), and it is projected that HT prevalence will increase to 29.2% for both men and women by 2025 (1). Studies investigating HT frequency in Turkey have been carried out since the 1960s, both

locally and on larger scales. The first comprehensive study to determine the prevalence of HT in Turkey was the Heart Diseases and Risk Factors in Turkish Adults (HDRFTA) study. The results from this study showed that the mean prevalence of HT in Turkey was 33.7%, increasing with age (8). Another study, whose focus was on HT in Turkey (PatentT), was conducted between 2002 and 2003 to particularly determine the frequency, distribution, awareness, treatment and control rates of HT in Turkey. The prevalence of HT in this study was determined as 31.8% (9). In the PatenT2 study conducted in Turkey in 2012, 30.3% of the participants were found to be hypertensive (32.3% in women, 28.4% in men) (10).

Recent socioeconomic and technological developments have led to significant changes in the way people live their lives; for example, people now tend to lead more sedentary lives, with unhealthy lifestyle habits. Therefore, the incidence of chronic diseases has increased across the world. However, it is known that positive lifestyle changes, such as regular physical activity, restriction of alcohol and cigarette use, and regular and balanced nutrition, serves to decrease CVD, HT and chronic diseases (8,10). A healthy lifestyle involves all the approaches used by an individual to take control of their own behavior and to improve the health levels of their daily activities (11,12). Walker et al. (1987) addressed healthy lifestyle behaviors under the headings of adequate and balanced nutrition, stress management, self-realization, regular exercise, interpersonal relationships, and sense of responsibility for protecting and improving individual health. It is necessary to apply health-promoting behaviors continuously to ensure life-long health. Therefore, for the development of community health, it is important that society as a whole adopt healthy lifestyle behaviors (13).

## Material and Methods

*Study Design*

This analytical cross-sectional study was conducted at a family health center between September 01, 2013 and February 01, 2014 to examine the relation between HT and healthy lifestyle behaviors in adults.

*Setting and sample*

The study universe consisted of individuals aged 30+ years who applied to the family health center during the research period; and the study sample included 400 individuals who had agreed to voluntarily participate in the study. The data were gathered using a questionnaire form, prepared by the researchers, and the Healthy Life Style Behaviors Scale, both of which were administered through face-to-face interviews. Also, blood pressure, Body Mass Indexes were taken.

*Measurements/Instruments*

*Questionnaire form:* This form prepared by the researchers on the basis of a literature (8,9,11,14,15) and consists of 43 questions to evaluate the patients' sociodemographic characteristics (8 questions) hypertension features (35 questions).

*Blood pressure measurement:* A cuffed sphygmomanometer and a stethoscope were used to measure patients' blood pressure. The patients were seated and in a rested state while the survey form was being filled out. Their blood pressure were measured twice in a 5-minute interval from the right arms while they were in a sitting position. All measurements were conducted according to the relevant principles and guidelines.

*Calculation of body mass indexes*

For assessment of the body-mass index of the participants (BMI, kg/ $m^2$), the limit values of <18.5 kg/$m^2$ for underweight, 18.5- 24.9 kg/$m^2$ for normal, 25.0-29.9 kg/$m^2$ for slightly overweight, 30.0- 39.9 kg/$m^2$ for overweight and >40.0 kg/$m^2$ for obese were adopted. To perform the body weight measurements, the participants, in light clothes with their shoes off, were placed on a scale sensitive to 0.5 kg; and to perform height measurements, a tape measurer was used to measure the participants as they stood against the wall, with their shoeless feet side by side.

*Healthy Lifestyle Behavior Scale (HLSB)*

The HLSB was developed by Walker, Sechrist and Pender (1987) (12). The validity and reliability of the scale made by Esin (1997) in Turkey, Cronbach's alpha value was found to be 0.91 (16). Questions on the scale are used to measure an individual's health-

promoting behaviors in relation to his/her lifestyle. Consisting of 48 items, the scale includes 6 subscales (self-realization: 13 items, health responsibility: 10 items, exercise: 5 items, nutrition: 6 items, interpersonal support: 7 items and stress management: 7 item), each of which may be used on its own independently. The total score on the scale is the sum of all points from the subscales constituting the HLSB. All items of the HLSB are positive. Responses to the items are made on a 4-point Likert scale (1=never, 2=sometimes, 3=frequently, 4=regularly). The lowest score possible for the whole scale is 48, while the highest possible score is 192. Higher scores obtained on the scale indicate that the individual applies stated health behaviors at a high level (12,16). In the present study, the Cronbach's alpha value was determined to be 0.95, which indicates high scale reliability. The reliability levels of the internal consistency coefficients of the subscales were 0.87 for self-actualization, 0.86 for health responsibility, 0.79 for stress management, 0.79 for interpersonal support, 0.76 for exercise, and 0.62 for nutrition.

*Ethical Consideration*

Before starting the study, written approval (dated 17.09.2013 and numbered 321) was obtained from the Scientific Ethics Committee. Written permission was also obtained from the Public Health Directorate for the questionnaire application. Participants were visited at home and informed about the study purpose by the researchers. The questionnaire and HLSB were applied to those who agreed to participate in the study, and their height, weight and blood pressure were measured and recorded.

*Data Analysis*

The SPSS 23.0 program was used for conducting analyses. A reliability analysis of the scale was performed to confirm its reliability. The arithmetic average, frequency, and percentages of the gathered data were calculated, and analysis of the data was carried out with Kruskal-Wallis H test, Mann-Whitney U test and correlation analysis. p values lower than 0.05 were considered significant.

## Results

Among the participants, 56.5% were female, 86.5% were married, 37.7% had an undergraduate or graduate degree, 28.7% were housewives and 52.8% had a balanced income. According to their BMIs, 50.0% were overweight, 59.3% ate three square meals a day, 41.0% consumed red meat frequently, 65.8% used olive oil in meals, 75.2% were not smokers, and 88.0% never used alcohol. In addition, 57.2% engaged in normal physical activity, and 68.3% had no chronic disease. To continue, 78.7% had not gone to the doctor due to HT in the last one year, 89.7% had a BP measurement at least once a year, and 22.7% used anti-hypertensive drugs. According to the BP measurements, 32.4% of the female participants and 31.0% of the male participants had a BP level of above ≥140 / 90 mmHg (Table 1).

**Table 1.** Baseline Characteristics of Study Participants (n=400)

| **Sociodemografic features** | **n (%)** |
|---|---|
| **Gender** | |
| Female | 226 (56.5) |
| Male | 174 (43.5) |
| **Marital status** | |
| Married | 346 (86.5) |
| Single | 54 (13,5) |
| **Education Status** | |
| Illiterate | 42 (10.5) |
| Primary | 82 (20.5) |
| Secondary school | 58 (14.5) |
| High school | 67 (16.8) |
| University and above | 151 (37.7) |
| **Occupation status** | |
| Housewife | 115 (28.7) |
| Officer | 137 (34.3) |
| Worker | 54 (13.5) |
| Retired | 41 (10.3) |
| Other | 53 (13.2) |
| **Monthly income** | |
| Low | 115 (28.7) |
| Medium | 74 (18.5) |
| High | 211 (52.8) |
| **BKI** | |
| <18.5 kg/m$^2$ | 4 (1.0) |
| 18.5-24.9 kg/m$^2$ | 124 (31.0) |
| 25-29.9 kg/m$^2$ | 200 (50.0) |
| 30-39.9 kg/m$^2$ | 66 (16.5) |
| >40 kg/m$^2$ | 6 (1.5) |

Continued ...

| Sociodemografic features | n (%) |
|---|---|
| **Going to doctor in the last year due to hypertension** | |
| Yes | 85 (21.3) |
| No | 315 (78.7) |
| **Measuring blood pressure until now** | |
| Yes | 359 (89.7) |
| No | 41 (10.3) |
| **High Blood pressure** | |
| Female | 153 (32.4) |
| Male | 142 (31.0) |
| **Daily eating frequency** | |
| One meal | 12 (3.0) |
| Two meal | 114 (28.5) |
| Three meal | 237 (59.3) |
| More often | 37 (9.2) |
| **Most consumed foods** | |
| Red meat | 164 (41.0) |
| Legumes | 111 (27.8) |
| Fish and chicken | 102 (25.5) |
| Cereal | 102 (25.5) |
| Vegetable and fruit | 53 (13.3) |
| **Physical activity status** | |
| Little | 126 (31.5) |
| Normal | 229 (57.2) |
| Enough | 45 (11.3) |
| **Presence of chronic illness** | |
| Yes | 127 (31.7) |
| No | 273 (68.3) |
| **Using antihypertensive drug** | |
| Yes | 91 (22.7) |
| No | 309 (77.3) |

**Table 2:** Means of Height, Weight, BKI, Blood Pressure and Age of Participants (n=400)

| | Min. | Maks. | M±SD |
|---|---|---|---|
| Height | 145.00 | 190.00 | 169.44±9.58 |
| Weight | 45.00 | 122.00 | 76.50±12.64 |
| BMI | 16.00 | 46.00 | 26.69±4.39 |
| Age | 27.00 | 85.00 | 43.92±12.04 |

The mean age of the participants was 43.92 ± 12.04 years, and their mean BMI was 26.69±4.39 (Table 2).

The mean HLSB score of the participants was found 121.86 ± 24.35, and high mean scores on the subscales of self-actualization (34.76 ± 7.14), nutrition (16.38 ± 3.34) and interpersonal support (19.08 ± 4.09), while the lowest mean score was obtained on the subscale of exercise (10.78 ± 3.58) (Table 3).

**Table 3:** Score Averages of Healthy Lifestyle Behavior Scale and Sub-Dimensions (n=400)

| | Min. | Max. | M±SD |
|---|---|---|---|
| Exercise | 5 | 20 | 10.78±3.58 |
| Nutrition | 7 | 24 | 16.38±3.34 |
| Health responsibility | 11 | 40 | 23.68±6.42 |
| Interpersonel support | 10 | 28 | 19.08±4.09 |
| Self-realization | 17 | 52 | 34.76±7.14 |
| Stress management | 7 | 28 | 17.20±4.18 |
| Total | 63 | 189 | 121.86± 24.35 |

The mean scores obtained by the male participants on the HLSB scale as a whole, as well as on the subscales of self-actualization, exercise, and stress management, were found statistically significantly higher than the female participants ($p < 0.05$).

Comparing the participants according to their eating habits, the participants who ate meals more frequently had statistically significantly higher mean scores on the HLSB scale as a whole, as well as on the subscales of self-actualization, health responsibility, exercise, nutrition, and stress management ($p<0.05$).

Comparing the participants according to their physical activity status, the participants with a good physical activity status obtained higher mean scores on the HLSB scale as a whole and on all its subscales ($p<0.05$).

The participants without a chronic disease obtained statistically significantly higher mean scores on the HLSB scale as a whole and on all its subscales than those of the participants with a chronic disease ($p<0.05$).

Comparing the participants according to their BP measurements, the participants with low BP measurements had higher mean scores on the HLSB scale as a whole and on the subscales of exercise, interpersonal support, and stress management ($p<0.05$).

The mean scores of the underweight and normal weight participants on the subscales of exercise and stress management were higher than those obtained by the overweight and obese participants ($p<0.05$).

**Table 4:** Comparision of Participants some Features and Healthy Lifestyle Behavior Scale and Sub-Dimensions Scores (n=400)

| Features | n | Self-realization | Health responsibility | Exercise | Nutrition | Interpersonal support | Stress management | Total |
|---|---|---|---|---|---|---|---|---|
| **Gender** | | | | | | | | |
| Female | 174 | 181.15 | 199.96 | 170.95 | 196.54 | 192.65 | 186.04 | 185.21 |
| Male | 226 | 215.40 | 200.92 | 223.25 | 203.55 | 206.55 | 211.63 | 212.27 |
| U | | 16295.000 | 19567.500 | 14519.500 | 18973.000 | 18295.500.232 | 17145.500 | 17002.000 |
| p | | .003 | .934 | .000 | .546 | | .028 | .020 |
| **Presence hypertension in family** | | | | | | | | |
| Yes | 175 | 177.70 | 189.95 | 175.74 | 186.23 | 180.30 | 174.25 | 177.04 |
| No | 225 | 218.23 | 208.70 | 219.76 | 211.60 | 216.21 | 220.92 | 218.74 |
| U | | 15698.00 | 17841.500 | 15354.000 | 17190.000 | 16152.500 | 15094.000 | 15582.500 |
| *p* | | .000 | 0.107 | .000 | .029 | .002 | .000 | .000 |
| **Daily eating frequency** | | | | | | | | |
| One meal | 12 | 173.08 | 180.67 | 157.00 | 149.96 | 186.63 | 151.63 | 158.63 |
| Two meal | 114 | 186.04 | 176.34 | 187.27 | 157.89 | 195.66 | 178.94 | 177.06 |
| Three meal | 237 | 200.01 | 204.13 | 200.43 | 216.48 | 195.99 | 207.01 | 205.03 |
| More often | 37 | 257.05 | 258.09 | 255.81 | 245.85 | 248.81 | 241.04 | 257.31 |
| KWH | | 11.337 | 14.785 | 11.736 | 28.245 | 7.237 | 11.474 | 15.563 |
| *p* | | .010 | .002 | .008 | .000 | 0.065 | .009 | .001 |
| **Physical activity status** | | | | | | | | |
| Little | 126 | 140.96 | 141.11 | 111.11 | 151.30 | 144.16 | 141.58 | 128.31 |
| Normal | 229 | 223.28 | 222.97 | 234.76 | 215.93 | 223.19 | 224.42 | 228.29 |
| Often | 45 | 251.26 | 252.43 | 276.47 | 259.71 | 242.78 | 243.73 | 261.22 |
| KWH | | 51.078 | 51.117 | 115.608 | 39.039 | 45.030 | 49.099 | 74.795 |
| *p* | | .000 | .000 | .000 | .000 | .000 | .000 | .000 |
| **Presence of chronic illness** | | | | | | | | |
| Yes | 127 | 165.33 | 181.95 | 165.81 | 172.81 | 169.80 | 160.68 | 164.30 |
| No | 273 | 216.86 | 209.13 | 216.64 | 213.38 | 214.78 | 219.02 | 217.34 |
| U | | 12868.500 | 14980.000 | 12930.500 | 13819.500 | 13437.00 | 12278.500 | 12738.000 |
| *p* | | .000 | .028 | .000 | .001 | .000 | .000 | .000 |
| **Using antihypertensive drug** | | | | | | | | |
| Yes | 91 | 151.54 | 178.12 | 156.60 | 160.86 | 159.23 | 150.50 | 152.99 |
| No | 309 | 214.92 | 207.09 | 213.43 | 212.17 | 212.65 | 215.22 | 214.49 |
| U | | 9604.500 | 12022.500 | 10065.000 | 10452.000 | 10304.000 | 9509.500 | 9736.500 |
| *p* | | .000 | .035 | .000 | .000 | .000 | .000 | .000 |
| **High Blood pressure** | | | | | | | | |
| Yes | 126 | 182.29 | 191.75 | 183.61 | 184.35 | 176.03 | 183.73 | 181.29 |
| No | 274 | 208.88 | 204.52 | 208.27 | 207.93 | 211.75 | 208.21 | 209.34 |
| U | | 14967.000 | 16160.000 | 15133.500 | 15227.500 | 14179.000 | 15149.00 | 14841.000 |
| *p* | | .032 | .304 | .047 | .057 | .004 | .049 | .024 |
| **BMI** | | | | | | | | |
| Underweight | 4 | 195.63 | 240.25 | 279.38 | 164.75 | 215.88 | 208.88 | 213.38 |
| Normal | 124 | 199.52 | 202.74 | 194.60 | 198.15 | 200.77 | 206.06 | 199.19 |
| Overweight | 200 | 206.96 | 200.64 | 208.45 | 202.05 | 205.02 | 203.51 | 205.95 |
| Obese | 66 | 193.80 | 205.27 | 196.52 | 209.94 | 196.49 | 192.11 | 197.89 |
| Morbid obese | 6 | 82.25 | 70.75 | 48.58 | 117.67 | 78.25 | 72.00 | 66.25 |
| KWH | | 7.153 | 8.210 | 13.656 | 4.024 | 7.207 | 8.249 | 8.635 |
| *p* | | .128 | .084 | .008 | .403 | .125 | .008 | .071 |

## Discussion

As a health problem, HT is responsible for the most deaths worldwide (15). In the present study, 32.4% of the female participants and 31.0% of the male participants were determined to have a BP level of above ≥140 / 90 mmHg. In the HDRFTA study, the prevalence of hypertension was reported to be 33.7% in 1990, whereas this rate increased to 36.3% in adult males and 49.1% in adult females in the period from 2001 to 2002 (8). The prevalence of hypertension throughout all of Turkey was determined to be 31.8% in the Turkish Hypertension Prevalence Study conducted by the Turkish Society of Hypertension and Renal Diseases in rural and urban areas in 26 provinces of Turkey in 2003 (14). Öztürk et al. (2011) reported the prevalence of HT as 31.7% (32.4% for females, 31.0% for males) in Kahramanmaraş, Turkey, and as 34.6% (42.9% for females, 24.4% for males) in Kayseri, Turkey (15). Wang et al. in their research, determined the prevalence of hypertension as 29.6% in total, with the prevalence being higher in men than in women (17). In a study conducted in Nepal, the overall age and sex-adjusted prevalence of hypertension was 28% (23% for females, 38% for males) (18).

Overweight and obesity, which are increasing globally, are known to pose risks for diseases such as CVD, type 2 diabetes, HT, dyslipidemia, and metabolic syndrome (19). In the present study, 50% of the participants were determined to be overweight and 16.5% to be obese. Çayır et al. found in their study of patients who visited a nutrition and dietetics outpatient clinic that 35.1% of women and 16.4% of men were obese (19). Kapelios et al. found in their study of lifestyle, diet and cardiovascular morbidity in rural areas that 29.6% of men and 37.8% of women were obese (BMI ≥30 kg/m$^2$) (20). In a study by Mehmood et al. on medical students, 15.8% of the women and 15.3% of the men were reported to have a BMI of between 25-45 kg/m$^2$ (4). A study with university students conducted by Tayem et al. found that 25% of the participants were overweight (31.1% males, 15.6% females) and 7.2% were obese. (21). The present study results indicated that HT is related to age, gender and BMI. In results from other studies, Tayem et al. found that BP was related to smoking and BMI, Peng et al. determined that BMI was associated with physical activity inadequacy and ischemic heart disease, and Ilow et al. reported that BMI, smoking and inadequate physical activity were associated with CVD. (21-23).

In the present study, the mean scores obtained by the male participants on the HLSB scale as a whole and on the subscales of self-actualization, exercise, and stress management were found to be higher than those of the female participants. However, Sivrikaya et al. determined no significant relationship between gender and HLSB (24). Yalçınkaya et al. found in their study of health workers that males had statistically significantly higher mean scores on the subscales of health responsibility and nutrition than those of females (25). Tambağ conducted a study with university nursing students and found that female students had higher mean scores on the subscales of health responsibility and nutrition than those of the male students, while male students obtained a higher mean score on the subscale of exercise than that of the female students (11). As can be seen from the above cited research, results related to the gender variable differ across studies.

It is largely people's own responsibility to be healthy and to maintain a healthy lifestyle, and this responsibility has an important role in preventing chronic diseases (13). The adoption of a healthy lifestyle, the reduction of unhealthy behaviors and habits to a minimum level, and the responsibility taken by individuals for their own health are necessary to lower the risk and to prevent chronic diseases. In the present study, underweight people had higher mean scores on the subscales of exercise and stress management than those in other weight groups. In addition, the participants who ate meals more frequently obtained higher mean scores on the HLSB scale as a whole and on the subscales of self-actualization, health responsibility, exercise, nutrition, and stress management. Yalçınkaya et al. found in their study of health workers that participants with a balanced diet obtained higher HLSB scores than those of other participant groups (25).

It is known that genetic structure and familial predisposition are effective in the formation of HT and other chronic diseases (2). In the present study, the participants with a history of HT in their family had statistically significantly higher mean scores on the HLSB scale as a whole and on all its subscales than

those of the participants without a history of HT in their family. In addition, the participants with low BP levels obtained higher mean scores on the HLSB scale as a whole and on the subscales of self-actualization, exercise, interpersonal support, and stress management than those in other groups. Further, the participants who were using anti-hypertensive drugs had statistically significantly higher mean scores on the HLSB scale as a whole and on all its subscales than those of the participants not using antihypertensive drugs. Şahin and Biçer determined in their study on hypertensive patients that participants had low HLSB scores (26). Level of physical activity and active lifestyle play an important role in preventing chronic diseases, especially hypertension. However, changes in the modern lifestyle and social structure have resulted in people leading more sedentary lifestyles. In the present study, the participants who were involved in more physical activity had higher mean scores on the HLSB scale as a whole and all its subscales than those in other groups. on the subscale of healthy lifestyle behaviors. Mete et al. found in their study of university students that students who regularly played sports had high scores on the HLSB scale (27).

## Conclusion

It has been determined that the male participants, the participants who ate meals more frequently, with higher levels of physical activity, without chronic diseases, not using antihypertensive drugs, the thin and normal weight, and the participants with normal blood pressure values had higher healthy lifestyle behaviors.

It is recommended that regular training on the prevention of chronic diseases be provided to communities, and that counseling services be integrated with primary care services, in order to increase awareness of hypertension. It is also recommended that supportive planning and programs on the prevention of hypertension and other chronic diseases be increased to promote the adoption of healthy lifestyle behaviors across society.

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ORIGINAL ARTICLE

# Comparative in vitro analysis of anti-diabetic activity of Indo-Pak black cardamom (*Amomum subulatum Roxb.)* and Chinese black cardamom *(Amomum tsao-ko Crevost et Lemaire)*

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**Summary.** Diabetes mellitus is a metabolic disorder of glucose metabolism. An indispensable strategy for the control of diabetes mellitus, especially diabetes type 2 and its harmful effects, is the efficient management of postprandial hyperglycemia. *Amomum subulatum Roxb.* and *Amomum tsao-ko Crevost et Lemaire* are considered useful for the treatment of diabetes mellitus in Indo-Pak region and P.R China respectively. In this study, all tested concentrations of aqueous (v/v) extracts of the seeds and rind showed significant inhibitory activity against α-amylase and α-glucosidase. It can be concluded that aqueous extracts of *Amomum subulatum Roxb* and *Amomum tsao-ko Crevost et Lemaire* dry fruit constitutes (seeds and rind) have the significant inhibitory activity against the two carbohydrate hydrolyzing enzymes and the presence of phytochemicals like flavonoids, saponins, and tannins etc may have contributed significantly to the inhibitory potential of plant extracts

**Key words:** α-amylase inhibitory activity, α-glycosidase inhibitory activity, *Amomum subulatum Roxb.*, *Amomum tsao-ko Crevost et Lemaire*, seed and rind, type 2 diabetes

## Introduction

Diabetes mellitus (DM) is the metabolic disorder characterized by increased blood glucose level (hyperglycaemia) with abnormality in carbohydrate, protein and fat metabolism. According to World Health Organization (WHO), there are 346 million people affected worldwide from diabetes and this number will be doubled by the year 2030 (1-2). It is such a progressive endocrine disorder of glucose metabolism that eventually leads to micro- and macro-vascular changes causing secondary complications that are incredibly challenging to manage (3). Type 1 diabetes arisen due to the inadequate synthesis of insulin by β-cells of the pancreas, while type 2 diabetes is regarded as primarily by insulin resistance (a condition in which peripheral cells do not respond normally to insulin) or β-cell dysfunction (4). The drugs which are mostly used to treat the diabetics are: insulin, sulfonylureas, biguanide, glycosidase inhibitors, aldosereductase inhibitor, carbamoylmethyl benzoic acid, thiazolidinediones (5, 6). Now-a-days, there are different kinds of therapeutic strategies for the treatments of diabetes in practice likewise: stimulation of endogenous insulin secretion, increase the activity of insulin at the target tissues and inhibition of α-amylase enzyme activity to reduce the degradation of starch and lower the blood glucose level (6-8). α-amylase is a well-known enzyme found in the pancreatic juice and saliva which break down large insoluble starch molecules into absorbable molecules. While mammalian α-glucosidase in the mucosal brush border of the small intestine catalyzed the last step of digestion of starch *i.e.* the breakdown of disaccharides that are ample in human diet (9, 1). The α-amylase and α-glucosidase involved in the di-

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gestion of carbohydrates can considerably decrease the postprandial increase of blood glucose after consumption of a mixed carbohydrate diet and thereby creating a dynamic platform in the management of postprandial blood glucose level in type 2 diabetic patients and borderline patients (11). Inhibitors of α-amylase enzyme (EC 3.2.1.1) and α-glucosidase enzyme (EC 3.2.1.20) postpone the breaking down of carbohydrates in the small intestine and reduce the postprandial blood glucose expedition (3, 12). α-amylase inhibitors are one of the anti-diabetic drug families, of which acarbose is the most prominent one. These drugs have a robust advantage and are suitable for healing non-insulin-dependent diabetes mellitus (type-2 diabetes) but also prompt the gastrointestinal side effects that reduce their use in a preventive approach (13, 14). Many researcher and nutritionist are extremely interested to fabricate a novel nutritional approach to perfectly control the postprandial glycaemia without inducing negative circumstances on the digestive system. In the aforesaid context, medicinal plants because of their easy accessibility and less side-effects have gaining special place in pharmaceutics to treat various kind of chronic diseases (15). Plant materials used as traditional medicine for the treatment of diabetes are deliberated as one of the good source for the drugs discovery or a lead to make a new chemical entity (16). Plant extracts or different folk plant preparations are being prescribed by the traditional practitioners and have also been accepted by the users for the treatment of diabetes and any other diseases in many countries all over the world especially in the third world countries (17). Currently more than 400 plants are being used in different forms to lessen the hyperglycaemic effects (11, 15). Several different inhibitors of α-amylase and α-glucosidase has been isolated from the medicinal plants to serve as an alternative drug with better potency and lesser adverse effects than existing synthetic drugs (18). Active compounds derived from the medicinal plants are not only the source of α-amylase and α-glycosidase inhibitors, moreover they also are the rich source of phenolic substances, and consequently they have great antioxidant activity (19). Amomum belongs to family Zingiberaceae is a genus of Rhizomatous terrestrial herb, distributed mainly in tropical Asia and Africa, found in the eastern Himalayas and cultivated in Nepal, northern West Bengal, Assam and Sikkim hills (20). The seeds are reported to possess stimulant, stomachic, alexipharmic and astringent properties, and are used in traditional medicine for the treatment of indigestion, vomiting, abdominal pains and rectal diseases. The seeds are found to promote the elimination of bile and are used to treat congestive jaundice; they are also used in gonorrhoea, while the rind has been reported to be useful in treating headache and stomatitis. The aromatic oil extracted from the seeds is useful to the eyes in cases of inflammation (21). *Amomum subulatum Roxb.* dry fruits locally regarded as black cardamom, have a strong camphor-like flavor, with a smoky character derived from the method of drying. It has many biological activities like: analgesic (22), anti-inflammatory (23), antimicrobial (24-27), antioxidant (28-31), antiulcer (32-36), cardioadaptogen (37), diuretic (38), and hypolipidaemic activity (39-41). While *Amomum tsao-ko Crevost et Lemaire* mostly used in Chinese cuisine and folk medicine preparations for the treatment of throat infection and stomach (42, 43). The methanol extract of *Amomum tsao-ko* had markedly influence on plasma glucose and thiobarbituric acid reactive substances (TBRAS) and antioxidant potential (43). However, the wide use of *Amomum subulatum Roxb.* and *Amomum tsao-ko Crevost et Lemaire* fruits as a medicinal plant has very less scientific evidences to attest its bioactive components as well as its usage as a medicinal source to treat diabetes. The current study is the first comparative report for in vitro evaluation of their aqueous seeds and rind extracts for bioactive compounds in relation to their anti-diabetic activity.

## 2. Materials and Methods

### *2.1. Plant material*

Dry fruits of *Amomum subulatum Roxb.* and *Amomum tsao-ko Crevost et Lemaire* were purchased from Punjab province, Pakistan and Shandong province, P.R China respectively. Their identification was authenticated by M. Jafar Jaskani from UAF. Pakistan and Haifang Xiao from SDUT. China.

### *2.2. Chemicals and reagents*

α-amylase from porcine pancreas, α-glucosidase from Saccharomyces cerevisiae, and para-nitrophenyl-

glucopyranoside were products of Sigma-Adrich Co., St Louis, USA, while soluble starch (extra pure) was obtained from J. T. Baker Inc., Phillipsburg, USA. Other chemicals and reagents were of analytical grade and water used was glass distilled.

*2.3. Preparation of aqueous extract of fruit's constituent*

Dried fruits (seeds with rind) of *Amomum subula-tum Roxb.* and *Amomum tsao-ko Crevost et Lemaire* were washed with water to remove all contaminants; these were dried under room temperature, the seeds were separated from rind, and then both fruits parts grounded separately to powder using disintegrator (Ultra Centrifugal Mill, MRK CO., Ltd., Tokyo Japan). 10 grams of the seeds and rind powder from both fruits were extracted by maceration in 100 mL of distilled water (v/v) for 3 days with frequent agitation speed of 280 rpm at 28°C in the dark. The supernatants were collected, filtered through Whatman No. 1 filter paper and the filtrate was then concentrated at 60°C using a rotary evaporator (BuchiLabortechnik, Flawil, Switzerland). Finally the concentrates were freeze dried (Labconco Corporation, Kansas City, MO, USA) to yield a dry powder. Dried extracts were weighed and dissolved in 10% dimethylsulphoxide (DMSO) to yield a stock solution from which lower concentrations were prepared.

*2.4. Phytochemical screening*

Phytochemical compositions of the seeds and rind of the said plants were determined using the methods previously described by Trease and Evans (44) and Sofowora (45).

*2.4.1. Test for anthraquinones:*

5 mL of chloroform was added to 0.5 g of the plant extracts of each specimen. The resulting mixture was shaken for 5 min after which it was filtered. The filtrate was then shaken with equal volume of 10 % ammonia solution. The presence of a bright pink color in the aqueous layer indicated the presence of anthraquinones.

*2.4.2. Test for flavonoids:*

A portion of the plant extract was heated with 10 mL of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 mL of the filtrate was shaken with 1 mL of dilute ammonia solution. Development of yellow coloration was an indication of the presence of flavonoids.

*2.4.3. Test for reducing sugar:*

To about 1 g of each plant extract in the test tube, 10 mL distilled water was added and the mixture boiled for 5 min. The mixture was filtered while hot and the cooled filtrate made alkaline to litmus paper with 20% sodium hydroxide solution. The resulting solution was boiled with an equal volume of Benedict qualitative solution on a water bath. The formation of a brick-red precipitate depicted the presence of reducing compound.

*2.4.4. Test for saponin:*

Approximately 2 g of plant extract was boiled in 20 mL of distilled water in a water bath and filtered. Next, 10 mL of the filtrate was mixed with 5 mL of distilled water and shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously again and then observed for the formation of emulsion as an indication of saponin.

*2.4.5. Test for steroids:*

In this test, 2 mL of acetic anhydride was added to 0.5 g of plant extract with 2 mL concentrated $H_2SO_4$. The color change from violet to blue or green is an indication of steroids.

*2.4.6. Test for Tannins:*

In the test for tannins, 0.5 g of plant extract was boiled in 20 mL of water in a test tube and filtered. Few drops of 0.1 % ferric chloride were added and observed for a brownish green or blue black coloration as an indication of tannins.

*2.4.7. Test for terpenoids:*

In brief, 0.5 g of plant extract was mixed with 2 mL chloroform and 3 mL $H_2SO_4$ was carefully added to form a layer. A reddish brown coloration of the interface was an indication of terpenoids.

*2.5 Determination of total phenolic compounds*

Total soluble phenolic compounds in the extracts

were determined with Folin-Ciocalteu reagent according to the method described by Hameed et al. (46). Briefly, 1 mL of extract (1000 μg/mL) in a volumetric flask was diluted with distilled water (46 mL). One mL of Folin-Ciocalteu reagent was added and the content of the flask was mixed thoroughly. After 3 min, $Na_2CO_3$ (3 mL, 2 % w/v) was added and then allowed to stand for 2 h with intermittent shaking. The absorbance was measured at 760 nm in a spectrophotometer. The total concentration of phenolic compounds in the extract determined as microgram of pyrocatechol equivalent by using an equation that was obtained from the standard pyrocatechol graph:

Absorbance = 0.0054 × total phenols (pyrocatechol equivalent) (μg) 0.0058.

*2.6 Assay for total flavonoid content*

Total flavonoid content was determined using the method given elsewhere (47, 48). Briefly, aluminium trichloride (1 mL, 2 % w/v) in methanol was mixed with the same volume of the extract (1 mL, 2000 μg/mL). Absorption readings at 415 nm were taken after 10 min against a blank sample consisting of an extract (1 mL, 2000 μg/mL) with methanol (1 mL) and without $AlCl_3$. The concentrations of flavonoid compounds were calculated according to the following equation that was obtained from the standard quercetin graph:

Absorbance = 0.0338 quercetin (μg) - 0.0002; R2= 0.9998.

*2.7. A-amylase inhibition assay*

The inhibition of α-amylase was determined using an assay modified from the Worthington Enzyme Manual (48). Aliquot 0-4 mg/mL in DMSO (v/v 1:1) of aqueous extract of fruit's constitutes was prepared and 500 μL of each concentration extract was mixed with 500 μL of 0.02 M sodium phosphate buffer (pH 6.9) containing α-amylase solution (0.5 mg/mL) and incubated at 25°C for 10 min. After pre-incubation, 500 μL of a 1.0 % starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube at timed intervals. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 mL of dinitrosalicylic acid color reagent. The test tubes were then incubated in a boiling water bath for 5 min and cooled to room temperature. The reaction mixture was then diluted by adding 15 mL of distilled water, and the absorbance was measured at 540 nm using a micro-plate reader (Thermomax, Molecular device Co., Virginia, USA). The experiments were performed in triplicate and the absorbance of sample blanks (buffer instead of enzyme solution) and a control (buffer in place of sample extract) were also recorded. The absorbance of the final extract was obtained by subtracting its corresponding sample blank reading. Acarbose was prepared in distilled water and used as positive control.

The percentage inhibition was calculated using the formula:

% Inhibition = {(Ac – Ae)/Ac} 100

where Ac and Ae are the absorbance of the control and extract, respectively.

$IC_{50}$ values (inhibitor concentration at which 50 % inhibition of the enzyme activity occurs) of seed and rind extracts were determined by plotting graph with varying concentrations of the said extracts against the percent inhibition.

*2.8. α-glucosidase inhibition assay*

The α-glucosidase was assayed using a method modified by Apostolidis et al. (49). Aliquot 0-4 mg/mL in DMSO (v/v 1:1) of aqueous extract of fruit's constitutes was prepared. 50 μL of each concentration extract was mixed well with 100 μL of 0.1 M phosphate buffer (pH 6.9) containing α-glucosidase solution (1.0 U/mL) and the mixtures were then incubated in 96-well plates at 25°C for 10 min. After pre-incubation, 50 μL of 5 mM p-nitrophenyl-α-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added to each well at timed intervals. The reaction mixtures were in-cubated at 25°C for 5 min. Before and after incubation absorbance readings were recorded at 405 nm using a micro-plate reader (Thermomax, Molecular device Co., Virginia, USA) and compared to a control which contained 50 μL of the buffer solution instead of the extracts. The experiments were performed in triplicate and the α-glucosidase inhibitory activity was expressed as percentage inhibition. Acarbose was prepared in distilled water and used

as positive control. The percentage inhibition was calculated using the formula:

$$\% \text{ Inhibition} = \{(Ac - Ae)/Ac\}\ 100$$

where Ac and Ae are the absorbance of the control and extract, respectively.

$IC_{50}$ values (inhibitor concentration at which 50 % inhibition of the enzyme activity occurs) of seed and rind extracts were determined by plotting graph with varying concentrations of the said extracts against the percent inhibition

*2.9. Statistical Result*

All the measurements were done in triplicate and results are expressed in terms of mean ± standard deviation and $IC_{50}$ values were calculated using Graph Pad Prism 5 version 5.01 (Graph pad software, Inc., La Jolla, CA, USA.) statistical software.

## 3. Results and discussion

To control the diabetes complications, the management of the blood glycemic (sugar) level is considerable and a novel approach. Inhibitors of carbohydrate hydrolyzing enzymes (*i.e.* α-amylase and α-glycosidase) have been practically valuable as oral hypoglycemic drugs for the control of diabetes especially in patients with type-2 diabetes mellitus (50-52). Inhibitors of α-glucosidase postpone the breaking down of carbohydrate in the small intestine and reduce the postprandial blood glucose expedition in a person suffering from diabetes (53). Several α-amylase inhibitors including acarbose, miglitol and voglibose are clinically useful to treat diabetes but these are expensive and have considerable clinical side effects. Medicinal plants have great potential to retard the absorption of glucose by inhibiting the saccharides hydrolyzing enzymes (54, 55). There is an attempt to explore the alternative drugs from medicinal plants with increased potency and less adverse effects than existing drugs (56-58).

Therefore, screening and isolation of inhibitors from plants for these enzymes are escalating.

The phytochemical composition of aqueous extracts of *Ammomum Subulatum Roxb.* fruit's constitutes (seeds and rind) indicated the presence of anthraquinones, flavonoids and tannins, in both the seed and rind part, while saponin, steroids and terpenoids were only present in rind extract and reducing sugar was only detected in its seed extract (Table 1). Whilst the phytochemical composition of aqueous extracts of *Ammomum tsao-ko Crevost et Lemaire* fruit's constitutes (seeds and rind) illustrated the presence of flavonoids and tannins, in its both seed and rind extract, while anthraquinones, steroids and reducing sugar were only present in seed extract and saponin and terpenoids were only detected in its rind extract (Table 2).

In the present study, anti-diabetic activity of the aqueous extracts from seeds and rind were evaluated with reference to α-amylase and α-glycosidase inhibi-

**Table 1.** Phytochemical composition of aqueous extract of *Ammomum subulatum Roxb.*

| Phytochemicals | Seed | Rind |
|---|---|---|
| Anthraquinones | + | + |
| Flavonoids | + | + |
| Reducing Sugar | + | - |
| Saponin | - | + |
| Steroids | - | + |
| Tannins | + | + |
| Terpenoids | - | + |

*(+): Present, (-) Not detected*

**Table 2.** Phytochemicals composition of aqueous extract of *Ammomum tsao-ko Crevost et Lemaire*

| Phytochemicals | Seed | Rind |
|---|---|---|
| Anthraquinones | + | - |
| Flavonoids | + | + |
| Reducing Sugar | + | - |
| Saponin | - | + |
| Steroids | + | - |
| Tannins | + | + |
| Terpenoids | - | + |

*(+): Present, (-) Not detected*

tion. It was found that the plants used in this study, *Amomum subulatum Roxb.* and *Amomum tsao-ko Crevost et Lemaire* fruit's constitutes showed potential anti-diabetic activities. The in vitro α-amylase and α-glucosidase inhibition study illustrated that the aqueous extract of *Amomum subulatum Roxb.* fruit's constitutes at concentrations of 4.0, 3.2, 2.4, 1.6 and 0.8 mg/mL inhibited α-amylase and α-glucosidase enzyme activities in a dose dependent manner. At the highest concentration of 4.0 mg/mL, the seed extract of *Amomum subulatum Roxb*. exhibited maximum α-amylase and α-glucosidase inhibitory activity of 87.1 % and 59.1 % respectively, while at the lowest concentration of 0.8 mg/mL showed a minimum inhibition of 67.3 % and 10.7 % respectively as well. The $IC_{50}$ values of aqueous seed extract of *Amomum subulatum Roxb*. were 1.70 mg/mL and 1.98 mg/mL respectively, while its rind extract at 4.0 mg/mL have highest α-amylase and α-glucosidase inhibitory activity of 86.9 % and 61.8 % respectively and at the lowest concentration of 0.8 mg/mL showed a minimum inhibition of 53.2 % and 22.9 % respectively as well (Figure 1, 2). The $IC_{50}$ values of rind extract of *Amomum subulatum Roxb*. were 1.10 mg/mL and 1.18 mg/mL respectively. Whilst acarbose at the concentration of 4.0 mg/mL, showed a maximum percentage inhibition of 74.3 % for α-amylase with an $IC_{50}$ of 2.1 mg/mL and 84.01 % for α- glycosidase with $IC_{50}$ 1.90 mg/mL value respectively. Our results suggested that the seed and rind extract of *Amomum subulatum Roxb*. have almost same percentage inhibition for α-amylase and α-glucosidase but higher than that of acarbose activity.

Likewise, the aqueous extract of *Amomum tsao-ko Crevost et Lemaire* fruit's constitutes (seeds and rind) also showed α-amylase and α-glucosidase inhibition in a dose dependent manner for concentrations of 4.0,

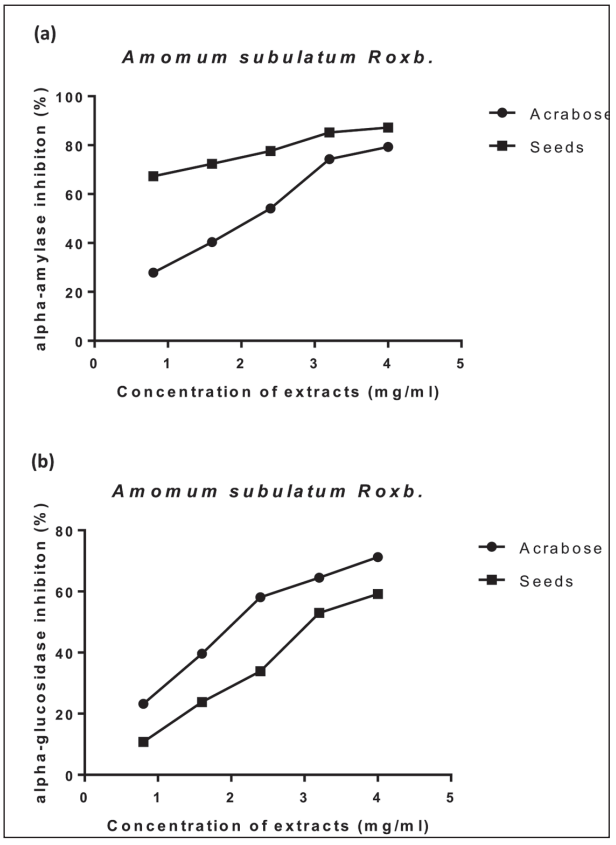


**Figure 1.** α-amylase inhibitory activities (a), α-glucosidase inhibitory activities (b), of seeds extracts of *Amomum subulatum Roxb.*

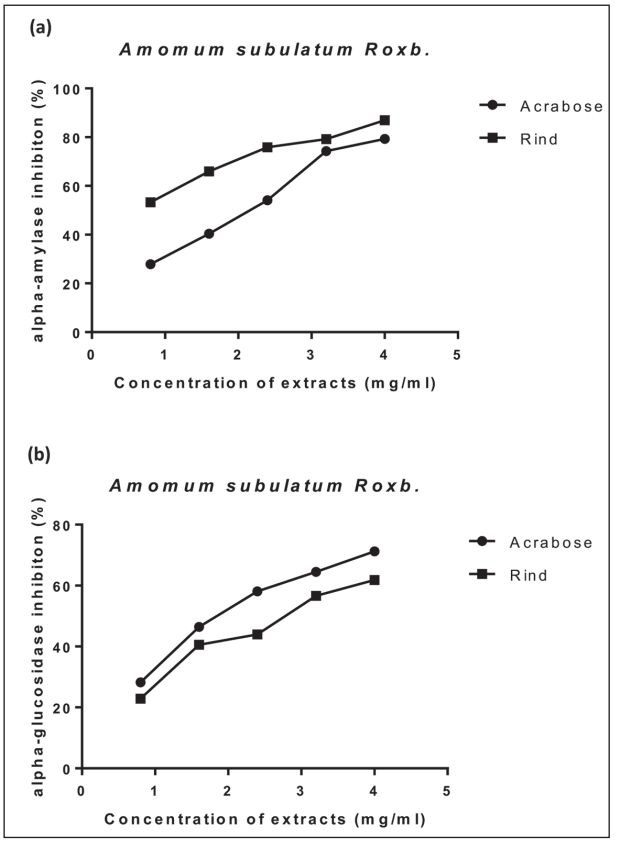


**Figure 2.** α-amylase inhibitory activities (a), α-glucosidase inhibitory activities (b), of rind extracts of *Amomum subulatum Roxb.*

3.2, 2.4, 1.6 and 0.8 mg/mL. At the highest concentration of 4.0 mg/mL, the seed extract of *Amomum tsao-ko Crevost et Lemaire* exhibited maximum α-amylase and α-glucosidase inhibitory activity of 83.9 % and 54.7 % respectively, while at the lowest concentration of 0.8 mg/mL showed a minimum inhibition of 52.5 % and 18.2 % respectively as well, with $IC_{50}$ values of 1.04 mg/mL and 1.4 mg/mL respectively. While its rind extract at 4.0 mg/mL have maximum α-amylase and α-glucosidase inhibitory activity of 69.3 % and 29.1 % respectively and at the lowest concentration of 0.8 mg/mL, rind extract showed minimum inhibition for α-amylase and α-glucosidase of 26.1 % and 4.02 % respectively as well (Figure 1, 2). The $IC_{50}$ values of aqueous rind extract of *Amomum tsao-ko Crevost et Lemaire* were 1.24 mg/mL and 2.4 mg/mL respectively. Among the two extracts of said plant, it is suggested that the seed extract of *Amomum tsao-ko Crevost et Lemaire* was more effective than the rind extract, and both extracts were relatively analogous to acarbose activity.

In the conclusive manner, it is suggested that seeds and rind extracts of *Amomum subulatum Roxb.* and seed extract of *Amomum tsao-ko Crevost et Lemaire* are much potent than acarbose on equal weight basis.

In this era of inquisitiveness, inventions and discoveries, it has now become indispensible to compare our findings with previous studies on different medicinal plants for inhibition against α-amylase and α-glucosidase activities. Kargbo et al. (59) suggested that at a concentration of 4.0 mg/mL, the ethanolic leaves extract of *Anisophyllea laurina* R. Br. ex Sabine exhibited α-amylase and α-glucosidase inhibitory activity of 78.5 % and 58.2 % respectively, with $IC_{50}$ values of 2.40 mg/mL and 3.11mg/mL respectively. The stem extracts showed α-amylase and α-glucosidase inhibitory activities of 69.5 % and 63.6 % respectively, with $IC_{50}$ values of 2.6 mg/mL and 3.5 mg/mL respectively. Dastjerdi et al. (60) found that α-amylase activity was inhibited by different *Teucrium* species. The $IC_{50}$ value of hydro-alcoholic extract of *T. polium* against α-amylase activity was 3.63 mg/mL. The $IC_{50}$ value of *T. oliverianum* and *T. Orientale* against α-amylase activities were 3.86 and 13.93 mg/mL, respectively. Kazeem et al. (61) worked on *Morinda lucida* Benth leaves, they revealed that the aqueous extract of *Morinda lucida* Benth leaves possesses $IC_{50}$ value of 2.30 mg/mL against α-amylase and $IC_{50}$value of 2.00 mg/mL against α-glucosidase activities. Mohamed et al. (62) investigated the α-amylase and α-glucosidase inhibitory activities of 50 % ethanolic extract of *Orthosiphon stamineus*, they found that $IC_{50}$ value of 36.70 mg/mL for α-amylase and $IC_{50}$ value 4.63 mg/mL for α-glucosidase. Balasubramaniam V. et al. (63) discovered that ethanol extract of *E. denticulatum* (red edible seaweed) at 10 mg/mL, significantly inhibited the α-amylase activity by 67 %. From this discussion, it is concluded that *Amomum subulatum Roxb.* and *Amo-mum tsao-ko Crevost et Lemaire* seed and rind extracts have best inhibitory activities against α-amylase and α-glucosidase in comparison with different medicinal plants stated above (Table 3).

Phytochemicals especially polyphenols has established increasing attention due to fascinating discoveries considering their biological activities (64). Previous findings proved that the methanolic extract of *Amomum subulatum Roxb.* fruit's constitutes showed high level of total phenolic content and total flavonoid content (65), it's essential oil demonstrated high level of total phenolic content due to presence of components like; 1,8-cineole, α-terpineol, terpinen-4-ol, spathulenol, α-pinene (66), Oleoresin of *Amomum subulatum Roxb.* seeds had moderate level of total phenoilc content, total flavonoid contant and total tannin content (67), its fruit's constitutes also had good antioxidant potential (68, 69). Many active compounds have already been explored in fruit's constitutes such as protocatechuic acid (70), petunidin-3,5-diglucoside, leucocyanidin-3-O-β-D-glucopyranoside, subulin, 1,8-cineole, α-terpinylacetate (71), protocatechualdehyde, 1,7-bis(3,4-dihydroxyphenyl) hepta-4E, 6E-dien-3-one, 2, 3, 7trihydroxy-5-(3,4-di-hydroxy-E-styryl)-6, 7, 8, 9-tetrahydro-5H benzocyclo-heptene (72), essential oil mainly consist of 1,8-cineole, lamonene, sabeinene, pinenes and terpinols (73), While earlier studies on *Amomum tsao-ko Crevost et Lemaire* fruit's constitutes demonstrated that it had moderate level of total phenolic content (TPC) (74,75) and total flavonoid content (TFC) (75). In the past, many phenolic compounds have been investigated in *Amomum tsao-ko Crevost et Lemaire* fruit's constitutes like; hannokinol, mesohannokinol, catechin, epicatechin, β-sitosterol, β-sitosterol 3-O-glucoside, 2,6 dimethoxyphenol, protocatechualdehyde, protocatechuic acid vanillic aci *p*-hydroxybenzoic acid

**Table 3.** $IC_{50}$ values for α-amylase and α-glucosidase inhibitory potential of different plants extracts

| Source Plants | $IC_{50}$ (mg/mL) | |
|---|---|---|
| | α-amylase | α-glucosidase |
| *Amomum subulatum Roxb.* (seed) | 1.7 | 1.9 |
| *Amomum subulatum Roxb.* (rind) | 1.1 | 1.1 |
| *Amomum tsao-ko Crevost et Lemaire* (seed) | 1.07 | 1.4 |
| *Amomum tsao-ko Crevost et Lemaire* (rind) | 1.24 | 2.4 |
| *Anisophyllea laurina* R. Br. ex Sabine (leaf) | 2.4 | 3.1 |
| *Anisophyllea laurina* R. Br. ex Sabine (stem) | 2.6 | 3.5 |
| *Teucrium polium* | 3.63 | – |
| *Teucrium oliverianum* | 3.86 | – |
| *Teucrium orientale* | 13.93 | – |
| *Morinda lucida* Benth (leaves) | 2.3 | 2 |
| *Orthosiphon stamineus* | 36.7 | 4.6 |

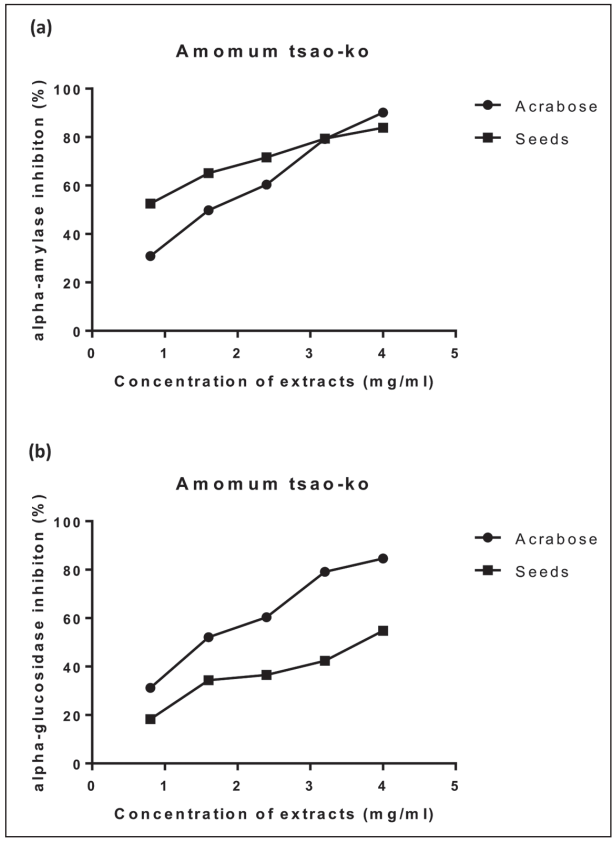


**Figure 3.** α-amylase inhibitory activities (a), α-glucosidase inhibitory activities (b), of seed extracts of *Amomum tsao-ko Crevost et Lemaire*

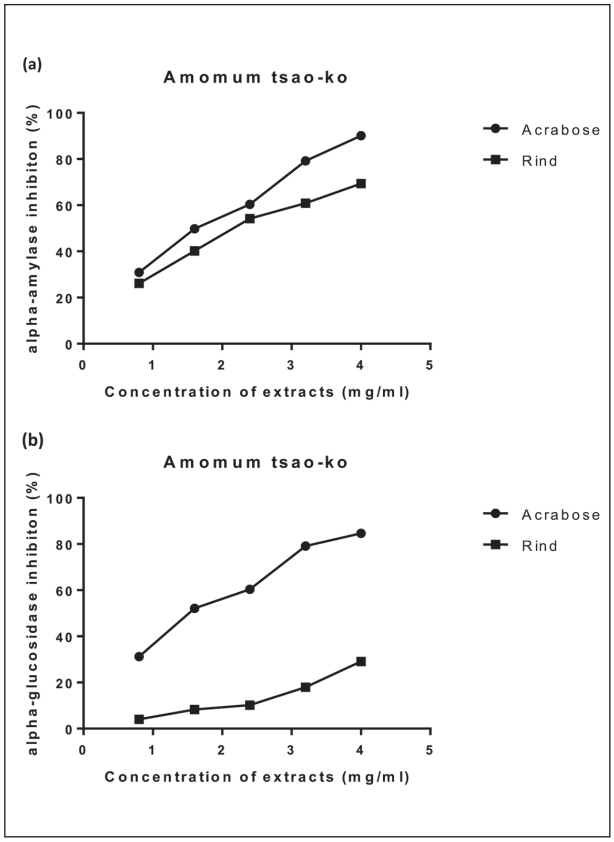


**Figure 4.** α-amylase inhibitory activities (a), α-glucosidase inhibitory activities (b), of rind extracts of *Amomum tsao-ko Crevost et Lemaire*

(76, 77), daucosterol, quercetin, quercetin-7-O-β-glucoside, quercetin-3-O-β-glucoside, catechol (78), 2-methoxy-1,4-biphenol-1-O-[6-O-(3-methoxy-4-hydroxybenzoyl)]-β-D-glucopyranoside, 3′, 5′-di-C-β-D-glucopyranosylphloretin, rutin, pyrogallic acid (79), and also fat soluble polar active components that might be responsible for decreased blood glucose and TBARS concentrations (80). The α-amylase and α-glucosidase inhibitory activities might be due to the individual or synergistic outcome of these bioactive compounds, the results of current work are very interesting, still sufficient in vivo studies (in rats or rabbits) are required to extrapolate its usage in humans.

## 4. Conclusion

In the present study, *Amomum subulatum Roxb.* and *Amomum tsao-ko Crevost et Lemaire* dry fruit's constitutes (seeds and rind) were evaluated to find out the possible mechanism for their anti-diabetic mode of action. *Amomum subulatum Roxb.* seeds and rind extract could inhibit starch digestion enzymes more efficiently than acarbose. Among the four aqueous extracts, seed extracts of both *Amomum subulatum Roxb.* and *Amomum tsao-ko Crevost et Lemaire* showed best inhibitory effect on α-amylase and better on α-glycosidase activities, whilst the rind extract of both *Amomum subulatum Roxb.* and *Amomum tsao-ko Crevost et Lemaire* showed good inhibitory potential for α-amylase and sufficient inhibitory potential for α-glycosidase activity. Based on the results presented in this study, it can be concluded that aqueous extracts of *Amomum subulatum Roxb.* and *Amomum tsao-ko Crevost et Lemaire* dry fruit's constitutes (seeds and rind) have high inhibitory effect on α-amylase and α-glycosidase activities. These activities might be attributed to presence of phenolic compounds earlier identified in these plants, which may have individual or synergistic consequence for such activities. Therefore our results suggested the potential use of these plants as a dietary supplement or in the manufacture of drugs for the control of increased blood glucose (sugar) level in the body. However, further studies are also needed to elucidate the active principle(s) constitutes in these plants which are responsible for such activities.

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Original article

# Simple tools for monitoring chlorophyll in broccoli raab and radish microgreens on their growing medium during cold storage

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**Summary.** Microgreens have been recently introduced as a new category of vegetables, with unexploited potential as functional foods. Due to containerized production, they can be commercialized while growing on the medium, ready for being harvested before use. The chlorophyll content of vegetables is important for both health benefits and visual appearance of the produce. This paper aims to evaluate the feasibility of using simple tools to monitor chlorophyll content in microgreens of two different species, broccoli raab (*Brassica rapa* L., Broccoletto group) and radish (*Raphanus sativus* L.), in varying stages of cold storage in their growing vessel. Image acquisition with a CCD camera, followed by image analysis using preset algorithms of an open source software (ImageJ) was the approach used. Image color analysis (median values of L*, a*, and b* indices) and textural parameters obtained from the gray-level co-occurrence matrix (GLCM) allowed to obtain regression models for chlorophyll content with satisfactory fitting parameters (adjusted $R^2$ was 0.765 and 0.843 for broccoli raab and radish, respectively). These results point out the possibility to set up low-cost, real time, non-destructive monitoring systems for microgreens quality during their growing as well as during storage.

**Key words:** *Brassica rapa* L., image analysis, GLCM, nutritional quality, *Raphanus sativus* L., ImageJ

## 1. Introduction

Microgreens are young and tender edible seedlings produced using the seeds of different species of vegetables, herbaceous plants, aromatic herbs and wild edible plants. Depending on the species that has been used, they can be harvested 7-21 d after germination, when the cotyledon leaves have fully developed and the first true leaves have emerged (1). Microgreens represent a new category of vegetables with different traits as compared to the already known sprouts and the common fresh-cut leafy vegetables. They are characterized by a wide range of colors, flavors, textures (2, 3). Due to high content of functional components such as antioxidants, vitamins and minerals etc., microgreens are considered as potential "functional foods" (1). Moreover, microgreens can contribute to preserve and valorize biodiversity, and recover and use many local varieties that are at risk of genetic erosion (1). Microgreens can be produced in open air as well as in protected environment, both on soil and soilless. The latter growing system allows also containerized production, which can result in commercialization of the product while growing on the medium, ready for being harvested just before use. Harvest and many postharvest issues can be avoided with this approach (1, 4).

Chlorophylls are pigments that give green color to vegetables and several fruits, where they play key

roles in photosynthesis. The chlorophyll content of vegetables is important for both health benefits and visual appearance of the produce (5). Its decrease is associated with cellular degradation and/or senescence, and it is often used to estimate quality loss of green vegetables (6, 7). In fact, strong relation of chlorophyll content with overall visual quality of vegetables has been reported (8). Moreover, chlorophyll can be considered a bioactive compound, since its dietary naturally occurring derivatives showed antioxidant and antimutagenic activity (9-11).

The measurement of quality parameters (i.e. chlorophyll content) is generally carried out using traditional analytical techniques whose application in the food industry poses several problems: they require very long times, are expensive and destructive.

Nondestructive analytical approaches would be therefore required for quality control during both production and storage of microgreens. To the purpose, visible imaging coupled to image analysis using open source software (ImageJ) can be a cheap, effective and simple approach, allowing to provide both color-related and texture-related information useful for food inspection, grading, detection (12-15).

As far as we know, no attempt has been made to evaluate the potential of visible imaging coupled to image analysis for monitoring of microgreens directly on their growing medium. This approach could take advantage of the almost flat surface of the microgreen crops and overcome the flaws of another simple nondestructive instruments such as colorimeter. In fact, analysis by colorimeter requires multiple readings that can be hindered by the small leaf surface, the contact with the sample and an equipment with relatively high cost (16, 17). Moreover, image analysis requires low-cost equipments and can be carried out using open source software, such as ImageJ (18).

The aim of the present research was to evaluate the feasibility of using such simple tools to monitor chlorophyll content in microgreens of two different species, broccoli raab (*Brassica rapa* L., Broccoletto group) and radish (*Raphanus sativus* L.), in varying stages of cold storage in their growing vessel.

## 2. Materials and methods

### *2.1. Microgreens production and storage*

Two different species were produced: broccoli raab (*Brassica rapa* L., Broccoletto group) also known as 'rappini' or 'rapini' and radish (*Raphanus sativus* L.). Seeds of a local variety ('*Sessantina*') produced by Puglia's hold-farmers were used for broccoli raab, while radish seeds cv Saxa were purchased (Riccardo Larosa company, Andria, Italy). The two genotypes were sown in four plastic trays (with holes at the bottom) filled with a mixture of peat (50% white-50% black peat mixture, Brill 3 Special, Brill Substrates, Georgsdorf, Germany), using a density of 3 seed $cm^{-2}$. Microgreens were grown in a growth chamber at controlled temperature (22°C) and relative humidity (85%). After germination, the seedlings were exposed for a 12 h photoperiod to a light irradiance of 200 $\mu mol\ m^{-2}\ s^{-1}$, determined by LICOR LI-190 (Li-Cor Inc., USA) quantum sensors. Seedlings were fertigated daily using a nutrient solution containing all the essential macro- and micro-nutrients at the following concentrations (mg $L^{-1}$): N 105, P 15, K 117, Ca 100, Mg 24, B 0.25, Cu 0.01, Fe 2.5, Mn 0.25, Zn 0.025, Mo 0.005.

Microgreen vessels were sampled ten days after germination, between fully development of cotyledons and first true leave. Four growing vessels per each species were sampled, put in low density polyethylene bags and stored in dark at 5°C. At day 0 and after 1, 2, 5 and 13 d of storage chlorophylls analysis, spectrocolorimetric determination and image acquisition were performed (n =4).

### *2.2. Chlorophyll analysis*

Total chlorophyll content was determined spectrophotometrically using the method of Lichtenthaler and Buschmann (19) with minor modifications. Excised leaves (0.5 g, corresponding to about twenty leaves, sampled throughout the vessel) were homogenized and added with 15 mL acetone (HPLC-UV grade, Pharmco-Aaper, Brookfield, CT, USA) and stirred for 20 min. The mixture was filtered (Grade 413 Filter Paper, Qualitative, VWR International, West Chester, PA, USA) and transferred into spectrophotometric cuvettes. Absorbance was read at 661.6 nm

and 644.8 nm with a Cary 60 UV-VIS (Agilent Technologies, Santa Clara, PA, USA) and total chlorophyll ($chl_{a,b}$, mg $L^{-1}$) was calculated as the sum of chlorophyll *a* ($chl_a$, mg $L^{-1}$) and chlorophyll *b* ($chl_b$, mg $L^{-1}$) calculated by the following formulas:

$$chl_a = 11.24\, A_{661.6} - 2.04\, A_{644.8}$$
$$chl_b = 20.13\, A_{644.8} - 4.19\, A_{661.6}$$

where $A_n$ was the absorbance of the extract at *n* nm of wavelength.

*2.3. Colorimetric analysis*

Colorimetric evaluations of lightness (L*), red index (a*), and yellow index (b*) were carried out under D65 illuminant by using a spectro-colorimeter CM-700d (Konica Minolta Sensing, Osaka, Japan) equipped with a pulsed xenon lamp. At least five readings were performed on different areas of each sample and the mean values were considered.

*2.4. Visible imaging*

Image acquisition was carried out using a low-cost equipment composed of the following elements: a black shooting box (50 x 42 x 28 cm) with two fluorescent lamps (40 W, 480 lm, 6400 K) and a small window on the top for the camera; a DMC-FS10 digital camera (Panasonic Corporation, Osaka, Japan) with a 12 Mpixel CCD, held above the light box by a moving mount at 28 cm from the bottom of the light box. Settings of the camera were in automatic mode (17).

*2.5. Image analysis: RGB measurement*

The acquired images were processed using the free ImageJ software (NIH, USA). Color thresholding was applied, in RGB color space, adjusting the parameters in order to select the microgreens and separate them from the background. The *RGB measure* plugin was run to obtain the mean RGB values of the image.

*2.6. Image analysis: L* a* b* measurement*

The image type was subsequently converted in a L* a* b* stack. Using the wand tool and changing tolerance parameters, the whole leaf area was selected and separated from the background. Then, the *measure* function allowed to measure mean, median, modal values and standard deviations for the selected pixels in all the three stacks.

*2.7. Image analysis: gray level co-occurrence matrix (GLCM)*

Tournier et al. (20) defined GLCM as the description of the second-order statistics in the images, permitting the calculation of textural features which are expected to represent the texture characteristics of the image studied. This approach allows to calculate how often pairs of pixels with specific values and in a specified spatial relationship occur in an image (21). Before calculating GLCM parameters, the original image was finally converted in a 8-bit gray scale image. The GLCM-texture plugin was then run to perform texture analysis. The displacement vector (D) was set with a distance of 1 pixel, while the angle was 0°. The following parameters were measured (22):

- angular second moment (ASM), describing the regularity of the image;
- inverse difference moment (IDM), describing the local homogeneity of the image;
- entropy (e), measuring the statistical randomness;
- contrast (c), also evaluating the local homogeneity.

For regression analysis, a preliminary screening was performed (data not shown) to select the variable subset giving the best results.

*2.8. Statistical analysis*

Regression models were built using Minitab 17 (Minitab Inc., State College, PA, USA). Full quadratic models including second order terms and first order interactions. Mean subtraction was applied as coding option, in order to reduce collinearity. Backward removal was applied for model selection, with p = 0.01 as removal threshold.

## 3. Results and discussion

*3.1. Chlorophyll content*

Figure 1 reports the variability of chlorophyll contents in the microgreens considered. Both species were characterized by quite high chlorophyll content (11,23–25), particularly radish which showed a medi-

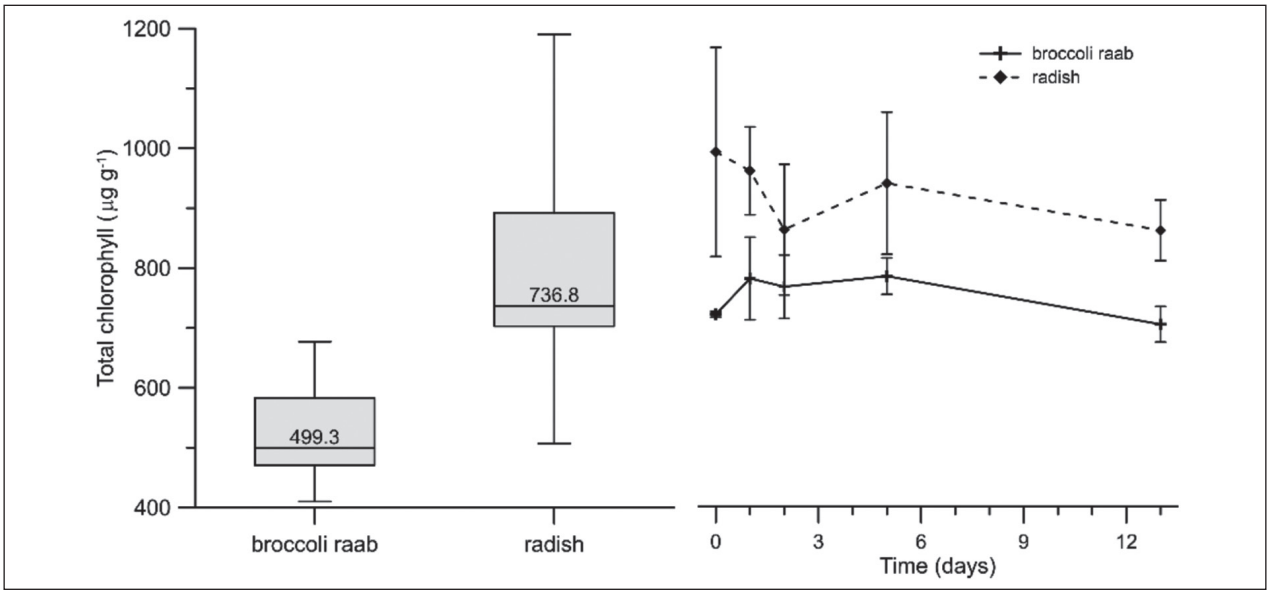


**Figure 1.** Box-Whisker and line plots of total chlorophyll ($chl_{a,b}$) content of microgreens during storage. Minimum, maximum, median, lower quartile, and upper quartile are reported in the Box-Whisker plot.

an content of 736 µg $g^{-1}$, while the median content in broccoli raab was 499 µg $g^{-1}$. The variability of data was higher for radish than for broccoli raab. Total chlorophyll did not show significant decreases during storage, contrarily to fresh cut produce stored at similar temperatures (3).

*3.2. Image analysis. Comparison of different algorithms for chlorophyll monitoring*

Figure 2 reports sample images of fresh and 13-days stored microgreens trays. Both broccoli raab and radish showed appreciable variations of visual aspect, such as incipient etiolation and chlorotic cotyledons. Therefore, sample images corresponded to a wide range of visual quality conditions.

Table 1 reports the comparison of the results of the regression models obtained for the chlorophyll content of microgreens as a function of image analysis parameters obtained from different algorithms. Bold characters in table highlight the model with the best performances. In fact, the models obtained showed quite different fitting performances. As regards broccoli raab, while colorimetric analysis did not allow to obtain a satisfactorily significant model, two out of the three image analysis algorithms provided significant

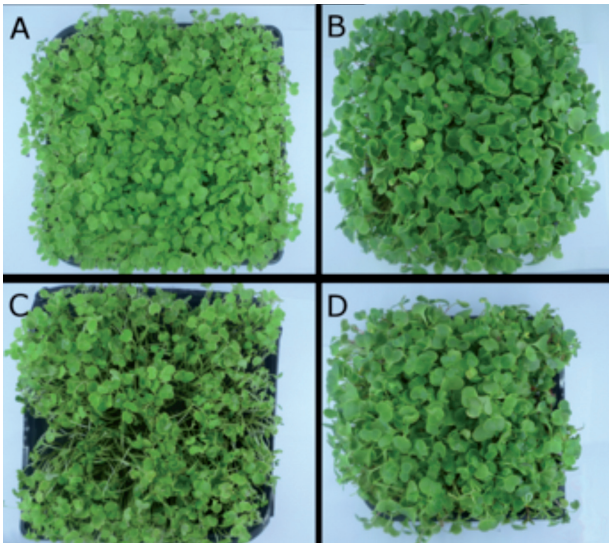


**Figure 2.** Representative samples of broccoli raab and radish microgreens after 1 d (A and B, respectively) and 13 d of storage (C and D, respectively).

models, though the best performances were obtained using L*, a*, b* median values: both adjusted $R^2$ and $R^2$ for prediction were by far higher than those of the other models.

**Table 1.** Comparison of the regression models for chlorophyll content[a]

| broccoli raab | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| method | algorithm | model parameters | model terms | *p*-value | adj. $R^2$ | $R^2$ pred. | MSE | SSE | PRESS |
| colorimeter | | L*, a*, b* | L,b | 0.081 | 0.233 | 0.108 | 4298 | 51577 | 69995 |
| image analysis | RGB | R, G, B | R,G,B,R*B,G*B | 0.039 | 0.502 | 0.149 | 2789 | 25105 | 66752 |
| | Lab | L*, a*, b* (mean value) | L,a,b,a*a,b*b,L*b,a*b | 0.018 | 0.700 | 0.623 | 1683 | 11780 | 73553 |
| | | L*, a*, b* (standard deviation) | L,L*L | 0.064 | 0.263 | 0.338 | 4130 | 49561 | 75786 |
| | | L*, a*, b* (modal value) | a,a*a | 0.032 | 0.344 | 0.000 | 3674 | 44083 | 80249 |
| | | **L*, a*, b* (median value)** | **L,a,b,a*a,b*b,L*b** | **0.004** | **0.765** | **0.488** | **1315** | **10523** | **40138** |
| | GLCM | ASM, contrast, IDM | ASM,c,IDM,ASM*IDM,c*IDM | 0.114 | 0.343 | 0.222 | 3682 | 33135 | 76702 |
| radish | | | | | | | | | |
| method | algorithm | model parameters | model terms | *p*-value | adj. $R^2$ | $R^2$ pred. | MSE | SSE | PRESS |
| colorimeter | | L*, a*, b* | L,a,b,L*L,a*a,b*b,L*b,a*b | 0.175 | 0.408 | 0.000 | 16240 | 97440 | 1215378 |
| image analysis | RGB | R, G, B | G,G*G | 0.011 | 0.449 | 0.367 | 15126 | 50505 | 243306 |
| | Lab | L*, a*, b* (mean value) | L | 0.098 | 0.135 | 0.000 | 23745 | 306688 | 464585 |
| | | L*, a*, b* (standard deviation) | - | - | - | - | - | - | - |
| | | L*, a*, b* (modal value) | - | - | - | - | - | - | - |
| | | L*, a*, b* (median value) | L | 0.093 | 0.140 | 0.000 | 23597 | 306767 | 462036 |
| | GLCM | **ASM, contrast, IDM** | **ASM,c,IDM,ASM*ASM,c*c,ASM*c** | **<0.001** | **0.843** | **0.668** | **4314** | **34513** | **127447** |

[a] Second order terms and first order interactions were included in the models. Mean subtraction was applied as coding option. Backward removal was applied for model selection, with $p = 0.01$ as removal threshold. Selected models are in bold.

The relative error of calibration (REC) was 3.7%, while the regression equation was the following:

$$\text{Total chlorophyll } (\mu g\ g^{-1}) = -4.165 \times 10^4 + 427.3\ L^* - 362\ a^* + 1.419 \times 10^3\ b^* - 6.59\ a^{*2} - 5.40\ b^{*2} - 14.44\ L^* \times b^* \quad \text{(eq. 3.1)}$$

Predicted data are plotted versus observed data in Figure 3, which also reports the regression residuals for the selected model for broccoli raab.

The reason why median values provided much better results than mean values can be explained considering that the differences between mean and median values changed during storage of broccoli raab. As regards lightness, median values were higher than mean values and the difference tended to a slight, linear increase ($p < 0.01$) during storage; as regards red index, the difference *mean value – median value* tended to increase ($p < 0.05$); finally, as regards yellow index, the difference *mean value – median value* was positive and tended to decrease ($p < 0.001$). These changes could be due to the increasing effect of outlying pixels deriving either from background or from individual leaves. As a consequence, being median values less affected by outliers than mean values, the regression resulted more powerful in modeling the overall content of total cholophyll in spite of possible imperfections of image segmentation.

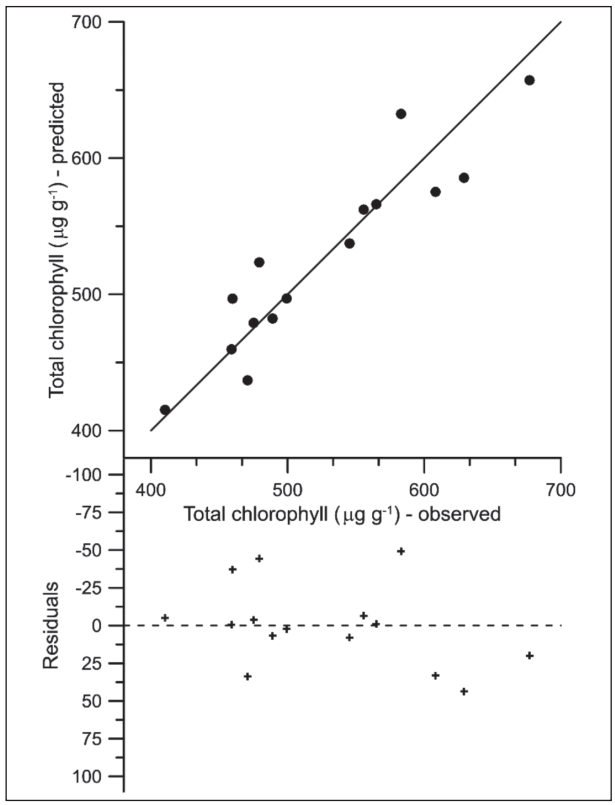


**Figure 3.** Regression analysis of total chlorophyll ($chl_{a,b}$) content in broccoli raab microgreens as a function of image analysis parameters. Plots of predicted values versus observed values (top) and residuals versus observed values (bottom). See Table 1 for regression indices (bold line) and equation 3.1 for regression equation.

A difference was observed between calibration performances of the model and its prediction capabilities, as can be observed comparing adjusted and prediction $R^2$, as well as SSE and PRESS. Model robustness could be increased by using larger sample sizes. Nevertheless, at this preliminary stage, algorithm comparison showed that image analysis can provide sufficient information to relate to the cholorphyll content. Further work is required for the improvement of the predictive capability of the selected model.

As regards radish, neither colorimeter nor L*, a*, b* image analysis algorithm provided significant models. On the other hand, the RGB algorithm allowed to obtain a significan regression ($p < 0.05$). Nevertheless, the best results were obtaind applying GLCM to the acquired images. Preliminary evaluation allowed to select, as starting variables, ASM, IDM and c. The best model showed values for both adjusted $R^2$ and $R^2$ for prediction equal to 0.843 and 0.668, respectively. The REC was 5.5% while the regression equation was the following:

$$\text{Total chlorophyll } (\mu g\ g^{-1}) = -6.750 \times 10^4 + 9.366 \times 10^7\ ASM + 858\ c + 1.861 \times 10^4\ IDM - 3.723 \times 10^{10}\ ASM^2 - 3.112\ c^2 - 6.048 \times 10^5\ \text{ASM} \times c \quad \text{(eq. 3.2)}$$

Predicted data are plotted versus observed data in Figure 4, which also reports the regression residuals for the selected model for radish (in bold in Table 1).

It clearly appears that GLCM texture analysis was particularly suited to assess total chlorophyll content in this species of microgreens. Previous applications of GLCM texture parameters of visible images mainly regarded classification or structural characterization of food samples (26). Most of the reported models reached an accuracy higher than 0.7, that is satisfactory for industrial applications (25). Values higher than those reported in the present study were obtained only for classification models. Few applications are reported of the use of GLCM texture for the assessment of chemical indices. Kondo et al. (27) coupled applied artificial neural networks to GLCM texture for determining sugar content in lyokan orange, gaining an accuracy of 0.84. Quevedo et al. (21) obtained highly significant ($R^2 > 0.976$) power law models for non enzymatic browning kinetics in avocado. The present application of GLCM texture on visible images of radish microgreens therefore expands the possibility of use of this algorithm for an effective chemical characterization of food samples. Also in this case, the differences between calibration and prediction pointed out the need to build more robust models with larger datasets. Nevertheless, the possibility to choose among several image analysis approaches and algorithms expands the potential of this technique and its adaptation to different crops.

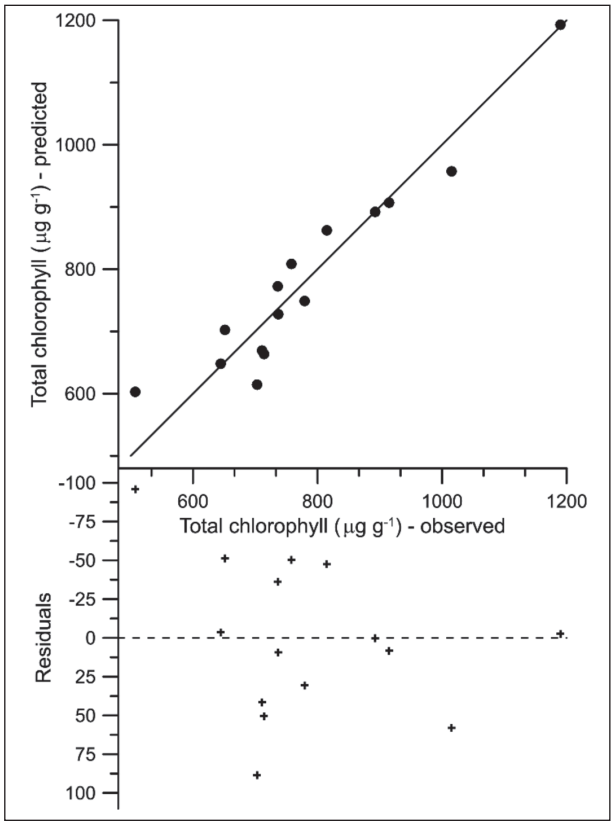


**Figure 4.** Regression analysis of total chlorophyll ($chl_{a,b}$) content in radish microgreens as a function of image analysis parameters. Plots of predicted values versus observed values (top) and residuals versus observed values (bottom). See Table 1 for regression indices (bold line) and equation 3.2 for regression equation.bold line) and equation 3.1 for regression equation.

## 4. Conclusion

Analysis of visible images of microgreens on their growing vessel resulted effective in monitoring a

chemical index (total chlorophyll content). The models described in this paper could be used for the automatic prediction of an important nutritional quality trait. This could be a significant achievement, since the strenght of commercialization of microgreens on their own growing vessel is strictly related to the possibility of keeping sensory and nutritional properties of the fresh product. The performances of statistical models relating image features with the monitored parameter can be optimized for each microgreens species. Different image analysis algorithms offer, to this scope, the opportunity of adopting the more appropriate model on the basis of the considered species, to allow the building up real time, non-destructive monitoring systems, using low-cost and simple pre-calibrated tools.

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Chemical compounds studied in this article
Chlorophyll (PubChem CID: 6449992)

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ORIGINAL ARTICLE

# The relation between meal frequency and obesity in adults

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**Summary.** *Objective:* To determine the relation between meal frequency and obesity in adults. *Methods:* A cross-sectional study was done among 1829 volunteer (520 men, 1309 women) selected through a multi-stage stratified random sampling method during 2015/2016. A standardized, confidential data collection sheet was used. It included socio-demographic factors, dietary behaviors, anthropometric measurements and energy-macro and micronutrient intakes. *Results:* The median meal frequency of women and men were 4 and 3, respectively. Approximately 57% of men and 61% of women have skipped meals and 76.8% of them were skipped their lunch. In addition, the individuals whose BMI were under and over 25 kg/m$^2$ (72.4%, 78.3%, respectively) often skipped lunch. The meal frequency positively correlated with waist to hip ratio in women ($p<0.05$). Additionally, there were positively significant correlations between meal frequency and saturated fatty acids, fiber, vitamin A, vitamin C, calcium and iron intake ($p<0.05$). *Conclusion:* This study indicated that increased meal frequency may have a beneficial effect on micronutrients intakes and some anthropometric measurements among adults.

**Keywords:** meal frequency, meal skipping, obesity

**Authors' Contributions**

SM, MÖ and MS: conceived, did data collection and statistical analysis and designed manuscript.
MAO did statistical analysis.
EK, SB, EY, BK, PT and AE did data collection.
EA, MT and GK did editing and finally approved manuscript.

## Introduction

Obesity is one of the most common multifactorial (genetic, metabolic, environmental, socioeconomics and behavioural factors) and epidemic disorders that imposes direct medical and indirect economic costs on society (1).Obesity is thought to be an imbalance between energy consumed and expended (2).For this reason, changes in dietary habits and physical activity have been implicated as potential causes of obesity. Therefore, effective strategies must be developed for the prevention of obesity (3). One of these strategies could be the meal frequency or meal skipping rates.

Several characteristics of dietary behavior such as eating frequency, temporal distribution of the meals throughout the day, skipping meal particularly breakfast and the frequency of having meals outside ('eating out') may influence body weight (4,5).However, studies about the influence of these factors on obesity is not conclusive: whereas some studies reported no association between frequency of meals and obesity, others reported an inverse association between meal frequency and the prevalence of obesity in adults and children (6-10).

Additionally, obese individuals are often considered to skip breakfast, consume little or no lunch and compensate by overeating during late afternoon and night (11,12).The present study aims to evaluate the associations between meal frequency or meal skipping and obesity levels among adults.

## Methods

*Study population*

This study was carried out in 2015 with 1829 volunteer adults, 520 men and 1309 women, aged between 40 and 64 years who applied a family health center with any reason in Ankara, Turkey. Inclusion criterias were age between 40 and 64 years, diagnosis of any chronic disease and ability to complete a questionnaire. The participants declare their consent through a written form. This study was approved by Baskent University Institutional Review Board (Project no: 94603339/18.050.01.08.01-699).

*Data collection tools*

A detailed questionnaire on socio-demographic factors and dietary behaviors including meal frequency and food frequency was used to data collection.

*Anthropometric measurements*

Weight and height were measured to the nearest 0.1 kg and 0.1 cm, respectively, with the patient wearing lightweight clothing and no shoes. Body Mass Index (BMI) was calculated as the individual's weight in kilograms divided by the square of their height in meters. Waist circumference (WC) was measured (to the nearest 0.1 cm) using a non-elastic tape at the umbilicus at the end of a normal expiration, with the participant in a standing posture. Hip circumference (HC) was measured at the most protruding part of the hips at the level of the greater trochanter of the femur. Waist-to-hip ratio (WHR) was calculated by dividing WC (cm) by HC (cm) (13,14). The participants' nutritional status was evaluated through the body mass index (BMI), waist and hip circumference -for-age and gender. BMI values under 25 kg/m$^2$ was accepted as normal and greater than or equal to 25 kg/m$^2$ was accepted as overweight and obese. As a risky WHR was defined as more than to 0.85 in women and as more than to 1.0 in men; waist circumference was defined as more than 88 cm in women and as more than 102 cm in men and waist to height ratio was defined as more than to 0.5 (13,14). In order to determine the nutritional status of individuals, Food Frequency Questionnaire (FFQ) including 70 items was used (15). Energy, macro and micro nutrients obtained by food frequency questionnaire, were analyzed using the Nutrient Data Base Program (16).

*Data analysis*

All continuous variables were presented as mean ± standard deviation. Frequencies and percentages were used for the presentation of categorical qualitative variables. Pearson Chi-Square ($\chi^2$) test was used in order to evaluate categorical variables. The normality of the distribution of data was evaluated by the one sample Kolmogorov–Smirnov test. A non-parametric Kruskal-Wallis tests were performed upon the data to establish if any differences were present between conditions. The linear correlations between variables were investigated with Pearson bivariate correlation for normal distributed data and Spearman bivariate correlation for non-normal distribution data. For the calculation of correlations between meal frequency and anthropometric measurements of the individuals participating in the study, "Partial Correlation Coefficient" was used to adjust the effects of energy, carbohydrate, protein, fat and fiber on these variables. Significant values of $p<0.05$ were considered to be statistically significant. The study data was analyzed using SPSS software (version 17.0, SPSS Inc., Chicago, IL).

## Results

The study included 1829 participants (28.4% men,71.6% women). The mean age was 53.0±7.46 years. Among participants, 24.9% of them had a BMI value of <25 kg/m$^2$, 75.1% of them had a BMI value of ≥25 kg / m$^2$ (The mean BMI value was 28.6±5.07 kg/m$^2$). It was determined that 94.0% of individuals were obese according to waist circumferences (>102 cm for men, >88 cm for women, respectively) and 95.5% of men and 89.5% of women had WHR above >0.5. Most of the participants consumed 3 or more meals (87.3% of men and 88.5% of women). The most skipped meal to both gender 71.7% of men and 78.7% of women was lunch. In underweight and normal BMI group, 88.7% of them had three or more meals per day. Similarly overweight and obese BMI groups, the frequency of consuming three or more meals per day was 88.1% ($p>0.05$).The study results showed that the individu-

als whose BMI value were under and over 25 kg/m$^2$ (72.4%, 78.3%, respectively) often skipped lunch.

The relationship between age and anthropometric measurements of the individuals and the frequency of meal was given in Table 1. According to Table 1, there was a linear correlation between meal frequency and WHR in women (r=0.055; p=0.049). In addition, there was a inverse correlation between meal frequency and weight and HC for men (p>0.05) (Table 1).

Statistically significant Spearman correlation coefficients between meal frequency and energy, macro and micro nutrients consumption are shown in Table 2. The strongest correlation was between meal frequency and saturated fatty acids (r=0.090,p=0.001), fiber (r=0.105,p=0.000), vitamin A (r=0.119, p=0.000), vitamin C (r=0.093, p=0.001), calcium (r=0.151, p=0.000) and iron (r=0.077,p=0.006) in women. In addition, there was statistically significant correlation between

**Table 1.** The relationship between age and anthropometric measurements of the individuals and the frequency of meals♯

| | Meal Frequency | | | |
|---|---|---|---|---|
| | Men (n:520) | | Women (n:1329) | |
| Age and Anthropometric Measurements | r | p | r | p |
| Age (years) | 0.047 | 0.305 | 0.050 | 0.072 |
| Weight (kg) | -0.016 | 0.720 | 0.020 | 0.482 |
| BMI (kg/m$^2$) | 0.049 | 0.284 | 0.019 | 0.500 |
| Waist circumference (cm) | 0.020 | 0.664 | 0.049 | 0.082 |
| Hip circumference (cm) | -0.007 | 0.876 | 0.022 | 0.440 |
| Waist to hip ratio | 0.032 | 0.481 | 0.055 | 0.049* |
| Waist to height ratio | 0.036 | 0.429 | 0.050 | 0.075 |

*p<0.05
♯ Adjusted for energy, carbohydrate, protein, fat and fiber.

**Table 2.** Correlations between meal frequency of the individuals and energy, macro and micro nutrients consumption

| | Meal Frequency | | | | | |
|---|---|---|---|---|---|---|
| | Men (n:520) | | Women(n:1329) | | Total (n:1829) | |
| Energy and Nutrients | r | p | r | p | r | p |
| Energy (kcal/day) | -0.015 | 0.733 | 0.062 | 0.026* | 0.016 | 0.490 |
| Carbohydrate (TE %) | 0.023 | 0.606 | -0.023 | 0.403 | -0.017 | 0.482 |
| Protein (TE %) | 0.011 | 0.807 | 0.034 | 0.217 | 0.018 | 0.443 |
| Total fat (TE %) | -0.023 | 0.610 | 0.007 | 0.804 | 0.010 | 0.672 |
| Saturated fatty acids (%) | 0.036 | 0.429 | 0.090 | 0.001* | 0.063 | 0.007* |
| Mono unsaturated fatty acids (%) | -0.010 | 0.825 | 0.070 | 0.012* | 0.036 | 0.126 |
| Poli unsaturated fatty acids (%) | -0.004 | 0.935 | 0.017 | 0.550 | 0.004 | 0.872 |
| Fiber (g) | 0.096 | 0.034* | 0.105 | 0.000* | 0.089 | 0.000* |
| Vitamin A (µg/RE) | 0.109 | 0.016* | 0.119 | 0.000* | 0.116 | 0.000* |
| Vitamin E (mg) | -0.015 | 0.740 | 0.002 | 0.940 | -0.010 | 0.683 |
| Folate (mcg) | 0.084 | 0.062 | 0.092 | 0.001* | 0.065 | 0.006* |
| Vitamin $B_{12}$ (mcg) | -0.025 | 0.586 | 0.086 | 0.002* | 0.043 | 0.067 |
| Vitamin C (mg) | 0.076 | 0.090 | 0.093 | 0.001* | 0.084 | 0.000* |
| Calcium (mg) | 0.062 | 0.167 | 0.151 | 0.000* | 0.116 | 0.000* |
| Iron (mg) | 0.062 | 0.168 | 0.077 | 0.006* | 0.056 | 0.018* |

*p<0.05

meal frequency and fiber (r=0.096, p=0.034), vitamin A (r=0.109, p=0.016) in men.

Table 3 was shown that the distribution of meal skipping according to consumption of energy, macro and micro nutrient values of the individuals. Meal skippers had significantly higher intakes for carbohydrate and vitamin E than non-meal skippers (p<0.05). On the other hand, meal skippers had significantly lower intakes for protein, vitamin $B_{12}$ and calcium than non-meal skippers (p<0.05).

## Discussion

Recent studies about obesity were focused on meal frequency and meal skipping. Generally, the main approaches to defining meals are: participant-identified, time-of-day, food-based classification and neutral. These definitions, along with examples from the literature and their respective advantages and disadvantages, are discussed (11,17,18). In this study, the number of meals was directly asked participant. So, our study primarily investigated meal frequency of individuals which showed 87.3% of men and 88.5% of women consumed three or more meals in a day.

Some studies have suggested that eating patterns, which describe eating frequency, the temporal distribution of eating events across the day, breakfast skipping, and the frequency of eating meals away from home, may be related to some anthropometric measurements that are predictors of obesity (3). Especially, some studies conclude that BMI or WC decreased with the increasing meal frequency (17,18) but some studies argue that increased energy intake depending on increased meal frequency associated with higher BMI or WC (19,20). In this study, we found that meal frequency was not significantly associated with change in body mass index and also it was significantly associated with WHR in women. Thus, in a meta-analysis study on this subject, it was determined that there was no difference between meal frequency and anthropometric measurements (21).

Cross-sectional studies have shown that meal skipping is associated with increased prevalence of overweight and obesity (5,22). For example, in a study have shown that WC and BMI values were significantly higher in people who skipped breakfast when compared with people who did not skip breakfast (22). In this study, we did not found statistically significant difference between meal skipping and BMI. Also

**Table 3.** The distribution of meal skipping according to consumption of energy, macro and micro nutrient values of the individuals

| | **Meal skippers (n:1090)** | **Non-meal skippers (n:724)** | |
|---|---|---|---|
| | **X̄±SD** | **X̄±SD** | **p** |
| Energy (kcal/day) | 1962.0±593.12 | 1926.3±585.02 | 0.131 |
| Carbohydrate (TE %) | 41.7±9.03 | 40.3±8.75 | 0.004* |
| Protein (TE %) | 16.1±3.36 | 16.9±3.73 | 0.000* |
| Total fat (TE %) | 42.1±8.36 | 42.6±8.15 | 0.370 |
| Saturated fatty acids (%) | 33.0±12.98 | 35.8±13.75 | 0.649 |
| Mono unsaturated fatty acids (%) | 35.6±14.20 | 35.8±13.75 | 0.615 |
| Poli unsaturated fatty acids (%) | 17.6±10.13 | 17.0±9.89 | 0.125 |
| Fiber (g) | 29.6±12.18 | 29.6±11.84 | 0.714 |
| Vitamin A (µg/RE) | 1034.9±1032.58 | 1359.8±1002.20 | 0.179 |
| Vitamin E (mg) | 15.8±9.95 | 15.1±9.61 | 0.038* |
| Folate (mcg) | 304.5±107.03 | 303.2±104.00 | 0.620 |
| Vitamin $B_{12}$ (mcg) | 4.7±4.07 | 4.8±9.44 | 0.024* |
| Vitamin C (mg) | 98.1±53.89 | 100.8±51.42 | 0.072 |
| Calcium (mg) | 905.6±352.49 | 930.6±314.47 | 0.009* |
| Iron (mg) | 12.7±4.88 | 12.8±4.56 | 0.456 |

*p<0.05

in our study, 59.8% of individuals reported that they skipped meals. The most skipped meal of the day was lunch for both men (71.7%) and women (78.7%). Also, 59.5% of overweight individuals skipped their meal.

Meal frequency and meal skipping also appears to be an important contributor to intakes of energy, macro and micronutrients among adults. For example two large population-based studies, one in US adults and the other in Swedish adults found that those who ate six or more times per day had higher intakes of carbohydrate and fibre but lower intakes of fat and protein compared with adults who ate once or twice per day or less than three times per day, respectively. Additionally, in these studies, a higher meal frequency was also associated with higher folate, vitamin C, iron and calcium (23,24). Among Puerto Rican adults, it was found that there was a linear relationship between total energy intake and meal frequency (25). In contrast among adults, it was found that the energy was negatively associated with meal frequency (especially snacking) (26). Studies based on meal skipping were examined. Some of them were identified the influence of breakfast skipping on nutrient intakes and some of them were identified the nutritional impact of omitting the lunch or dinner meal. These results suggest that meal skipping was consistently associated with lower micronutrient intakes especially calcium, vitamin C, folate, vitamin A, magnesium (27). While all these results were evaluated, the evidence to support associations between meal frequency and nutrient intake was less consistent.

In our study, we found that higher meal frequency had significantly correlated higher saturated fatty acids, fiber, vitamin A, vitamin C, calcium and iron. Also, we examined the distribution of meal skipping according to consumption of energy, macro and micro nutrient values of the individuals. So, we found that meal skippers had significantly higher intakes for percentage of carbohydrate and vitamin E than non-meal skippers. On the other hand, meal skippers had significantly lower intakes for protein, vitamin $B_{12}$ and calcium than non-meal skippers.

*Limitations of the study*

Firstly, our study population was conducted in age between 40-64 years, so the findings may not apply to other age groups. Secondly, the heterogeneity of meal definitions were a major impediment to the interpretation of findings in this study.

## Conclusion

The results from our study suggest that meal frequency were associated with obesity based on waist to hip ratio. Additonally we found that meal frequency and meal skipping significiantly correlated with many macro and micronutrients intake. Further research should be conducted to examine the dietary profiles of eating patterns and their relations with obesity to encourage healthy eating and meal-based guidelines should be improved.

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Original Article

# The relation between eating behaviours and obsession among university students

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**Summary.** *Aim:* To investigate the relation between obsession and eating behaviours among university students including some factors that affected this relation. *Methods:* This cross-sectional study was conducted with 1754 university students. The study sample was selected using the stratified sampling method. Basic demographic data were collected. Eating attitudes were determined using the Eating Attitude Test (EAT)-40, and obsessive-compulsive behaviours were determined using the Maudsley Obsessive Compulsive Inventory (MOCI). Anthropometric measurements were also examined. *Results:* Females (16.2%) were observed to have higher risks of eating disorder than males (11.4%) ($p<0.05$). Eating disorder risk was also higher among students in the educational sciences (20.4%), health sciences (14.4%), applied sciences (11.8%) and social sciences (10.4%) ($p<0.05$). A positive relation was found between eating behaviour and obsession ($r=0.337$, $p<0.001$). *Conclusion:* The study found a relationship between eating behaviour and obsession. It also concluded that gender and education were the factors that affected eating behaviour and obsession.

**Key words:** eating behaviours, eating disorders, obsession, obsessive-compulsive disorder, university students

## Introduction

Eating disorders are defined by the 10th revision of the International Classification of Disorders (ICD)-10 as "behavioural syndromes associated with physiological disturbances and physical factors" (1). Research has shown that eating disorders are related with distress, functional impairment, depression, suicide attempts, anxiety disorders, substance abuse, increased obesity risk and morbidity (2). According to the Diagnostic and Statistical Manual of Mental Disorders (DSM)-V, eating disorders are classified as Anorexia Nervosa (AN), Bulimia Nervosa (BN) and Binge Eating Disorder (BED). AN is characterized by the maintenance of a body weight below a minimum level that is considered to be normal for the relevant age, gender, developmental state and physical health; the practice of sustained energy intake restrictions; extreme fear of weight/fat gain; and the feeling of distress about self-perceived weight/shape. BN is described as recurrent episodes of binge eating, recurrent improper compensatory behaviours to prevent weight gain and excessive preoccupation with body image (shape/weight). Binge eating involves the consumption of larger amounts of food within a short period of time than most people consume within the same time period. A diagnosis of BED is based on the recurrence of binge eating episodes at least once a week for 3 months (3). Eating disorders are observed in 5-10% of young people (4). Approximately 90% of eating disorder cases are observed

among people under the age of 25 (5), the peak age for AN is between 15-18 (6). The lifetime prevalence of AN and BN among females can be 4% and 2% at the most, respectively (7). The risk of an eating disorder can be associated with familial, biological, social and cultural factors. Additionally, other psychiatric disorders, such as depression, anxiety, and obsessive-compulsive disorder (OCD), also increase the risk of an eating disorder (4). A cross-sectional study conducted with 1960 females showed that frequent use of Facebook further increased the risk of eating disorders, and state anxiety compared to other online activities (8).

ICD-10 defines OCD as "recurrent obsessional thoughts or compulsive acts". This definition is retained in ICD-11. OCD is equally common among males and females. It usually emerges in childhood or early adulthood (9). The prevalence of OCD among children and adolescents is 1-3%. Body dysmorphic disorder, AN, BN and binge eating are considered to be OCD spectrum disorders (10). Thus, OCD and AN have similar cognitive, behavioural and personality pathologies (11). This relationship can be better explained through phenomenological, neurobiological and family studies. Personality disorders, mood disorders, anxiety and OCD are comorbidities of eating disorders while obsession and perfectionism are predisposing factors for OCD (12, 13). AN- and OCD-related comorbidities are frequently observed (14). Clinical studies have shown that the prevalence of OCD is high among individuals with AN, and vice versa. OCD is the most common anxiety disorder of AN and BN. Almost 40% of patients with BN and 10-60% of patients with AN have OCD excluding food obsession and compulsions (15). Stress and anxiety levels tend to increase during university life, therefore eating problems can emerge. This is particularly the case for students who have competitive or perfectionist characteristics (16). This study aimed to determine the relation between obsession and eating behaviours among university students.

## Material and Methods

*Participants*

The study was conducted between February and April, 2013 with the voluntary participation of 1754 students. Of them, 760 were females (mean age: 21.3±2.3 years) and 994 were males (mean age: 22.2±2.6 years). The students were between the ages of 17 and 33 and studied at Near East University in Nicosia, North Cyprus. The sample was determined using the stratified sampling method which was based on the selection of students from different academic departments categorized into four main education fields.

*Data collection*

The study data were collected through a questionnaire and face-to-face interviews. Basic demographic data (e.g. age, gender, education field), eating attitudes, obsessive compulsive behaviours and anthropometric measurements were examined.

*Eating Attitude Test (EAT)-40*

EAT-40 was developed by Garner and Garfinkel to assess eating disorder risk. The test consists of 40 items, arranged on a 6-point Likert scale, with responses ranging from "always" to "never". The test scores range between 0 and 120 points. Test scores of 30 and above indicate high risk (abnormal eating behaviour) while those of below 30 indicate low risk (17). The Turkish validity and reliability of the test was tested by Savaşır and Erol in Turkey (18).

*Maudsley Obsessive Compulsive Inventory (MOCI)*

This inventory was designed by Hodgson and Rachman to identify obsessive compulsive indications for both healthy and psychiatric patients. The inventory includes four sub-scales: checking, washing, doubt and slowness (19). Erol and Savaşır adapted the inventory into Turkish. A fifth subscale 'rumination' was added into the adapted version. After applying a factor analysis, three instead of four factors were identified: washing, obsessive thinking, and slowness and control. The inventory includes 37 items and the scores range between 0-37 points. Higher scores indicate increased number of obsessive-compulsive indications (20).

*Anthropometric measurements*

Weight (kg) and height (cm) were obtained using standard techniques and equipment. Weight was measured using a basic digital scale, sensitive to 0.1 g, while height was measured without shoes, to the nearest 0.1

cm, using measuring tape. Body mass index (BMI) was calculated dividing weight in kilogram by height in square meter (kg/m$^2$). The BMI cut-off points were divided into following categories: underweight (<18.5 kg/m$^2$), normal weight (18.5-24.9 kg/m$^2$), overweight (25.0-29.0 kg/m$^2$) and obese (≥30.0 kg/m$^2$) (21).

*Statistical data analysis*

The study data were statistically analysed using the Statistical Packages for Social Sciences (SPSS) for Windows, version 18.0. Descriptive statistics were given as mean (χ), standard deviation (SD), median (med) and interquartile range (IR) for continuous variables; and as numerals and percentages for categorical variables. The normality of data distribution was determined using the Kolmogorov-Smirnov test. This test showed that a nonparametric test should be used for analysis. To compare BMI, EAT-40, and MOCI scores according to the field of education and EAT-40 and MOCI scores according to BMI classification, Kruskal-Wallis variance analysis was used. To further compare the binary difference of these variables, Bonferroni correction of the Mann Whitney U test was used. In addition, the Mann Whitney U test was used for binary comparison of eating disorder risk groups according to MOCI and BMI classification. The differences between gender groups' BMI, EAT-40 and MOCI mean values were investigated using the Z-test. Eating disorder risk for different education fields and BMI classification were compared using the Chi-square test. Finally, the correlation between EAT-40 and MOCI scores were determined using Spearman's correlation analysis, and a scatter plot was drawn to better illustrate the correlation. A statistical significance level of p<0.05 was used for all analyses.

## Results

BMI, eating attitudes and obsessive behaviours of students according to gender and education fields are shown in Table 1. The mean BMI values of males and females were found to be 24.4±3.99 and 21.8±4.35 kg/m$^2$, respectively (p<0.001). Figure 1a shows that eating disorder risk percentages were found to be higher in females (16.2%) than in males (11.4%) (p<0.05).

**Table 1.** BMI, EAT-40 and MOCI scores according to sex and education fields

| | BMI (kg/m$^2$) | Z | p | EAT-40 | Z | p | MOCI | Z | p |
|---|---|---|---|---|---|---|---|---|---|
| **Sex** | **$\chi^2$±S [med (IR)]** | | | **$\chi^2$±S [med (IR)]** | | | **$\chi^2$±S [MED (IR)]** | | |
| Male (n=994) | 24.4±3.99 [24.1(4.1)] | -15.918 | <0.001 | 17.1±11.95 [14.0(12.0)] | 5.331 | <0.001 | 15.6±6.63 [16.0(9.0)] | 6.440 | <0.001 |
| Female (n=760) | 21.8±4.35 [21.2(4.1)] | | | 19.3±11.99 [16.0(13.0)] | | | 17.9±6.56 [18.0(9.0)] | | |
| **Education field** | | | **p** | | | **p** | | | **p** |
| Educational sciences (n=277) | 22.7±3.91 [22.4(5.0)] | | | 21.0±13.51 [17.0(13.0)] | | | 18.6±6.76 [19.0(10.0)] | | |
| Social sciences (n=557) | 23.5±3.76$^{a,b}$[23.4(4.9)] | 25.057 | <0.001 | 16.7±11.66$^b$[13.0(12.0)] | 28.574 | <0.001 | 16.0±6.52$^b$[16.0(10.0)] | 31.336 | <0.001 |
| Applied sciences (n=391) | 23.7±5.07$^{a,b}$[23.3(4.8)] | | | 17.7±10.79$^b$[15.0(12.0)] | | | 16.3±6.82$^b$[16.0(10.0)] | | |
| Health sciences (n=529) | 22.8±4.47 [22.4(4.7)] | | | 18.1±12.6$^b$[15.0(13.0)] | | | 16.3±6.55$^b$[17.0(9.0)] | | |
| Total (n=1754) | 23.2±4.34 [22.9(4.9)] | | | 18.0±12.01 [15.0(12.0)] | | | 16.6±6.69 [17.0(9.0)] | | |

*$^{a,b}$ p<0.05 (Bonferroni correction Mann Whitney U test); $^a$: refers to statistically significant differences between health sciences ; $^b$: refers to statistically significant differences between educational science; med: Median; IR: Interquartile range; BMI: Body mass index; EAT-40: Eating Attitudes Test-40; MOCI: Maudsley Obsessive Compulsive Inventory*

The BMI mean values of the students in the fields of health sciences and educational sciences were lower than those in the fields of social sciences and applied sciences ($p<0.05$) (Table 1). Higher EAT-40 scores indicate increased abnormal eating behaviours, which also refers to an increased risk of an eating disorder. Moreover, higher MOCI scores indicate increased obsessive-compulsive behaviour or increased risk of obsessive-compulsive disorder. Both EAT-40 and MOCI mean scores of the females were higher than those of the males ($p<0.001$). In addition, the mean EAT-40 and MOCI scores of the students in the educational sciences were higher than those in other education fields ($p<0.001$) (Table 1). The increased eating disorder risk percentages of students in the educational sciences, health sciences, applied sciences and social sciences were 20.4%, 14.4%, 11.8% and 10.4%, respectively ($p<0.05$). The educational sciences, followed by the health sciences, had the highest percentages of students with increased abnormal eating behaviours (Figure 1b).

Underweight students had the lowest mean scores on the EAT-40 (17.8±9.72), while obese students had the highest mean scores (18.9±11.71). However, no statistically significant difference was observed between BMI groups ($p>0.05$) (Table 2). The percentage of students with obesity who were at an increased risk of an eating disorder was 16.2%, while the percentage was 11.2% for underweight students ($p>0.05$) (Figure 1c). Although EAT-40 scores were found to not be statistically different between BMI groups, overweight students had the lowest mean scores on the MOCI (15.8±6.66). These scores were found to be statistically different from the MOCI scores obtained by underweight, normal weight and obese groups ($p<0.05$) (Table 2).

The total MOCI score and its subscale scores were determined to be higher among the group with increased abnormal eating behaviours compared to the group with lower abnormal eating behaviours ($p<0.001$) (Table 3). A positive relation was observed between EAT-40 score and total MOCI score ($r=0.337$, $p<0.001$) (Figure 2). A positive relation was also observed between EAT-40 score and MOCI subscale scores ($p<0.001$) (Table 4).

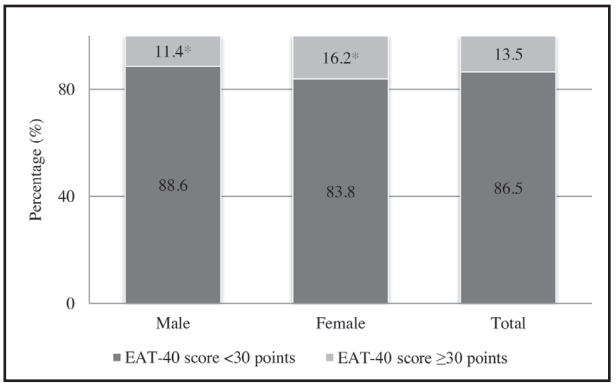


**Figure 1a.** Eating disorder risk percentages according to sex *p<0.05. EAT-40: Eating Attitudes Test-40

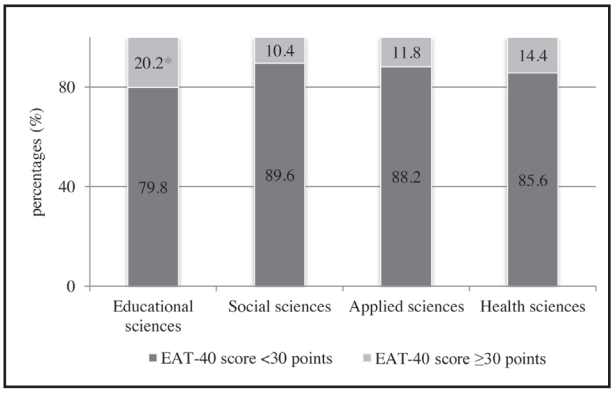


**Figure 1b.** Eating disorder risk percentages according to education fields. *p<0.05, refers to statistically significant difference compare to other education fields. EAT-40: Eating Attitudes Test-40

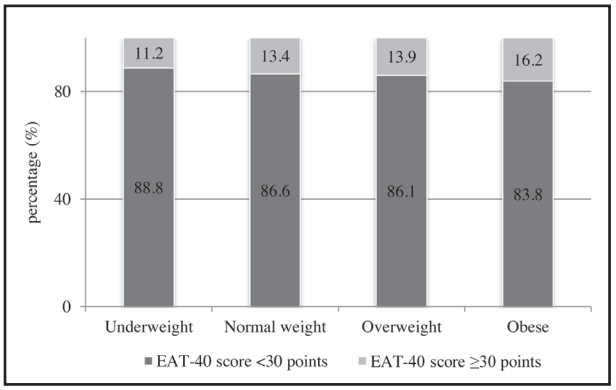


**Figure 1c.** Eating disorder risk percentages according to BMI classification. EAT-40: Eating Attitudes Test-40
BMI: Body Mass Index

**Table 2.** EAT-40 and MOCI scores according to BMI classification

| | EAT-40 $\chi^2$±S [med (IR)] | $\chi^2$ | p | MOCI $\chi^2$±S [med (IR)] | $\chi^2$ | p |
|---|---|---|---|---|---|---|
| Underweight | 17.8±9.72 [16.0(10.2)] | 2.377 | 0.498 | 17.5±6.89* [18.0(9.2)] | 8.642 | 0.034 |
| Normal | 18.0±12.25 [15.0(12.0)] | | | 16.7±6.64* [17.0(9.0)] | | |
| Overweight | 18.0±12.10 [15.0(13.0)] | | | 15.8±6.66 [16.0(10.0)] | | |
| Obese | 18.9±11.71 [15.5(11.0)] | | | 17.5±6.87* [18.0(9.0)] | | |
| Total | 18.0±12.01 [15.0(12.0)] | | | 16.6±6.69 [17.0(9.0)] | | |

** MOCI scores of overweight students differ among other BMI classification groups (p < 0.05, Bonferroni correction Mann Whitney U test); med: Median; IR: Interquartile range; EAT-40: Eating Attitudes Test–40; MOCI: Maudsley Obsessive Compulsive Inventory*

**Table 3.** MOCI scores according to eating disorder risk percentages

| | EAT-40 <30 point $\chi^2$±S [med (IR)] | EAT-40 ≥30 points $\chi^2$±S [med (IR)] | $\chi^2$ | p |
|---|---|---|---|---|
| **MOCI Total score** | 16.0±6.64 [16.0(10.0)] | 20.4±5.66 [20.0(7.0)] | -9.557 | <0.001 |
| **MOCI Subscales scores** | | | | |
| MOCI Rumination | 3.5±2.40 [3.0(3.0)] | 4.9±2.07 [5.0(2.0)] | -8.344 | <0.001 |
| MOCI Doubt | 3.7±1.42 [4.0(2.0)] | 4.1±1.45 [4.0(2.0)] | -4.268 | <0.001 |
| MOCI Slowness | 2.4±1.65 [2.0(3.0)] | 3.4±1.69 [3.0(3.0)] | -8.015 | <0.001 |
| MOCI Washing | 4.9±2.32 [5.0(4.0)] | 6.0±2.27 [6.0(3.0)] | -6.873 | <0.001 |
| MOCI Checking | 3.1±2.25 [3.0(4.0)] | 4.5±2.13 [4.0(3.0)] | -8.788 | <0.001 |

*med: Median; IR: Interquartile range; EAT-40: Eating Attitudes Test–40; MOCI: Maudsley Obsessive Compulsive Inventory*

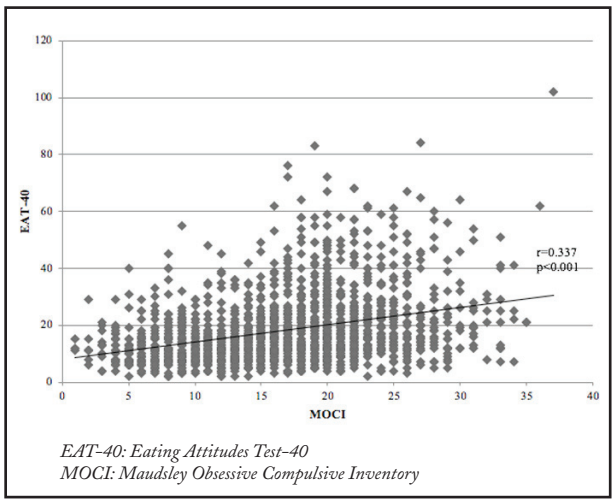


**Figure 2.** Correlation among EAT-40 and MOCI scores. EAT-40: Eating Attitudes Test-40. MOCI: Maudsley Obsessive Compulsive Inventory

**Table 4.** EAT-40 and MOCI scores correlation

| | EAT-40 score | |
|---|---|---|
| | r | p |
| **MOCI Total score** | 0.337 | 0.000* |
| MOCI Subscales' scores | | |
| MOCI Rumination | 0.269 | 0.000* |
| MOCI Doubt | 0.201 | 0.000* |
| MOCI Slowness | 0.3 | 0.000* |
| MOCI Washing | 0.25 | 0.000* |
| MOCI Checking | 0.298 | 0.000* |

*EAT-40: Eating Attitudes Test–40*
*MOCI: Maudsley Obsessive Compulsive Inventory*

## Discussion

Eating disorders are more common among young people and females than in males (5). The reasons for eating disorders are complex and not well understood. Nonetheless, genetic predisposition and social, familial, psychological and biological factors were revealed to influence the risk of eating disorders (7, 22). In the present study, the EAT-40 mean scores of the females (19.3±11.99) were higher than those of the males (17.1±11.95) (p<0.001). These results were similar to those of a pilot study conducted in 2011 with 152 students at the same university. In this pilot study, the EAT-40 mean scores of the females (19.1±12.34) were found to be higher than those of the males (17.0±10.07) but the difference was not statistically significant (p>0.05)

(23). In another study, EAT-40 mean score was found to be 18.67±11.30 for female students, and 16.79±10.73 for male students ($p<0.05$) (24).

The present study found that 13.5% of the students were at higher risk, and the females (16.2%) were observed to be at higher risk than the males (11.4%). Similarly, three different studies conducted with university students in Turkey reported an increased risk of eating disorders at the rates of 13.3%, 13.8%, and 14.0%, respectively, among the students (25-27). In France, the prevalence of eating disorders among university students is also high (20.5%) and is associated with stress, depression and other behaviour risks (28). During university education, challenging factors regarding courses, increased responsibilities and higher self-demand can potentially lead to the development of stress and depression among students.

Moreover, the social emphasis on body image and body shape affects the body weight perception of young people. In Western culture, the image of ideal beauty is associated with being thin, which results in the engagement in different weight loss activities and an increased risk of eating disorders (29). The spread of this image of ideal beauty through Western advertising can influence the conception of Eastern body ideals (30, 31). A study comparing the risk of eating disorders among college students in the United States and the Philippines showed that Filipino students had a 10.9 times higher risk of eating disorders compared to American students (32). In a different study, female university students in the United States demonstrated more body dissatisfaction than Bosnian female university students (33). Another study reported that university students in Japan, compared to those in Korea, had a more pronounced desire to have lower body weight than their actual weight (34). These results indicate that cultural beliefs and attitudes influence the development of eating disorders. Moreover, some ethnic groups or regions have higher prevalence of eating disorders (35, 36).

Both EAT-40 and MOCI mean scores of the students in the educational sciences were higher than those of the students in other fields. The percentages of students with an increased eating disorder in the educational sciences, health sciences, applied sciences and social sciences were 20.4%, 14.4%, 11.8% and 10.4%, respectively ($p<0.05$). A study conducted in Turkey reported that the mean EAT-26 scores of students in the social sciences (16.34±7.43) and health sciences (17.64±8.02) were similar and below the risk cut off point ($p<0.05$) (37). Another study from Turkey found that 8.4% of fine arts students had an increased risk of eating disorders. In a study from Pakistan, 23.3% of business students were reported to have the same risk (38, 39). A study on eating behaviours of students in different study fields, conducted in Turkey again, considered the eating behaviour risk to be higher among students in physical education and sports (10.7%) compared to nutrition and dietetics (2.9%) and social sciences (0.4%) ($p<0.05$) (40). Studies on eating disorder risk with respect to education field, conducted in various countries, have largely focused on students in health sciences, particularly medical and nursing departments. The percentages of eating disorder risk among health sciences students in various countries were reported to be 22.7% in Pakistan, 15.3% in Turkey, 10% in Brazil, and 7.8% in India (38, 41-43). These data suggest a relationship between education field and eating behaviour implying that eating behaviour within the same education field varies by country. Therefore, eating behaviour can be associated with cultural and ethnic differences.

The difference between genders regarding the risk of eating disorders is also an important issue. In the present study, the mean BMI values of male students were higher than those of the females ($p<0.001$), and the percentages associated with the risk of eating disorders were higher in the females (16.2%) than in the males (11.4%). Other studies also suggested a relationship between the risk of eating disorder and gender, and revealed different results. For example, some indicated that the risk of eating disorders was higher among female students than among males, while others suggested the opposite. However, no statistical significance was found in the latter case (25, 27). Other studies conducted with university students in Turkey showed that the eating disorder risk of female students was higher than that of male students ($p<0.05$) (44; 45; 46). Eating disorders are highly prevalent, and female students are at higher risks of eating disorders than males in China (9.9% females, 2.0% males), Sarawak, Malaysia (13.7% females, 5.6% males), Spain (20.85% females, 14.9% males) and Bangladesh (40.2% females, 34.3% males) (47-49). Furthermore, a study conducted in Ja-

pan reported that 5-10% of female students had eating disorder symptoms, and the increasing prevalence of eating disorders among female students was thought to be associated with body perception and the fear of weight gain (50, 51). The difference between genders regarding the risk of eating disorders can be attributed to the fact that females place greater importance to body image and aesthetics than males (52). The risk of eating disorders is associated with self-esteem, family history, child abuse and neglect (53).

Eating disorders among female university students are also associated with weight-related teasing (54). Various studies indicate that underweight and overweight individuals, and those with obesity have an increased risk of eating disorders. In this study, underweight students had the lowest mean EAT-40 score (17.8±9.72), while students with obesity had the highest mean EAT-40 score (18.9±11.71). However, the difference was not statistically significant between BMI groups ($p>0.05$). Furthermore, the percentage of students with obesity who had an increased risk of eating disorders was 16.2%, while the percentage was 11.2% for underweight students ($p>0.05$). In a pilot study conducted in 2011 with 152 students at the same university, 18.2% of underweight and 13.5% of overweight students had an increased risk of eating disorders. These results support the present study ($p<0.05$) (23). Another study reported that 17.2% of underweight and 21.2% of overweight university students had a higher risk of eating disorders (44). In a study conducted with university students, eating disorder risks of overweight students, and those with obesity were two times higher than those of normal weight students, and the risk of eating disorders among underweight students were 2.9 times lower than those of normal weight students (24). However, another study conducted with university students found no statistical difference between BMI groups with respect to eating disorders (26). In addition to body dissatisfaction and dieting, BMI is also considered to have an effect on the risk of eating disorders. Studies show that increased BMI indicate higher likelihood of body dissatisfaction and dieting (55, 56).

Obsessive-compulsive behaviours and BMI are considered to be main predictor variables of increased abnormal eating behaviour among female university students (57). Therefore, in addition to BMI, other psychological factors can affect eating behaviour. For example, depression, anxiety, anger, stress, and sadness are known to negatively affect eating behaviour. A study conducted with university students found that eating disorders and subjection to weight-related teasing resulted in psychological distress (58). Personality traits play a key role in the comorbidities associated with eating disorders and obsessive-compulsive disorder (59). Personality attributes, such as perfectionism and impulsivity, also have an impact on obsessive-compulsive disorders and eating behaviours. Although obsessive-compulsive disorders and eating disorders are in different categories, they are considered to have mutual predispositions (13). Eating disorders mainly include food- and weight-related obsessive compulsive dietary restrictions (11). In this study, total/subscale MOCI scores were higher among the students included in increased abnormal eating behaviour group compared to those in lower abnormal eating behaviour group ($p<0.001$). There was a positive relation between the EAT-40 score and total/subscale MOCI scores ($p <0.001$). A study conducted with nursing students found a positive correlation between EAT-40 score and total MOCI score, which supported present study (60). A study conducted with patients who had obsessive-compulsive disorder reported increased abnormal eating behaviours among them (61). Perfectionism and obsession are associated with increased eating disorder disease symptoms (15). Another study conducted in Turkey reported that the most common comorbidity of eating disorders was obsessive-compulsive personality disorders (62).

## Conclusions

This study indicated that female university students had higher tendency to abnormal eating behaviours and obsessions than males. Furthermore, students in the field of educational sciences, followed by students in the health sciences, had the highest risks of eating disorders. Students with higher levels of abnormal eating behaviour also had higher levels of obsession compared to students with lower levels of abnormal eating behaviour. In conclusion, a relationship was found between eating behaviour and obsession. Gender and education field were also considered to be the factors that affected eating be-

haviour and obsession. Although there are studies on the relationship between gender, and eating behaviours and obsession, further studies are needed on the reasons of the differences in the education fields.

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ORIGINAL ARTICLE

# Nutritional status and food intake are related to malnutrition risk and length of stay in hospitalized patients

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**Summary.** *Objective:* The aim was to evaluate the relationship between food intake and malnutrition risk of hospitalized patients. *Methods:* In this study 192 hospitalized patients were included. Food intake was performed on 24 h recall dietary method Nutritional Risk Screening-2002 has been used to evaluate the nutritional status of patients. Odds ratios with 95% confidence intervals were computed using a univariate and multivariate stepwise logistic regression model with malnutrition risk as the response variable. *Results:* The mean age of individuals was 50.3±16.35 years, 29.4% of males and 20.0% of females were at risk group. The patients with malign neoplasms had the highest malnutrition risk score. The overall coverage of the energy, protein, fibre, vitamin C, vitamin B12, calcium, iron and the other micronutrients of the malnutrition risk group were significantly lower than well-nourished patients ($p<0.05$). This difference was more remarkable among the patients who were younger than 65 years. Recent weight loss increased the malnutrition risk, 1.1 times in the last three and 6 months ($p=0.003$), in the last two months 1.7 times ($p=0.000$) and in the last one month was 1.5 times ($p=0.002$). *Conclusion:* The factors associated with malnutrition can be identified as food intake, recent weight loss, length of stay and anthropometric measurements. Patients who were malnourished by screening tool presented decreased food intake and had longer length of stay. A comprehensive nutritional evaluation that will allow adequate intervention and nutritional therapy is needed to avoid hospital malnutrition.

**Key words:** food intake, length of stay, malnutrition, NRS-2002, weight loss

**Abbreviations**

ASPEN: American Society of Clinical Nutrition and Metabolism, BMI: Body mass index, DRI: Dietary recommended intake, ESPEN: European Society of Clinical Nutrition and Metabolism, LOS: Length of stay in hospital, MDC: Main diagnostic categories, MUFA: Mono unsaturated fatty acids, NRS-2002: Nutritional Risk Screening-2002, PUFA: Poly unsaturated fatty acids, SFA: Saturated fatty acids, SPSS: Statistical Package for Social Sciences, WHO: World Health Organization

## Introduction

Besides the impressive increase in the prevalence of obesity and its associated diseases, malnutrition is a widespread and unrecognized problem in hospitalized patients (1, 2). Malnutrition prevalence is seen between 15-70% of hospitalized patients (3, 4). It was found that one-in-three malnourished patients and one-in-five well-nourished patients consumed nothing or up to 25% of the provided food (5). Many studies have suggested that in comparison to well-nourished patients, malnourished patients exposed to worse outcomes. Malnourished patients have worse treatment response and increased rates of outcomes such as prolonged length of stay in hospital (LOS), increased readmissions and mortality (6-9).

Hospital malnutrition can be caused by disease or treatments. Physiological changes resulting from the

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disease (e.g. fever, gastrointestinal symptoms), dietary modifications (e.g. protein or fat restricted diet) and clinical examinations (e.g. colonoscopy) may increase the nutritional requirements or reduce nutrient intakes of the individuals. Hospital malnutrition can also have attributed to other causes, such as inadequate meal service in hospital and inadequate quality and flexibility of hospital catering (10).

There are limited studies evaluating how the hospital malnutrition and LOS is affected by nutritional status and nutrient intake. The main purpose of this study was to evaluate the effect of food intake, anthropometric measurements, LOS and main diagnostic categories (MDC) on the malnutrition risk of hospitalized patients.

## Methods

A nonrandomized cross sectional design was used to compare malnutrition status and nutritional intake of hospitalized patients in Adult Hospital and Oncology Hospital of Hacettepe University. At least 150 participant planned to enrol the study according to the power analysis. This study was conducted among 192 adult (>18 years) volunteers between March-July 2014. Patients with cognitive impairment, oedema or dehydration, pregnant/lactating women and clinically unstable patients were excluded from the study. Ethical approval of this study has been granted by the regional ethics committee of the university (February 13, 2014; GO 14/67-02).

Data were collected by face to face interviews using a standard questionnaire. Food intake was performed on 24 h recall dietary method. BEBIS program (Pasifik Company, İstanbul, Turkey) were used to determine average daily energy and nutrient intake. Nutritional status of patients were evaluated by Nutritional Risk Screening-2002 (NRS-2002). NRS-2002 classifies patients' nutritional status based on body mass index (BMI), percentage of recent weight loss and recent change in food intake and severity of disease. Being nutritionally "at risk" was defined as a NRS-2002 score ≥3. This tool is recommended by the European Society of Clinical Nutrition and Metabolism (ESPEN) for hospital nutritional screening (11, 12). Dietary types were classified as regular diet, specific diet (diabetic, low salt, lipid and cholesterol diets etc.), restricted (protein, potassium or phosphorus restricted diets, test diets etc.) and enteral nutrition.

The body weights of individuals with minimal clothing without shoes were measured with a body analyser (Tanita HA622). Height was measured with a stable stadiometer. BMI was calculated for each individual. BMI was calculated as weight (kg)/height ($m^2$) and all participants were classified into four BMI categories according to the World Health Organization (WHO) 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 obesity (≥30.0 kg/$m^2$) (13). The mid arm circumferences were measured with a fiber-glass tape which was sensitive to 0.1 cm. All measurements were obtained as described above (14).

SPSS (Statistical Package for Social Sciences Inc., Chicago, IL, United States) for Windows 15.0 program was used to analyse the data. The results were presented as the mean and standard deviation ($\bar{\chi}$±S) values. The table of percentage points was given for qualitative data. Mann Whitney-U test was used to compare the differences between two groups. Pearson chi-square test was performed to evaluate the categorical variables. Odds ratios with 95% confidence intervals were computed using a univariate and multivariate stepwise logistic regression model with malnutrition risk as the response variable. For all statistical procedures, a-*P* value of less than 0.05 was considered significant.

## Results

A total of 192 hospitalized patients were enrolled in the study. Gender and nutritional risk specific distributions of BMI, LOS, weight loss and mid arm circumference are described in Table 1. The male to female ratio of our sample was 1.1 (102/90) while mean age and BMI were 50.3±16.35 years and 27.0±5.92 kg/$m^2$. According to malnutrition risk evaluated with NRS 2002; 29.4% of males and 20.0% of females were at risk group. Also one in every four screened patients (25%) had risk of having malnutrition.

Malnutrition risk by age, MDC, appetite status and dietary type were shown in Figure 1. The age de-

**Table 1.** Malnutrition risk by anthropometric measurements and length of stay (LOS) by main diagnostic categories

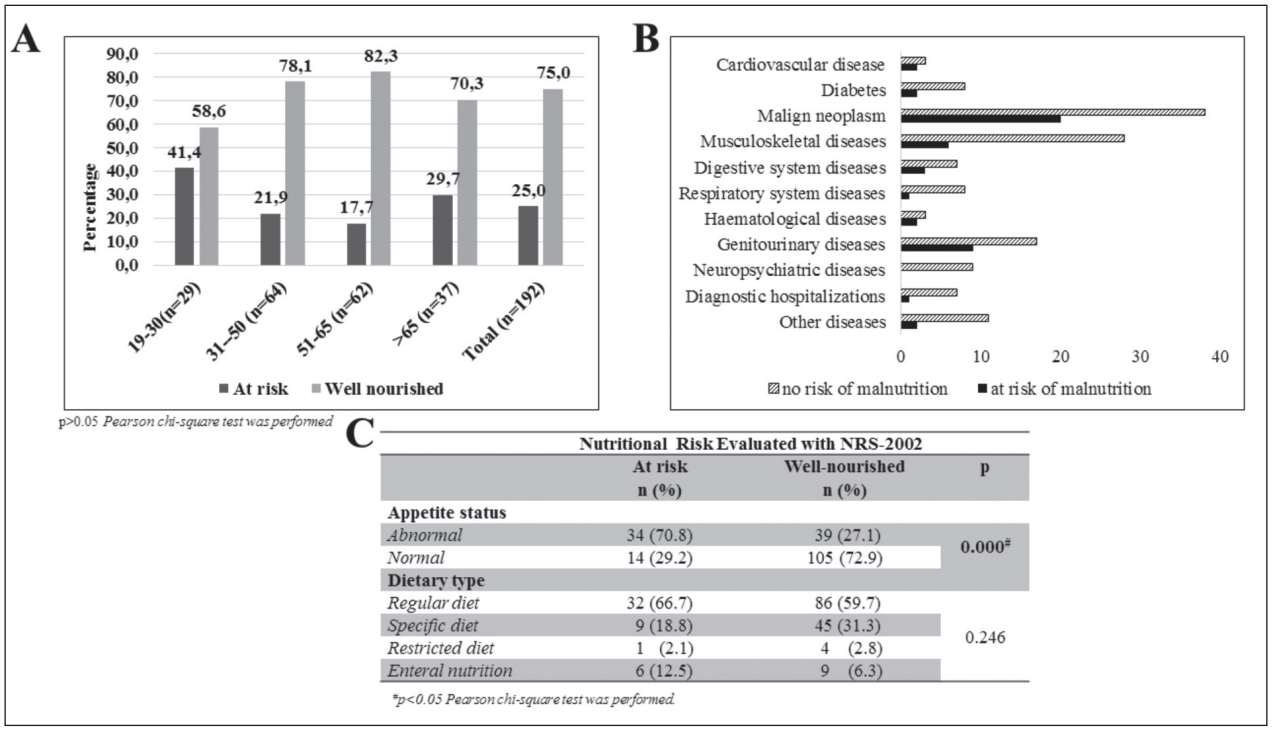
| Anthropometric measurements and LOS ($\bar{\chi}$±S) | Nutritional Risk Evaluated with NRS-2002 | | Total (n=192) | p-value |
|---|---|---|---|---|
| | At risk (n=48) | Well-nourished (n=144) | | |
| **BMI (kg/m²)** | | | | |
| Female | 27.9±7.58 | 28.9±6.74 | 28.7±6.88 | 0.313 |
| Male | 23.0±3.80 | 26.5±4.29 | 25.5±4.42 | **0.000#** |
| Total | 24.9±5.95 | 27.7±5.95 | 27.0±5.92 | **0.000#** |
| **Mid arm circumference (cm)** | | | | |
| Female | 28.7±4.39 | 30.6±4.62 | 30.3±4.12 | 0.208 |
| Male | 27.0±3.63 | 29.6±3.51 | 28.8±3.72 | **0.003*** |
| Total | 28.7±4.39 | 30.6±4.62 | 29.5±4.22 | **0.001#** |
| **Weight Loss (%)** | | | | |
| Last six months | 11.1±6.57 | 6.6±5.05 | 8.6±6.16 | **0.001#** |
| Last three months | 9.6±5.89 | 5.8±4.99 | 7.7±5.74 | **0.001#** |
| Last two months | 8.1±5.16 | 3.7±1.80 | 5.9±4.43 | **0.000#** |
| Last one month | 6.1±3.37 | 3.5±1.90 | 4.8±2.98 | **0.000#** |
| **Mean LOS (days) by MDC** | | | | |
| Other diseases | 2.5±2.12 | 3.4±2.54 | 3.3±2.43 | 0.641 |
| Diagnostic hospitalizations | 4.0±0.00 | 9.7±7.23 | 9.0±6.99 | 0.500 |
| Neuropsychiatric diseases | -- | 6.2±4.41 | 6.2±4.41 | -- |
| Genitourinary diseases | 13.2±22.40 | 3.5±2.55 | 6.9±13.67 | 0.107 |
| Haematological diseases | 8.5±0.71 | 8.7±3.51 | 8.6±2.51 | 0.800 |
| Respiratory system diseases | 9.0±0.00 | 8.0±12.01 | 8.1±11.24 | 0.667 |
| Digestive system diseases | 22.0±11.26 | 4.1±4.60 | 9.5±10.80 | **0.033*** |
| Musculoskeletal diseases | 3.3±1.97 | 7.6±9.20 | 6.9±8.53 | 0.494 |
| Malign neoplasm | 10.8±14.45 | 4.4±4.78 | 6.6±9.68 | **0.036*** |
| Diabetes | 2.0±2.83 | 12.5±20.60 | 10.4±18.72 | 0.237 |
| Cardiovascular disease | 11.0±00 | 7.7±11.55 | 8.5±9.57 | 1.000 |
| **Total** | 10.0±14.26 | 6.22±8.20 | 7.2±10.13 | **0.040*** |

**p<0.05, #p≤0.001 Mann Whitney U test was performed. BMI: body mass index, MDC: main diagnostic categories*

pendent distribution of the NRS-2002 score did not differed significantly among the age groups (p=0.083) as it is shown in figure 1A. According to MDC, the patients with malign neoplasms had the highest malnutrition risk score (Figure 1B). According to NRS-2002 scores, the loss of appetite was 70.8% of the patients at risk of malnutrition while 27.1% of well-nourished patient reported loss of appetite (p=0.000) (Figure 1C). There was no significant difference between dietary type and malnutrition status (p=0.246).

BMI and mid arm circumference of men who were at risk of malnutrition was lower than well-nourished group while there were no differences in women (Table 1). At malnutrition risk group the mean percentage of weight loss in the last six, three, two and one months were significantly higher than well-nourished group (p<0.005). Participants who were at malnutrition risk group had longer LOS compared to well-nourished participants (p=0.040). Similarly, the LOS was significantly higher in malign neoplasm and digestive system disease patients at risk of malnutrition (p<0.05).

Energy and protein consumption percentage were 79.9% and 75.9%, respectively. According to Table 2, the overall coverage of the energy, protein, fibre and micronutrients of the malnutrition risk group were significantly lower than well-nourished patients (p<0.005). This difference was more remarkable among the patients who were younger than 65

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| Nutritional Risk Evaluated with NRS-2002 | | | |
|---|---|---|---|
| | At risk n (%) | Well-nourished n (%) | p |
| **Appetite status** | | | |
| *Abnormal* | 34 (70.8) | 39 (27.1) | 0.000# |
| *Normal* | 14 (29.2) | 105 (72.9) | |
| **Dietary type** | | | |
| *Regular diet* | 32 (66.7) | 86 (59.7) | |
| *Specific diet* | 9 (18.8) | 45 (31.3) | 0.246 |
| *Restricted diet* | 1 (2.1) | 4 (2.8) | |
| *Enteral nutrition* | 6 (12.5) | 9 (6.3) | |

*p<0.05 Pearson chi-square test was performed.

**Figure 1.** Participants' malnutrition risk according to NRS-2002. A. Malnutrition risk by age, B. Malnutrition risk by main diagnostic categories, C. Malnutrition risk by appetite status and dietary type

years (p<0.005). In the malnutrition risk group saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) and cholesterol coverage of patients older than 65 years were higher but this difference was not statistically significant (p>0.05). In contrast, patients under the age of 65 in the malnutrition risk group had a lower percentage of SFA, MUFA, PUFA and cholesterol but only SFA coverage was statistically significant (p=0.016).

Seventy-one patients (n=71, 39%) did not meet their energy needs while 149 patients (57.3%) consumed less protein depending on the Dietary Recommended Intake (DRI) threshold (Figure 2). Both energy and protein intakes of 68 patients (35.4%) were below their nutritional needs. Also one in every five screened patients (20.8%) consumed both energy and protein over their needs.

When the variables tested as determinants of nutritional status were assessed by univariate logistic regression model as predictors of malnutrition risk; LOS, weight loss and some anthropometric measurements including BMI, mid-arm and calf circumferences were identified as important determinants but not dietary types (Table 3). Malnutrition risk was increased 1.032 fold by the increase of LOS (p=0.039). Similarly, malnutrition risk was significantly associated with weight loss in the last six (p=0.003), three (p=0.009), two (p=0.000) and one months (p=0.002). Malnutrition risk did not differ according to dietary type. When malnutrition risk evaluated by BMI; being underweight increased the risk of malnutrition up to 6 fold (p=0.035) compared to normal weight. In contrast, malnutrition risk decreased by the increase of BMI but this was statistically significant only in obese individuals (OR: *0.307, 0.113-0.838, p=0.021)*. There was a protective effect of increased mid arm circumference up to 15% (p=0.001) while calf circumference was 11% protective against malnutrition risk (p=0.004).

## Discussion

Malnutrition is a major global health concern and mostly affects hospitalized patients. Impaired appetite,

**Table 2:** Percentage of patients' coverage of the nutritional needs according to malnutrition status

| Energy and Nutrients | Age groups (years) | | | | | | |
|---|---|---|---|---|---|---|---|
| | ≤ 65 years | | | > 65 years | | | Total |
| | At risk ($\bar{\chi}$±S) | Well-nourished ($\bar{\chi}$±S) | p | At risk ($\bar{\chi}$±S) | Well-nourished ($\bar{\chi}$±S) | p | p value |
| Energy (kcal) | 68.9±28.24 | 77.8±21.53 | 0.057 | 87.4±26.02 | 102.4±30.36 | 0.150 | **0.050*** |
| Protein (g) | 98.0±49.71 | 113.4±35.68 | 0.066 | 107.3±49.54 | 121.4±46.46 | 0.242 | **0.039*** |
| SFA (g) | 197.2±89.20 | 234.1±122.57 | **0.016*** | 234.7±122.07 | 209.9±49.32 | 0.635 | 0.052 |
| MUFA (g) | 123.3±77.25 | 137.2±59.83 | 0.089 | 147.1±59.60 | 121.6±36.17 | 0.170 | 0.339 |
| PUFA (g) | 117.7±63.29 | 130.1±57.40 | 0.265 | 139.3±70.29 | 115.5±49.79 | 0.270 | 0.691 |
| Cholesterol(mg) | 111.8±74.09 | 129.9±63.90 | 0.129 | 136.2±77.76 | 128.4±60.86 | 0.883 | 0.230 |
| Fibre (g) | 56.7±31.11 | 76.5±28.68 | **0.001#** | 75.4±43.33 | 88.3±40.34 | 0.384 | **0.001#** |
| Vitamin C (mg) | 72.4±58.03 | 119.7±75.15 | **0.000#** | 93.8±80.22 | 142.4±99.85 | 0.108 | **0.000#** |
| Vitamin B1 (mg) | 64.2±31.67 | 81.2±25.96 | **0.002*** | 76.9±35.58 | 87.2±31.20 | 0.300 | **0.002*** |
| Vitamin B2 (mg) | 105.2±48.30 | 128.8±42.08 | **0.006*** | 112.9±56.94 | 144.0±58.31 | 0.108 | **0.001#** |
| Niacine (mg) | 149.4±87.82 | 175.8±68.57 | 0.070 | 164.3±84.05 | 193.5±88.14 | 0.270 | **0.033*** |
| Vitamin B6 (mg) | 84.5±47.24 | 109.4±44.10 | **0.004*** | 84.7±38.99 | 101.4±45.12 | 0.216 | **0.001#** |
| Folate (mcg) | 56.9±23.36 | 78.9±2.99 | **0.000#** | 69.8±32.14 | 80.0±34.45 | 0.316 | **0.000#** |
| Vitamin B12 (mcg) | 163.1±122.30 | 205.1±100.55 | **0.008*** | 216.7±134.44 | 234.5±124.66 | 0.832 | **0.018*** |
| Calcium (mg) | 60.9±23.60 | 73.3±29.24 | **0.047*** | 55.7±25.21 | 78.4±34.91 | 0.055 | **0.007*** |
| Phosphorus (mg) | 137.4±60.31 | 165.7±51.17 | **0.012*** | 157.1±67.73 | 186.1±63.40 | 0.181 | **0.005*** |
| Iron(mg) | 73.8±46.24 | 94.3±43.89 | **0.009*** | 99.5±40.96 | 109.4±54.81 | 0.635 | **0.019*** |
| Magnesium (mg) | 52.7±26.78 | 69.1±22.87 | **0.001#** | 63.7±33.94 | 72.0±30.46 | 0.300 | **0.000#** |

**p<0.05, #p≤0.001 Mann Whitney U test was performed. MUFA: Mono unsaturated fatty acids, PUFA: Poly unsaturated fatty acids, SFA: Saturated fatty acids*

inadequate food intake, recent weight loss and reduction in anthropometric measurements were identified as major contributors of malnutrition (15). This study evaluates the association between malnutrition risk and anthropometric data, nutrition related parameters, LOS and MDC in hospitalized patients. Weight loss, LOS and anthropometric measurements were found as major contributors to malnutrition risk while dietary type was not related to malnutrition. Additionally, it was found that energy and nutrient intake of patients who were at risk of malnutrition were significantly lower than well-nourished patients. In the study population one in every four screened patients (25%) had risk of having malnutrition. Also 29.4% of males and 20.0% of females were nutritionally at risk group. Other studies reported similar results (16,17). This may be because of men demand healthcare services later than women. Thus, men may have more probability of being malnourished than women.

In the present study the loss of appetite was significantly lower in at risk group versus well-nourished group. It is known that in appetence is an important variable related to malnutrition (18, 19).

BMI is a simple and objective measurements for determining the nutritional status and is an important component of several malnutrition screening tools (19). Malnutrition can be underestimated when assessed by BMI alone (20, 21). Because clinical signifi-

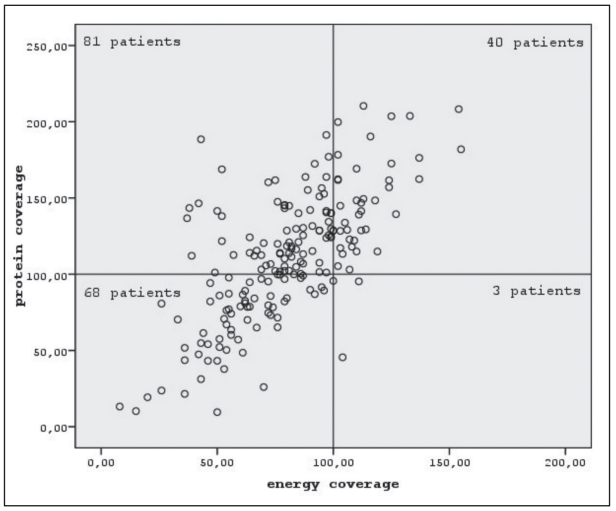


**Figure 2.** Coverage of the recommended nutritional needs. Needs were calculated according to Dietary Guidelines

**Table 3.** Bivariate logistic regression analysis of the association between factors related to nutritional status and malnutrition risk

| | Odds ratio (95%CI) | p |
|---|---|---|
| **LOS (days)** | 1.032 (1.002-1.064) | **0.039*** |
| **Weight loss (%)** | | |
| Last six months | 1.142 (1.046-1.247) | **0.003*** |
| Last three months | 1.147 (1.035-1.271) | **0.009*** |
| Last two months | 1.685 (1.258-2.256) | **0.000#** |
| Last one month | 1.507 (1.166-1.948) | **0.002*** |
| **Dietary type** | | |
| Regular diet | 1 | Reference |
| Specific diet | 0.538 (0.236-1.224) | 0.139 |
| Enteral nutrition | 0.672 (0.072-6.239) | 0.727 |
| Restricted diet | 1.792 (0.591-5.436) | 0.303 |
| **Anthropometric measurements** | | |
| ***BMI (kg/m²)*** | | |
| <18.5 | *6.143 (1.141-33.066)* | ***0.035**** |
| 18.5-24.9 | 1 | Reference |
| 25.0-29.9 | *0.521 (0.241-1.125)* | *0.097* |
| >30 | *0.307 (0.113-0.838)* | ***0.021**** |
| ***Mid arm circumference (cm)*** | 0.850 (0.775-0.933) | **0.001#** |
| ***Calf circumference (cm)*** | 0.890 (0.822-0.963) | **0.004*** |

**p<0.05, #p≤0.001 Odds ratios were computed using a bivariate logistic regression model BMI: body mass index, LOS: length of stay*

cant weight changes and the reduction of oral intake that occur before removal to the hospital are ignored in the BMI calculation. In addition, a patient may have a high BMI and might be malnourished depending on reduced food intake caused by an underlying disease. On the other hand, decreased BMI does not indicate the individuals are malnourished. Furthermore, it was shown that BMI alone is insufficient in assessing an obesity risk factor for individuals (20). In our study the BMI of men who were at risk of malnutrition was lower than well-nourished group (p=0.000). On the other hand, there was no difference on BMI of women between two groups. In parallel with dos Santos et al. (22), being underweight increased the risk of malnutrition up to 6 fold (OR: 6.143, *1.141-33.066,* p=0.035) compared to normal BMI. In contrast, there is a reverse association between obesity and malnutrition risk. Similarly, other studies reported that obesity is inversely associated with clinical outcome (23, 24). It is likely that there is a wide variation in body composition and nutritional status in the overweight and obese populations (25). All these findings support the concepts that greater body stores confer survival advantages in catabolic conditions.

Similar to BMI; other anthropometric measurements such as mean handgrip strength, mid arm and calf circumferences may be related to malnutrition risk. Mean handgrip measurements of females and males were respectively 18.9±6.39 kg and 31.1±8.99 kg. Vanitha et al. (26) reported that the mid arm circumference was higher in well-nourished group. In our study, mid arm circumference of females and males were respectively 30.3±4.12 cm and 28.8±3.72 cm. Mid arm circumference was significantly higher in well-nourished men while there was no difference among women. There was a protective effect of increased mid arm circumference up to 15% while calf circumference was 11% protective against malnutrition risk by the bivariate logistic regression analysis.

Weight loss is one of the main nutritional assessment indicators associated with long-term mortality in numerous studies (6, 21, 27). BMI cut off points, the amount and duration of weight loss are related to malnutrition. Clinically significant weight loss has previously been found to be associated with morbidity and mortality (21, 25). American Society of Clinical

Nutrition and Metabolism (ASPEN) recommends a weight loss below 10% in the last six months, 7.5% in the last three months and 5% in the last month to avoid malnutrition (28). In our study the weight loss was higher than ASPEN recommendations at malnutrition risk group (respectively 11.1±6.57%; 9.6±5.89%; 6.1±3.37%). Also, recent weight loss of malnourished group was significantly higher in at risk group versus well-nourished group. Recent weight loss increased the malnutrition risk 1.1 times in the last 3 and 6 months and in the last 1 month was 1.5 times.

LOS has been thought as a factor that effects patients' well-being during hospital treatment (29). In general patients with malnutrition have longer LOS associated with prolonged duration of treatment and increased morbidity (25, 29). Lim et al. (8) demonstrated that malnutrition was an independent risk factor for longer LOS. We found that being at risk group significantly increased mean LOS compared to well-nourished group. In parallel, one study conducted in Switzerland reported higher LOS among undernourished patients compared to well-nourished patients (30). Also our results showed that malnutrition risk was increased 1.032 fold by the increase of LOS.

The relationship between malnutrition and LOS was reported in many studies (31-34) and can be considered an independent risk factor related to other complications and mortality (35). Reducing the LOS, therefore, has the potential to improve patients' quality of life by decreasing the risk of infections and other hospital-acquired diseases (29).

In our study, the LOS was significantly higher in malign neoplasm and digestive system disease patients at risk of malnutrition ($p<0.05$). According to MDC the patients with malign neoplasms had the highest malnutrition risk score. Similarly, other studies have found that cancer patients had higher malnutrition rates than non-oncologic patients (2, 36, 37). Cancer patients are particularly vulnerable to nutritional deficiencies due to the combined effects of malignancy and its treatments (38, 39). It is known that many treatment methods including chemotherapy, radiotherapy and surgery negatively affect the nutritional status related to commonly experienced side effects such as nausea, vomiting, anorexia, lethargy, diarrhea, esophagitis and dysphagia (40,41). These are strong reasons to avoid malnutrition by monitoring the nutritional status of all cancer patients throughout their illness.

Although many hospital diets provide sufficient energy and nutrients, previous studies showed that patients failed to meet their energy and protein needs in parallel with our study (15, 34, 42). However, studies evaluating only energy and protein intake may be inadequate to assess patients' nutrient intake. A study reported no significant differences for energy and nutrients and for intakes below 1/3 of dietary recommendations from nutritionally-at-risk and well-nourished patients (42) but in our study malnutrition risk group had significantly lower intake and this difference was more remarkable among the patients who were younger than 65 years.

DRI is used to evaluate nutritional requirements of healthy individuals, but the needs of hospitalized patients may be increased because of their clinical outcomes. So patients' food intake should be monitored even they cover their needs according to DRI, not to underestimate malnutrition risk.

Our single-center study population was heterogeneous by age group and MDC. Although its overall big sample size it involves a rather small sample in different age groups. 24 h recall dietary method may have variable outcomes in terms of hospital menu, LOS, stage of medical investigations, disease and treatment to evaluate nutritional intakes.

Although these limitations, our study has some strengths. First of all, energy and nutrient requirements were calculated individually by age group and gender. Moreover, there was no notification about the study which could have influence the behavior of patients. Patients were evaluated by their dietary type. So malnutrition risk was not overestimated because of less energy and nutrient intakes provided by restricted diets. The nutritional screening was performed in different departments of hospital and this lead to evaluate malnutrition rates of several diagnostic categories in different departments.

## Conclusion

In conclusion, malnutrition prevention and treatment is a major challenge. A proper diagnosis is es-

sential for the nutritional therapy to be started as soon as possible, allowing an efficient dietetic-therapeutic intervention. Early intervention with additional nutritive treatment can lower malnourished associated complications and LOS. So, malnutrition should be adequately screened and documented. Nutritional intervention in patients at risk of malnutrition leads to a better prognosis, reducing the morbidity and mortality, improving quality of life. In conclusion, not only the physicians and dietitians but also the hospital managers, nurses and food service staff have to understand that good nutrition is a prerequisite for preventing hospital malnutrition.

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Original article

# The role of muscle in disease-related malnutrition. Decalogue of Good Practices

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**Summary.** *Introduction:* Disease-related malnutrition (DRM) is a major problem in Western societies, and the consequences pose a high economic burden. DRM affects not only muscle mass but also muscle function. However, there are currently few tools and strategies to enable a proper assessment of muscle and estimate muscle loss associated with malnutrition. *Objective:* To highlight the current situation of deficiency in the assessment of muscle mass and function in malnourished patients and to develop recommendations for the inclusion of muscle assessment as an element of measurement in the nutritional recovery of patients with DRM. *Methods:* A multidisciplinary expert panel comprised of twenty-one physicians from Spain was convened to review, refine and summarize current recommendations on the assessment, diagnosis, and nutritional and functional treatment of malnourished patients. *Results:* The experts highlighted the impact of DRM on muscle, as well as the crucial role of this organ in the correct nutritional, metabolic and functional recovery of patients. The lack of strategies for the assessment of muscle in DRM led to a proposed Decalogue of Good Practices for the optimal treatment of these patients. *Conclusion:* The review is intended to guide in the nutritional and functional recovery of patients with DRM, based both on consensus opinions of experts and on the most recent scientific evidence in the field. The proposed Decalogue provides a comprehensive approach to the management of the malnourished patient.

**Key words:** disease-related malnutrition, nutritional assessment, muscular assessment

## Abbreviations

DRM: Disease-related malnutrition;
HMB: β-hydroxy-β-methylbutyrate;
NRM: Nutrition, Recovery and Muscle;
FIM: Functional Independence Measure;
ONS: Oral nutritional supplement.

## 1. Introduction

Disease-related malnutrition (DRM), can be defined as the "subacute or chronic state in which several degrees of overnutrition or undernutrition are combined with an inflammatory pattern, resulting in changes in body composition and functionality" (1). It is a problem of enormous proportions in Western societies, where the cost exceeds € 120 billion per year in the European Union (2). A high percentage of hospitalized patients suffer from malnutrition, reaching 23% in Spain (37% among patients over 70 years of age), (3) although this is a universal problem recognized by the European Council in the ResAP resolution (4).

DRM affects not only muscle mass, but also its function. The most evident sign of malnutrition is weight loss and since muscle constitutes a significant percentage of body dry weight, it is one of the organs most affected by adaptive changes as a result of DRM. At present there are a scarce number of tools and strategies, both anthropometric and biochemical, that allow an accurate evaluation of both muscle mass and function, to estimate the muscular involvement associated with malnutrition (5).

This guide describes the current situation related to the deficiency in the assessment of muscle mass and function in malnourished patients and proposes the optimal parameters to include, incorporating muscular assessment as an element of measurement in the nutritional recovery of patients with DRM. The guide aims to serve as a reference for health professionals involved in the management of patients with DRM, in order to help improve their clinical situation; as well as providing information to the administrative entities of the hospital sector involved in the care of these patients. It also proposes a Decalogue of Good Practices to guide professionals in the comprehensive treatment of malnourished patients.

## 2. Methods

A multidisciplinary working group, called "Nutrition, Recovery and Muscle (NRM)" was created, consisting of 21 Spanish physicians (endocrinologists, rehabilitators, geriatricians and sports medicine) with extensive experience in nutritional and functional assessment, diagnosis and treatment of malnourished patients. Basing on the literature review regarding the present status of DRM treatment and the experience in clinical practice, the group reviewed, refined and summarized current recommendations on the assessment, diagnosis, and nutritional and functional treatment of malnourished patients. The consensus document includes a series of recommendations about the role of muscle in DRM and presents a Decalogue of Good Practices, which proposes muscle assessment as an integral part of the patient's treatment.

## 3. Results

### *3.1. Implications of malnutrition*

In the presence of malnutrition, muscular plasticity acts as a compensatory mechanism of the organism, allowing the metabolic balance to be altered (6). However, sustained protein-calorie malnutrition leads not only to a deterioration of the structural functions of the muscle but also to the metabolic ones, which, if not properly treated, can make it difficult to recover nutritional and functional status.

Correct diagnosis and an appropriate treatment plan play a key role in patient recovery.

### *3.2. DRM: Methods of screening and nutritional assessment*

Nutritional screening is the starting point needed to ensure that all individuals who can benefit from nutritional support are easily identified and that those who cannot benefit are not unnecessarily treated. The nutritional screening tools validated in adults include: Nutritional Risk Screening (NRS), Malnutrition Universal Screening Tool (MUST), Mini Nutritional Assessment (MNA) (5). These tools do not include muscle assessment, with the exception of partial assessment of the MNA scale.

Nutritional assessment aims to characterize the nutritional status of patients with malnutrition or suspected risk of malnutrition, in order to establish a therapeutic plan (7). The procedure is based on a joint use of techniques (clinical and dietary history, physical examination, anthropometry, biochemical parameters and evaluation of interactions between medicines, nutrients and disease) (7). Nutritional assessment scales include some indirect measurement of lean mass. Basic anthropometry does not evaluate lean mass, except for the muscular circumference of the arm; while advanced anthropometry evaluates the muscle indirectly (impedance measurement) or directly (DEXA), with the latter being little used and not standardized in clinical practice. There are also no biochemical parameters that allow an easy evaluation of muscle mass.

As part of the nutritional assessment, hand grip dynamometry is an indirect method that is being in-

troduced to assess muscular functionality. However, reference values are only available for some subpopulations in Spain (8). In geriatric and rehabilitation consultations the *Short Physical Performance Battery* (9) is also used as functional test.

Thus, the assessment of the muscle compartment is scarce in screening and nutritional assessment methods, and there is generally a shortage of anthropometric and biochemical tools that allow a correct evaluation of the muscle mass and function in the malnourished patient.

### *3.3. The role of muscle in the context of the DRM*

Malnutrition affects muscle function even before changes in mass are perceived, so alteration in nutrient intake, digestion or absorption can have a significant impact on muscle health, regardless of changes in muscle mass (10).

The description and redefinition of the muscle as a secretory organ releasing anabolic and catabolic peptides expressed and produced by the muscle fibers, called myokines, has provided a conceptual basis for understanding how skeletal muscle communicates with other organs or tissues to maintain the body homeostasis (11). This shows that in addition to the functions related to movement, power generation and postural maintenance, muscle has a metabolic and even endocrine function (12, 13); as well as an important role in the beneficial effects of exercise on health, and on the pathogenesis of several diseases, such as obesity, sarcopenia and diabetes (11). Therefore, skeletal muscle is increasingly recognized as one of the main regulators of energy and protein metabolism through its communication network with different organs of the body (14).

If the muscle's ability to maintain homeostasis is diminished due to malnutrition, muscle function, including strength or metabolic balance, may be affected. Thus, muscular deterioration or atrophy not only affects the motor function of the muscle, but also the metabolic function, mainly due to the loss of mitochondria and their enzymes and of muscular capillaries (12).

During the hospital stay, important changes occur in the patient due to altered nutrient intake, immobilization and inflammation, which can be included within the concept of metabolic atrophy. Thus, in the absence of contraction, atrophy occurs in both muscle size and function, decreasing not only fiber thickness but also capillary density and muscle metabolic activity (13). DRM is also commonly associated with an onset of chronic or acute disease and the presence of an inflammatory component. To compensate this situation, the organism undergoes an adaptive response. Due to the plasticity capacity of the muscle, this tissue is one of the first to act as a defense against malnutrition.

Muscle plasticity implies a change in the flow and utilization of carbohydrates and proteins as metabolic substrates. Changes related with plasticity include not only the export and use of muscle proteins (as a source of energy to support major functions), but also a decrease in the availability of amino acids throughout the body (mainly glutamine), which affects metabolic functions, oxidative response, protein turnover, muscle mass and strength/contractility. If sustained malnutrition is not adequately treated, the muscle's buffering capacity can be critically overridden. Under these conditions, and as a result of the general body deterioration (e.g. muscular insulin resistance), patients can enter a vicious circle of complications that affect quality of life, prolong hospitalization, and increase risk of readmission, morbidity and healthcare expenses (Figure 1).

### *3.4. Functional muscle assessment in malnourished patients*

There is a strong association between malnutrition and disability that opens the possibility of multiple interrelations between nutritional improvement and motor recovery (14). Several tools are available to determine the patient functional status, which can be grouped into generic and designed for specific conditions. The generic ones assess the functional situation independently of the cause of disability. As generic scales, the following stand out: *Patient Evaluation and Conference System* (PECS), *Katz Activities of Daily Living Scale* (KADLS), *Barthel Index*, Level of Rehabilitation Scale (LRS) and *Functional Independence Measure* (FIM). The Barthel index (15) is widely used in the geriatric setting to assess the patient's functional capacity. This index contains 10 items and its total score varies between 0 (total dependence) and 100 (complete independence).

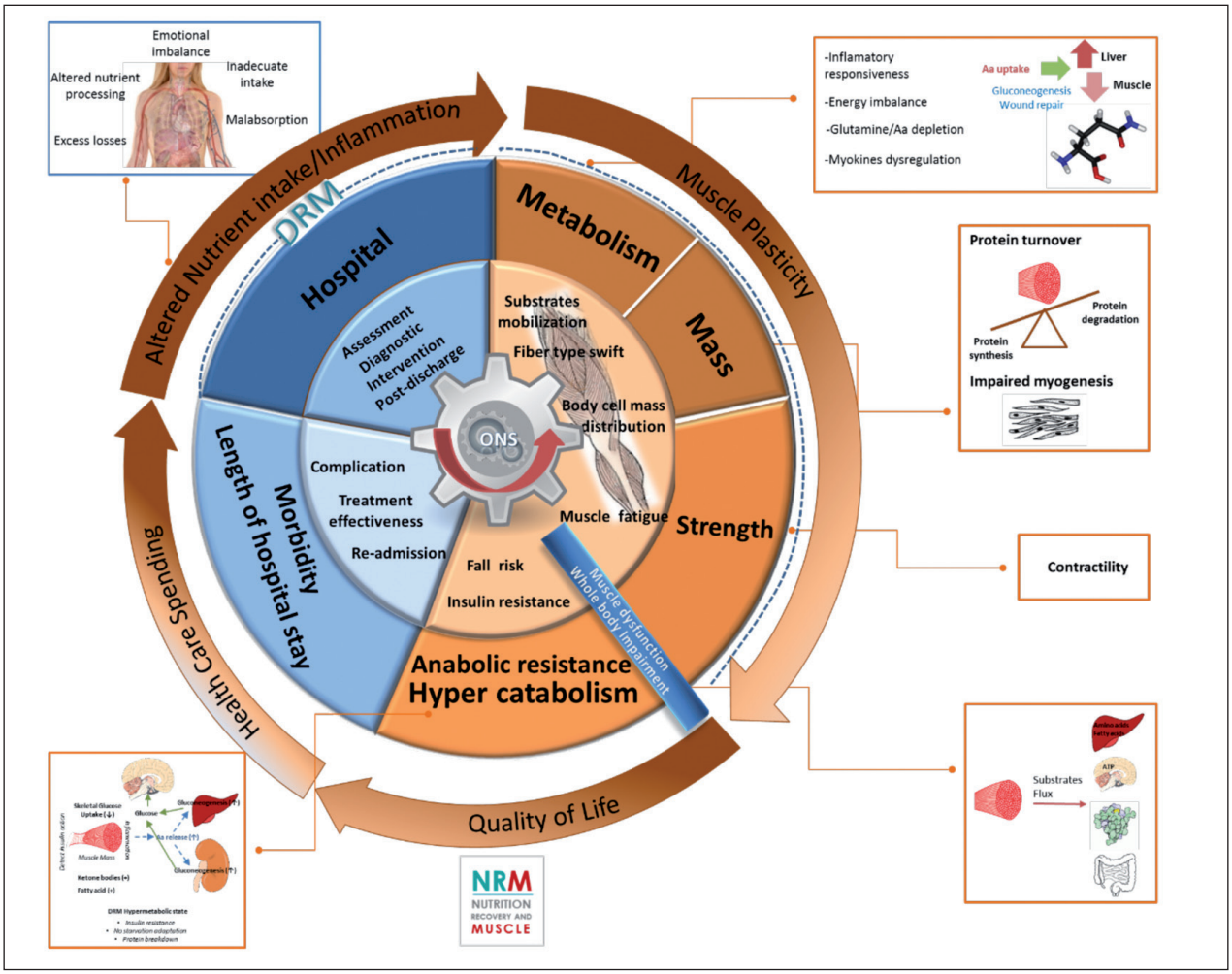


**Figure 1.** Response cycle in the DRM. If DRM is not properly diagnosed and treated, patients may enter a vicious circle of dysregulation of muscle function that will ultimately affect the nutritional and functional recovery of patients, their quality of life and, at the same time, add to health expenses due to morbidity and increased hospital stays generated by malnutrition. Figure abbreviations: Aa: Amino acids, ATP: Adenosine Triphosphate, DRM: Disease-related Malnutrition, ONS: Oral Nutritional Supplements

In the rehabilitation field, the FIM scale has displaced other generic scales as a global measure of functional disability, having created a benchmark between hospital rehabilitation services in the United States and in other countries. The importance of the FIM scale (16) and Barthel index is that they allow to establish a progression in the acquisition of functional abilities that depend largely on muscular training.

Also, in the assessment of the patient's functional status is important to explore muscle strength, either through measurement of the function of a muscle group (e.g. lower limb while standing or walking), or more analytically by a muscular balance (manual or instrumental).

In outpatients, timed tests, such as the 6-minute walk test (17) and timed up and go (18), among others, allow assessment of muscle mobility and function. It is important to note that these tests have shown a high correlation with important outcome variables, such as survival and quality of life.

### *3.5. Treatment plan*

Optimal nutritional status and energy balance are essential for the rehabilitation of muscle mass and function. Therefore, the integral approach of the malnourished patient should include an adequate nutritional

treatment and a physical exercise plan adapted to the individuals' clinical situation, in order to avoid muscle and energy metabolism deterioration, promoting overall health, well-being, and recovery from disease (14).

The main objective of nutritional support is to minimize the protein-calorie imbalance in order to achieve the maximum possible functional independence. Moreover, there is scientific evidence demonstrating the efficacy of nutritional support with specific nutrients to improve certain aspects of DRM, such as the anti-inflammatory (19) or immune-modulatory effects (20) of some nutrients regarding inflammation (1) and impaired immune system function associated with DRM (3).

In the area of muscle recovery, there is still a significant lack of scientific knowledge regarding the pathophysiological mechanisms that relate nutrition and muscle in the disease, as well as clinical studies that demonstrate the joint effect of nutrition and exercise on the main determinants of the disease.

*3.5.1 Nutritional and functional support*

The broad spectrum of malnutrition in clinical practice makes it necessary to determine the protein, calorie and specific nutrient requirements according to each clinical situation. Furthermore, the nutritional support focused on the muscle implies a whole morphological, metabolic and functional study within the context of malnutrition.

Loss of muscle mass is very important clinically because it leads to decreased strength and exercise capacity. The key to the development of an optimal treatment plan aimed at skeletal muscle recovery and muscle strength as key factors of clinical response lies in clinically distinguishing the causes of muscle loss (21).

*Energy requirements*

For the calculation of energy requirements, as well as for the selection of the enteral nutrition formula, the classical therapeutic targets should be considered, such as the recovery of lost weight or the improvement of analytical parameters, together with specific targets of muscle recovery, such as lean mass recovery (impedance, arm circumference) and functional recovery (dynamometry/gait test) of the patient.

*Protein requirements*

There are no specific nutritional recommendations related to the requirements for protein intake in situations of loss of muscle mass or muscle function. The general recommendations of protein intake in adults are around 1.0 -1.2 g of protein/kg of weight/day. Older adults with an acute or chronic pathology need a higher intake of protein (1.2 - 1.5 g/kg body weight/day). This requirement may reach 2.0 g/kg body weight/day in severe malnutrition and severe stress (22).

*Specific nutrients*

*- Amino Acids*

The utility of certain supplements, such as glutamine or arginine, in patients with severe acute diseases to improve physical performance and assist in the restoration of lean mass and neuromuscular function, is an area of research that needs more placebo-controlled studies to determine the efficacy and optimal dosing of these (23).

Dietary enrichment with branched-chain amino acids, including leucine, appears to have positive effects on specific signaling pathways for muscle protein synthesis. There are studies in elderly sarcopenic patients with administration of 2.5 and 2.8 g of leucine/day that show an improvement in muscle mass or in physical performance, but not in muscle strength. Also, it has been observed that leucine supplementation in combination with resistance exercise training improves leg muscle mass and muscle strength, but not physical performance (24).

*- Omega-3 Fatty Acids*

There is extensive literature on the effects of omega-3 fatty acids on muscle parameters in cachexia, with favorable results in certain clinical circumstances, especially in patients with cancer (25). The anti-inflammatory properties of omega-3 fatty acids could improve muscle anabolic resistance in older adults, improving the rate of muscle protein synthesis (26).

*- Supplementation with β-hydroxy-β-methylbutyrate (HMB)*

HMB is an active metabolite of leucine, present in some foods (avocado, citrus, cauliflower, alfalfa). It is produced naturally in the body, but insufficiently in

situations of metabolic stress. HMB acts as a substrate for cholesterol synthesis in the muscle cell and in turn also regulates protein metabolism, inhibiting its degradation and stimulating its synthesis (27).

HMB has shown positive effects on lean body mass and strength following exercise, and in disease-related muscle wasting. In clinical trials, the administration of HMB is able to attenuate the decrease of muscle mass in patients submitted to 10 days of absolute rest and 8 weeks of later recovery, which emphasizes its potential effect regardless of the physical activity, aspect of special interest in the field of hospital malnutrition, since it frequently involves prolonged periods of rest (28). A growing body of evidence suggests HMB may help slow the muscle loss experienced in sarcopenia and improve measures of muscle strength (29). Moreover, in elderly malnourished patients, the use of a high-protein oral nutritional supplement (ONS) containing HMB is associated with a 50% significant reduction in mortality risk at hospital discharge (4.8% vs 9.7%; p = 0.018), compared with placebo (30).

In those clinical circumstances that involve a nutritional and muscular deterioration, the use of specific ONS to target muscular recovery can support improvements in nutritional, metabolic and muscular parameters; especially when associated with a therapeutic exercise plan.

*3.5.2 Therapeutic exercise*

The treatment plan should always include therapeutic exercises, even in patients with a poor nutritional and functional status. Muscle contraction, even when small, contributes to improved muscle protein synthesis and decreased atrophy.

Three types of exercise can be used to increase muscle strength: isometric, isotonic and isokinetic. In isometric exercise, the muscle maintains a constant length when a resistance is applied, without changes in the joint position. With aging, the isometric contraction decreases (31). Isotonic exercise is performed at constant power and is the best known and practiced. These contractions occur along with amplitude of movements against resistance, the velocity is not stationary and is divided into two phases: a) concentric or positive phase: contraction with shortening of the muscle and b) eccentric or negative phase: contraction with elongation of the involved muscle. Isotonic training is effective for improving strength and should be considered as a key part of increasing muscle strength. To do this, there is a great variety of methods and equipment: weights, fixed resistors, cables and pulleys, machines of constant and variable resistance, as well as devices with elastic, hydraulic or robotic resistance; all having as a common goal achieve a voluntary contraction during training, according to the patient's situation (32). For its part, the isokinetic exercise tries to mobilize the maximum force-generating capacity of a muscle along a complete joint path at constant speed, for which specific machinery is needed.

A training program should include (in patients whose condition allows): warm-up, aerobic exercise, strength training and a cooling period. In very impaired patients, only the act of contracting the muscles and trying to "force", will serve to enhance muscular trophism and its beneficial effects. Aerobic training will include low-impact activities: walking, pedaling, swimming, or climbing stairs. Strength training should target the large muscle groups, which are important for the activities of daily living: legs, trunk, shoulders, triceps, biceps, leg triceps, abdominals and waist. Strength training can be done at low, medium or high intensity. This intensity is defined by maximum repetition (MR). To achieve muscular enhancement, it is recommended to train at 60 to 80% of 1 MR, in three sets of 8 to 12 repetitions, three times a week (33).

It is important to remember that only strength training, that is, muscular contraction against resistance, is the one that can stop or reverse sarcopenia. There is a loss of muscle strength of 5-10% per week if muscle specific strength training is ceased (34).

## 4. Conclusion

The achievement of an adequate nutritional status and energy balance is fundamental for the recovery of mass and muscle function in the malnourished patient. Appropriate nutritional supplementation should, in those cases where it is possible, be accompanied by an exercise plan to maintain trophism and muscle protein synthesis. There is evidence of the benefit of this combined intervention. All this shows the need to im-

plement strategies and methods for measuring muscle mass and function that allow documentation of the impact caused by DRM on these parameters, as well as the evolution of their recovery.

**Decalogue of Good Practices:**

1. The diagnosis of malnutrition and muscular deficit is basic to improve the nutritional management of patients, since both worsen prognosis.
2. Nutritional screening, through any of the available validated tools, should provide an estimate of the risks associated with malnutrition. The 'MUST' tool is useful in ambulatory and hospitalized patients, both in hospitals and the home setting.
3. Muscle evaluation should be integrated in the nutritional assessment process, including body composition data (impedance measurement), function (dynamometry), or any other tool that assesses muscle function.
4. Complete muscular functional assessment should include evaluation of muscle strength through measurement of a muscle, or muscle group, by muscle balance (manual or instrumental) and timed tests in outpatients.
5. The integrated approach that supports the nutritional and functional recovery of the patient must be based on an intervention that includes nutritional treatment and an adequate exercise plan. For correct protein synthesis, the muscle needs the stimulation via muscular contraction, as well as proper nutrition.
6. The objective of the intervention is to achieve an overall improvement of the disease in out-patients, as well as to improve muscle composition and functionality during hospital admission.
7. The provision of nutritional support should meet the patients' macro- and micronutrient requirements, and should be adapted to each clinical situation.
8. The recommendation for the administration of other specific nutrients should be based on relevant health outcomes with proven scientific evidence.
9. An exercise training program must adapt, in its different phases, to the characteristics and clinical situation of patients, including aerobic and strength training exercises, combined with nutritional supplementation.
10. Clinical follow-up of patients should include anthropometric, biochemical and muscular functionality parameters to monitor adequacy of nutritional and functional recovery.

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Original article

# Nutrition literacy as a determinant for diet quality amongst young adolescents: a cross sectional study

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**Summary.** The purpose of the present study was to assess the association between nutrition literacy and diet quality among young adolescents. In this cross-sectional study, 388 adolescents aged 13-15 were selected from secondary schools, Shiraz, Iran, using cluster random sampling method. The Revised Children's Diet Quality Index (RCDQI) was assessed using a validated Food Frequency Questionnaire (FFQ) and nutrition literacy was measured via a 3-dimensional questionnaire. Ordinal regression was used to examine the association between nutrition literacy and the quartiles of RCDQI as well as its components. RCDQI mean and standard deviation and total nutrition literacy (T-NL) were 65.19 ± 8.96 and 52.98 ± 7.15 among all the participants. Diet quality scores were higher in boys. Sources that were mostly used to collect nutritional information included the Internet (18.6%), families (15.2%) and books (13.1%). Among boys, an increase in T-NL (OR: 1.049; CI 95% 1.001-1.098), interactive nutrition literacy (OR: 1.13; CI 95% 1.033-1.236), and critical nutrition literacy (OR: 1.086; CI 95% 1.016-1.161) could enhance diet quality. Furthermore, increase in functional nutrition literacy was associated with lower sugar intake and better energy balance in boys and higher dairy intake in girls. Since there was an association between health literacy and diet quality amongst adolescents, health policy-makers should develop new strategies with focus to increase understanding of nutrition literacy during adolescence years.

**Key words:** health literacy, nutrition, dietary habits, diet quality, adolescence

## Introduction

Proportional intake of healthy foods plays a significant role in growth and development of young individuals. On the other hand, unhealthy eating habits can leave adolescents predisposed to reduce learning ability and academic underachievement (1), in addition to prevalence of chronic disease (2).

Health literacy has been defined as the capacity to raise an individual health awareness to cope with personal requirements, which plays a critical role in health-related decisions and behaviors (3). Low health literacy in adolescence increases the chance of improper health status that reduces health-promoting behavior, especially in relation to nutrition (4). As an important aspect of health literacy, nutrition literacy is defined as the ability of an individual to obtain, process and understand nutritional information and services required to make proper (nutrition) decisions in their lives (5). Nutrition literacy falls into three categories of Functional Nutrition Literacy (FNL), Interactive Nutrition Literacy (INL) and Critical Nutrition Literacy (CNL) (6). In previous studies, nutrition-based health literacy were negatively associated with fat intake (7), and unhealthy food consumption (8). Also, by reviewing the literature we can see how nutrition literacy has shaped youth eating habits (8).

Exploring the nutrition literacy status and its relation with quantity and quality of dietary intake among youths might help to adopt effective strategies for promoting nutritional health among this critical age group.

Studies on nutrition literacy, especially on adolescents in Iran are very limited. Therefore, the purpose of this study was to assess the association between nutrition literacy and diet quality among young adolescents.

## Method

*Subjects*

In this cross-sectional study, using cluster random sampling, 420 adolescents aged 13-15 were selected from fourteen private and public secondary schools from 4 educational districts in Shiraz, the largest city in southern Iran. One class (average of 30 students) was selected randomly from each school. Foreign students and adolescents with chronic disease or special diets were excluded from the study. Since completing all the 3 questionnaires was not possible in one day (due to tiredness), for this reason, data were collected through face to face interviews in 2 separate sessions with one week interval. A briefing session was held for parents prior to the research, and a written informed consent was obtained from families/ guardians.

*Measurements*

General and Anthropometrics questionnaire: The participants were asked about demographic characteristics such as age, gender and parents' education level and occupation. Height was measured without shoes to the nearest of 0.1 cm using a non-stretchable tape. Weight was measured in light clothing to the nearest of 0.1 kg using a digital scale (Seca, Germany). BMI was calculated as body weight (kg) divided by height square (m2). Based on World Health Organization (WHO) the participants' body mass index (BMI) percentile value was assigned to one category of either overweight or obese, normal and underweight (9).

Nutrition assessment: Adolescents dietary intakes were estimated based on the previous year using a validated food frequency questionnaire (FFQ) (10). RCDQI scores were calculated based on the studies by Kranz (11, 12). In relation to scoring, full points were assigned to adolescents with intakes within the recommended levels (ranging from 2.5 - 10 depending on the component), with reductions made proportionally for suboptimal intake and overconsumption.

Some index components were not compatible with the Iranian dietary pattern. For example, whole grain breads are not easily accessible in some districts, as they are only sold in specific supermarkets and bakeries. On the other hand, daily consumption of natural juices is not a routine dietary habit among Iranians, hence, no one had a juice intake higher than 360 ml (12 Oz) per day to be scored as "excess juice" in the index (12). Thus, juice intake was included in the "fruit" category (13). Therefore, the scores for these two food items were considered zero in the RCDQI scoring.

Total physical activity (adolescent physical activity and recall questionnaire-APARQ) (14) was an indicator for energy balance in adolescents. Following classification of physical activity into "sedentary", "moderate" and "vigorous" categories, each individual energy intakes was evaluated to estimate their energy requirements (EER) ±10%, appropriate for age, gender and 3 levels of physical activity, and then they were scored for both under- and over-consumption of energy.

The total RCDQI score was 90 and the adolescents' dietary intakes were assessed using Nutritionist-4, which was modified based on the Iranian food composition table (15) for Persian foods.

Knowledge: General nutrition knowledge questionnaire (GNKQ) (16) was used to assess the participants' nutritional knowledge. Scoring system was based on 1 and zero point for correct and incorrect/ "I don't know". The questionnaire reliability was assessed in the present study (Cronbach's $\alpha$= 0.76).

Nutrition literacy questionnaire (NLQ-20): this is a 34-item, five-point Likert scale ranging from 1 (strongly agree) to 5 (strongly disagree) including the three main domains of nutrition literacy. In addition to 20-item NLQ, some additional descriptive questions were used as well. (17).

Its content validity was confirmed by a panel of experts (n=7). Face validity was assessed in a group of 25 adolescents. After omitting 3 items, the 7-item construct of functional nutrition literacy was validated through principal component analysis (loading factor> 0.4, eigenvalue>1 and varimax rotation) and its reliability was confirmed by calculating Cronbach's alpha coefficient ($\alpha$=0.63). Similarly, through this process the construct validity and reliability of final 6-item interactive NL (alpha=0.65) and 7-item critical NL

(alpha=0.74) were confirmed. In total, the NLQ-20 scores ranged from 20 to 100.

In the descriptive part, adolescents were asked about possible sources of information related to nutrition that they have used recently (books, pamphlets, family, friends and classmates, doctors or health care providers, Internet, library, magazines, newspapers, radio, television programs, traditional herbal drug sellers). Secondly, we assessed adolescents' self-efficacy in obtaining information that they required (How confident are about you getting nutrition-related advice or information if you needed it?) using a 5-point Likert scale ranging from 1 (not confident at all) to 5 (completely confident). Third, the participants were asked to answer the question: "how much do you trust the information about nutrition, diet or food coming from each of the following sources?"; the 13 sources included doctors, nurses or health care providers, dietitians, family, friends, books, newspapers and magazines, Internet, television, radio, public clinics or hospitals, private clinics or hospitals and international organizations.

*Statistical analysis*

Descriptive analysis was done to assess demographic and anthropometric characteristics, as well as the descriptive part of nutrition literacy. All covariates with p values < 0.2 under single variable analysis were entered into the regression analysis. Ordinal regression was used to evaluate the association between nutrition literacy and the quartiles of RCDQI, as well as its components. P value <0.05 was considered to be statistically significant. Data were analyzed via SPSS (ver.24) and Nutritionist-4 (modified for Persian food) was used to assess dietary intakes.

*Ethics*

This study was conducted according to the Declaration of Helsinki and all procedures involving human subjects were approved by the local ethics committee of Shiraz University of Medical Sciences (IR-SUMS.REC.1395.S133). Written informed consents were obtained from all parents/guardians.

## Results

Total of 388 adolescents participated in this study (response rate= 92.38%), out of which 64.2% were female and total of 26% were overweight or obese. Mean and standard deviation of T-NL equaled to 65.19 ± 8.96 among all the participants. FNL was higher in girls (p=0.001); however, CNL was significantly higher in boys (p=0.015). Of all the participants, 68.1% had mentioned that they looked for information about nutrition diet or food, and the most visited sources included the Internet (18.6%), family (15.2%) and books (13.1%). Total of 26.3% were completely, and 41.3% somewhat confident that they could get nutrition-related information if they needed it. There was a significant weak and positive correlation between trust and CNL (r=0.132, p=0.013). Moreover, the barriers had a negative relationship with FNL (p=0.001) and CNL (p=0.046). Although knowledge score was significantly higher in girls compared to boys (p=0.001), their diet quality score was not accordant and mean RCDQI score was higher among boys (p=0.002). Demographic and anthropometric characteristics and general information are reported in Table 1.

Increases in T-NL enhanced diet quality in boys (OR: 1.049; CI 95% 1.001-1.098), and increases in INL had increased the odds for being in the higher quartiles of the RCDQI score by 1.13 times (CI 95% 1.033-1.236). Furthermore, increase in CNL was associated with better diet quality (OR: 1.086; CI 95% 1.016-1.161); however, FNL had no association with the RCDQI score. In girls, no associations were observed between diet quality and total nutrition literacy or its components (Table 2).

As shown in Table 3, further analysis revealed the association between intake of food items and nutrition literacy. Among boys, an increasing FNL was associated with higher sugar score quartile, which showed lower intake of sugar (OR, 1.071; 95% CI, 1.002-1.146). In addition, increase in this nutrition literacy component enhanced dairy intake in girls (OR, 1.049; 95% CI, 1.001-1.098) and improved energy balance in boys by 1.082 times (95% CI, 1.011-1.159). Increases in INL raised the odds for energy score by 8% in boys (OR, 1.080; 95% CI, 1.011-1.154) and increased CNL improved vegetable intake in this gender group (OR, 1.080; 95% CI, 1.011-1.154). Augmentations in T-NL could also lead to increased vegetable intake in male adolescents (OR, 1.043; 95% CI, 1.001-1.087).

**Table 1.** Demographic and anthropometric characteristics, and nutrition literacy, knowledge and diet quality score among study subjects

| **Demographic characteristics** | **Boys (n=139)** | **Girls (n=249)** |
|---|---|---|
| Age, mean (SD) | 14.37 (0.91) | 13.64 (0.92) |
| **Education district, n (%)** | | |
| District 1 (medium to high socio-economic status) | 92 (66.2) | 143 (57.4) |
| District 2 (low socio-economic status) | 47 (33.8) | 106 (42.6) |
| Mother education, n (%) | | |
| Illiterate & Primary education | 14 (10.6) | 26 (11.3) |
| High school & diploma | 106 (80.3) | 151 (65.3) |
| University education | 12 (9.1) | 54 (23.4) |
| **Father education, n (%)** | | |
| Illiterate & Primary education | 10 (7.7) | 18 (7.9) |
| High school & diploma | 91 (70) | 137 (60.1) |
| University education | 29 (22.3) | 73 (32.0) |
| **Anthropometric characteristics** | | |
| BMI, n (%) | | |
| Underweight | 26 (18.7) | 32 (12.9) |
| Normal weight | 73 (52.5) | 156 (62.7) |
| Overweight and Obese | 40 (28.8) | 61 (24.4) |
| **General information** | | |
| Nutrition literacy, mean (SD) | | |
| FNL | 21.17 (2.93) | 22.28 (3.68) |
| INL | 20.05 (3.39) | 19.75 (4.25) |
| CNL | 24.21 (4.20) | 22.95 (5.05) |
| T-NL | 65.35 (7.26) | 65.10 (9.83) |
| Confidence to get required nutrition information | | |
| Completely confident | 38 (27.9) | 62 (25.4) |
| Very confident | 20 (14.7) | 43 (17.6) |
| Somewhat confident | 60 (44.1) | 97 (39.8) |
| A little confident | 5 (3.7) | 17 (7) |
| Not confident at all | 13 (9.6) | 25 (10.2) |
| Barriers, mean (SD) | 20.74 (7.97) | 21.61 (6.20) |
| Trust, mean (SD) | 43.34 (9.73) | 43.37 (9.31) |
| Knowledge, mean (SD) | 52.50 (18.44) | 58.89 (16.23) |
| RCDQI score, mean (SD) | 54.50 (6.34) | 52.12 (7.44) |

*BMI, body mass index; FNL, functional nutrition literacy; INL, interactive nutrition literacy; CNL, critical nutrition literacy; T-NL, total nutrition literacy; RCDQI, revised children diet quality index.*

## Discussion

In the present study, there was a significant association between nutrition literacy and diet quality amongst adolescents, and it was shown that increase in T-NL and its components such as INL, and CNL could enhance diet quality among boys. Furthermore, increases in FNL were associated with lower sugar intake and better energy balance in boys and higher dairy intake in girls. Results are discussed in detail.

**Table 2.** Components of RCDQI, mean score, and mean intake based on score quartiles (lowest and highest quartiles) among boys and girl adolescents

| Component | Scoring criteria | Max score | Boys | | | Girls | | |
|---|---|---|---|---|---|---|---|---|
| | | | Mean score (SD) | Intake based on score quartiles, Mean (SD) | | Mean score (SD) | Intake based on score quartiles, Mean (SD) | |
| | | | | lowest | highest | | lowest | highest |
| Added sugar % | ≤10% of total energy intake | 10 | 5.88 (3.51) | 17.39 (3.71) | 10.64 (3.68) | 6.53 (3.37) | 16.16 (10.57) | 10.63 (3.34) |
| Total fat % | 25%-35% | 2.5 | 1.87 (1.01) | 34.07 (4.85) | 32.56 (3.31) | 1.91 (0.98) | 34.43 (4.15) | 32.41 (3.47) |
| Linoleic acid % | ≤ 5%-10% | 2.5 | 1.70 (1.12) | 9.90 (5.49) | 8.42 (4.23) | 1.46 (1.21) | 11.46 (6.45) | 8.04 (3.60) |
| Linolenic acid % | 0.6%-1.2% | 2.5 | 2.18 (0.53) | 0.59 (0.26) | 0.73 (0.28) | 2.14 (0.59) | 0.70 (0.32) | 0.75 (0.27) |
| DHA+EPA% | ≤10% of ALA | 2.5 | 2.40 (0.43) | 4.57 (2.91) | 3.75 (3.15) | 2.41 (0.44) | 3.27 (3.18) | 3.71 (2.93) |
| Total grain (OZ) | | | | | | | | |
| Female | 5-6 OZ | 5 | 1.30 (1.01) | 13.95 (4.97) | 12.59 (3.18) | 1.46 (1.18) | 10.79 (4.07) | 11.01 (2.39) |
| Male | 6-7 OZ | | | | | | | |
| Fruit (Cup) | | | | | | | | |
| Female | 1.5 cup | 10 | 8.68 (2.43) | 1.98 (1.27) | 3.40 (1.43) | 8.69 (2.49) | 1.81 (1.49) | 3.05 (1.35) |
| Male | 2 cup | | | | | | | |
| Vegetable (Cup) | | | | | | | | |
| Female | 2.5 cup | 10 | 6.27 (2.39) | 1.66 (0.82) | 2.13 (0.63) | 7.02 (2.70) | 1.36 (0.78) | 2.28 (0.82) |
| Male | 3 cup | | | | | | | |
| Dairy (Cup) | 3 | 10 | 5.46 (2.68) | 1.26 (0.70) | 1.98 (0.81) | 4.96 (2.89) | 0.96 (0.76) | 2.12 (0.85) |
| Iron | EAR* | 10 | 8.84 (2.50) | 13.81 (5.54) | 16.16 (3.38) | 6.08 (3.30) | 12.31 (4.93) | 14.64 (4.03) |
| Energy | Energy ± 10% of EER | 10 | 8.57 (1.48) | 2790.69 (995.78) | 2549.23 (640.09) | 8.50 (1.81) | 2158.19 (814.18) | 2206.70 (478.89) |

*RCDQI, revised children diet quality index; DHA, Docosahexaenoic acid; EPA, Eicosapentaenoic acid; ALA, α-linolenic acid; EAR, Estimated Average Requirement; EER, Estimated Energy Requirement.*

** ≤EAR = 0 points, EAR-RDA = 5 point, ≥RDA = 10 point*

**Table 3.** Association between RCDQI score quartile and nutrition literacy among boy and girl adolescents using the ordinal logistic regression (Odds ratio (OR) and 95% confidence interval (CI))

| Variables | Boys | | | Girls | | |
|---|---|---|---|---|---|---|
| | OR | 95% CI | | OR | 95% CI | |
| | | lower | upper | | lower | upper |
| FNL | 0.974 | 0.913 | 1.040 | 1.000 | 0.955 | 1.046 |
| District | | | | | | |
| 1 | 1.414 | 0.733 | 2.727 | 1.829* | 1.140 | 2.933 |
| 2 | 1.000 | | | 1.000 | | |
| knowledge | 0.976** | 0.959 | 0.993 | 1.002 | 0.988 | 1.016 |
| INL | 1.130** | 1.033 | 1.236 | 1.019 | 0.970 | 1.071 |
| District | | | | | | |
| 1 | 1.336 | 0.678 | 2.635 | 1.843* | 1.144 | 2.967 |
| 2 | 1.000 | | | 1.000 | | |
| knowledge | 0.976* | 0.959 | 0.994 | 1.000 | 0.986 | 1.015 |
| CNL | 1.086* | 1.016 | 1.161 | 1.015 | 0.978 | 1.053 |
| District | | | | | | |
| 1 | 1.241 | 0.619 | 2.489 | 2.014** | 1.243 | 3.262 |
| 2 | 1.000 | | | 1.000 | | |
| knowledge | 0.972* | 0.955 | 0.989 | 1.001 | 0.986 | 1.016 |
| T-NL | 1.049* | 1.001 | 1.098 | 1.012 | .987 | 1.037 |
| District | | | | | | |
| 1 | 1.391 | 0.685 | 2.823 | 2.083** | 1.260 | 3.442 |
| 2 | 1.000 | | | 1.000 | | |
| knowledge | 0.985 | 0.967 | 1.003 | 0.999 | 0.983 | 1.015 |

*RCDQI, revised children diet quality index; FNL, functional nutrition literacy; INL, interactive nutrition literacy; CNL, critical nutrition literacy; T-NL, total nutrition literacy.*

*District 1: medium to high socio-economic status, District 2: low socio-economic status*

**p<0.05, **p<0.01*

**Table 4.** Association between RCDQI components score quartile and nutrition literacy among boy and girl adolescents using the ordinal logistic regression (Odds ratio (OR) and 95% confidence interval (CI))

| | Sugar score | | Dairy score | | Vegetable score | | EER score | |
|---|---|---|---|---|---|---|---|---|
| | OR | CI 95% | OR | CI 95% | OR | CI 95% | OR | CI 95% |
| **Boys** | | | | | | | | |
| FNL | 1.071* | 1.002-1.146 | 0.950 | 0.888-1.016 | 1.012 | 0.947-1.082 | 1.082* | 1.011-1.159 |
| INL | 1.038 | 0.952-1.131 | 1.062 | 0.974-1.157 | 1.057 | 0.969-1.154 | 1.089* | 1.082-1.098 |
| CNL | 1.012 | 0.949-1.080 | 1.047 | 0.981-1.117 | 1.080* | 1.011-1.154 | 0.968 | 0.907-1.033 |
| T-NL | 1.041 | 0.998-1.086 | 1.013 | 0.973-1.054 | 1.043* | 1.001-1.087 | 0.994 | 0.955-1.034 |
| **Girls** | | | | | | | | |
| FNL | 0.958 | 0.914-1.003 | 1.049* | 1.001-1.098 | 1.020 | 0.974-1.068 | 0.999 | 0.954-1.046 |
| INL | 1.000 | 0.952-1.050 | 1.028 | 0.979-1.080 | 0.970 | 0.924-1.019 | 1.052 | 1.001-1.106 |
| CNL | 1.008 | 0.971-1.046 | 0.992 | 0.956-1.030 | 0.997 | 0.961-1.035 | 1.036 | 0.996-1.076 |
| T-NL | 0.995 | 0.974-1.016 | 1.012 | 0.991-1.034 | 0.996 | 0.975-1.017 | 1.018 | 0.997-1.040 |

*RCDQI, revised children diet quality index; EER, Estimated Energy Requirements; FNL, functional nutrition literacy; INL, interactive nutrition literacy; CNL, critical nutrition literacy; T-NL: total nutrition literacy.*

*Knowledge, district (distric 1, district 2), BMI entered the ordinal analysis as covariates.*

**p<0.05*

*Diet quality in adolescents*

Our RCDQI score was lower compared to other studies (13, 18), mainly because we did not have a score for whole grains and excess fruit juice consumption.-

In our study, mean percentage of added sugar was higher than the amount recommended by dietary guideline (less than 10% of calories per day), which is in line with other studies in Iran (19-21). Although mean intake of total fat met the recommended amount, it was higher than the optimal level of 30%. It seems that fat intake has increased in recent years among Iranian adolescents, which is in line with the consumption of fast-foods and processed foods (22, 23). The main staple food in Iran are refined wheat and rice, forming the main portion of our carbohydrate intake (24). Therefore, policy-makers should make whole grain products more accessible and affordable for everyone in order to reach the dietary recommendations of whole grains as a preventive measure to reduce the increasing rate of non-communicable diseases.

In this study, vegetable intake was very low amongst the adolescent population, which was congruent with a systematic review in Iran (25). Despite schools' free-milk-distribution program implemented by the Iranian Ministry of Health and Medical Education, present study shows that mean intake of dairy products is lower than the recommended amount, and in 2014, Iran's per capita consumption of milk and dairy products was announced at almost half the world average (26).

Overall, most of the RCDQI components assessing adolescents' diet quality requires more attention and improvement that should be considered in nutritional education programs and policies.

*Nutrition literacy in adolescents*

In spite of relative enhancement of nutrition knowledge following educational interventions, limited improvements were observed in dietary behaviors (27). This may be due to the failure of those interventions to improve nutrition literacy as an important mediator between nutrition-related knowledge and practice (8). Different aspects of nutrition literacy are discussed separately as follow.

Knowledge: Knowledge was higher among girls, which was not in line with their T-NL and diet quality in our study. In a systematic review, four out of nine studies revealed that females had greater food knowledge than males, and one found that females had poorer dietary practices despite their greater nutritional knowledge, which is consistent with the present study (8). Evidence indicates that knowledge alone is usually not enough to change individual behaviors such as dietary choices (8).

Sources of knowledge: In our population, the most visited sources of nutrition and food information included the Internet, family and books. In Cash's study, dietitians, nutritionists and general physicians were the three most preferred sources, and were considered as most trustworthy, credible and effective. However, in line with our study, the most utilized sources of nutrition information were the Internet, friends, family and magazines (28). Zoellner et al., reported that the Internet is not a frequently used source of nutrition information among adults (29), and other studies found the major sources of food knowledge to be the family among adults (30-32) and relative classes among undergraduate students (31).

National surveys in the United State identified the Internet as the most popular source of health information (29). There is a huge amount of scientific and non-scientific information and biased advertising and news on Internet, but teenagers hardly ever refer to scientific data. These facts suggest that adolescents should be educated on how and where to find valid information.

Trust: In present study, there was significant, weak, positive correlation between trust and CNL. In Zoellner's study (29), nutrition literacy was significantly associated with the level of trust toward information sources, and the Internet was identified as the least trusted source of nutritional information by adults.

Confidence: Only about one forth (26.3%) of the adolescents were completely confident that they could get nutrition-related information if they needed it. Nutrition literacy was higher in people who reported a higher level of confidence, but the relationship was not statistically significant. Our result was in agreement with Zoellner's study in which adults with lower literacy level had less confidence in their ability to obtain nutrition information, but the trend was not sig-

nificant (29). However, in Ghaddar's study, health literacy was positively associated with self-efficacy (33). It seems that planners and policy-makers should raise confidence levels among this age-group in order to inspire better choice of nutritional information sources.

Barriers: There were significant negative relationships between functional and critical NL with the barriers to find food and nutrition information in our study. Zoellner et al., noted that adults with lower nutrition literacy rated higher for barriers to seek nutrition information than those with adequate literacy, but the trend was not significant (29).

Functional, Interactive and critical nutrition literacy: In this study, increases in INL, CNL, and T-NL had significantly enhanced diet quality among boys, but FNL was not associated with the RCDQI score. In girls, no association was observed between diet quality and T-NL or its components. In recent years, nutritional information has been delivered through textbooks and school health programs. In addition, access to various educational resources such as health channels and social networks has helped the teenagers to increase their knowledge, and consequently their FNL. Nonetheless, this knowledge by itself cannot improve nutritional behavior as it was not designed to affect their skills, motivation or behavior.

There was an association between food item intake and nutrition literacy in the present study. Increased FNL was associated with lower sugar intake and improved energy balance in boys and enhanced dairy intake in girls. Increased INL could increase the energy score and increases in CNL and T-NL could lead to increased vegetable intake. Since osteoporosis has become a public health concern in recent years, especially among women, many health and nutrition education programs have been assigned to this issue. The same is true for obesity and its related problems in both genders; therefore, it is expected that adolescents have better nutrition literacy in these contexts.

In a systematic review of 9 studies, only one had assessed the relationship between nutritional skills and dietary intake, and found an association between more frequent food preparation (as an interactive literacy), and increased fruit consumption in young boys, as well as increased fruit and vegetable intake in girls. It also had a negative association with the consumption of junk food items such as soft drinks in girls and fried foods in boys (8). INL such as frequency of reading food label was not associated with dietary intake in Huang et al., study (34). In another study, nutrition-based health literacy predicted lower fat intake, but it was not a significant predictor of fruit and vegetable intake among college students (7).

Few studies have assessed the relationship between nutrition literacy and food behavior in children and adolescents. Different tools were used to examine nutrition literacy in those studies, which mostly did not separate the various dimensions of nutrition literacy as we did. Thus, the studies are not easily comparable. Although the field of health literacy has grown immensely, it is still relatively new and there are still ongoing debates regarding its construct and measurement. Thus, further studies using multi-dimensional nutrition literacy questionnaire similar to what we used is highly recommended.

## Conclusion

In recent years, nutritional education programs have increased among adolescents, and they have been successful in increasing nutritional knowledge. However, they have not been sufficient in promoting nutritional behavior. Furthermore, with increased access to information and communication technologies, the expected plan is to go beyond just delivering nutritional information, but also to develop higher cognitive and behavioral skills to promote healthy eating habits. Nutrition literacy, as a combination of knowledge, cognitive and behavioral skills, has the potential to resolve this issue and improve healthy decision-making regarding eating habits.

Nutrition literacy and its components have significant association with diet quality in adolescents, hence, public healthcare planners and policy-makers should develop new public health strategies with a focus on increased understanding of food literacy among adolescents, especially girls.

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Original article

# Determination of obesity, stunting, and nutritional habits in disabled children and adolescents

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**Summary.** *Objective:* This study was conducted to determine obesity, stunting and nutritional habits in children and adolescents with disabilities. *Methods:* The study was carried out in 612 disabled children and adolescents in a 2-19 age group enrolled in 8 special education practice and rehabilitation institutions in Kırıkkale city center in Turkey. The general characteristics and eating habits of the participants were examined and body mass indexes (BMI) were calculated (n=527) to determine obesity and stunting. *Results:* Of the participants (n=612), 39.2% were female and 60.8% were male, of whom 39.4% were mentally disabled, 37.1% were physically disabled, 12.3% were mentally and physically disabled, and 11.3% were suffering other types of disabilities (speech disorders, learning disability, etc.). Of the participants (n=527), 18.8% were overweight and 17.8% were obese. The rate of overweight (Male:18.5%, Female:19.2%) and obesity (Male:19.1%, Female:15.9%) was higher in males compared to females ($p>0.05$). The correlation between BMI classification and disability type, disability level, and income level was not statistically significant ($p>0.05$). The rate of stunting was 24.5%, which was lower in males compared to females (Male:20.4%, Female:30.8%) ($p>0.05$). There was no statistically significant difference between height-for-age classifications and the type of disability ($p>0.05$); however, the difference between disability level and income level was significant ($p<0.05$). It was observed that 50.0% of the participants had eating problems and 45.5% had the habit of skipping breakfasts. *Conclusion:* In this study, it was observed that overweight, obesity, and stunting are very high in disabled children and adolescents. This field warrants further research.

**Key words:** disabled children and adolescents, obesity, stunting, nutrition

## Introduction

It was reported by the World Health Organization that approximately 15% of the world population have disabilities, and approximately 93 million children (5.1%) under the age of 15 have a moderate and severe disability (1). Due to several factors, such as unhealthy eating habits and inadequate physical activity, overweight and obesity are seen more frequently in mentally and/or physically disabled children and adolescents compared to their non-disabled peers (2). In addition to obesity, undernutrition (low weight, stunting) is also an important health problem for disabled children and adolescents (3, 4).

The proportion of the disabled population to the total population in Turkey is 12.3%, of which, 4.2% is in the 0-9 age group and 4.6% is in the 10-19 age group (5). There exist few studies reporting obesity, stunting, and eating habits in disabled children and adolescents in Turkey (3,6,7).

This study was conducted to determine obesity, stunting, and nutrition habits of children and adolescents with disabilities.

## Materials and Methods

This is a descriptive study, which depicts the participants in an accurate way, involving disabled individuals who are enrolled in all special education, practice, and rehabilitation institutions in Kırıkkale city center

and it was carried out under the project supported by the Ministry of Family and Social Policy, The Support Program for the Disabled (EDES) in cooperation with the Provincial National Education Directorate under the coordination of Kırıkkale Governorship. The project was a comprehensive effort to determine the nutritional status of the disabled individuals and their families, and some of the data on disabled children and adolescents were reported in this article.

The universe of the study was 679 disabled children and adolescents in the age range of 2-19 years attending special education practice and rehabilitation institutions in Kırıkkale city center in 2016. Since the disabled children and adolescents have different types of disabilities, such as mental, hearing, visual, orthopedic, Down syndrome, and autism spectrum disorder, no sampling was carried out and the whole universe was covered by the complete count method. In total, 612 children and adolescents who had provided informed consent from their parents and 305 mothers who answered the questionnaire were included in the study (Table 1).

The heights and body weights of the participants were measured. However, some of them were uneasy when taking the measurements and did not allow a complete measurement taking process, and some others could not be measured at all due to their disability status. For this reason, the obesity and stunting status of 527 children and adolescents in whom both height and body weight measurements were taken together were evaluated.

TANITA BC418MA and TANITA BC545N (for < 7 years old) scales for body weight measurements and a TANITA portable stadiometer for height measurement were used for measurements, which were made in accordance with proper techniques (8). As no international comparable reference values were available in the assessment of anthropometric measurements for disabled children and adolescents, obesity and stunting were determined using the BMI and height-for-age percentiles for healthy children and adolescents according to WHO (9,10). Based on these criteria, the following categories were developed: <3. percentile underweight/stunted, ≥3.- <15. percentile risk of underweight/short, ≥15.-<85. percentile normal, ≥85.-<97. percentile overweight / tall, and ≥97. percentile obese/very tall.

In the study, the types of disabilities were classified as "mentally disabled", "physically disabled", "mentally and physically disabled", and "other". In the "other" group, those who are not mentally or physically disabled, but with language and speech disorders, special learning difficulties, or pervasive developmental disorders were included.

Monthly income status of the families was investigated and evaluated according to the official minimum wage in Turkey. The official net minimum wage as of 2016 in Turkey was 1300.99 TL (Turkish liras) ($ 433.66) (11). Based on this number, lower than 1000 TL was classified as "very low", 1000-2000 TL as "low", 2001-4000 TL as "medium", and above 4000 TL as "high" income level.

The data were assessed based on the responses to the questionnaire and anthropometric measurements. Descriptive statistics are provided as number and percentage for categorical variables and median (Interquartile Range-IQR) for continuous variables that do not fit normal distribution.

Diagonal tables were created to analyze categorical variables and Chi-square values were calculated on the appropriate tables. The Mann-Whitney U test was used for comparisons by gender, and the Kruskal-Wallis non-parametric variance analysis was used for comparisons by age groups for continuous variables. Mann-Whitney U test with Bonferroni correction was applied in post-hoc binary comparisons to determine the different age group when the variance analysis revealed a difference. For statistical analysis and calculations, IBM SPSS Statistics 22.0 (12) and MS-Excel 2010 programs were used. Statistical significance level was accepted as $p < 0.05$.

This study was approved by Kırıkkale University Ethical Committee of Social Sciences and Humanities Research.

## Results

Among the children and adolescents participating in the study (n=612), 39.2% (n=240) were female and 60.8% (n=372) were male, and the median age was 11.0 (IQR=6.0) for both genders. Of the participants, 39.4% were mentally, 37.1% were physically, and 12.3%

were mentally and physically disabled, and 11.3% had other types of disabilities (language and speech impairment, special learning difficulty, etc.) (Table 1).

Based on the BMI values, 18.8% of the participants were overweight and 17.8% were obese. While overweight (M: 18.5%, F: 19.2%) and obesity (M: 19.1%, F: 15.9%) rates were higher in males than females, the difference was not statistically significant ($\chi^2$=2.156, p=0.707). There was no statistically significant difference between the BMI classification and age groups ($\chi^2$=14.924; p=0.246). Of the participants, 14.2% had short stature and 24.5% were stunted. Short stature (M: 13.8%, F: 14.9%) was higher in males and stunting was higher in females (M: 20.4%, F: 30.8%), but the difference was not statistically significant ($\chi^2$= 8.169, p=0.086). No statistically significant difference was found between height-for-age classifications and the age groups (p> 0.05) (Table 2).

Obesity (21.2%) was higher in mental and physical disabilities, and overweight (21.3%) was higher

**Table 1.** Demographic data of the disabled children and adolescents participating in the study

| | | Female n (%) | Male n (%) | Total n (%) | Female vs Male |
|---|---|---|---|---|---|
| Gender | | 240 (39.2) | 372 (60.8) | 612 (100.0) | |
| Age (Year) | 2-5 | 25 (10.4) | 35 (9.4) | 60 (9.8) | $\chi^2$=0.602; p=0.896 |
| | 6-9 | 62 (25.8) | 92 (24.7) | 154 (25.2) | |
| | 10-14 | 94 (39.2) | 157 (42.2) | 251 (41.0) | |
| | 15-19 | 59 (24.6) | 88 (23.7) | 147 (24.0) | |
| | **Total** | 240 (100.0) | 372 (100.0) | 612 (100.0) | |
| | **Median (IQR)** | 11.0 (6.0) | 11.0 (6.0) | 11.0 (6.0) | Z=0.079; p=0.937 |
| Disability Type | Mental | 95 (39.6) | 146 (39.3) | 241 (39.4) | $\chi^2$=4.332; p=0.229 |
| | Physical | 97 (40.4) | 130 (34.9) | 227 (37.1) | |
| | Mental and physical | 22 (9.2) | 53 (14.2) | 75 (12.3) | |
| | Others | 26 (10.8) | 43 (11.6) | 69 (11.3) | |
| | **Total** | 240 (100.0) | 372 (100.0) | 612 (100.0) | |
| Disability Level (%)* | 0-25 | 14 (5.8) | 24 (6.5) | 38 (6.3) | $\chi^2$=1.514; p=0.679 |
| | 26-50 | 100 (42.0) | 136 (37.1) | 236 (39.0) | |
| | 51-75 | 62 (26.1) | 105 (28.6) | 167 (27.6) | |
| | 76-100 | 62 (26.1) | 102 (27.8) | 164 (27.1) | |
| | **Total** | 238 (100.0) | 367 (100.0) | 605 (100.0) | |
| Education Level*§ | Illiterate | 42 (44.7) | 57 (40.1) | 99 (41.9) | $\chi^2$=2.321; p=0.677 |
| | Literate | 20 (21.3) | 37 (26.1) | 57 (24.2) | |
| | Primary school | 17 (18.1) | 32 (22.5) | 49 (20.8) | |
| | Secondary school | 9 (9.6) | 10 (7.0) | 19 (8.1) | |
| | High school | 6 (6.4) | 6 (4.2) | 12 (5.1) | |
| | **Total** | 94 (100.0) | 142 (100.0) | 236 (100.0) | |
| Income level* | Very low | 45 (33.8) | 52 (28.1) | 97 (30.5) | $\chi^2$=2.972; p=0.396 |
| | Low | 58 (43.6) | 90 (48.6) | 148 (46.5) | |
| | Middle | 24 (18.0) | 39 (21.1) | 63 (19.8) | |
| | High | 6 (4.5) | 4 (2.2) | 10 (3.1) | |
| | **Total** | 133 (100.0) | 185 (100.0) | 318 (100.0) | |

**: Only those participated in the survey were included in the calculations.*

*§ : 6-year and younger children were not included.*

**Table 2.** The distribution of the disabled children and adolescents participating in the study based on BMI values by age group and gender, height by age classification

| Age (Year) | Gender (n) | Total n | BMI | | | | | | Height-for-age | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Underweight n (%) | Risk of underweight n (%) | Normal n (%) | Overweight n (%) | Obese n (%) | $\chi^2$; p | Stunted n (%) | Short n (%) | Normal n (%) | Tall n (%) | Very tall n (%) | $\chi^2$; p |
| 2-5 | Female | 18 | 4 (22.2) | 0 (0.0) | 7 (38.9) | 5 (27.8) | 2 (11.1) | N/A | 10 (55.6) | 1 (5.6) | 6 (33.3) | 1 (5.6) | 0 (0.0) | N/A |
| | Male | 28 | 3 (10.7) | 1 (3.6) | 16 (57.1) | 4 (14.3) | 4 (14.3) | | 9 (32.1) | 2 (7.1) | 13 (46.4) | 3 (10.7) | 1 (3.6) | |
| | Total | 46 | 7 (15.2) | 1 (2.2) | 23 (50.0) | 9 (19.6) | 6 (13.0) | | 19 (41.3) | 3 (6.5) | 19 (41.3) | 4 (8.7) | 1 (2.2) | |
| 6-9 | Female | 53 | 5 (9.4) | 5 (9.4) | 27 (50.9) | 7 (13.2) | 9 (17.0) | 0.697; 0.952 | 18 (34.0) | 6 (11.3) | 20 (37.7) | 8 (15.1) | 1 (1.9) | 0.348; 0.555 |
| | Male | 79 | 5 (6.3) | 7 (8.9) | 39 (49.4) | 12 (15.2) | 16 (20.3) | | 16 (20.3) | 15 (19.0) | 43 (54.4) | 2 (2.5) | 3 (3.8) | |
| | Total | 132 | 10 (7.6) | 12 (9.1) | 66 (50.0) | 19 (14.4) | 25 (18.9) | | 34 (25.8) | 21 (15.9) | 63 (47.7) | 10 (7.6) | 4 (3.0) | |
| 10-14 | Female | 83 | 5 (6) | 8 (9.6) | 37 (44.6) | 19 (22.9) | 14 (16.9) | 0.829; 0.935 | 20 (24.1) | 16 (19.3) | 44 (53.0) | 1 (1.2) | 2 (2.4) | 2.722; 0.099 |
| | Male | 135 | 9 (6.7) | 15 (11.1) | 55 (40.7) | 28 (20.7) | 28 (20.7) | | 25 (18.5) | 17 (12.6) | 82 (60.7) | 9 (6.7) | 2 (1.5) | |
| | Total | 218 | 14 (6.4) | 23 (10.6) | 92 (42.2) | 47 (21.6) | 42 (19.3) | | 45 (20.6) | 33 (15.1) | 126 (57.8) | 10 (4.6) | 4 (1.8) | |
| 15-19 | Female | 54 | 2 (3.7) | 6 (11.1) | 29 (53.7) | 9 (16.7) | 8 (14.8) | 4.795; 0.309 | 16 (29.6) | 8 (14.8) | 30 (55.6) | 0 (0.0) | 0 (0.0) | 3.187; 0.074 |
| | Male | 77 | 7 (9.1) | 14 (18.2) | 28 (36.4) | 15 (19.5) | 13 (16.9) | | 15 (19.5) | 10 (13.0) | 48 (62.3) | 3 (3.9) | 1 (1.3) | |
| | Total | 131 | 9 (6.9) | 20 (15.3) | 57 (43.5) | 24 (18.3) | 21 (16.0) | | 31 (23.7) | 18 (13.7) | 78 (59.5) | 3 (2.3) | 1 (0.8) | |
| Total | Female | 208 | 16 (7.7) | 19 (9.1) | 100 (48.1) | 40 (19.2) | 33 (15.9) | 2.156; 0.707 | 64 (30.8) | 31 (14.9) | 100 (48.1) | 10 (4.8) | 3 (1.4) | 8.535; 0.074 |
| | Male | 319 | 24 (7.5) | 37 (11.6) | 138 (43.3) | 59 (18.5) | 61 (19.1) | | 65 (20.4) | 44 (13.8) | 186 (58.3) | 17 (5.3) | 7 (2.2) | |
| | Total | 527 | 40 (7.6) | 56 (10.6) | 238 (45.2) | 99 (18.8) | 94 (17.8) | | 129 (24.5) | 75 (14.2) | 286 (54.3) | 27 (5.1) | 10 (1.9) | |

*N/A: incalculable*

in physical disabilities, but no statistically significant difference was found between BMI classification and the type of disability, disability level, and income level ($p>0.05$). Stunting was the highest (30.3%) in participants with mental and physical disabilities, and there was no statistically significant difference between height-for-age classifications and the type of disability ($\chi^2=15.699$; $p=0.205$).

On the other hand, there was a significant difference between height classification and disability and income levels ($\chi^2=27.782$; $p=0.006$ and $\chi^2=8.208$; $p=0.004$, respectively). It was observed that stunting rate in those with over 50% disability level was significantly higher than that of those with 50% or less disability level ($\chi^2=19.903$; $p=0.001$). Similarly, average height was lower in those with low or very low income compared to those with middle or high income level ($\chi^2=10.219$; $p=0.037$) (Table 3).

Fifty percent of the participants had eating problems, 38.5% of them had a habit of skipping a main meal, the most frequently skipped meal was breakfast (45.5%), and 18.8% of them consumed rice-pasta, 18.8% potato chips-French fries, 13.2% chocolate-wafers, and as drinks, 37.6% consumed milk-ayran (yoghurt with water), 27.4% cola-carbonated beverages, and 24.0% consumed prepackaged fruit juices. In addition, 8.5% of the participants were found to be using nutritional supplements (Table 4).

## Discussion

In this study, the majority of the participants were male, and mental disability (39.4%) was higher than physical disability (37.1%), although not statistically significant, and the level of disability of the 54.7% of the participants were over 50%. Similarly, it was reported by Kaya et al. that

**Table 3.** The distribution of the disabled children and adolescents based on BMI values by disability type, disability level, and income level, and height-for-age.

| | | | BMI | | | | | | Height-for-age | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Total n | Under weight (%) | Risk of underweight (%) | Normal n (%) | Over weight n (%) | Obese n (%) | $\chi^2$; p | Stunted n (%) | Short n (%) | Normal n (%) | Tall n (%) | Very Tall (%) | $\chi^2$; p |
| Disability Type | MD | 223 | 16 (7.2) | 24 (10.8) | 103 (46.2) | 35 (15.7) | 45 (20.2) | 20.072; 0.066 | 41 (18.4) | 31 (13.9) | 133 (59.6) | 14 (6.3) | 4 (1.8) | 15.699; 0.205 |
| | PD | 183 | 21 (11.5) | 15 (8.2) | 82 (44.8) | 39 (21.3) | 26 (14.2) | | 53 (29.0) | 23 (12.6) | 96 (52.5) | 6 (3.3) | 5 (2.7) | |
| | MPD | 66 | 1 (1.5) | 6 (9.1) | 33 (50.0) | 12 (18.2) | 14 (21.2) | | 20 (30.3) | 13 (19.7) | 29 (43.9) | 4 (6.1) | 0 (0.0) | |
| | Others | 55 | 2 (3.6) | 11 (20.0) | 20 (36.4) | 13 (23.6) | 9 (16.4) | | 15 (27.3) | 8 (14.5) | 28 (50.9) | 3 (5.5) | 1 (1.8) | |
| | Total | 527 | 40 (7.6) | 56 (10.6) | 238 (45.2) | 99 (18.8) | 94 (17.8) | | 129 (24.5) | 75 (14.2) | 286 (54.3) | 27 (5.1) | 10 (1.9) | |
| Disability Level (%) | 0-25 | 31 | 2 (6.5) | 3 (9.7) | 17 (54.8) | 5 (16.1) | 4 (12.9) | 16.456; 0.171 | 3 (9.7) | 3 (9.7) | 20 (64.5) | 5 (16.1) | 0 (0.0) | 27.782; **0.006** |
| | 26-50 | 223 | 13 (5.8) | 33 (14.8) | 100 (44.8) | 39 (17.5) | 38 (17.0) | | 39 (17.5) | 34 (15.2) | 132 (59.2) | 14 (6.3) | 4 (1.8) | |
| | 51-75 | 142 | 8 (5.6) | 10 (7.0) | 66 (46.5) | 31 (21.8) | 27 (19.0) | | 42 (29.6) | 21 (14.8) | 70 (49.3) | 6 (4.2) | 3 (2.1) | |
| | 76-100 | 126 | 17 (13.5) | 10 (7.9) | 52 (41.3) | 23 (18.3) | 24 (19.0) | | 42 (33.3) | 17 (13.5) | 63 (50.0) | 2 (1.6) | 2 (1.6) | |
| | Total | 522 | 40 (7.7) | 56 (10.7) | 235 (45.0) | 98 (18.8) | 93 (17.8) | | 126 (24.1) | 75 (14.4) | 285 (54.6) | 27 (5.2) | 9 (1.7) | |
| Income Level | Very low | 69 | 8 (11.6) | 4 (5.8) | 35 (50.7) | 9 (13.0) | 13 (18.8) | 19.228; 0.083 | 25 (36.2) | 14 (20.3) | 29 (42.0) | 1 (1.4) | 0 (0.0) | 8.208; **0.004** |
| | Low | 105 | 6 (5.7) | 12 (11.4) | 48 (45.7) | 22 (21.0) | 17 (16.2) | | 31 (29.5) | 7 (6.7) | 63 (60.0) | 3 (2.9) | 1 (1.0) | |
| | Middle | 53 | 3 (5.7) | 0 (0.0) | 23 (43.4) | 14 (26.4) | 13 (24.5) | | 19 (35.8) | 3 (5.7) | 26 (49.1) | 2 (3.8) | 3 (5.7) | |
| | High | 9 | 0 (0.0) | 1 (11.1) | 3 (33.3) | 3 (33.3) | 2 (22.2) | | 0 (0.0) | 2 (22.2) | 4 (44.4) | 1 (11.1) | 2 (22.2) | |
| | Total | 236 | 17 (7.2) | 17 (7.2) | 109 (46.2) | 48 (20.3) | 45 (19.1) | | 75 (31.8) | 26 (11.0) | 122 (51.7) | 7 (3.0) | 6 (2.5) | |

*MD: Mental Disability, PD: Physical Disability, MPD: Mental and Physical Disability, Others: Speech impairment, learning disability, etc.*

most of the disabled individuals under 18-year age group were males, the rate of mental disability was higher than that of other types of disabilities, and 53.4% of the participants had over 60% disability level (13). The World Health Organization reported that disability is more common at low socio-economic levels (1). In this study, similarly, the majority of the families (77.0%) were found to have "very low" and "low" income levels.

Rimmer et al. reported that overweight and obesity were seen more frequently in male children and adolescents with disability (14), Llyod et al. reported that they were seen more frequently in girls (15), and Mikulovic et al. reported that there is no difference between genders (15). In addition, overweight and obesity have been reported to increase with age (3,14,15). Overweight and obesity were reported to be similar in 8-11 and 12-18 age groups (15). In the present study, obesity was found to be higher in males than females, although not statistically significant, which is consistent with the literature. In terms of age groups, obesity was higher in 10-14 age group compared to the other age groups.

In addition to obesity, low weight and stunting are also health problems seen in disabled children and adolescents (3,4). In Iran, it was reported that stunting rate was high in 6-12 year-old physically disabled children and it was more common in females (F:46.3%, M:38.5%) (4). In another study, it was stated that the rate of stunting in 10-18 year age group mentally handicapped individuals was 18.6%, it increased with age, and it was higher in females (F: 37.5%, M: 21.7%) (3). In the present study, general stunting frequency was found to be high (24.5%) and it was higher in females, but the difference was not statistically significant ($p>0.05$). It is considered that the reasons for the high incidence rate of stunting in the present study may be the nutritional problems that adversely affect normal growth and development in children and adolescents with disabilities and poor income levels of families. In addition, 10.6% (n=56) of the participants were found to be both overweight/obese and stunting/short, and 23.1% (n=122) were found to be normal in terms

**Table 4.** The distribution of the eating habits of the disabled children and adolescents (n=201)*

| | | n (%) |
|---|---|---|
| Eating problems (lack of appetite, chewing, swallowing problems, being obsessive /addictive about certain foods etc.) | | 100 (50.0) |
| Skipping main meals | | 77 (38.5) |
| The most frequently skipped meal (n=85) | Breakfast | 35 (45.5) |
| | Lunch | 36 (46.7) |
| | Dinner | 6 (7.8) |
| Habit of having snacks | | 173 (86.5) |
| The number of having snacks(times/day) (n=203) | 1 | 48 (27.7) |
| | 2 | 68 (39.4) |
| | 3 | 41 (23.7) |
| | >3 | 16 (9.2) |
| | Dairy (Yoghurt, cheese) | 9 (7.0) |
| | Meat, chicken, fish | 16 (12.5) |
| | Soudjouk, salami, sausage | 6 (4.7) |
| | Egg | 6 (4.7) |
| Food types preference (n=144) | Rice, pasta, soup | 24 (18.8) |
| | Pie, pastry | 6 (4.7) |
| | Fresh fruit | 15 (11.7) |
| | Dessert (rice pudding, pudding etc.) | 5 (3.9) |
| | Chocolate, wafers | 17 (13.2) |
| | Potato chips, French fries | 24 (18.8) |
| | Milk, ayran | 55 (37.6) |
| | Water | 9 (6.2) |
| Drink types preference (n=164) | Tea | 7 (4.8) |
| | Prepackaged fruit juices | 35 (24.0) |
| | Cola, carbonated beverages | 40 (27.4) |
| The use of nutritional supplements (n= 17) | | 17 (8.5) |
| The type of supplement used (n=17) | Mineral supplement | 2 (11.8) |
| | Vitamin supplement | 8 (47.1) |
| | Mineral-vitamin supplement | 4 (23.5) |
| | Other (omega-3, herbal products, etc.) | 3 (17.6) |

**Only those participated in the survey were included in the calculations.*

of height and body weight with regard to age and sex. This outcome suggests that more effective efforts should be carried out to improve the nutritional status of children and adolescents with disabilities.

It was reported that the prevalence of obesity is higher in children and adolescents with disabilities compared to their peers (17). In a meta-analysis study conducted by Maiano et al., mentally disabled adolescents were reported to have a 1.54 fold higher risk of overweight than their non-disabled peers, while obesity was reported to be 1.80 fold more likely to occur in the same group (18). In this study, there was no statistically significant difference between BMI classifications and the type of disability. Banks reported that poor living conditions brought by poverty, especially in low and middle-income countries, increased the risk

of disability (19). Overweight and obesity prevalence in this study is higher in high-income families in the present study, but this result is not statistically significant ($p>0.05$). Similarly, stunting was higher in both mentally and physically disabled participants compared to the other three disability types (30.3%), and there was no statistically significant difference between height-for-age values and disability types ($p>0.05$). In a previous study, stunting was observed in 12.8% of the healthy children in a 7-15 year age group, and stunting rate was reported to be higher in a school with a low socioeconomic level compared to a school with a high socioeconomic level (20).

In the present study, there was a statistically significant difference between height-for-age and disability and income levels ($p<0.05$). Stunting in children and adolescents with over 50% disability level was significantly higher compared to those with 50% or less disability level ($p<0.05$). On the other hand, children with "very low" and "low" income levels were found to be shorter than children with "middle" and "high" income levels ($p<0.05$).

The eating problems of the disabled people, such as difficulties in chewing and swallowing, being addictive to certain food types, and being obsessive about certain food types, cause them to take inadequate or excessive energy (21). In the present study, it was observed that 50.0% of the participants had various eating problems. Inadequate and unbalanced eating habits of the disabled children and adolescents have been shown in various studies (4,22,23). In this study, the high prevalence of stunting and obesity also suggest that the disabled children and adolescents may not have adequate and balanced nutrition. Inadequate quality and quantity of dietary intake of the disabled people affects their health negatively (22). It is reported that 3 main meals should be consumed for adequate and balanced nutrition, and it is also declared that if necessary, snacks should be consumed, and because breakfast is the first source of energy in a day, it should not be skipped for continuation of cognitive and physical performance (24). In the present study, it was observed that the majority of the disabled children and adolescents had 3 main meals and 2 snacks. Of those who skipped the main meal, 45.5% skipped the breakfast, the most important meal of the day. Banta et al. reported that mentally disabled children in the age range of 5-11 years consume more soda/sugary drinks, fried potatoes and fast-food compared to their mentally non-disabled peers (23). In this study, In the present study, it was observed that carbohydrate foods, such as rice and pasta, and oily foods (potato chips, French fries) were preferred by the disabled children and adolescents, followed by sugary (chocolate, wafer) foods. It was determined that milk and ayran were the most preferred beverages, and the second most frequent beverage group was cola and carbonated beverages.

It is known that some families have their children use vitamin / mineral supplements because of the dietary problems their children are suffering. In a previous study, 56% of the children with autism spectrum disorder received multivitamin / mineral supplement (25). In the present study, the consumption of nutritional supplementation was not common (8.5%).

In the present study, it was observed that overweight, obesity, and stunting are very high among the disabled children and adolescents. In order to prevent this, families should gain consciousness about adequate and balanced nutrition, and feeding of the disabled, and should cooperate with a multidisciplinary health team including a dietitian. There is a need for large-scale research regarding the assessment criteria of the anthropometric parameters and the assessment of the nutritional status of the disabled children and adolescents.

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Original Article

# Is the design of dietary based studies enough with a quantitative description?

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**Summary.** *Objective:* A proper diet, from a macronutrients quantitative point of view, does not imply it is qualitatively correct. We demonstrate that quantitative dietary advice may be qualitatively deficient. *Design and setting:* A search for suitable recommendations of macronutrients contribution to total energy intake was performed. Two similar quantitative (15-25% proteins, 45-60% carbohydrates and 20-35% fat) but qualitatively different weekly menus were designed using DIAL software. Menu A provides more fibre, MUFAs, vitamins, minerals and minor components of the diet than menu B and PUFAs/SFA index is higher. In menu B, SFA, cholesterol, glucose and sucrose levels are higher. *Results:* Menu A has better qualitative contribution of carbohydrates, fat, vitamins and minerals; it is better adjusted to the dietary reference values, and provides more phytochemicals components. It also has more fibre and less simple sugar. Menu B provides plenty of fructose mainly from soft drinks. *Conclusions:* Diet may be deficient if only planned taking in consideration the quantitative aspect. All of this can generate wrong study analyses and conclusions.

**Key words:** diet habit, nutrients, qualitative evaluation, quantitative evaluation

## Introduction

The analysis of the populations' dietary patterns is a topical issue. The traditional Mediterranean diet (MD) pattern (1), and somewhat less known, Japanese model (2), arouse interest of experts for their protective action against chronic diseases (cardiovascular diseases (CVD) (1, 2), cancer (3), diabetes (4, 5), neuronal and degenerative diseases (6). However, it must be kept in mind that the key to these two dietary patterns is part of a healthy lifestyle (7, 8). Thus, efforts over the past 50 years have led to set of science-based nutritional requirements and dietary guidelines, in order to be transmitted to professionals in the field and society. Scientific authorities are responsible for issuing these nutritional recommendations, understood by Mataix-Verdú (2009) (9) as a series of mainly quantitative parameters, which if followed by individuals, allows them have an "optimal" health. In this case, three recommendations of organisms of great recognition in Spain and Europe have been selected: Spanish Society for the Study of Obesity (SEEDO), Fundación Española de la Nutrición (FEN) and European Food Safety Authority (EFSA).

SEEDOs recommendations are collected in the Federación Española de Sociedades de Nutrición, Alimentación y Dietética (*FESNAD)-SEEDO* Consensus (2011) (10), gathered after the review of existing scientific data to make recommendations to the maximum evidence, and whose principal objective is to prevent obesity and its co-morbidities. In the document, it is recommended that consumption of carbohydrates equals or exceeds 50% of total energy intake (recommendation grade C, according to the SIGN system) (11); most of complex carbohydrates type (more than 25g/day fibre, and less than 10% of simple sugars). Regarding the lipids, the document recognizes as rigorous limit the one issued by the EFSA: between 20-35% (if olive oil is consumed) of total energy intake (12). It adds the recommendation to limit con-

sumption of trans fats. As for proteins, the evidence regarding their consumption is insufficient to draw any recommendation, but limits the consumption of meat and meat products to prevent weight gain (recommendation grade C) (10).

*El libro blanco de la nutrición en España* (13), produced by the FEN, issues recommendations based on reviews of sources of information concerning the nutritional status of the Spanish population. As a recommendation for carbohydrates, it states that they should contribute between 55-60% of total energy intake, preferably being of complex type. It emphasizes the importance of dietary fibre, counselling the consumption of 25-30 g/day of non-absorbable carbohydrates and increase the consumption of products made of whole wheat flour, legumes, vegetables, fruit and vegetables, and decrease the consumption of pastries. Lipids should provide between 20-35% of total energy intake, less than 10% of saturated fatty acids (SFA), and less than 7% of polyunsaturated fatty acids (PUFAs). As for proteins, it recommends providing 0.8 g of protein/Kg of body weight/day, representing 8-15% of the contribution to the total energy intake. It is important to obtain proteins through high biological value proteins.

Finally, the EFSA sets dietary reference values (DRV), providing a recommendation for carbohydrates of 45-60% of total energy intake, with important fibre intake (25 g/day) (14). However, the expert committee considers that there is insufficient evidence to set a minimum limit of carbohydrate intake, so this is an illustrative percentage (14). As for proteins and lipids, it recommends the consumption of 13-20% of proteins of total energy intake (15), and 20-35% of lipids (12). It stresses the importance of limiting consumption of SFA (less than 10% of total energy intake) and trans fats, because it claims that there is sufficient evidence to confirm that a high consumption of both causes an increase in plasma cholesterol and, consequently, the risk of CVD. Adults must pay attention to consumption of omega-3 (ω3) polyunsaturated fatty acids, consuming 250 mg/day, to reduce the risk of CVD.

Some studies (16-18) have gone into more detail within these official recommendations. The contributions of each macronutrient to the total energy values are not static, they may vary through stages of life, especially during first years (16) and old age (17, 18).

These recommendations are used by professionals in nutrition and health for setting standards in the correct nutrition, through food and health, of the population they serve, either in public health services, private services or managing quality control of scientific studies and recommendations of public health and community nutrition (9).

Despite all this, a study has shown that the quantitative character of a diet (proportion of energy provided by each macronutrient) is not comparable to its qualitative nature (quality of macronutrients) (16). A diet based on whole grains, as opposed to a refined grain-based diet is an example. Empirical evidence has shown that increasing obesity is associated with increased consumption of foods based on refined products. This is due to the 50% decrease of postprandial energy expenditure compared to whole grain foods, according to Barr and Wright (19), while consumption of whole grains has been linked in several studies with a decrease in weight and body mass index (BMI) (20, 21); getting itself to manifest, in some cases, a decrease of up to 22% probability of becoming overweight just with a regular consumption of whole grain breakfast cereals (22). Some authors suggest (23, 24) that these whole grains protective actions versus refined grains, owing to grains fibre, contains, along with fibre from fruits, vegetables and legumes, phytochemicals extracts that are responsible for important antioxidant, anticancer and anti-inflammatory functions beneficial for health. The amount of plentiful and varied phytochemicals in grains could be the reason of the many protective effects of whole grains versus refined grains, and one of the reasons that could explain the differences between the quantitative and the qualitative diet. However, such protection is attributed to a synergistic effect of the complex mixture of phytochemicals in plant foods and/or whole grain foods, and not to a specific isolated phytochemical. Its mechanism and even interactions remains a challenge (25).

In order to establish indicative of the quality values of the diet, Diet Quality Index (DQI) algorithms have been developed to evaluate their overall quality (based on quantities of certain nutrients, foods, or both) and to categorize individuals according to their

more or less healthy eating pattern, and in that way determine risk factors for non-communicable chronic diseases (26). It is important to use accepted and validated methods by the international community to estimate food consumption as accurately as possible and avoid biases (for invalidity) and random errors (for inaccuracy) (27).

It is hypothesized that studies based on dietary treatments, and/or based on diets with different characteristics, show incomplete information to understand the study's design and results.

Due to the emergence of important studies in the field of nutrition, we believe that a more detailed methodology conducted in such studies is necessary to avoid bias in future studies and for better understanding. A quantitative diet is not enough to understand and determine whether a dietary treatment or a food pattern is appropriate or not.

The aim of this study is to demonstrate that dietary advice only from a quantitative point of view may be wrong or deficient in quality.

## Methods

A search for suitable recommendations of the contribution of macronutrients to total energy intake, issued by the FESNAD (10), FEN (13) and EFSA (12) was performed; besides a bibliographical search in the main databases (PubMed, EMBASE, SciELO).

Two standard weekly menus (menu A or "healthy" and menu B or "unhealthy"), with a similar quantitative profile, but qualitatively different were designed. The quantitative profile used to make the menus is recommended by the EFSA (12): 15-25% of total energy should be provided by proteins, 45-60% by carbohydrates and 20-35% by fat. The DIAL software was used for nutritional calibration of menus (28). For their evaluation, we used the recommendations of the EFSA (12) one of Europe's greatest scientific rigor agencies. DRV of FESNAD (29) were used to inform recommendations of specific micronutrients for the Spanish population; for a healthy male aged 20-29 years.

## Results

Table 1 lists the nutritional evaluations of menu A (Table 2) and menu B (Table 3, according to the EFSA recommendations (12) and the DRV of the FESNAD (29). These two menus, despite being normocaloric and having a quantitative profile according to the EFSAs guidelines, have some notable qualitative differences. According to the results obtained through the DIAL software, the amounts of sugars in both cases are much higher than recommended (less than 10% of total energy intake). However, it is noteworthy that this recommendation applies, according to World Health Organization (WHO), to all added sugars and sugars naturally present in foods such as honey, syrups and fruit concentrates, but not fruits as such. The software also incorporates mono- and disaccharides in these products to the calculated amounts of sugars, as well as vegetables or leafy vegetables. Furthermore, it can be seen in Table 1, how levels of glucose and sucrose are substantially higher in menu B; although in menu A fructose levels are higher (mainly due to abundant consumption of fruits). Fructose levels in menu B are also high (23.9 g/day) due to a high cola consumption. The contribution of fibre in menu A is four times higher (93 g/day) than in menu B (18.9 g/day), which barely reaches the recommended intake (25 g/day). It is mostly insoluble fibre in both menus.

Although the lipids profile of both menus follows the recommendations, levels of MUFAs are higher in menu A (39.6 g/day). In menu B, SFA levels, even being within the recommendations (less than 10% of total energy intake), are higher than in menu A (8.9% and 6.8%, respectively). The same happens with cholesterol (294 mg/day in menu B versus 174 mg/day in menu A), which are also at the boundary of the recommendations (300 mg/day). In addition, the PUFAs/SFA index is higher in menu A (0.87 vs. 0.71, respectively), although in both cases it is within the reference values (0.5, according to the EFSA), so the quality of dietary fat is better.

As for the content of micronutrients, menu A provides more vitamins B1, B2, B6 and folate, where the B menu does not reach the recommended DRV (300 ug/day), as well as vitamin C (60 mg/day), and vitamin E (15 ug/day); but does reach DRV for vita-

min K (120 ug/day). However, the menu B provides more vitamin B12, vitamin A and vitamin D, although none reach its DRV (5 ug/day). It must be emphasized that, despite menu B provides more vitamin A, menu A provides more carotenes. Within the mineral intake, it can be seen in Table 1, how menu A provides greater quantity of all minerals analyzed (calcium, iron, magnesium, zinc, potassium and phosphorus), except for sodium and selenium that are more abundant in menu B. Neither menu covers the DRV for iodine (150 ug/day), but it is slightly higher in menu A (119 ug/day). The calcium provided by menu B is less than calcium supplied by the other menu, and is close to the established DRV limit (900 mg/day). Finally, it can be seen in Table 1 that menu A also provides greater amounts of minor components of the diet, such as b-sitosterol, campesterol, estigamsterol, and oxalic, malic and citric acids, although there are no recommendations or DRV for them.

## Discussion

Paying attention to diets quality is critical because all its nutrients and minor components together not only decrease the risk of certain diseases, but also decrease of mortality from cardiovascular causes, diabetes mellitus (22%, approximately) (2) and cancer (15% less, approximately), as well as for a reduction in overall mortality from any cause (30). Phytochemicals are compounds of plant products (31) with important antioxidant, anticarcinogenic and anti-inflammatory effects (25).

Phytochemicals appear to be responsible for the benefit of the intake of certain foods in larger quantities in humans. A review conducted in 2004 (24) about the preventive effect of phytochemicals on cancer, suggests that there is strong epidemiological evidence on the reduction of cancer risk (lung, colon, liver, esophagus, cervix, oral cavity, stomach, bladder, pancreas and ovary) with a regular intake of fruits and vegetables, due to its phytochemicals content. Furthermore, it was found that the risk of cancer was twice as high in people with low intake of fruits and vegetables. Although the antioxidant activity of phytochemicals and their action against free radicals is clear, the mechanisms of action are still being debated. These include a potential effect of the regulation of expression and cell differentiation, antiviral and antibacterial action, and even enzymes and immune system modulation. Among the more than 5000 identified phytochemicals in fruits, vegetables and grains (24), one of the most studied are flavonoids. Its intake is known as a preventive factor for CVD (25). Flavonoids are significantly inversely related with myocardial infarction, coronary artery disease and low-density-lipoproteins of cholesterol (LDL-c) levels of plasma, apparently due to a modulation of the synthesis and the absorption of cholesterol, among other actions (25, 32).

Effects of phytochemicals have also been shown on legumes consumption. In a recent review of the preventive effect of legume consumption in chronic diseases (23), it was observed that consumption may provide protection against CVD, type 2 diabetes, hypertension, and even inflammation but, as said above, the synergistic effects of these compounds still remains a challenge. Since phytochemicals are more plentiful and varied in the outer layers of the grains (33), the protective effect is also observed in products rich in fibre, such as whole grains. In a cohort study (34) over 289,900 women without measurable disease, followed for 10 years, was observed that there were, within the group consuming whole grains (2-4 servings/day), minor cases of hypertension and CVD, against those who preferentially consumed refined grains, showing a possible role of whole grains in prevention of diseases. Others have also shown a significant risk reduction of type 2 diabetes (relative risk (RR) of 3 servings/day of whole grains = 0.68) compared to foods based on refined grains (RR 3 servings/day of refined grains = 0.95) (35). Furthermore, whole grains consumption has been associated with a decreased risk of mortality of up to 17% compared to those who ate predominantly refined grains (33).

Another essential part of the diets quality are sugars added to foods. The recommendations on consumption of organizations such as the EFSA (29), indicate that sugars should not exceed 10% of total caloric intake. The WHO, however, in their new recommendations (36) reduces sugar intake to < 5% of the total caloric intake (about 25 g/day). This applies to monosaccharides and disaccharides added to foods by manufacturers or consumers; and naturally present

**Table 1.** Menus A and B nutritional assessment.

| | Recomendatıon[15,26] | Menu A or "Healthy" | | Menu B or "Unhealthy" | |
|---|---|---|---|---|---|
| **Energy (Kcal)** | | 2180 | | 2208 | |
| **Proteins (%)** | 15-25 | 104 g | 19.1% | 84.2 g | 15.3% |
| **Carbohydrates (%)** | 45-60 | 265.96g | 48.8% | 281 g | 52.8% |
| **Lipids (%)** | 20-35 | 79.5 g | 32.1% | 77.4 g | 31.4% |
| **Simple sugars (g)** | | 103 | | 165+ | |
| **Total fibre (g)** | >25 | 93^ | | 18.9* | |
| **Soluble fibre (g)** | | 10.1^ | | 3.5 | |
| **Insoluble fibre (g)** | | 24.6^ | | 9.6 | |
| **Fructose (g)** | | 27.1 | | 23.9 | |
| **Glucose (g)** | | 19.7 | | 30.8^ | |
| **Sacarose (g)** | | 19.6 | | 75.1^ | |
| **Cholesterol (mg)** | <300 | 174 | | 297* | |
| **SFA (%)** | <10 | 16.7 g | 6.8% | 21.8 g | 8.9% |
| **MUFAs (%)** | 15-20 | 39.6 g | 16.4% | 32.4 g | 13.2% |
| **PUFAs (%)** | 6-11 | 14.5 g | 6% | 15.6 g | 6.4% |
| **Tiamin (mg)** | 1.2 | 2.1^ | | 1.6 | |
| **Riboflavin (mg)** | 1.6 | 2.8^ | | 1.8 | |
| **Niacin (mg)** | 18 | 44 | | 44.4 | |
| **Vit. $B_6$ (mg)** | 1.5 | 2.9^ | | 2.2 | |
| **Vit. $B_{12}$ (mg)** | 1.5 | 9.6 | | 10.9 | |
| **Folate (mg)** | 300 | 450^ | | 260* | |
| **Vit. C (mg)** | 60 | 180^ | | 53* | |
| **Vit. A (mg)** | 700 | 740 | | 1175^ | |
| **Retinol** | | 140 | | 910^ | |
| **Carotene** | | 3385^ | | 1570 | |
| **Vit. D (mg)** | 5 | 3.5* | | 4.6* | |
| **Vit. E (mg)** | 15 | 10.8* ^ | | 5.4* | |
| **Vit. K (mg)** | 120 | 535^ | | 123 | |
| **Calcium (mg)** | 900 | 1260^ | | 920 | |
| **Iron (mg)** | 9 | 31.6^ | | 15.6 | |
| **Iodine (mg)** | 150 | 119* ^ | | 81.1* | |
| **Magnesium (mg)** | 350 | 706^ | | 390 | |
| **Zinc (mg)** | 9.5 | 15.5^ | | 10.8 | |
| **Sodium (mg)** | 1500 | 1725 | | 1870 | |
| **Potassium (mg)** | 3100 | 5940^ | | 3450 | |
| **Phosphorus (mg)** | 700 | 2200+ | | 1795 | |
| **Selenium (mg)** | 55 | 135 | | 140 | |
| **B-sitosterol** | | 82.2^ | | 16.9 | |
| **Campesterol** | | 6.7^ | | 1.7 | |
| **Estigmasterol** | | 8.5^ | | 1.5 | |
| **Oxalic acid** | | 0.84^ | | 0.14 | |
| **Malic acid** | | 2.5^ | | 0.37 | |
| **Citric acid** | | 3.8^ | | 0.75 | |

*Nutrient data accompanying the symbol "*" do not reach the marked recommendations. Nutrient data with the "+" symbol provide 20% more than the opposite menu and nutrient data with the "^" symbol contribute 30% or more than the opposite menu.*

**Table 2.** Menu A or "healthy"

| | DAY 1 | DAY 2 | DAY 3 | DAY 4 | DAY 5 | DAY 6 | DAY 7 |
|---|---|---|---|---|---|---|---|
| **Breakfast** | Green tea with skimmed milk (100 ml) and stevia (10 g). Whole wheat bread (60 g), orange (150 g), olive oil (10 g), natural tomato (30 g) | Green tea with skimmed milk (250 ml) and stevia (10 g), whole wheat bread (60 g), tomato (30 g), olive oil (10 g), pineapple (200 g) | Green tea with skimmed milk (250 ml) and stevia (10 g), whole wheat bread (60 g), avocado (30 g), olive oil (10 g), apple (200 g) | Green tea with skimmed milk (250 ml) and stevia (10 g), whole wheat bread (60 g), orange (150 g), olive oil (10 g), natural tomato (30 g) | Green tea with skimmed milk (250 ml) and stevia (10 g), whole wheat bread (60 g), tomato (30 g), olive oil (10 g), pineapple (200 g) | Green tea with skimmed milk (250 ml) and stevia (10 g), whole wheat bread (60 g), light Philadelphia cheese (30 g), avocado (30 g), apple (200 g) | Green tea with skimmed milk (250 ml) and stevia (10 g), whole wheat bread (60 g), olive oil (10 g), natural tomato (30 g), orange (150 g) |
| **Mid-morning snack** | Apple (200 g), walnuts (20 g) | Raspberry (100 g), fresh cheese (50 g), honey (10 g) | Banana (150 g), skimmed plain yogurt (125 g) | Pear (200 g), hazelnuts (20 g) | Honey (10 g), fresh cheese (50 g) | Almonds (20 g), dark chocolate (>80%) (25 g) | Banana (200 g), fresh cheese (50 g) |
| **Lunch** | Chickpeas salad (70 g) with tomato (200 g) and onion (50 g). Pork fillet (150 g), olive oil (10 g), flax seeds (20 g), whole wheat bread (50 g), skimmed plain yogurt (125 g) | Eggplant tempura (200 g), flour (10 g), olive oil (10 g), fresh hake (200 g), mushroom (80 g), whole wheat bread (50 g), kiwi (100 g) | Fry lightly whole wheat pasta (70 g) with zucchini (80 g), natural tomato (80 g), olive oil (10 g), oregano and basil. whole wheat bread (50 g), mandarin (100 g) | Fry lightly whole wheat rice (70 g) with mushrooms (60 g), onion (30 g) and soy sprouts (50 g). Olive oil (10 g), whole wheat bread (50 g), pineapple (200 g) | Noodles soup (30 g), with chickpeas (40 g) and hen (50 g), grilled salmon (120 g), carrot (50 g), potato (120 g), olive oil (10 g), whole wheat bread (50 g), raspberries (150 g) | French beans salad (70 g) with spinach (50 g), onion (50 g) and tuna (60 g), olive oil (10 g), whole wheat bread (50 g), cherries (150 g) | Avocado (200 g) filled with potato (100 g), tomato (50 g), sardines (150 g), lettuce (30 g), cucumber (80 g) and onion (50 g), olive oil (20 g), vinegar (5 g), whole wheat bread (50 g), pear (150 g) |
| **Afternoon snack** | Green tea with skimmed milk (100 ml) and stevia (10 g), quince (20 g), fresh cheese (50 g) | Skimmed yogurt (125 g), oat (20 g) | Green tea with skimmed milk (100 ml) and stevia (10 g), dark chocolate (25 g) | Dried dates (40 g), green tea with skimmed milk (100 ml) and stevia (10 g) | Green tea with skimmed milk (100 ml) and stevia (10 g), natural almonds (15 g) | Skimmed yogurt (125 g), oat (20 g), strawberries (150 g) | Green tea with skimmed milk (100 ml) and stevia (10 g), dark chocolate (25 g) |
| **Dinner** | Artichoke (150 g), fresh mackerel (125 g), baked potato (200 g) with oregano, olive oil (10 g), whole wheat bread (50 g), kiwi (100 g) | Endive (70 g) with avocado (50 g), apple (60 g) and grilled asparagus (70 g), olive oil (10 g), whole wheat bread (50 g), plum (150 g) | Boiled cauliflower (100 g), potato (200 g), grilled chicken fillet (150 g), olive oil (10 g), vinegar (5 g), whole wheat bread (50 g), apricot (200 g) | Tomato salad (80 g), lettuce (30 g), onion (50 g), raisins and blueberry (50 g), steak (150 g), olive oil (20 g), whole wheat bread (50 g), plain yogurt (125 g) | Egg omelette (120 g), boiled green beans (80 g) with carrot (50 g), potato (200 g), olive oil (10 g), whole wheat bread (50 g), loquat (80 g) | Boiled potatoes (200 g) with mushrooms (150 g). Lettuce (30 g), tomato (80 g), onion (50 g) and pine nuts salad (30 g). Olive oil (20 g), vinegar (5 g), whole wheat bread (50 g), apple (200 g) | Steamed mussels (100 g), White rice with peas (100 g) and Brussels sprouts (100 g), olive oil (10 g), whole wheat bread (50 g), plum (200 g) |

**Table 3.** Menu B or "unhealthy"

| | DAY 1 | DAY 2 | DAY 3 | DAY 4 | DAY 5 | DAY 6 | DAY 7 |
|---|---|---|---|---|---|---|---|
| **Breakfast** | Coffee with whole milk (250 ml), sugar (10 g), chocolate cereals (50 g) | Coffee with whole milk (250 ml), sugar (10 g), whole wheat bread (50 g) with ham (30 g) | Coffee with whole milk (250 ml), sugar (10 g), biscuits (50 g) | Coffee with whole milk (250 ml), sugar (10 g), chocolate cereals (50 g) | Coffee with whole milk (250 ml), sugar (10 g), whole wheat bread (50 g) with ham (30 g) and olive oil (10 g) | Coffee with whole milk (250 ml), sugar (10 g), biscuits (50 g) | Coffee with whole milk (250 ml), sugar (10 g), chocolate cereals (50 g), walnuts (30 g) |
| **Mid-morning snack** | Cola drink (333 ml) | Cola drink (333 ml) | Cola drink (333 ml) | Cola drink (333 ml) | Cola drink (333 ml) | Cola drink (333 ml) | Cola drink (333 ml) |
| **Lunch** | Steak (200 g) with baked potato (200 g), Lettuce (30 g) and tomato (80g) salad, olive oil (10 g), whole wheat bread (50 g), natural yogurt (125 g) | Chard (150 g), fresh hake (200 g), mushrooms (50 g) with garlic (5 g), olive oil (10 g), whole wheat bread (50 g), Cola drink (333 ml) | Egg pasta (70 g), fry lightly with zucchini (80 g) and walnuts (20 g), olive oil (10 g), whole wheat bread (50 g) | White rice (70 g) fry lightly with mushrooms (30 g), onion (20 g) and Emmental cheese (10 g), olive oil (10 g), whole wheat bread (50 g), Cola drink (333 ml) | Noodles soup (30 g), with chickpeas (40 g) and hen (50 g), sole (120 g), olive oil (10 g), whole wheat bread (50 g) | Baked turkey thigh (250 g), onion (100 g), apple (100 g), white wine (150 ml), olive oil (10 g), whole wheat bread (50 g), Cola drink (333 ml) | White rice (70 g), fried tomato (50 g), swordfish (150 g), olive oil (10 g), whole wheat bread (50 g) |
| **Afternoon snack** | Cola drink (333 ml), loaf of bread (40 g) with sliced cheese (10 g) and ham (30 g) | Cola drink (333 ml), natural yogurt (125 g) | Cola drink (333 ml), loaf of bread (40 g) with pate (40 g) | Cola drink (333 ml), natural yogurt (125 g) | Cola drink (333 ml), loaf of bread (40 g) with ham (40 g) | Cola drink (333 ml), natural yogurt (125 g) | Cola drink (333 ml), loaf of bread (40 g) with sliced cheese (10 g) and ham (30 g) |
| **Dinner** | Artichokes (50 g) with *Serrano* ham (8 g), fresh mackerel (125 g), olive oil (20 g), whole wheat bread (50 g), natural yogurt (125 g), Cola drink (333 ml) | Endives (70 g) with Roquefort cheese (45 g), apple (60 g) and walnuts (20 g), turkey ham (20 g), whole wheat bread (50 g) | Fry lightly cauliflower (100 g), grilled chicken fillet (150 g), olive oil (15 g), whole wheat bread (50 g), natural yogurt (125 g), Cola drink (333 ml) | Tomato (80 g), onion (50 g), sweet corn (30 g) and sardines (150 g) salad, balsamic vinegar (5 g), olive oil (10 g), whole wheat bread (50 g) | Cheese omelette: egg (120 g) and Emmental cheese (20 g), boiled green beans (80 g) with carrot (50 g), olive oil (10 g),whole wheat bread (50 g), plain yogurt (125 g), Cola drink (333ml) | Boiled potatoes (200 g) with pine nuts (30 g), pork fillet (150 g), olive oil (10 g), whole wheat bread (50 g) | Steamed mussels (150 g), white rice (60 g) and stir fried artichokes (100 g) with garlic, olive oil (10 g), whole wheat bread (50 g), natural yogurt (125 g), Cola drink (333 ml) |

in honey, syrups and fruit concentrates, yet do not refer to fruits, vegetables and other plant products.

One of the monosaccharides in the spotlight is fructose. A high intake of fructose has been linked to an increased risk of obesity, type 2 diabetes, CVD, metabolic disease, nonalcoholic fatty liver disease and fructose malabsorption, which may secondarily alter the flora and intestinal motility (37-39). Drinks with added sugars, such as soft drinks, are the main source of added sugars in the diet (37). Most of sugars in them are fructose, added in the form of 'high in fructose corn syrup (37-39). The fructose added from this com-

pound is directly linked to an increased risk of CVD derived from an increased fat deposition in viscera and atherogenic dyslipidemia (37). The main problem of excess fructose stems from the prolonged consumption of these type of foods and not from eating foods that contain it naturally (like fruits and honey), in a healthy diet. Furthermore, in the case of fruit, it is presented as a protective factor in the development of obesity and cardiovascular disease (38, 39). One of the most healthful eating patterns studied is the MD, and this is mainly due to its high quality. The MD is characterized by a high consumption of plant foods (minimally processed), fruit as the typical dessert, olive oil as the primary fat and moderate consumption of dairy products, fish, eggs and poultry. Follow up on MD can significantly reduce the risk of chronic disease and even reduce the risk of mortality (10). As seen in the results, menus A and B are clear examples of how the quantitative value of the diet does not always correlate with quality points that have been discussed above. Menu A or "healthy" has lower amounts of added sugars, higher amounts of fibre (and thus phytochemicals), better quality of fat and increased amounts of micronutrients.

The ENIDE study (40), conducted between 2009-2010 on the nutritional assessment of the Spanish diet (in terms of energy and macronutrients), threw unsatisfactory results in terms of diets quality in the Spanish population. Most had an unbalanced calorie profile (42% of total dietary intake as fat, 40% as carbohydrate and between 16-18% as protein), and the same happened with the lipid profile (more than 10% of the contribution was in form of SFA, 4.6% as PUFAs, and 15-20% in the form of monounsaturated fatty acids (MUFAs). There was, also, a high consumption of meat and meat products, and products high in sodium, fat and added sugars, facing a very low intake of vegetables, fruits and vegetables, and low consumption of cereals, predominantly refined. However, the situation seemed to partially improve in 2013, according to data of food consumption in Spain from the Ministry of Agriculture, Food and Environment (41). An increased consumption of basic foods, such as bread, rice, pasta and legumes, was observed (although not specified "whole grain" or "refined grain"), as well as vegetables and fish, reducing the consumption of meat and meat products, but also the consumption of fruits.

It is therefore necessary to continue investing efforts to inform and advise the society on the diets quality. Promote the consumption of whole grains (at least 3 servings per day) (35), fruits (3 servings per day) and vegetables (2 servings per day) (13), dried fruits, nuts and fish. Also reduce consumption of simple sugars (less than 10% of total energy intake (12), although some agencies, such as the WHO, have begun to recommend less than 5% of the total energy intake (36) and solid fats. On the other hand, one should not lose sight of the importance of determining if the population does or not follow a healthy or quality diet and the costs in which it may incur (30).

These factors are important considering relevant studies published in high impact journals in this science field, in which the effectiveness of different diets are compared (42-48).

## Conclusion

According to the results obtained in the menus, it becomes clear how diet may be deficient in terms of quality, if only planned taking into account the quantitative aspect. High qualitative value diets have been linked to the prevention of chronic diseases such as obesity, CVD, type 2 diabetes, hypertension and cancer, as well as to a possible decrease in mortality derived from them and even overall mortality from any cause. Therefore, dietary advice should focus not only on establishing an adequate caloric profile, but also pay look closely to the type of carbohydrates and fats, and minority dietary components such as vitamins, minerals and phytochemicals.

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Original article

# Macronutrient composition and Body Mass Index vary by season in college students

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**Summary.** *Objective:* To describe seasonal variation in food intake, body weight, body mass index (BMI) and mid- upper arm circumference (MUAC) in college students. *Methods:* In this follow-up study, 120 male and 180 female college students aged 18-35 years were enrolled. All participants were visited every 3 month (four sampling points: baseline and three consecutive quarters) for 1-year period. Dietary intakes were assessed using seven- to nine-day food records on randomly selected days per quarter. Anthropometric measurements were performed by a trained dietetic according to standard protocol at four time points (12 to 15 weeks apart). *Results:* Daily caloric and carbohydrate intakes were higher by 193 kcal and 8% of total daily calorie intake during the summer compared to the winter. Fat intake was consumed in greater amount in the winter rather than other seasons. The highest weight and BMI were observed in the summer (72.9±4.6 kg and 26.22±5.3 kg/m2, respectively), whereas the lowest values were in the winter (68.7±3.6 kg and 24.7±3.8 kg/m$^2$, respectively). The greatest difference for MUAC was found between fall and winter (-2.80±0.25 cm; P=0.001). *Conclusions:* There are seasonal variations in diet and anthropometric measurements among college students. Therefore, it must be taken into account when counseling individuals about healthy habits as well as when designing nutritional epidemiology studies.

**Key words:** seasonal variation, obesity, body mass index, diet, macronutrient composition.

## Abbreviation

MUAC: Mid- upper arm circumference; BMI: Body mass index.

## Introduction

The obesity prevalence has been gradually increased over the past 3 decades (1,2). According to the statistics, 13% of the world's adult population (11% of men and 15% of women) are obese and 39% are overweight (38% men and 40% women) (3). Corresponding values among Iranian adults are 12.6- 25.9% and 27- 38.5%, respectively (4).

Anthropometric measurements can be influenced by genetic, epigenetic and environmental factors (e.g. dietary intakes and physical activity) (5,6). Researchers are increasingly recognizing the importance of the environmental factors in facilitating weight gain and obesity (5,7). The association of seasonal variation as an environmental variable and weight change is a new concept in nutritional epidemiology which has been poorly investigated (8-11). Seasonal variation may be related to anthropometric measurements including fat and muscle mass and body weight by affecting physical

activity and dietary intakes. A large-scale monitoring population study among Netherland adults showed that subjects had higher body mass index (BMI) and waist circumference (WC) during winter compared to summer, and seasonal variation was greater for abdominal obesity rather than general obesity (12). Another short-term longitudinal study revealed that subjects had greater weight gain during the festive season holiday than other seasons (9-12).

Weight change might be associated with changes in muscle and fat mass. Therefore, it is possible that weight change by seasonal variation would be followed by changes in fat and muscle mass over the year. Indeed, participants may have higher physical activity level during summer than winter and have higher calorie intake and lower physical activity level during winter (8, 9, 13). These differences may lead to different change in fat and muscle mass. Because of differences in physical activity and dietary intakes changes among different age groups through the seasons, it is relevant to investigate such association in all age groups. To the best of our knowledge, there is little evidence in this context and no report from Iran and Asia, as a less developed country. In the current study, we aimed to examine anthropometric changes over the year and distinguish session where people eat more, active less, and gain weight.

## Methods

*Subjects*

Participants were recruited from the students of Isfahan University of Medical Sciences. Individuals were recruited if they were 18 to 35 years old, not be on weight-control diets, be free of metabolic disorders which may affect weight status (e.g. Cushing's syndrome or hypothyroidism or hyperthyroidism) and not being pregnant or lactating. To provide a random sampling, multistage cluster random sampling method was used. First, we considered the number of all schools (n=9) and departments (n=30) in Isfahan University of Medical Sciences, and then some students were randomly selected from each department. Based on the suggested formula for cross-sectional study, 300 participants would provide adequate power (β= 80%) for the current study. However, to increase the power of study, we enrolled 500 students between February 2014 and February 2015, with at least 10 individuals from each department. Individuals were excluded if their daily energy intake was <800 or >4200 kcal (n=8). Subjects who had less than eight dietary records or had not fully attended in every five visits were also excluded (n=20). Finally, statistical analysis was conducted among 300 individuals. All subjects declared their willingness to participate in the research by providing a written informed consent. The present study was approved by the research council and ethical committee of the School of Nutrition and Food Science, Isfahan University of Medical Sciences, Isfahan, Iran.

*Data collection*

Demographic characteristics including sex, age and socio-economic status were collected by a self-administered questionnaire at baseline. Body weight and height of participants were measured with wearing light clothing and no shoes. The measurement of height was recorded to the nearest 0.5 cm and weight was recorded to the nearest 100 gr. BMI was calculated by dividing weight in kilogram by the square of height in meter. Overweight and obesity were defined according to BMI (154). Overweight was defined as 25≤BMI≤29.9, whilst obesity was considered as BMI≥30 kg/m$^2$ (8, 15).

To measure the Mid- upper arm circumference (MUAC), participant should have bent the right arm to a 90˚ angle at the elbow and the upper arm was parallel to the chest. Then the midpoint of right arm was ascertained and marked at the skin. The insertion tape was pulled around the marked midpoint of the arm, but not too tight that the tissue was compressed (16). The accuracy of measurement was 1 mm.

At the end of every three months weight, MUAC and BMI were measured (totally five visits). The participants were asked to recall hours they spent the previous day at different types and intensities of activities. MET values were obtained from a physical activity compendium published by Ainsworth et al.(17). All interviews were conducted by trained dietitians.

*Dietary intake assessment*

Dietary intakes were monthly assessed by a 3-day food record. Totally, we had at least nine weighted

food records for each season. Participants were asked to record their dietary intakes in 2 non-consecutive weekdays and one weekend day except for special days (celebration and party). All participants were educated how to record their dietary intakes, meals and snacks with exact portions and standard serving sizes during 24 hours. The average number of dietary records was 36 per subject. The accuracy of food records were checked by trained nutritionists and ambiguous items were declared via phone interview. Household measurements were converted to grams. Energy and nutrient intakes were estimated by using NUTRITIONIST IV (N4) software, which was adopted for Iranian foods.

*Statistical analyses*

Normal distribution of dietary intakes and anthropometric measurements were tested by Kolmogorov–Smirnov. General characteristics of study population were described as means and percentages by using descriptive statistics. One-way analysis of variance (ANOVA) was performed for multiple sample comparison for continuous variables (dietary intakes, anthropometric measurements and their mean differences between seasons) across the seasons. Independent sample t-test was done to compare continuous variables between men and women in each season. Mean differences in anthropometric measurements between seasons were also compared in adjusted models for sex, physical activity and socioeconomic status by using analysis of covariance (ANCOVA). All statistical analyses were performed by Statistical Package for Social Sciences (SPSS, Inc., Chicago IL, USA; version 20). P< 0.05 was considered significant in all statistical analyses.

## Results

**Table 1** shows demographic characteristics of participants at baseline. Participants' average age was 21.7 (s.d.=2.3) years and 40 % of participants were male. The participant's means height and weight were 169.0 (s.d =21.0) cm and 71.2 (s.d =3.6) kg, respectively. Almost 50% of participants were normal weight and around 11.0% were obese. The average BMI was 25.6 (s.d. = 2.3) kg/m$^2$, and the average MAC was 22.4 (s.d =2.1) cm.

**Table 2** shows means of dietary intakes and physical activity of participants by season. In the winter, fat had the highest contribution in total energy intake (36 ±2% of daily energy intake), and in the summer, carbohydrate had the highest contribution (60 ± 5% of daily energy intake). There were no significant differences in seasonal variation of dietary intakes and physical activity between male and female.

**Table 3** indicates means of anthropometric measurements by season in the entire study population, and each gender. The peaks of body weight and BMI were in the summer and the peak of MUAC was in the fall. There were no significant differences in seasonal variation of body weight, MUAC and BMI between male and female.

**Table 1.** Characteristics of study participants at baseline.

| Variable | Male | | Female | |
|---|---|---|---|---|
| | Mean/frequency | s.d. | Mean/frequency | s.d. |
| Age (years) | 21.90 | 2.60 | 21.56 | 2.39 |
| Male (%) | 40 | - | 60 | - |
| Never smoker (%) | 100 | - | 100 | - |
| Height (cm) | 1.76 | 0.29 | 1.63 | 0.23 |
| Weight (kg) | 79.6 | 3.7 | 62.5 | 3.1 |
| MUAC (cm) | 28.93 | 2.9 | 16.91 | 2.5 |
| BMI[1] classification (%) | | | | |
| Normal (18.5–24.9) | 52.5 | - | 46.93 | - |
| Overweight (25–29.9) | 38.5 | - | 40.97 | - |
| Obese ( ≥ 30) | 10.01 | - | 12.01 | - |

[1] MUAC: Mid- upper arm circumference; BMI: Body mass index

**Table 2.** Relevant dietary intakes by season

| Variables | Winter mean | Spring mean | Summer mean | Fall mean | P-value[1] |
|---|---|---|---|---|---|
| Energy (kcal/d) | 2000 ± 21 | 2006 ± 22 | 2193 ± 36 | 2034 ± 27 | < 0.05 |
| Male | 2009±19 | 2012±20 | 2220±22 | 2084±23 | <0.01 |
| Female | 1998±20 | 2000±19 | 2165±23 | 1981±21 | <0.05 |
| P-value[2] | 0.18 | 0.21 | 0.16 | 0.15 | - |
| Carbohydrate (%) | 52 ± 5 | 55 ± 4 | 60 ± 5 | 56 ± 5 | < 0.05 |
| Male | 53±6 | 55±4 | 61±5 | 57±5 | <0.05 |
| female | 51±5 | 55±4 | 59±4 | 55±5 | <0.05 |
| P-values | 0.24 | 0.58 | 0.34 | 0.42 | - |
| Fat (%) | 36 ± 2 | 31 ± 2 | 29 ± 2 | 24 ± 2 | < 0.05 |
| Male | 35 ±2 | 31±2 | 28±2 | 23 | <0.05 |
| female | 37±2 | 31±2 | 30±2 | 25 | <0.05 |
| P-values | 0.34 | 0.76 | 0.65 | 0.43 | - |
| Protein (%) | 12 ± 1 | 14 ± 2 | 11 ± 1 | 15 ± 3 | 0.09 |
| Male | 12±1 | 14±2 | 11±1 | 15±2 | 0.11 |
| Female | 12±1 | 14±2 | 11±1 | 15±2 | 0.10 |
| P-value | 0.81 | 0.76 | 0.67 | 0.83 | - |
| Saturated fatty acids (%) | 10 ± 2 | 8 ± 1 | 8 ± 1 | 8 ± 1 | 0.18 |
| Male | 11 ±3 | 8 ± 1 | 8 ± 1 | 8 ± 1 | 0.09 |
| female | 9±2 | 8 ± 1 | 8 ± 1 | 8 ± 1 | 0.17 |
| P-values | 0.23 | 0.67 | 0.75 | 0.84 | - |
| Total physical activity (MET-h/d) | 8.9 ± 2.1 | 8.5 ± 2.0 | 9.3 ± 2.1 | 8.7 ± 2.2 | 0.08 |
| Male | 9.2 ±2.3 | 8.7±2.1 | 9.5±2.2 | 8.8±2.3 | 0.09 |
| female | 8.6±2.0 | 8.2±2.0 | 9.1±2.0 | 8.6±2.1 | 0.07 |
| P-values | 0.11 | 0.25 | 0.16 | 0.43 | - |

[1] This P-value compares the seasons and derived from one-way ANOVA test.
[2] This P-values compares the values between men and women and derived from independent sample t-test.

**Table 4** indicates the comparison of anthropometric measurements between seasons in the entire study of population in crude and adjusted models. The means of BMI, MUAC and weight were significantly different between all seasons (P<0.05). Maximum difference for weight and BMI was between summer and winter (P=0.001). Maximum difference for MUAC was between fall and winter (-2.80±0.32; P=0.01) as well as fall and summer (-2.80±0.25; P=0.001). All relationships remained significant even after adjustment for sex, physical activity and socioeconomic status.

## Discussion

This study suggests significant seasonal fluctuations in weight, BMI and MUAC as well as dietary intakes including energy, fat and carbohydrate. Greater energy and carbohydrate intakes in summer concurred with greater BMI and weight. No seasonal fluctuations were observed in physical activity and protein intake. The results of this study illustrate that increased carbohydrate intake has more effect on calorie intake and weight gain than increased fat intake.

It is well established that there is seasonal fluctuations in anthropometric measurements, particularly

**Table 3.** Relevant anthropometric measurements and physical activity by season

| Variable | Winter mean | Spring mean | Summer mean | Fall mean | P-value[1] |
|---|---|---|---|---|---|
| Weight (kg) | 68.7 ± 3.6 | 70.3 ± 3.3 | 72.9 ± 4.6 | 71.9 ± 5.2 | < 0.05 |
| Male | 70.1±3.9 | 72.2±3.7 | 74.8±4.9 | 73.8±5.7 | <0.05 |
| female | 66.9±3.1 | 68.1±3.0 | 70.6±4.5 | 69.3±5.1 | <0.05 |
| P-values[2] | 0.03 | 0.01 | 0.03 | 0.01 | - |
| MUAC[3] (cm) | 20.3 ± 5.6 | 21.4 ± 4.6 | 22.3 ± 5.1 | 23.1 ± 4.6 | < 0.05 |
| Male | 22.1±5.8 | 22.3±4.7 | 23.9±5.3 | 24.5±4.6 | <0.05 |
| Female | 18.5±5.4 | 19.2±4.5 | 21.0±5.0 | 21.6±4.5 | <0.05 |
| P-values | 0.04 | 0.05 | 0.07 | 0.09 | - |
| BMI[3] (kg/m$^2$) | 24.7 ± 3.8 | 25.28 ± 4.1 | 26.22 ± 5.3 | 25.8 ± 5.9 | < 0.01 |
| Male | 26.1 ±3.9 | 26.1±5 | 27.7±5.4 | 26.5±5.8 | <0.01 |
| female | 22.9±3.5 | 24.3±5.1 | 25.0±5.2 | 24.2±5.4 | <0.01 |
| p-values | 0.01 | 0.09 | 0.10 | 0.08 | - |

[1] This P-value compares the seasons and derived from one-way ANOVA.
[2] This P-value compares the values between men and women and derived from independent sample t-test.
[3]MUAC: Mid- upper arm circumference; BMI: Body mass index

**Table 4.** Comparison of anthropometric measurements between seasons

| Variables | Spring | | | | | | Summer | | | | fall | | Season |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Summer | | fall | | Winter | | fall | | Winter | | Winter | | P value |
| | Mean difference | P value | Mean difference | P value | Mean difference | P value | Mean difference | P value | Mean difference | P value | Mean difference | P value | |
| Weight (kg) | | | | | | | | | | | | | |
| Crude | 2.59±0.23 | 0.01 | 1.54±0.11 | 0.01 | -1.60±0.24 | 0.01 | -1.02±0.12 | 0.03 | -4.12±0.50 | 0.001 | -3.21±0.42 | 0.001 | 0.001 |
| Adjusted model[1] | 2.50±0.21 | 0.02 | 1.49±0.10 | 0.03 | -1.50±0.22 | 0.01 | -1.00±0.13 | 0.04 | -4.10±0.48 | 0.001 | -3.20±0.44 | 0.001 | 0.001 |
| BMI (kg/m$^2$) | | | | | | | | | | | | | |
| Crude | 0.95±0.24 | 0.01 | 0.52±0.19 | 0.05 | -0.54±0.13 | 0.03 | -0.42±0.07 | 0.02 | -1.55±0.39 | 0.001 | -0.97±0.12 | 0.01 | 0.01 |
| Adjusted model | 0.92±0.21 | 0.01 | 0.50±0.14 | 0.05 | -0.51±0.11 | 0.04 | -0.40±0.06 | 0.03 | -1.49±0.34 | 0.001 | -0.95±0.16 | 0.01 | 0.01 |
| MUAC | | | | | | | | | | | | | |
| Crude | 0.95±0.23 | 0.01 | 1.71±0.23 | 0.01 | 0.90±0.28 | 0.01 | -2.80±0.32 | 0.01 | -2.06±0.31 | 0.01 | -2.80±0.25 | 0.001 | 0.001 |
| Adjusted model | 0.90±0.27 | 0.01 | 1.69±0.20 | 0.01 | 0.87±0.25 | 0.01 | -2.72±0.33 | 0.01 | -2.00±0.26 | 0.01 | -2.76±0.27 | 0.001 | 0.001 |

[1] adjusted for sex, physical activity, socioeconomic status.

weight and BMI. However, it seems depending on study population and behavioral factors, the pattern and magnitude of these fluctuations might be different. For example, in college students, due to differences in dietary intakes and physical activity levels during different seasons, larger fluctuations might be expected in comparison with a middle-aged population who have similar schedule in different seasons of the year. Findings from Ma's study support this assumption. Consistent with our findings Ma revealed seasonal variation in body weight in middle-aged adults (47.6 yr), but their fluctuations were considerably less than ours (18).

Our findings indicated MUAC is related to weight and BMI, it is compatible whit Banik's study(19). In our study all of them were at minimum level in winter, but findings from Lemma's study indicated MUAC was more related to another factor named maximum voluntary contraction [MVC] than BMI (20). Previ-

ous reports proposed that circumference measurements specially limb circumferences like MUAC might be sensitive indirect measurements of peripheral muscle; therefore, it would be more appropriate to look into the changes in MUAC as a measurement of declining of muscle mass than BMI (21-23). As shown in our study, peak of MUAC was in the fall as well as the peak of protein intake; therefore, it could be inferred that muscle mass would be greater in the fall rather than other seasons in this study. The results of Jahnset al's study confirmed that dietary intake vary by season but they reported energy intake was not different(24). Their objective group was different with us.

Findings regarding fluctuations in energy and macronutrient intakes are controversial. Whereas some evidence does not confirm such variations either in calorie (25-27) or in macronutrients intake (25, 28), others have reported variations in energy and macronutrient intakes by season (8). In spite of considerable agreement regarding the fluctuation in fat and carbohydrate intakes by season (18, 29), there is debate for protein. In line with our findings, most of earlier studies (18, 26, 27, 30, 31), but not all (32), showed that protein intake did not vary by season, though its changes were marginally significant in our study. Although there is agreement regarding the variation in energy, fat and carbohydrate by season (8), there are differences in the peak of fat or carbohydrate intake between studies. Inconsistent with our findings showing a peak intake of carbohydrate in summer and a peak intake for fat in winter, Ma and colleagues revealed a peak for carbohydrate and fat in winter and summer, respectively (18). However, the intake of fat was larger in winter in male industrial employees who had greater BMI in winter rather than summer (8). Greater consumption of carbohydrate during summer in our study might be related to higher intake of ice cream, fruits and drinks rich in sugar in the summer than other seasons to compensate loosing body water (33).

An explanation underlying greater body weight and BMI during summer might be related to dietary macronutrient composition. In spite of similar protein consumption by seasons, fat and carbohydrate intakes were differently consumed in summer and winter. There is evidence supporting the satiating properties of high fat/low carbohydrate diet rather than a low carbohydrate/high fat diet and its favorable effect on short-term weight loss (34). Moreover, because our participants were college students who were studying in three seasons except for summer, we hypothesize that greater anthropometric measurements in summer might be related to lower psychological stress, which consequently could be associated with higher intake of carbohydrates and energy. Pagels's study showed there was difference in physical activity intensity by weather variation, their objective group was school children(35).

Our study has some strength. First of all, larger sample size of this study rather than previous studies in this context let us to extract more accurate findings (25, 26, 36). Second, our study population was healthy college students who aged 18-35 years, and therefore, had more similar lifestyle rather than other studies' population which conducted on a wide age range of participants. Although this could limit the generalizability of our findings, it could remove the potential source of residual confounders, especially physical activity, and make more reliable our findings. Third, using 24-h dietary record to assess dietary intakes provide more accurate data than food frequency questionnaires (FFQs) and recall which used in earlier studies (37). The limitations of our investigation were: Diet and physical activity information were obtained from self-reported 24-h records. Although there is always the potential for misclassification due to error in self-reporting, the error is minimized by training the participants.

## Conclusion

In conclusion, the present study indicated that seasonal variations of daily caloric intake, fat and carbohydrate intakes could be concurred with variations in body weight, MUAC and BMI among college students. These fluctuations were not different between male and female. No significant fluctuations were observed in physical activity and protein intake.

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ORIGINAL ARTICLE

# Major dietary patterns and their associations with diet quality indices in Iranian adults

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**Summary.** *Background:* Limited data on the association of dietary patterns with nutrient intakes and diet quality indices are available. *Objective:* We examined the relation of dietary patterns and diet quality indices and nutrient intakes among Iranian. *Methods:* This cross-sectional study was conducted among 389 Isfahani adults. Dietary intakes were estimated using a validated semi-quantitative food frequency questionnaire. Dietary patterns were identified using factor analysis method. Diet quality indices [nutrient adequacy ratio (NAR), Mediterranean (MED) score, healthy eating index (HEI), dietary diversity score (DDS) and dietary energy density (DED)] were calculated according to standard methods. *Results:* We identified four dietary patterns: healthy, high animal fat and protein, traditional and Western patterns. Participants in the top tertile of healthy dietary pattern, in comparison with those in the first tertile, had greater NARs for all nutrients of concern, HEI (73.5±6.8 vs.67.7±6.3; P<0.0001) and MED score (5.5±1.2 vs. 3.5±1.5; P<0.0001). Higher scores of high animal fat and protein dietary pattern were more nutrient-dense, while greater adherence to the traditional dietary pattern was associated with greater MED score (4.8±1.5 vs. 4.2±1.7; P=0.004), but lower NARs. Individuals in the top tertile of Western dietary pattern had more energy-dense diet than those in the first (0.9±0.2 vs. 0.8±0.1; P=0.002) *Conclusion:* Dietary patterns are differently related to nutrient intakes and diet quality indices. Further studies are needed to determine the quality of dietary patterns to determine the best pattern.

**Key words:** diet quality, dietary patterns, nutrients, nutritional status

## Introduction

Given the increasing prevalence of various chronic diseases, dietary intakes have been considered as a pivotal factor in the etiology of various non-communicable diseases. Although many investigations assessed the role of individual foods and nutrients, dietary pattern, as a holistic approach, has been emerged to consider the potential interactions between foods and their components. Therefore, it seems that dietary patterns provide more reliable association between diet and diseases.

Two approaches, posterior and priori, have been suggested to identify dietary patterns. To date, the relevance of both approaches in health has been widely investigated. There is much evidence from epidemiological studies that showing posterior dietary patterns are related to various cardiometabolic risk factors (e.g. insulin resistance, obesity, inflammation, dyslipidemia and impaired glucose tolerance) (1-6) as well as priori dietary patterns. Mediterranean dietary pattern (MED) (7-9) and healthy eating index (HEI) (10-13), as two priori dietary patterns, are inversely correlated with the risk of chronic diseases, whilst dietary energy density (DED) is positively related (14-17). Hitherto, we are aware of no study which assessed the associations of posterior dietary patterns with priori dietary patterns.

This issue could be important because of unique characteristics of dietary patterns in each population. For example, it is probable that loaded factors in a specific dietary pattern (e.g. healthy pattern) to be different from one population to another one. Therefore, the interactions, diet quality indices and thereby the effects of dietary patterns on health status might be different in each population. It is clear that healthy dietary pattern is a nutrient-dense and high quality pattern whilst Western pattern is a nutrient-poor and low quality; however, there might be some other patterns that their quality is not easily discernible. Additionally, it is clinically useful to know which nutrient deficiencies are more probable with specific dietary patterns. Despite a nutrition transition in our country, like other developing countries, a recent systematic assessment indicated no changes in diet quality of Iranians from 1999 to 2010, because of an increment in consumption of both healthy and unhealthy foods (18). However, it is not clear what were the impacts of these changes on nutritional status of Iranians. In this cross-sectional study, we aimed to find major dietary patterns in a sample of Isfahani adults and evaluate their associations with diet quality indices as well as nutrient adequacy ratios (NAR).

## Subjects and Methods

*Participants*

This cross-sectional study was performed in a sample of Isfahani adults aged 24-71 y and working in Esfahan Steel Company (n=400). To provide a random sampling, after considering all parts of company (n=14), we randomly selected some phone numbers using multistage cluster random sampling method. All subjects declared their willingness to participate in the research by providing written informed consent. The present study was approved by the research council and ethical committee of Isfahan University of Medical Sciences, Isfahan, Iran. We excluded participants if their energy intake was out of range 800-4200 kcal/d (n=7), and all statistical analysis was performed on 393 individuals.

*Dietary intake assessments*

Usual dietary intake was assessed by using a valid semi-quantitative food frequency questionnaire (FFQ). FFQ consisted of 168 food items with standard serving sizes commonly used by Iranians. Participants were asked to report the frequency of each food item according to their consumption during the last year. All FFQs were administered by a qualified dietitian. Daily intake of each food item were estimated based on the frequency of consumption, and then were converted to gram by using household measures (19). Daily energy and nutrient intakes were estimated by using NUTRIONIST IV which modified for Iranian's food. We assigned each food item into one of 38 described food groups, because of large numbers of the food items relative to the numbers of subjects (Table 1). We allocated a food item in a specific food group based on the similarity of nutrients content. Some food items were individually allocated in a food group either because of their unique nutrient contents or their contribution in a specific dietary pattern.

*Dietary Diversity Score (DDS)*

In order to calculate DDS, we used the method of Kant et al. (20). According to U.S. Department of Agriculture's Food Guide Pyramid, all food items were categorized in five main food groups (bread–grains, vegetables, fruit, meats, and dairy) (21). These main groups also divided to 23 subgroups to assess the diversity score of diets (22). Seven subgroups were considered for the bread-grain group (refined bread, biscuits, macaroni, whole bread, corn flakes, rice, and refined flour). Fruit and vegetables comprised two (fruit and fruit juice, berries and citrus) and seven subgroups (vegetables, potato, tomato, other starchy vegetables, legumes, yellow vegetables, and green vegetables), respectively. Four (red meat, poultry, fish, and eggs) and three (milk, yogurt, cheese) subgroups for meat and dairies were considered. According to the Food Guide Pyramid quantity, participants who consumed each group per day were defined as the consumers of that group. Maximum and minimum scores of each main group were 2 and 0. Total diversity score was calculated by the summation of the scores of the five main groups. Therefore, total dietary diversity score was ranged between 0-10. We used the same method for calculating each food group diversity score. For example, if a person consumed potato, yellow and green vegetables in each day, his vegetables' diversity score would be $(3 \div 7) * 2 = 0.85$.

**Table 1**. Food groups used in the factor analysis

| Food groups | Food items |
|---|---|
| Refined grains | White breads (lavash, baguettes), noodles, pasta, rice, toasted bread, milled barley, sweet bread, white flour, starch, biscuits |
| Whole grains | Dark breads (Iranian), barley bread, popcorn, cornflakes, wheat germ, bulgur |
| Potatoes | Potatoes |
| Tomatoes | Tomatoes, tomato sauce, tomato pasta |
| Yellow vegetables | Carrots |
| Green leafy vegetables | Spinach, lettuce |
| Cruciferous vegetables | Cabbage, cauliflower, Brussels sprouts, kale |
| Other vegetables | Cucumber, mixed vegetables, eggplant, celery, green peas, green beans, green pepper, turnip, corn, squash, mushrooms, onions |
| Garlic | Garlic |
| Olive | Olive, olive oil |
| Legumes | Beans, peas, lima beans, broad beans, lentils, soy |
| Nuts | Peanuts, almonds, pistachios, hazelnuts, roasted seeds, walnuts |
| Fruit | Pears, apricots, cherries, apples, raisins or grapes, bananas, cantaloupe, watermelon, oranges, grapefruit, kiwi, strawberries, peaches, nectarine, tangerine, mulberry, plums, persimmons, pomegranates, lemons, pineapples, fresh figs and dates dried figs, dried dates, dried mulberries, other dried fruit |
| Fruit juices | Apple juice, orange juice, grapefruit juice, other fruit juices |
| Fish | Canned tuna fish, other fish |
| Poultry | Chicken with or without skin |
| Red meats | Beef, lamb |
| Processed meats | Sausages, hamburger |
| Organ meats and animal fats | Beef liver, animal fats |
| Eggs | Eggs |
| Low-fat dairy products | Skim or low-fat milk, low-fat yogurt |
| High-fat dairy products | High-fat milk, whole milk, chocolate milk, cream, high-fat yogurt, cream yogurt, cream cheese, other cheeses, ice cream |
| Yogurt drink | Doogh |
| Vegetable oils | Vegetable oils (except for olive oil) |
| Hydrogenated fats | Hydrogenated fats, margarine |
| Butter | Butter |
| Mayonnaises | Mayonnaises |
| Broth | Broth |
| Pizza | Pizza |
| Salt and pickles | Salt and pickles |
| Snacks and French fries | Potato chips, corn puffs, crackers, popcorn, French fries |
| Condiments, sweets and deserts | Jam, jelly, honey, chocolates, cookies, cakes, confections |
| Soft drinks | Soft drinks |
| Sugars | Sugars, candies, gaz (an Iranian confectionery made of sugar, nuts, and tamarisk) |
| Tea | Tea |
| Coffee | Coffee |
| Pickle | Pickle |
| Curd | Curd |

*Dietary energy density (DED)*

To calculate DED, we divided each subject's self-report of total daily energy intake (kcal/day) into the total weight of foods consumed (g/day), excluding beverages (23). The weight of foods (excluding beverages) estimated by summing the weight of food items. We did not include the weight of drinks, since earlier studies have shown that changing in weight of drinks could not alter effects of DED on body weight (24).

*Healthy Eating Index (HEI)*

We used Kennedy et al' method to calculate HEI (25). According to this method, HEI contains 10 various components. Components 1 to 5, including grains, vegetables, fruit, milk, and meat, were scored based on the consumed proportion of each food group compared with recommended amounts (26). Thereby, after removing outlier values, participants who consumed at or above the recommended amounts received a score of 10. In contrast, individuals with no serving consumption would be scored 0. Other consumers were scored proportionally between 0-10. Components 5 to 10 contain percentages of total fat and saturated fatty acids consumption, the amounts of cholesterol intake, dietary diversity score, and the amounts of sodium intake, respectively. A diet containing less than 30% of total energy from fat, less than 10% of total energy from saturated fat, less than 300 mg cholesterol and no added table salt according to the FFQ, were awarded a full score of 10 points.

*Mean adequacy ratio and nutrient adequacy ratios (MAR & NAR)*

Nutrient adequacy ratio (NAR) was defined as the ratio of daily nutrient intakes to standard recommended amounts according to age and gender categories for each person (27). The values of NAR for 15 main nutrients including zinc, iron, calcium, magnesium, vitamin $B_1$, vitamin $B_2$, vitamin $B_3$, vitamin $B_5$, vitamin $B_6$, vitamin $B_9$, vitamin $B_{12}$, biotin, vitamin A, vitamin C, and vitamin D, were calculated. We divided the summation of NARs by the number of nutrients (n= 15) to calculated mean adequacy ratio (MAR) (28).

*Creation of MED scores:* For the calculation of MED dietary score, we considered a maximum of 9 points, counting 1 point if: the daily serving of fruits, fish, vegetables, whole grains, legumes, nuts and ratio of the gram of MUFA to saturated fatty acids (SFA) were equal or more than the median intake of study population and also the daily serving of meats (red meat, poultry and processed meats) and dairy products were less than median intake of the study population. Energy adjustment, using the residual method, was done for all food groups before the score ranking. Finally, we categorized participants according to the tertiles of their scores (29).

*Assessment of anthropometric measures:* Weight was measured by using digital scales while participants were minimally clothed and not wearing shoes. Weight was recorded to the nearest 100 grams. Height was measured by using a fixed-wall tape while the participants were standing, without shoes and shoulders were in normal position. Body mass index was calculated as weight (kg) divided by height ($m^2$). Waist circumference (WC) was measured at the narrowest level between the lowest rib and iliac crest over light clothing by using an unstretched tape measure and recorded to the nearest 0.5 cm. All measurements were taken by the same dietitian to reduce measurement error.

*Assessment of biomarkers:* To assess biochemical markers, 10 ml venous blood samples were drawn after an overnight (12 h) fast. Plasma concentrations of glucose and serum lipid profiles were measured by using commercially available enzymatic reagents (Pars Azmoon, Tehran, Iran) adapted to a Selectra-2 autoanalyzer (Vital Scientific, Spankeren, The Netherlands. Fasting blood sugar (FBS) was measured on the day of blood collection via enzymatic colorimetric method. Serum triglyceride levels were determined using enzymatic colorimetric tests with glycerol phosphate. Serum levels of HDL-C were assessed with phosphotungstic acid after precipitation of the apolipoprotein B-containing lipoproteins.

*Assessment of other variables:* Blood pressure was measure after a 15-min rest by using a standard mercury sphygmomanometer 2 times while participants were sitting. The mean of two measurements was recorded as subject's blood pressure. Demographic characteristics including age, sex, smoking habits, socioeconomic status, medical history, current use of medications was obtain with questionnaires.

*Definition of terms:* Obesity was defined as BMI more than 30 $kg/m^2$ (30). Abdominal obesity was considered as WC ≥88 cm for women and ≥102 cm for men (31).

*Statistical analysis*

Major dietary patterns were identified using principle component analysis and the factors were rotated by orthogonal transformation. Eigenvalues>1.6 and the Scree test were considered to retain important factors (32). After the fourth factor, the eigenvalues of the factors fell considerably and changed slightly after the fifth factor. Based on our interpretation of the data and available evidence, we labeled the derived factors (dietary patterns). The scores of the dietary patterns were computed by summing intakes of food groups weighted by their factor loadings, and a factor score was given to each participant for each recognized pattern (32). Participants were categorized based on the tertile of dietary pattern scores. To detect the significant differences in quantitative variables (e.g. age, BMI, WC, NARs of different nutrients and diet quality scores), one-way analysis of variance was done. Chi-square tests were performed to compare the distribution of qualitative variables. We used SPSS software (version 9.05; SPSS Inc, Chicago IL) for all statistical analyses.

## Results

Four dietary patterns were recognized by using factor analysis: the healthy dietary pattern (loaded by various kinds of vegetables, low fat dairy products and nuts), the traditional dietary pattern (loaded by refined grains, high-fat dairy products, sugars, legumes, tea, salt and pickles, eggs, vegetable oils and hydrogenated oils, but low in whole grains), the high animal fat and protein dietary pattern (loaded by organ meats and animal fats, red meats, broth, potatoes and processed meats) and the Western dietary pattern (loaded by soft drinks, pizza, processed meats, mayonnaises, snacks and French fries, butter, condiments, sweets and deserts). Table 2 shows the factor-loading matrixes for identified dietary patterns are shown in. Other identified but minor dietary patterns were not included in the subsequent analysis, because they explained only small variances.

General characteristics and dietary intakes of participants are shown in Table 3. Participants in the highest tertile of Western dietary pattern were younger (P=0.06), less likely to be married and low socio-economic status. Participants in the highest tertile of traditional dietary pattern were more likely to be smoker or ex-smoker. Compared with individuals in the highest tertile of high animal fat and protein dietary pattern, those in the lowest tertile were more likely to be married. Weight was not significantly different across the tertiles of different dietary patterns.

Age- and sex-adjusted energy intake was less in the first tertile of all dietary patterns compared with the top tertile. Individuals in the third tertile of healthy dietary pattern had lower carbohydrate and cholesterol but greater protein and fiber intake. Individuals in the first tertile of traditional dietary pattern had higher protein, fat, saturated fatty acids (SFA) and fiber intake, but carbohydrate was not significantly different across the tertiles. Conversely, protein, fat, cholesterol and SFA consumed in greater amounts by individuals in the third tertile of high animal fat and protein dietary pattern, whilst carbohydrate was consumed in less amounts. Compared with individuals in the first tertile of Western dietary pattern, those in the highest tertile consumed greater protein and SFA.

The NAR values of different nutrients across tertiles of dietary pattern scores are presented in Table 4. Higher healthy dietary pattern scores were associated with greater NARs for all nutrients. Traditional dietary pattern scores were inversely related to NARs of all nutrients except for B12. Participants in the first tertile of high animal fat and protein consumed less amounts of Zn, Fe, B1, B2, B3, B6, b12 and vitamin A. other nutrients were not significantly correlated with this pattern. Higher adherence to Western dietary pattern was associated with lower consumption of B2, Ca and Zn. Other nutrients were significantly different across tertiles of Western pattern. MAR was greater in the top tertile of high animal fat and protein, but no significant difference was found across tertiles of Western and healthy patterns.

Table 5 indicates the associations of priori diet quality indices and identified dietary pattern scores. Individuals with higher scores of healthy dietary pattern had greater HEI, DDS and MED scores. HEI was inversely related to other dietary pattern scores. Higher scores of both Western and high animal fat and protein dietary patterns were more energy-dense in comparison with lower scores. However, ED was

**Table 2**. Factor-loading matrix for major dietary patterns[1]

| | Dietary patterns | | | |
|---|---|---|---|---|
| **Food groups** | **Healthy** | **Traditional** | **High animal fat and protein** | **Western** |
| Green leafy vegetables | 0.61 | | | |
| Other vegetables | 0.61 | | | |
| Tomatoes | 0.59 | | | |
| Olive | 0.55 | | | |
| Yellow vegetables | 0.53 | | | |
| Cruciferous vegetables | 0.52 | | | |
| Fish | 0.42 | | | 0.2 |
| Pickle | 0.36 | | | |
| Curd | 0.35 | | 0.28 | 0.22 |
| Garlic | 0.34 | | | |
| Yogurt drink | 0.33 | | | |
| Low-fat dairy products | 0.31 | | | -0.21 |
| Nuts | 0.24 | | | |
| Coffee | 0.21 | | | |
| Sugars | | 0.65 | | 0.21 |
| Legumes | | 0.58 | | |
| Tea | | 0.51 | | |
| Refined grains | | 0.42 | | |
| Salt and pickles | | 0.40 | | |
| Poultry | | 0.33 | | |
| Whole grains | | -0.30 | | |
| Eggs | | 0.296 | | |
| High-fat dairy products | | 0.28 | 0.22 | 0.21 |
| Vegetable oils | | 0.27 | | |
| Hydrogenated fats | | 0.27 | | |
| Organ meats and animal fats | | | 0.91 | |
| Red meats | | | 0.89 | |
| Broth | | 0.26 | 0.32 | -0.22 |
| Potatoes | | | 0.23 | |
| Soft drinks | | | | 0.57 |
| Pizza | | | | 0.51 |
| Processed meats | | | 0.33 | 0.49 |
| Mayonnaises | | | | 0.38 |
| Snacks and French fries | | | | 0.37 |
| Butter | | | | 0.34 |
| Condiments, sweets and deserts | | | | 0.32 |
| Fruit juices | | | | 0.25 |
| Fruit | | | | 0.20 |
| *Percentage of variance explained (%)* | *0.083* | *0.072* | *0.53* | *0.045 1* |

*Values<0.20 were excluded for simplicity.*

**Table 3**. Characteristics and dietary intakes of study participants across the tertile of dietary pattern scores.

| | Healthy | | | | Traditional | | | | High animal fat and protein | | | | Western | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Tertile 1 | Tertile 2 | Tertile 3 | P[1] | Tertile 1 | Tertile 2 | Tertile 3 | P | Tertile 1 | Tertile 2 | Tertile 3 | P | Tertile 1 | Tertile 2 | Tertile 3 | P |
| n | 129 | 130 | 130 | | 129 | 130 | 130 | | 129 | 130 | 130 | | 129 | 130 | 130 | |
| Age (y) | 37.77± 0.74 | 38.06± 0.69 | 38.92± 0.42 | 0.5 | 37.95± 0.81 | 38.72± 0.63 | 38.08± 8.22 | 0.7 | 37.79± 0.75 | 38.75± 8.0 | 38.22± 0.42 | 0.6 | 39.13± 0.71 | 38.77± 0.81 | 36.87± 0.62 | 0.06 |
| Weight (kg) | 78.69± 1.21 | 79.10± 1.15 | 81.06± 1.17 | 0.3 | 77.59± 1.20 | 80.58± 1.19 | 80.69± 1.12 | 0.1 | 81.40± 1.25 | 79.04± 1.18 | 78.44± 1.09 | 0.2 | 79.64± 1.04 | 80.76± 1.20 | 79.62± 1.27 | 0.4 |
| Married (%) | 90.7 | 90.8 | 93.8 | 0.6 | 89.1 | 94.6 | 91.5 | 0.3 | 93.0 | 95.4 | 86.9 | 0.04 | 92.2 | 96.2 | 86.9 | 0.02 |
| Low-socioeconomic status (%) | 16.1 | 16.3 | 13.1 | 0.5 | 12.7 | 18.1 | 14.6 | 0.7 | 10.2 | 14.8 | 20.5 | 0.2 | 19.2 | 15.5 | 10.9 | 0.04 |
| Smoker or ex-smoker (%) | 27.1 | 30.0 | 28.4 | 0.7 | 13.9 | 31.6 | 40.0 | <0.0001 | 31.8 | 21.5 | 32.3 | 0.2 | 24.0 | 30.7 | 30.8 | 0.6 |
| **Dietary intakes** | | | | | | | | | | | | | | | | |
| Energy (kcal/d) | 1970.27± 45.26 | 2320.09± 53.13 | 2642.44± 32.73 | <0.0001 | 1969.77± 54.64 | 2280.28± 45.12 | 2682.74 ±51.87 | <0.0001 | 2249.62 ±55.82 | 2123.94± 47.97 | 2561.38± 59.03 | <0.0001 | 2105.04± 48.42 | 2190.23± 52.87 | 2638.56± 57.14 | <0.0001 |
| Carbohydrate (% of total energy) | 64.48± 0.58 | 64.78± 0.55 | 62.50± 0.58 | 0.01 | 62.83±0.59 | 64.39± 0.55 | 64.52± 0.59 | 0.096 | 66.20± 0.52 | 64.39± 0.53 | 61.19± 0.54 | <0.0001 | 63.37± 0.57 | 64.34± 0.56 | 64.04± 0.59 | 0.5 |
| Fat (% of total energy) | 24.58± 0.54 | 24.45± 0.51 | 25.78± 0.54 | 0.2 | 26.11± 0.54 | 24.59± 0.51 | 24.12± 0.54 | 0.04 | 23.61± 0.50 | 24.30± 0.05 | 26.89± 0.51 | <0.0001 | 24.59± 0.52 | 24.34± 0.51 | 25.88± 0.54 | 0.1 |
| Protein (% of total energy) | 13.17± 0.28 | 14.10± 0.26 | 16.40± 0.28 | <0.0001 | 15.57± 0.30 | 14.05± 0.28 | 14.07± 0.30 | <0.0001 | 13.54± 0.28 | 14.73± 0.28 | 15.40± 0.28 | <0.0001 | 15.26± 0.29 | 14.71± 0.28 | 13.72± 0.29 | 0.002 |
| Cholesterol (mg/d) | 212.01± 10.27 | 195.17± 9.68 | 175.19± 10.22 | 0.05 | 197.29± 10.48 | 186.20± 9.78 | 198.77± 10.44 | 0.6 | 136.34± 8.13 | 161.19± 8.18 | 284.25± 8.28 | <0.0001 | 194.03± 10.06 | 192.80± 9.83 | 195.40± 10.32 | 0.98 |
| SFA (% of total energy) | 7.06± 0.20 | 7.10± 0.19 | 6.84± 0.20 | 0.6 | 7.70± 0.20 | 6.94± 0.18 | 6.36± 0.20 | <0.0001 | 6.50± 0.19 | 7.04± 0.19 | 7.45± 0.19 | 0.002 | 6.47± 0.19 | 6.74± 0.18 | 7.78± 0.19 | <0.0001 |
| Dietary fiber (g/d) | 15.0± 0.42 | 18.16± 0.40 | 21.23± 0.42 | <0.0001 | 19.69± 0.47 | 17.93± 0.44 | 16.81± 0.47 | <0.0001 | 17.94± 0.45 | 18.05± 0.45 | 18.43± 0.46 | 0.7 | 18.64± 0.46 | 18.20± 0.45 | 17.60± 0.47 | 0.3 |

*[1]ANOVA for quantitative variables and chi-square test for qualitative variables.*
*[2] Mean±SE (all such values), unless indicated.*

**Table 4**. The mean ± SE of diet mean adequacy ratio (MAR) and nutrients adequacy ratio (NAR) across the tertiles of dietary patterns.

| | Healthy | | | | Traditional | | | | High animal fat and protein | | | | Western | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Tertile 1 | Tertile 2 | Tertile 3 | P [1] | Tertile 1 | Tertile 2 | Tertile 3 | P | Tertile 1 | Tertile 2 | Tertile 3 | P trend | Tertile 1 | Tertile 2 | Tertile 3 | P |
| MAR | 1.4±0.07 | 1.4±0.06 | 1.5±0.07 | 0.4 | 1.4±1.0 | 1.3±0.7 | 1.5±0.7 | <0.0001 | 1.1±0.05 | 1.1±0.05 | 2.0±0.05 | <0.0001 | 1.4±0.06 | 1.4±0.06 | 1.4±1.06 | 0.9 |
| NARs of nutrients | | | | | | | | | | | | | | | | |
| Zn | 0.7±0.02 | 0.7±0.02 | 0.9±0.03 | <0.0001 | 0.9±0.02 | 0.7±0.02 | 0.7±0.02 | <0.0001 | 0.74±0. 02 | 0.76±0. 02 | 0.85±0.02 | <0.0001 | 0.84±0.02 | 0.78±0.02 | 0.73±0.02 | 0.005 |
| Ca | 1.1±0.04 | 1.2±0.04 | 1.5±0.04 | <0.0001 | 1.4±0.04 | 1.3±0.04 | 1.2±0.04 | 0.01 | 1.3±0. 04 | 1.3±0. 04 | 1.3±0. 04 | 0.5 | 1.4±0. 04 | 1.3±0. 04 | 1.1±0. 04 | <0.0001 |
| Fe | 1.5±0.04 | 1.6±0.04 | 1.8±0.04 | <0.0001 | 1.7±0.04 | 1.6±0.04 | 1.5±0.04 | 0.04 | 1.4±0. 04 | 1.6±0. 04 | 1.8±0. 04 | <0.0001 | 1.6±0. 04 | 1.7±0. 04 | 1.6±0. 04 | 0.4 |
| Mg | 0.6±0.02 | 0.7±0.02 | 0.9±0.02 | <0.0001 | 0.88±0.02 | 0.73±0.02 | 0.68±0.02 | <0.0001 | 0.8±0. 02 | 0.8±0. 02 | 0.5±0. 02 | 0.6 | 0.8±0.02 | 0.8±0.02 | 0.7±0.02 | 0.3 |
| B1 | 1.1±0.03 | 1.2±0.02 | 1.4±0.03 | <0.0001 | 1.4±0.03 | 1.3±0.03 | 1.2±0.03 | <0.0001 | 1.2±0. 03 | 1.28±0. 03 | 1.33±0. 03 | 0.001 | 1.3±0. 03 | 1.3±0. 03 | 1.2±0. 03 | 0.4 |
| B2 | 1.7±0.05 | 1.8±0.04 | 2.2±0.05 | <0.0001 | 2.1±0.05 | 1.9±0.05 | 1.8±0.05 | <0.0001 | 1.8±0. 05 | 1.9±0. 05 | 2.0±0. 05 | 0.04 | 2.0±0. 05 | 1.9±0. 05 | 1.8±0. 05 | 0.001 |
| B3 | 1.0±0.02 | 1.1±0.02 | 1.2±0.02 | 0.003 | 1.15±0.02 | 1.09±0.02 | 1.05±0.02 | 0.02 | 1.0±0.02 | 1.1±0.02 | 1.2±0.02 | 0.001 | 1.0±0.02 | 1.1±0.02 | 1.1±0.02 | 0.09 |
| B5 | 0.6±0.01 | 0.7±0.01 | 0.8±0.01 | <0.0001 | 0.74±0.3 | 0.68±0.2 | 0.65±0.2 | <0.0001 | 0.7±0.01 | 0.7±0.01 | 0.7±0.01 | 0.3 | 0.7±0.01 | 0.7±0.01 | 0.7±0.01 | 0.9 |
| B6 | 1.2±0.04 | 1.4±0.04 | 1.6±0.04 | <0.0001 | 1.6±0.04 | 1.3±0.04 | 1.2±0.04 | <0.0001 | 1.29±0. 04 | 1.32±0. 04 | 1.6±0. 04 | <0.0001 | 1.4±0. 04 | 1.4±0. 04 | 1.4±0. 04 | 0.9 |
| B9 | 0.8±0.04 | 0.9±0.04 | 1.1±0.04 | <0.0001 | 1.1±0.04 | 0.92±0.04 | 0.88±0.04 | 0.003 | 1.0±0. 04 | 0.9±0. 04 | 1.0±0. 04 | 0.2 | 1.0±0. 04 | 0.9±0. 04 | 1.0±0. 04 | 0.9 |
| B12 | 7.0±0.9 | 5.1±0.8 | 3.4±0.9 | 0.02 | 6.8±0.9 | 4.5±0.8 | 4.2±0.9 | 0.08 | 1.0±0.7 | 1.3±0.7 | 13.1±0.7 | <0.0001 | 5.2±0.8 | 4.8±0.8 | 5.5±0.8 | 0.9 |
| Biotin | 0.26±0.02 | 0.33±0.02 | 0.39±0.02 | <0.0001 | 0.4±0.02 | 0.3±0.02 | 0.2±0.02 | <0.0001 | 0.3±0.02 | 0.3±0. 02 | 0.3±0. 02 | 0.1 | 0.3±0.02 | 0.3±0. 02 | 0.3±0. 02 | 0.8 |
| Vitamin C | 1.7±0.1 | 2.2±0.1 | 3.0±0.1 | <0.0001 | 2.9±0.1 | 2.1±0.1 | 1.8±0.1 | <0.0001 | 2.5±0.1 | 2.2±0.1 | 2.1±0.1 | 0.08 | 2.1±0.1 | 2.2±0.1 | 2.5±0.2 | 0.2 |
| Vitamin A | 1.2±0.05 | 1.5±0.05 | 2.0±0.05 | <0.0001 | 1.8±0.0.6 | 1.5±0.0.6 | 1.3±0.0.6 | <0.0001 | 1.45±0.06 | 1.55±0.06 | 1.7±0.06 | 0.01 | 1.6±0.06 | 1.6±0.06 | 1.6±0.06 | 0.9 |
| Vitamin D | 0.16±0.01 | 0.20±0.01 | 0.23±0.01 | 0.003 | 0.25±0.01 | 0.20±0.01 | 0.15±0.01 | <0.0001 | 0.2±0. 01 | 0.2±0.01 | 0.2±0. 01 | 0.06 | 0.2±0. 01 | 0.2±0. 01 | 0.2±0. 01 | 0.5 [1] |

*Resulted from ANCOVA. Adjusted for age, sex and energy intake.*

**Table 5**. The mean ± SD of diet quality indices across the tertiles of dietary patterns.

| | Healthy | | | | Traditional | | | | High animal fat and protein | | | | Western | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Tertile 1 | Tertile 2 | Tertile 3 | P [1] | Tertile 1 | Tertile 2 | Tertile 3 | P | Tertile 1 | Tertile 2 | Tertile 3 | P trend | Tertile 1 | Tertile 2 | Tertile 3 | P |
| HEI | 67.7±6.3 | 69.3±6.4 | 73.5±6.8 | <0.0001 | 72.6±7.7 | 68.9±6.4 | 69.1±6.0 | <0.0001 | 70.5±7.2 | 71.4±7.1 | 68.6±6.2 | 0.004 | 71.5±6.9 | 71.0±6.4 | 68.1±7.0 | <0.0001 |
| DDS | 5.6±0.8 | 5.6±0.7 | 5.9±0.8 | 0.002 | 5.8±0.6 | 5.5±0.8 | 5.8±0.8 | 0.004 | 5.5±0.8 | 5.8±0.7 | 5.9±0.7 | 0.001 | 5.7±0.8 | 5.8±0.8 | 5.6±0.7 | 0.4 |
| DED | 0.8±0.2 | 0.8±0.1 | 0.8±0.1 | 0.5 | 0.8±0.2 | 0.8±0.1 | 0.8±0.1 | 0.7 | 0.8±0.1 | 0.8±0.2 | 0.9±0.1 | <0.0001 | 0.8±0.1 | 0.8±0.2 | 0.9±0.2 | 0.002 |
| Mediterranean score | 3.5±1.5 | 4.3±1.5 | 5.5±1.2 | <0.0001 | 4.2±1.7 | 4.3±1.6 | 4.8±1.5 | 0.004 | 4.4±1.5 | 4.3±1.6 | 4.5±1.6 | 0.5 | 4.4±1.6 | 4.3±1.5 | 4.5±1.7 | 0.5 [1] |

Resulted from one-way ANCOVA. Adjusted for age, sex and energy intake.

not significantly related to traditional and healthy dietary patterns. Individuals in the higher tertile of traditional pattern had greater MED scores. Subjects in the higher tertile of high animal fat and protein were more probably to have higher diversity scores.
We did not find any differences in the prevalence of general and abdominal obesity as well as lipid profile abnormalities across the tertiles of different dietary patterns (Figure 1).

## Discussion

The results of the current study illustrated that healthy dietary pattern was a high quality pattern whilst Western and traditional dietary patterns were associated with lower diet quality indices. Higher scores of healthy pattern were more nutrient-dense in comparison with higher scores of traditional and Western dietary patterns.

Dietary patterns have attracted much attention during last decade; however, to the best of our knowledge, no study has assessed their associations with diet quality indices. Due to differences in dietary patterns in each region, it is relevant to determine their associations with diet quality indices to choose the best one separately in each population. On the other hand, it is probably that loaded factors in healthy dietary patterns differ from one population to another and thereby affect diet quality indices as well as health outcomes (6, 33, 34). Although it is expected that healthy dietary pattern to be high quality diet and Western dietary pattern to be low-quality diet, it is of interest to know which nutrients are less likely to be met by specific dietary pattern. Additionally, other identified dietary patterns in different populations might be differently related to diet quality indices. It would be clinically useful to know which nutrients are deficient in each dietary pattern.

In the present study, high animal fat and protein dietary pattern was loaded by some unhealthy foods like potatoes, high fat dairy products and animal protein and fats, but higher scores of this pattern were associated with greater NARs of different nutrients

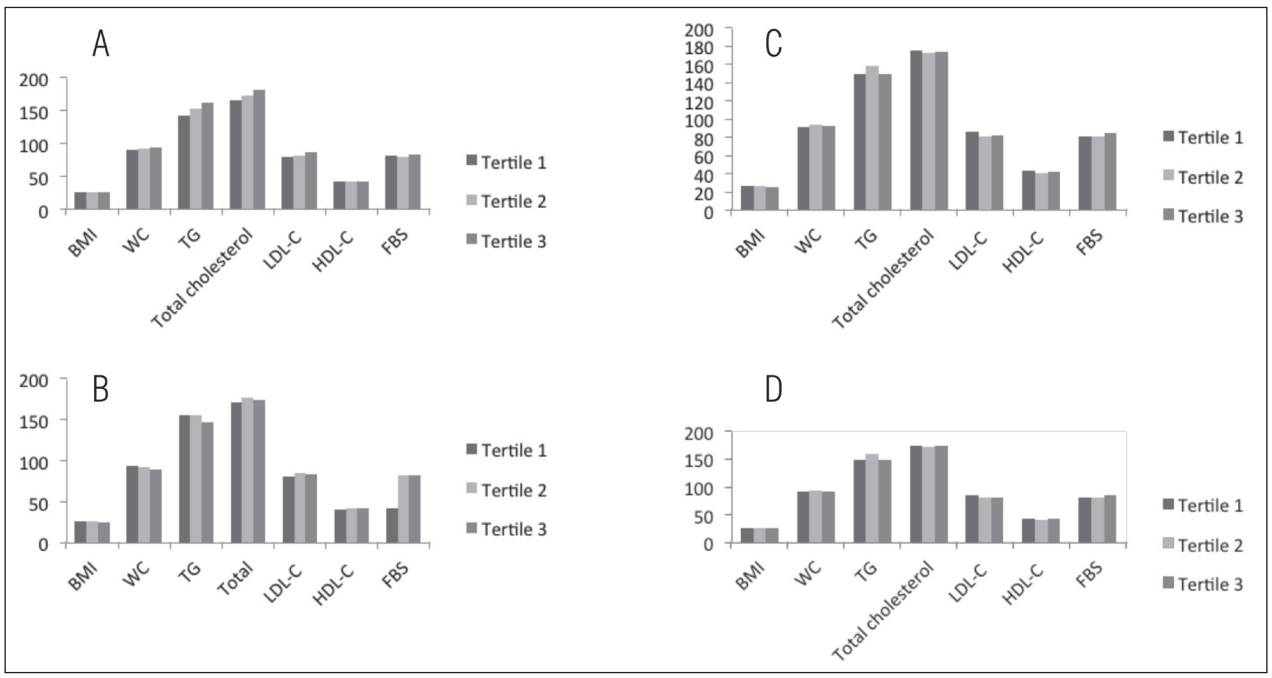


**Figure 1.** Mean of anthropometric measures, lipid profile and fasting blood sugar across tertiles of dietary patterns (A-healthy dietary pattern, B- Traditional dietary pattern, C- High animal fat and protein dietary pattern, D- Western dietary pattern). BMI: body mass index, WC: waist circumference, TG: triglyceride, LDL-C: low density lipoprotein, HDL-C: high density lipoprotein, FBS: fasting blood sugar.

including Zn, Fe, B1, B2, B3, B12 and vitamin A, in comparison with traditional and Western dietary patterns. This result is in line with the results of a current analysis on individuals participating in NHANES. They showed that the consumption of animal-based protein sources contributes to greater intakes of several nutrients of concern (e.g. Zn, Fe and B12) (8, 9). On the other side, we observed that traditional dietary pattern was mainly loaded by refined carbohydrates and inversely related to all NARs. Therefore, this might be concluded that high animal fat and protein are preferred to the traditional pattern to provide adequate intakes of different micronutrients. However, with considering their associations with SFA and cholesterol intake, it seems that traditional dietary pattern is healthier than high animal fat and protein.

Moreover, we found that higher adherence to the high animal fat and protein dietary pattern was associated with lower HEI scores and no significant difference in MED score, whilst traditional dietary pattern was positively related to MED score and negatively to HEI score. Therefore, it is difficult to determine the superior pattern between two dietary patterns, since higher scores of high animal fat and protein are more nutrient-dense whilst traditional dietary pattern contained lower amounts of SFA and cholesterol besides higher scores of MED pattern.

Other relevant findings of this study are related to determining nutrients of concern, including Mg, Zn, vitamin D, biotin and B5. Additionally, Ca and B2 deficiencies are two prevalent nutrient deficiencies among Iranian persons (35, 36). Our results suggested that their adequacy ratios significantly decreased according to each increased tertile of Western dietary pattern score.

In contrast with earlier publications, we did not observe any significant differences in cardiometabolic risk factors (1, 3, 4, 6, 37, 38). This discrepancy between our study and others might be attributable to differences in study population. Our participants were younger than the participants of Esmaillzadeh et al.'s study (6). Additionally, it was conducted among female teachers and controlled for the role of physical activity, whilst we did not. However, a new cohort study indicated that dietary patterns in older persons could not predict the risk of related-deaths to CVDs events and cancer (34). This study was not a representative sample and most of our participants were men. Therefore, the external validity of our findings might be limited, but its internal validity is acceptable, because of random sampling method.

In conclusion, healthy dietary pattern is favorably associated with NARs and different priori diet quality indices, but Western is not significantly related to these indices. Traditional and high animal fat and protein dietary patterns are differently related to the diet quality indices. Whilst higher scores of high animal fat and protein dietary pattern were more nutrient-dense, greater adherence to the traditional dietary pattern was associated with greater Med score. Further studies are needed to determine the quality of dietary patterns to determine the best pattern.

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L.A. conceptualized and designed the study. F.H. performed statistical analyses and drafted the manuscript, and interpreted data. N.R.P.F. participated in data collection and entry. M.K. and M.H.B. participated in data collection and took measurements. All authors approved the final manuscript for submission.

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ORIGINAL ARTICLE

# An evaluation of the effects of Ramadan fasting on anthropometric, metabolic and endocrine parameters

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**Summary.** *Objective:* The aim of this study was to examine the effects of the energy and nutrients intake at Ramadan fasting on anthropometric measurements, metabolic and endocrine parameters. *Methods:* This prospective study included a total of 80 healthy volunteers, aged 19-50 years, who were fasting during the month of Ramadan. Anthropometric measurements, blood samples were taken and nutritional intake was recorded of all participants at 5 days before Ramadan(BR) and on the 27th day at the end of Ramadan (AR). The anthropometric measurements comprised height, weight, body mass index(BMI), hip-waist circumference, body fat ratio and fat mass measurements. From the blood samples, analysis was made of fasting glucose, insulin, total cholesterol, LDL-C, HDL-C and TG. From the records of food intake, energy and nutrients were calculated. The anthropometric measurements, metabolic and endocrine parameters, energy and nutrients BR and AR were compared generally. *Results:* In the period from BR to AR in the values, a decrease was determined in body weight (77.4±11.0, 76.2±10.7kg, p<0.01), BMI (26.1±2.6, 25.7±2.5kg/m2, p<0.001), waist circumference (94.5±9.0, 91.8±8.3cm p<0.001) and body fat ratio (24.4%±6.4%, 23.8%±5.9%, p<0.01). An increase was determined in the values of fasting glucose (83.5±7.4, 91.1±12.3mg/dl, p<0.01), insulin (8.8±4.1, 10.1±4.5μU/ml, p<0.01) and HOMA-IR (1.8±0.9, 2.2±1.1, p<0.01). There was a decrease in HDL-C levels (p<0.001). *Conclusion:* Ramadan fasting was observed to have positive effects on anthropometric measurements such as body weight, BMI, fat mass and waist circumference, which are cardiovascular risk factors, but no similar positive effect was seen on endocrine and metabolic parameters.

**Key words:** Ramadan fasting, anthropometric measurement, metabolic and endocrine parameters

## 1. Introduction

Obesity is a significant health problem in modern societies and according to World Health Organisation (WHO) data, 13% of the adult population worldwide are obese (1, 2). In the formation of obesity, genetic, environmental and physiological factors change the balance between energy intake and consumption (3). Healthy eating has been shown to have positive effects on obesity, and the number and timing of daily meals is also important (1). Increasing the duration of fasting by reducing the number of daily meals is known as intermittent fasting. The beneficial effects of intermittent fasting on glycaemic control, metabolism, cardiovascular risk, cancer and life expectancy have been researched in recent studies (4).

Although previous studies report different results, Ramadan Fasting is appropriate model for researching beneficial effects of intermittent fasting (5). In the ninth month of the Islamic calendar (the month of Ramadan), Muslims fast without eating or drinking from sunrise to sunset. Throughout this month, nutritional intake is obtained from meals in the evening and during the night, and thus the duration of sleep is shortened (6). Energy and glucose metabolism, appetite and hormonal responses are changed by prolonged fasting periods and the changes in nutritional habits also effects lipid profile and body composition during Ramadan (7, 8).

Some previous studies have examined the effects of Ramadan fasting on body composition and biochemical parameters (6). However, the majority of those studies have been conducted on a limited number of subjects. In this study, an examination was made of the effects of Ramadan fasting on anthropometric, metabolic and endocrine parameters and the energy and nutrients intake of individuals who were fasting.

## 2. Method

The study was prospectively conducted on healthy volunteers, aged 19-50 years, who were fasting during the month of Ramadan 2017. Approval for the study was granted by the Local Ethics Committee (decision no: May 2017, 38/27). Subjects were excluded from the study if they had diabetes mellitus, hypertension, were smokers, had a >5% increase or decrease of body weight in the previous 3 months, or if they were obese (BMI ≥30 kg/m$^2$).

Nutritional intake was recorded, and blood samples and anthropometric measurements were taken of all participants at 5 days before Ramadan (BR) and on the 27th day at the end of Ramadan (AR). The metabolic and endocrine data obtained from the BR and AR blood samples, the BR and AR anthropometric measurements and the BR and AR nutritional elements intake were evaluated separately for all the individuals. The subjects who had sahur fasted for the 27 days of Ramadan without eating and drinking for approximately 15 hours per day. Apart from the duration of fasting, the subjects ate without calorie restriction.

All participants were contacted by telephone on days 7, 14, 21 and 27 of the month of Ramadan to confirm whether or not they were complying with the study protocol. During the follow-up period, any subjects who had stopped fasting for any reason, who had an interval of not fasting (apart from females who could not fast during the menstrual cycle) were withdrawn from the study.

For all subjects, measurements were taken of body weight, height, waist-hip circumference, body mass index (BMI) and body composition. Body weight was measured with a Tanita MC 780 device, which was sensitive to 0.1 kg, with the individual in a fasting state and without shoes. Height was measured with a rigid measure, sensitive to 0.1 cm. Hip circumference was measured in cm from the highest point of the measure with the subject standing side-on. BMI was calculated with the formula of body weight (kg) / height (m)$^2$. On the day of measurements, the individual had not had anything to eat or drink for at least 8 hours previously, had not had a bath or sauna and had not done any sport. When taking the measurements, the barefoot individual stood on the metal plate of the device and held the appropriate parts with the hands with the arms parallel to the body. The body fat ratio percentage and body fat mass, as kg, were recorded.

Blood samples were collected after 8-10 hours fasting. Analysis of the serum glucose, insulin, total cholesterol, high density lipoprotein cholosterol (HDL-C), low density lipoprotein cholosterol (LDL-C) and triglyceride (TG) levels was made using a Saturna 300 Crony device. For insulin resistance, the homeostasis model evaluation (HOMA-IR) was used. The formula of "serum insulin level (IU/ml) x serum glucose level (mg/dl) /405" was used in the HOMA-IR calculation.

Energy and nutrients intake was determined with a 3-day record of food consumption. The subjects were instructed how to record 3 days of food intake as 2 consecutive weekdays and one weekend day. Portion sizes were explained using household measures (spoons, plates, slices, water glass, etc). The nutrients and amounts in the food and drink consumed throughout 3 days were calculated using standard meal recipes and the daily amount of consumed nutrition was determined by dividing each nutrient by 3 (9). The

values of the mean energy and nutrients consumed were analysed using the "Computer-assisted nutritional program, nutritional information system".

Data obtained in the study were analysed using SPSS 11.5 software. In the descriptive statistics, continuous variables were stated as mean±standard deviation (SD), median, minimum and maximum values and numerical variables as number (n) and percentage (%). In the comparison of the BR and AR body composition and laboratory values of the individuals fasting during Ramazan, conformity of the data to normal distribution was assessed with the Paired Samples t-test and the Wilcoxon test. A value of $p<0.05$ was accepted as statistically significant.

## 3. Results

A total of 85 healthy individuals were enrolled in the study and the evaluations were completed with 80 (94.1%) subjects. Due to health reasons, 5 subjects stopped fasting and were withdrawn from the study. The 80 subjects who completed the study comprised 52 (65%) males and 28 (35%) females with a mean age of 37±7.2 years (Table 1).

***Anthropometric results;*** In the measurements of subjects, a significant decrease was determined in body weight (77.4±11.0, 76.2±10.7 kg, $p<0.01$), BMI (26.1±2.6, 25.7±2.5 kg/m2, $p<0.001$), waist- hip circumference (94.5±9.0, 91.8±8.3 cm/ 105.8±5.0, 102.7±4.7 cm, $p<0.001$) and body fat ratio (24.4%±6.4%, 23.8%±5.9%, $p<0.01$) (Table 1). Also, a significant decrease was determined in the BR and AR body fat ratio (24.4±6.4%, 23.8±5.9%, $p<0.01$) and fat mass (18.7±5.0 kg, 18.0±4.8 kg, $p<0.001$).

***Metabolic and endocrine results;*** In the individuals, a significant increase was determined in the period from BR to AR in the values of fasting glucose (83.5±7.4, 91.1±12.3 mg/dl, $p<0.01$), insulin (8.8±4.1, 10.1±4.5 μU/ml, $p<0.01$) and HOMA-IR (1.8±0.9, 2.2±1.1, $p<0.01$) (Table 2). Ramadan fasting was not observed to have any significant effect on total cholesterol, LDL-C or triglycerides ($p>0.05$). A significant decrease was determined in HDL values (52.1±16.5, 46.3±13.9, $p<0.05$) (Table 2).

***Energy and nutrients intake results;*** In the evaluation of the data obtained from the records of food intake for 3 days, a significant decrease was determined

**Table 1.** Demographic data and anthropometric measurements before and after Ramadan

| | **Sahur** | **Without sahur** | **Total** |
|---|---|---|---|
| Age (year) | 37±7.3 | 36.42±6.8 | 37±7.2 |
| Gender | | | |
| Male | 42 (% 63.6) | 10 (% 71.4) | 52 (% 65) |
| Female | 24 (% 36.4) | 4 (% 28.6) | 28 (% 35) |
| | **Before Ramadan** | **After Ramadan** | **P value** |
| Weight (kg) | 77.4±11.0 | 76.2±10.7 | 0,003 |
| BMI | 26,1±2,6 | 25.7±2.5 | 0.000 |
| WC (cm) | 94.5±9.0 | 91.8±8.3 | 0.000 |
| HC (cm) | 105.8±5.0 | 102.7±4.7 | 0.000 |
| W/H | 0.8±0.07 | 0.8±0.06 | 0.921 |
| BF (%) | 24.4±6.4 | 23.8±5.9 | 0.005 |
| BFM (kg) | 18.7±5.0 | 18.0±4.8 | 0.005 |

*BMI, body mass index; WC, waist circumference; HC, hip circumference; W/H waist to hip ratio; BF, body fat percentage; BFM, body fat mass*

from BR to AR in respect of energy, carbohydrate, fat and fibre intake (p<0.001). Furthermore, polyunsaturated fatty acids (PUFAs) (23.6±10.5 g, 17.4±7.2 g, p<0.05), monounsaturated fatty acids (MUFAs) (29.4±8.9 g, 23±10.6 g, p<0.05), n-3 polyunsaturated fatty acids (n-3) (2.3±1.4 g, 1.6±0.7g, p<0.004) and n-6 polyunsaturated fatty acids (n-6) (20.9±9.8 g, 15.1±7.4 g, p<0.002) intakes decreased significantly and no change observed at n-6/n-3 ratio (10.3±5.4 g, 10.4±5.9 g, p>0.5) and saturated fatty acids (SFAs) intake (28.5±9.1 g, 25.4±13.8 g, p>0.05) (Table 3).

## 4. Discussion

The results of this study based on fasting during Ramadan showed that, i) there was a reduction in the anthropometric measurements of body weight, BMI, waist circumference and body fat ratio, ii) the metabolic and endocrine parameters of fasting glucose, insulin and HOMA-IR values increased and with the exception of a decrease in HDL-C levels, there was no change in the blood cholesterol levels, and iii) all the parameters of energy and nutrients intake decreased.

**Table 2.** Metabolic and endocrine parameters before and after Ramadan

| | Before Ramadan | After Ramadan | P value |
|---|---|---|---|
| FBG (mg/dl) | 83.5±7.4 | 91.1±12.3 | 0,003 |
| Insulin (μU/ml) | 8.8±4.1 | 10.1±4.5 | 0.004 |
| HOMA-IR | 1.8±0.95 | 2.2±1.1 | 0.004 |
| TC (mg/dl) | 191.4±35.2 | 187.1±33.7 | 0.062 |
| LDL-C (mg/dl) | 116.6±34.2 | 115.9±33.0 | 0.897 |
| HDL-C (mg/dl) | 52.1±16.5 | 46.3±13.9 | 0.000 |
| TG (mg/dl) | 106.8±56.7 | 121.7±88.0 | 0.521 |

*FBG, fasting blood glucose; HOMA-IR, homeostatic model assessment of insulin resistance; TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C high density lipoprotein cholesterol; TG, triglycerides*

**Table 3.** Energy and nutrients intake before and after Ramadan

| | Before Ramadan | After Ramadan | P value |
|---|---|---|---|
| Energy (kcal) | 1964.3±760.3 | 1567.7±512.9 | 0.000 |
| Carbohydrate (g) | 207.8±94.8 | 175.9±69.1 | 0.008 |
| Protein (g) | 73.3±24 | 56.1±14.7 | 0,002 |
| Fat (g) | 86.5±34.7 | 69.1±27.1 | 0.001 |
| Fiber (g) | 22.7±8.2 | 15.9±5.7 | 0.000 |
| SFAs (g) | 28.5±9.1 | 25.4±13.8 | 0.207 |
| PUFAs (g) | 23.6±10.5 | 17.4±7.2 | 0.002 |
| MUFAs (g) | 29.4±8.9 | 23±10.6 | 0.004 |
| n-3 (g) | 2.3±1.4 | 1.6±0.7 | 0.004 |
| n-6 (g) | 20.9±9.8 | 15.1±7.4 | 0.002 |
| n-6/n-3 | 10.3±5.4 | 10.4±5.9 | 0.950 |

SFAs, saturated fatty acids; PUFAs, polyunsaturated fatty acids; MUFAs, monounsaturated fatty acids; n-3, n-3 polyunsaturated fatty acids; n-6, n-6 polyunsaturated fatty acids

Body fat ratio and excessive body weight are known to be risk factors for cardiovascular disease (10). The lack of fluid intake together with food intake during Ramadan fasting could cause total weight loss by increasing loss of body fluid (11). However, in the current study, together with the reduction in total body weight, the body fat mass was also observed to have decreased. Therefore, it is not correct to explain the body weight loss by fluid loss only. Furthermore, as there was seen to be a reduction in energy intake at AR, it can be thought that the total weight loss occurred more through the oxidation of fat and the reduction in fat mass.

Morbidity and mortality associated with cardiovascular disease are known to be affected by total body fat and the accumulation of abdominal body fat (12). In recommendations for healthy living given by Sadiya et al, a significant decrease was shown in the waist circumference values of patients with metabolic syndrome who were monitored during Ramadan (13). In addition, a study investigating the effects of Ramadan fasting reported significant reduction in body fat percentage of nonalcoholic fatty liver disease patients (14). On the other hand fat oxidation was increased because of Ramadan fasting (15). But, Lessan et al showed no significant change on resting metabolic rates of healthy nonobese volunteers (7). So, Ramadan-induced body fat and abdominal fat loss can be attributed to a reduction in energy intake. Although a systematic rewiev concluded that Ramadan fasting has neutral effects on cardiovascular risk factors (16), Syam et al showed significant reduction in body fat without protein loss in Ramadan (6). We found a significant decrease in abdominal fat and total body fat due to intermittent fasting. This decrease can be attributed to energy deficit in Ramadan fasting. So, in the current study a significant reduction was observed in abdominal fat mass and waist circumference values supports the view that Ramadan fasting has positive effects on cardiovascular health in particular.

Conflicting results have been reported from studies that have investigated the effects of Ramadan fasting on lipid profiles (17, 18). Changes in nutritional habits in the month of Ramadan, reduced physical activity and cultural factors could affect these results (19). In the current study, while no difference was seen in the BR and AR total cholesterol, LDL-C and triglyceride values, a signifcant difference was determined in the HDL-C values, which were observed to have reduced from BR to AR. In contrast, in another study that was conducted on a small number of healthy individuals, a 30% increase was shown in HDL-C values (20). Ziaee et al reported that Ramadan fasting increased LDL-C and this could have been an indicator of the difference between BR and AR energy and fat intake (19). High consumption of fatty acids is associated with atherosclerosis and the structures of this fatty acids determine the real impact (21). The SFAs are more likely to contribute CVD, but PUFAs are known to be antiatherogenic (22). SFAs were shown to raise serum TC levels, so dietary guidelines recommend limiting amounts of saturated fat intake (23). On the other hand, a diet low in n-6/n-3 ratio are known to have beneficial effects on cardiovascular risk and data shows lowering n-6/n-3 ratio decreases TG and TC levels even a high fat diet (24). In the current study, nutrient consumption was calculated from the 3-day food intake records and according to these, energy intake was reduced compared to BR. Therefore, the reduction in HDL-C values could be related to lower levels of physical activity during Ramadan rather than reduced energy intake. Also, in our study we observed any change in n-6/n-3 ratio and SFAs intake but significant decreases occured in PUFAs, MUFAs, n-3 and n-6 fatty acids consumption. Altough the consumption of SFAs remain similar levels according to BR, the percent of energy coming from SFAs increase because of declining total energy intake. Both the increasing energy percent from SFAs and decreasing intake of PUFAs could influence significantly decreased HDL-C and increased TG levels in our study.

In a study by Unalacak et al, the fasting glucose levels in obese individuals were shown to be significantly reduced compared to those of subjects who were not obese (25). Another study of 115 subjects showed that fasting glucose levels were significantly reduced with Ramadan fasting (26). The results of the current study showed a similar effect of Ramadan fasting on fasting glucose and insulin levels. Ramadan fasting was observed to have significantly increased the fasting glucose, insulin and HOMA-IR levels in healthy individuals. The nutrient intake records in the current

study showed a significant reduction in fibre intake. This suggests that the increasing glucose and insulin levels were associated with reduced fibre intake and the associated reduced digestive energy expenditure**.** In addition, as the evening meal taken before the blood sample collection was not standard, this could have affected the fasting glucose and insulin values.

Insulin resistance is known to play a role in the development of type 2 DM, hypertension, hyperlipidemia and atherosclerosis (27). In a study by Gnanou et al, it was reported that HOMA-IR values were reduced together with glucose and insulin levels in healthy subjects fasting during Ramadan (28). In contrast, Unalacak et al showed that in individuals with normal body weight HOMA-IR values were increased by Ramadan fasting (25). The high HOMA-IR values of the current study are similar to the findings of that study. When it is taken into consideration that the participants of both studies were from the same sociocultural background, the elevated HOMA-IR levels can be explained by the similarity of nutritional habits.

In the evaluation of the 3-day food intake records in the current study, the AR energy intake was observed to have reduced. In contrast, previous studies have shown an increase in energy intake and Al-Hourani et al observed no significant change (29-32). Despite no calorie restrictions, that the carbohydrate and energy intake was reduced suggests that it could have been due to the month of Ramadan occuring in the summer and thus the time for eating and drinking was shorter. Similarly, there was a reduction in fibre intake and it can be said that this contributed to the reduced energy requirement.

Limitations of this study were primarily that there was no measurement of the resting energy expenditure of the participants at BR and AR, and levels of physical activity were not recorded.

In conclusion, the results of the study showed that there were positive effects of Ramadan fasting on anthropometric measurements such as body weight, BMI, fat mass and waist circumference, which are cardiovascular risk factors, but similar positive effects were not observed on endocrine and metabolic parameters.

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Original Article

# Elevated neutrophil-to-lymphocyte ratio in the diagnosis of subacute thyroiditis

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**Summary.** Subacute thyroiditis (SAT) is a self-limiting inflammatory condition of the thyroid gland in which multinucleated giant cells constitute a key histological finding. The neutrophil-lymphocyte ratio (NLR), determined from peripheral blood, is accepted as an available and practical indicator of the systemic inflammation. The purpose of this study was to determine the neutrophil-to-lymphocyte ratio (NLR), a novel marker of inflammation, in patients with SAT and to compare these values with those from healthy subjects. A total of 150 participants were included in the study, 75 SAT patients and 75 healthy volunteers. Retrospectively, demographic and laboratory data of the subjects were obtained from our institution's database. Patients with active infection, diabetes mellitus, malignancy, other chronic inflammatory diseases and hematologic disorders were excluded from the study. Values for complete blood count (CBC) and serum laboratory parameters of SAT patients were the baseline values obtained at the time of SAT diagnosis. Control subjects consisted of healthy volunteers who visited our institution for a routine check-up. A total of 75 subacute thyroiditis patients 54 (72%) were female and 21 (28%) were males and 50 (66.6%) were female and 25 (33.3%) were male and 75 were healthy adults were included. The mean age was 39.95±14.2, years for patients with SAT and 37.53±13.45 years for the control group. There was no significant difference between the age for groups ($P$ = 0.13). NLR levels were found to be 3,56 ± 2,64 in patients with SAT; NLR levels were found to be 1.41 ± 0.9 in the control group. NLR levels were significantly higher in patients with SAT compared to the control group. Our study showed that ıncreased NLR may be useful as an indicator of the presence of SAT, especially in complicated cases. The assessment of neutrophil-lymphocyte ratio in conjunction with radiological and clinical findings will assist in the achievement of an accurate diagnosis. Larger, prospective studies are required to determine its usefulness in assessing diagnostic potential and treatment outcomes in SAT patients.

**Key words:** Subacute thyroiditis, neutrophil-to-lymphocyte ratio, diagnosis

## Introduction

Subacute granulomatous thyroiditis (SGT), also known as De Quervain's thyroiditis, is a self-limiting, inflammatory disease of the thyroid that is believed to be caused by a systemic viral infection (1-7). It was first diagnosed in 1825, and 18 cases were reported as "thyroiditis acuta simplex" until 1895. This pathology was compiled by the Swiss surgeon De Quarvein in 1904 and 1936 (4). It typically occurs in the area of the gland in mid-aged hyperthyroid women complaining of pain, tenderness, fatigue and mild fever (1,2,5,6,7).

Complete blood count (CBC)-derived parameters and their relation to certain diseases have recent-

ly received attention from researchers. One of these CBC parameters is the neutrophil-to-lymphocyte ratio (NLR). NLR is considered to be a marker of inflammation and, due to its simplicity and low cost, has been studied in many medical conditions. An elevated neutrophil count in a CBC predicts ongoing inflammation and decreased lymphocyte count is considered to be an indicator of poor prognosis, so a combination of these two measures is generally accepted to be predictive of an inflammatory situatio (8). NLR reflects both inflammatory burden (by neutrophil count) and regulatory mechanisms (by lymphocyte count) in inflammatory disease (9,10). Studies suggest NLR is associated with occult inflammation in certain conditions (11-15). In this retrospective study, NLR was determined in 75 SAT subjects and investigated the possible association between SAT and NLR by comparing these values to NLR determined in a healthy population.

Since there is a strong association between inflammation and SAT, and between inflammation and NLR, we aimed to compare NLR values of patients with SAT to those of healthy volunteers.

## Materials and Methods

*Patients*

Patients with diagnosis of SAT who were followed up in the endocrinology clinic of our institution were enrolled to present retrospective study. Diagnosis of SAT was established with a combination of relevant history and findings in physical examination that were supported by characteristic findings on ultrasound scan (diffuse enlargement of the gland, decreased echo pattern and diminished thyroid blood in the doppler) and decreased uptake in thyroid scintigraphy. The study protocol was approved by the ethics committee of Dicle University, Faculty of Medicine, Diyarbakir, Turkey. The study was conducted in accordance with the Declaration of Helsinki.

*Biochemical Measurements*

Fasting venous blood samples were collected in the morning after 8 h of fasting. The assays were performed at the laboratory in Dicle University's Faculty of Medicine and Firat University's Faculty of Medicine using a biochemical analyzer (ABX Pentra DX 120; HORIBA, Ltd.). Hemograms were determined with an autoanalyzer (Coulter® LH 780 hematology system; Beckman Coulter, Inc.). The blood samples were processed within 30 min after blood collection.

CBC and serum parameters used in this study were the baseline laboratory findings that were recorded in our database at the time of SAT diagnosis. Control subjects consisted of healthy volunteers who visited our institution for a routine check-up. General characteristics and laboratory data of all participants were obtained from the computerized database of our clinics. White blood cell count (WBC), neutrophil count (Neu), lymphocyte count (Lym), hemoglobin (Hb), hematocrit (Htc), mean corpuscular volume (MCV) and platelet count (PLT) were recorded for all participants. NLR was calculated simply dividing the Neu value by the Lym value.

*Exclusion Criteria*

Patients with cardiovascular disease, hepatic or renal failure, previously detected malignancies, diabetes mellitus, hyperthyroidism, pregnancy, chronic obstructive pulmonary disease, or who were smokers or using anticoagulant–antiplatelet medications were excluded from the study. Hemoglobin <13 g/dL for males and <12 g/dL for females, and white blood cell counts >12,000 cells and <4000 cells were ignored.

*Statistical Analysis*

All statistical analyses were performed with the SPSS version 15.0 (SPSS Inc., Chicago, IL, USA). The significance of the mean differences between groups was assessed by Student's *t* test. Also, paired *t* test was used for repeated data in the patient group. Data were presented as mean ± standard deviation. Relationships between variables were tested using Pearson's correlation analysis. Receiver operating characteristic (ROC) curve graphics were used in the comparison of sensitivity and specificity. *P* values less than 0.05 were regarded as significant.

## Results

*Demographic Findings*

A total of 75 subacute thyroiditis patients (54%) were female and 21 (28%) were males and 50 (66.6%) were female and 25 (33.3%) were male (Figure 1) and 75 were healthy adults were included. Mean ages of the SAT and control groups were 39.95±14.2 and 37.53±13.45 years, respectively. The difference was not statistically significant (p>0,05). There was no significant difference between the two groups in terms of age and gender (p> 0.05). Mean age was 37.56 ± 10.17 years in female patients with subacute thyroiditis and 46.05 ± 15.07 years in male patients (Figure 2).

*Laboratory Findings*

NLR levels were found to be 3,56 ± 2,64 in patients with SAT; NLR levels were found to be 1.41 ± 0.9 in the control group. NLR levels were significantly higher in patients with SAT compared to the control group.

When the laboratory findings of patients with subacute thyroiditis were compared with the control group, the sedimentation value was significantly higher in the patient group (53.9 ± 14.24, 11.04 ± 6.86, p <0.01, respectively). C reactive protein value was significantly higher in the diseased group (7.08 ± 5.65, 0.44 ± 0.38, p <0.01, respectively). The leucocyte, neutrophil, neutrophil percentage, lymphocyte percentage and platelet counts were significantly higher in the patient group (Table 1).

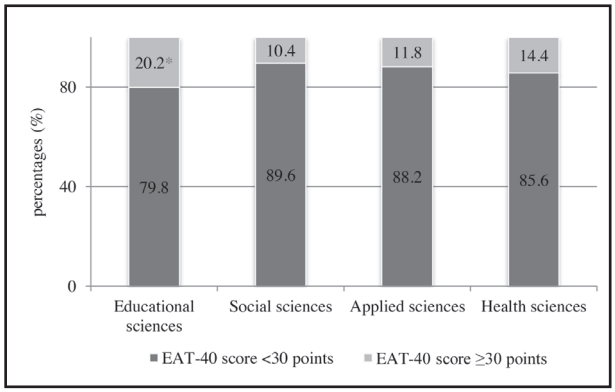


**Figure 1.** Gender distribution

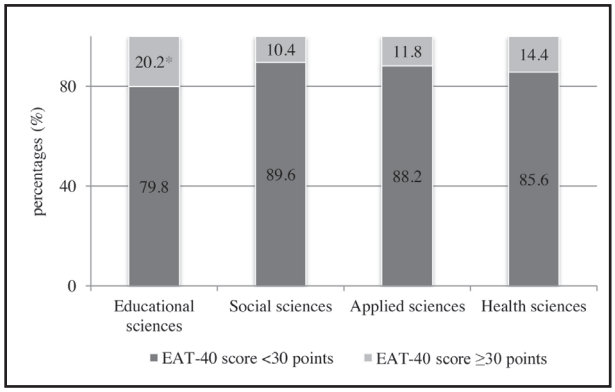


**Figure 2.** Gender age distribution

**Table 1.** Laboratory data of the study population.

| | SAT group | Control group | P value |
|---|---|---|---|
| ESR (mm/h) | 53,9±14,24 | 11,04±6,86 | 0,001 |
| | n:75 | n:75 | |
| CRP (mg/L) | 7,08±5,65 | 0,44±0,38 | 0,001 |
| | n:75 | n:75 | |
| WBC (u/mm$^3$) | 9,94±3,49 | 7,92±1,82 | 0,002 |
| | n:75 | n:75 | |
| NEU % | 66,9±9,59 | 58,8±9,16 | 0,001 |
| | n:75 | n:75 | |
| LYM % | 23,64±8,29 | 31,11±8,19 | 0,001 |
| | n:75 | n:75 | |
| NEU/LYM | 3,56±2,64 | 1,41±0,9 | 0,001 |
| | n:75 | n:75 | |
| HEMOGLOBIN | 12,12±1,46 | 13,81±1,58 | 0,001 |
| | n:75 | n:75 | |
| PLATELET | 367,63±117,14 | 283,37±82,01 | 0,001 |
| (u/mm$^3$) | n:75 | n:75 | |
| MPV (fl) | 7,81±1,1 | 8,78±1,35 | 0,001 |
| | n:75 | n:75 | |
| PDW (fl) | 17,77±1,19 | 20,19±1,11 | 0,001 |
| | n:75 | n:75 | |
| FT3 (pg/mL) | 11,03±5,45 | 4,86±0,5 | 0,001 |
| | n:75 | n:75 | |
| FT4 (ng/ml) | 42,18±17,67 | 16,67±2,53 | 0,001 |
| | n:75 | n:75 | |
| TSH (uIU/mL) | 0,03±0,05 | 1,6±1,25 | 0,001 |
| | n:75 | n:75 | |

*ESR, Erytrocyte Sedimentation Rate; CRP, C-Reactive Protein; WBC, White Blood Cell; NEU, Neutrophil; LYM, Lymphocyte; MPV, Mean Platelet Volume; PDW, Platelet Distrubition Width; FT3,Free T3 ; FT4, Free T3; TSH,Tiroid Stimulating Hormon*

## Discussion

The main finding of our study is that NLR was elevated in SAT patients compared with the healthy control subjects. This is the first reported association between SAT and NLR.

NLR is a hematologic parameter that is studied the most. Like other hematological inflammatory markers, most studies focus on its prognostic power (16). Shimada et al. proposed NLR as a reliable predictor of inflammatory burden (17). C-reactive protein (CRP), which responds immediately to infectious or inflammatory stimulus, is one of the most well-established inflammatory markers and, interestingly, NLR was found to correlate with CRP (18,19). There are a number of reports studying NLR in various thyroid diseases. Researchers from Taiwan showed that NLR correlated with the size of thyroid tumors (20). Moreover, elevated NLR was proposed as a negative prognostic factor for survival in subjects with papillary thyroid cancer (21). Aside from thyroid neoplasm, NLR has also been found to correlate with other types of neoplasms (22,23). Inflammation plays a critical role in tumor development, progression, clinical presentation and prognosis of cancer (24). SAT is also characterized by a prominent inflammatory burden, which is consistent with neutrophilic and lymphocytic inflammation of the thyroid gland; (25) therefore, the increased NLR seen in SAT patients compared with controls in this study is likely to be a result of acute inflammation. In our study, when the laboratory findings of patients with subacute thyroiditis were compared with the control group, the sedimentation value was significantly higher in the patient group. C reactive protein value was significantly higher in the diseased group. The leucocyte, neutrophil, neutrophil percentage, lymphocyte percentage and platelet counts were significantly higher in the patient group.

Fair number of Multinucleated Giant Cells, epithelioid cell granulomas, inflammatory cells (lymphocytes, macrophages and neutrophils), degenerated follicular epithelial cells, and a dirty background composed of cellular debris, naked, degenerated nuclei and thick colloid are the key cytological characteristics for the diagnosis of SAT (26). As in our study, potent inflammatory markers such as high-level NLRs give strong insight into the diagnosis and progression of the SAT.

Elevated NLR has been reported in patients with familial Mediterranean fever (FMF) and has emerged as a valuable predictor of the development of amyloidosis (27). Both thyroid follicular cells and inflammatory cells, involved in HT, are capable of producing cytokines that may exacerbate the autoimmune process and the inflammatory response; (28,29) therefore, mechanisms similar to those seen in FMF may induce elevated NLR.

On gross examination, the thyroids affected by SAT are typically asymmetrically enlarged and firm with a tan-white cut surface and ill-defined nodularity (30). The histological appearance of SAT varies with the phase of the disease. The early phase, which correlates with a hyperthyroid status, is characterized by destruction of follicular epithelial cells with colloid extravasation and colonization of follicles predominately by neutrophils forming microabscesses. As the disease progresses the acute inflammation is gradually replaced by lymphocytes, histiocytes, and characteristically multinucleated giant cells engulfing colloid. Histologically, the late phase is remarkable for increasing amounts of interfollicular fibrosis and corresponds to hypothyroid phase (30, 31). In our study; In order to increase the value of the study, In the acute phase of the SAT, the cases were taken to study.

In healthy populations, NLR is increased in the elderly; however, the mean age of the 75 SAT patients was not different from the healthy controls in our study so the increase in NLR seen in our study cannot be attributed to this age-related correlation.

Limitations of study; the number of total patient considered in the study were 75, however future studies needs to be undertaken involving more number of cases along with nuclear, hormonal and radiological correlation. The follow up of the patients were also not conducted as it was a retrospective study.

In conclusion, NLR could prove to be an important tool for measuring systemic inflammation in SAT patients since it is cost effective, readily available, and easily calculated. Our study showed that ıncreased NLR may be useful as an indicator of the presence of SAT, especially in complicated cases. The assessment of neutrophil-lymphocyte ratio in conjunction

with radiological and clinical findings will assist in the achievement of an accurate diagnosis. Larger, prospective studies are required to determine its usefulness in assessing diagnostic potential and treatment outcomes in SAT patients.

## Acknowledgements

The study protocol was approved by the ethics committee of Dicle University, Faculty of Medicine, Diyarbakir, Turkey. The study was conducted in accordance with the Declaration of Helsinki.

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Original article

# Preoperative intravenous ibandronate for treating severe hypercalcemia associated with primary hyperparathyroidism: an effective and low cost

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**Summary.** Primary hyperparathyroidism (PHPT) is a prevalent mineral metabolism disorder usually caused by a single parathyroid adenoma. Although PHPT is the most frequent cause of hypercalcemia, severe hypercalcemia cases are rarely encountered. Severe hypercalcemia results in fatal complications unless immediately treated; moreover, it causes delays in surgery for PHPT, the primary treatment. Some patients admitted because of hypercalcemia require intravenous bisphosphonate treatment. Aimed to investigate the efficacy of intravenous ibandronate, which is a relatively cheap drug than other intravenous bisphosphonates, in the preoperative treatment of symptomatic hypercalcemia in patients with PHPT. Also, there are some difference in the total cost of treatment for patients treated with ibandronate and zolendronate. The medical records of patients operated at Dicle University Department of General Surgery between 2010 and 2017 due to PHPT were retrospectively evaluated. Patients who were admitted because of hypercalcemia associated with parathyroid adenoma and underwent minimally invasive surgery subsequent to the lowering of calcium levels via preoperative intravenous ibandronate and zolendronate were included. Totally, 20 of 167 patients received a preoperative bisphosphonate due to hypercalcemia associated with PHPT. Seven patients treated with zoledronate only. Thirteen were treated with ibandronate only. There was no difference in hypercalcemia correction between the groups. Percentage of patients with hypocalcemia was less in the ibandronate group. The hypocalcaemic period was shorter in patients receiving ibandronate. Cost of hospital stay in patients receiving ibandronate is cheaper than zolendronate (780±462 USD versus 1765±1537 USD). Ibandronate use reduces the cost of hypercalcemia treatment by 55% in comparison with zolendronic acid. Intravenous ibandronate for treating severe hypercalcemia associated with PHPT is an effective and relatively cheap drug.

**Key words:** hyperparathyroidism, hypercalcemia, ibandronate

## Introduction

Primary hyperparathyroidism (PHPT) is a common mineral metabolism disorder caused by excessive secretion of parathyroid hormone (PTH) from a parathyroid adenoma. Typically, biochemical outcomes of autonomously active parathyroid tissue are persistently elevated levels of PTH despite the presence of hypercalcemia (1).

Although PHPT is the most frequent cause of hypercalcemia in patients presenting to polyclinics, it is rarely the reason for patients who are hospitalized due to hypercalcemia. Severe hypercalcemia results in fatal complications unless immediately treated; moreover, it causes delays in undergoing surgery for PHPT, which is the primary treatment (2-4).

Severe hypercalcemia is one of the indications to perform surgery for treating PHPT (5). During sur-

gical preparation, intravenous fluids and furosemide are administered. In most patients hospitalized due to severe hypercalcemia, intravenous bisphosphonate treatment is required. Bisphosphonates reduce calcium levels by inhibiting osteoclastic activity (6). In case of severe hypercalcemia, zoledronate is frequently used due to its high efficacy. Ibandronate has been used and demonstrated to be efficient for treating hypercalcemia of malignancy (7, 8).

In the present study, we aimed to investigate the efficacy of intravenous ibandronate, which is a cheaper drug than other intravenous bisphosphonates, in the preoperative treatment of severe hypercalcemia in patients with PHPT. Also, is there any difference in the total cost of treatment for patients treated with ibandronate and zolendronate?

## Materials and Methods

The medical records of all patients who were operated at Dicle University School of Medicine, Department of General Surgery, between 2010 and 2017 due to PHPT were retrospectively evaluated. Patients who were admitted to the hospital due to hypercalcemia associated with parathyroid adenoma and underwent minimally invasive surgery after the lowering of calcium levels because of preoperative intravenous ibandronate and zoledronate were included. A total of 20 patients were included in the study. In our laboratory, the PTH measurement method is chemiluminescent (Roche Cobas E601) and the normal calcium range is 8.4-10.2 mg/dl. Preoperative calcium, phosphorous, and PTH levels were recorded. The duration for calcium levels to normalize after ibandronate/zoledronate treatment were recorded. The cost of hospital stay for each patient was recorded in both groups. Postoperative calcium, phosphorous, and PTH levels were assessed. All patients underwent minimally invasive surgery. The pathology reports of all patients reported the presence of adenoma. One patient had cystic parathyroid adenoma. Patients who took ibandronate/zoledronate due to hypercalcemia associated with PHPT but who were not operated, patients who used bisphosphonates other than ibandronate/zoledronate to treat hypercalcemia, and patients who were treated with hemodialysis and/or calcitonin along with ibandronate/zoledronate were excluded. Our treatment scale for hypercalcemic PHPT: calcium 10.4-12 mg/dl fluid and diuretic, 12-14 mg/dl + bisphosphonates, >14 mg/dl + calcitonin, altered consciousness, cardiac effects + hemodialysis.

From hospitalization to exernation to the hospital, we have set up the cost calculations for PHPT treatment.

Calcium normalization time was calculated as (day) when calcium was ≤12 mg/dl after the patient was given bisphosphonate.

Statistical analyses were performed by using SPSS software, version 22. Non-parametrical variables were assessed using Wilcoxon signed-rank test. A p-value of <0.05 was considered statistically significant.

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. The local ethics committee has approved this study (Dicle University 198/2017).

## Results

Totally, 20 of 167 patients (11.9%) who were operated underwent preoperative bisphosphonate treatment due to severe hypercalcemia associated with PHPT. Among them, 7 (29.1%) (five female and two male) were treated with zoledronate only, 13 (54.2%) were treated with ibandronate only. Among these 13 patients, seven were female and six were male. The mean age of the patients was 56±18 (range, 18-89) years (62±19 ibandronate group/57±17 zolendronate group). All patients were of Caucasian origin and exhibited significant hypercalcemia symptoms. The mean calcium level in patients before ibandronate treatment was 14.31±0.92 mg/dL, and the mean duration of calcium regulation after ibandronate treatment was 3.3±1 (range, 2-5) days. Mean calcium levels after ibandronate treatment was 10.19±0.89 mg/dl (p=0.001); mean preoperative PTH levels were 585.69±389.95 pg/ml, and mean postoperative PTH levels were 44.13±43.15 pg/ml (p=0.001) The mean calcium level in patients before zolendronate

treatment was 14.6±0,96 mg/dl, and the mean duration of calcium regulation after zolendronate treatment was 2,43±0,78 (range, 1-3) days. Mean calcium levels after zolendronate treatment was 11,02±1.18 mg/dl (p=0.01); mean preoperative PTH levels were 1351±580 pg/ml, and mean postoperative PTH levels were 28.86±1.58 pg/ml (p=0.01) (Table 1). There was no difference in hypercalcemia correction between the groups. The hypocalcaemic period was shorter in patients receiving ibandronate. Percentage of patients with hypocalcemia was less in the ibandronate group (23% versus 71%). Three patients (23%) had postoperative hypocalcemia in ibandronate groups, and five patients (71%) in zoledronate groups. Cost of hospital stay in patients receiving ibandronate is cheaper than zolendronate (780±462 USD versus 1765±1537 USD). Ibandronate use reduces the cost of hypercalcemia treatment by 55%.

## Discussion

Severe hypercalcemia is a rare disorder accounting for 2-5% of patients with PHPT. Severe hypercalcemia is an emergency medical condition and may be life threatening. Therefore, it should be immediately treated (9-11). As severe hypercalcemia is usually associated with a single parathyroid adenoma, surgery is the primary treatment. Patients are usually operated following the regulation of calcium levels with intravenous bisphosphonates. Bisphosphonates reduce surgical complications associated with hypercalcemia and shorten preoperative period (12, 13).

In our series of 13 patients, we successfully treated severe hypercalcemia using 3 mg of intravenous ibandronate, and the patients were operated and cured. In a literature review, we did not find data on ibandronate use for treating hyperparathyroidism with severe hypercalcemia.

There are some studies in the literature covering few cases of hyperparathyroidism with severe hypercalcemia, which is a rare medical condition. The study with the most cases was a series by Sarfati et al. that included 59 patients (4, 14, 15).

Phitayakorn et al. normalized preoperative calcium levels by administering pamidronate to six patients and zoledronate to one patient (12).

Starker et al. treated eight patients with bisphosphonate and preoperatively achieved normocalcemia. However, the bisphosphonates used were not specified (13).

Lv et al. administered zoledronate to nine patients due to hypercalcemia associated with PHPT and observed significant reduction in calcium levels (16).

We observed that ibandronate, which is cheaper than other bisphosphonates, is effective.

Our study showed that ibandronate was non-inferiority to zolendronate for treatment PHPT hypercalcemia.

**Table 1.** Characteristic of patients groups

| | **Ibandronate group n =13** | **Zoledronate group n=7** | **P value** |
|---|---|---|---|
| Age (year) | 62±19 | 57±17 | N.S |
| Sex (female/male) | 7/6 | 5/2 | N.S |
| Calcium (mg/dl) before treatment | 14.3±0.92 | 14.6±0.96 | N.S |
| Calcium (mg/dl) after treatment | 10.19±0.89 | 11.02±1.18 | N.S |
| PTH (pg/ml)before surgery | 585±398.95 | 1351±580 | N.S |
| PTH (pg/ml)after surgery | 44±43.15 | 28.86±1.58 | N.S |
| Post-bisphosphonate hypocalcemia (yes/n) | 3/13 (23%) | 5/7 (71%) | |
| Calcium <12 mg/dl (day) | 3,3±1 (2-5) | 2,48±0,78 (1-3) | |
| Hospital stay cost (USD) | 780±462 | 1765±1537 | |

*N.S: Non significance*

In PHPT, the total hospital cost of patients receiving ibandronate for hypercalcemia was lower than in zoledronate

Cost of hospital stay in patients receiving ibandronate is cheaper than in zolendronate (780±462 USD versus 1765±1537 USD). Ibandronate use reduces the cost of hypercalcemia treatment by 55%.

One of the undesired outcomes of effective bisphosphonates is the occurrence of hypocalcemia after treatment. Corsello et al. reported hypocalcemia over a three-month period in a patient they followed up after zoledronic acid treatment (17). In our study, we observed a lower rate of hypocalcemia in patients treated with ibandronate. It was thought to be due to shorter duration of action of ibandronate. In our study, the mean duration of calcium regulation after ibandronate treatment was 3.31±1.03 (range, 2-5) days.

In hypercalcemia of malignancy, zoledronate, which is highly effective, may be preferred. We believe that ibandronate is a reliable alternative for treating hypercalcemia associated with hyperparathyroidism.

The retrospective nature of our study and the low number of patients are the limitations of our study. Double-blind prospective studies that include larger number of patients are required.

To conclude, although hypercalcemic crisis associated with hyperparathyroidism is rare, it is a life-threatening condition. Intravenous ibandronate may be an effective and relatively cheap drug in the surgical preparation and treatment of patients with hypercalcemic crisis associated with PHPT. Ibandronate use reduces the cost of hypercalcemia treatment by 55% in comparison with zolendronic acid.

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Original article

# The weight and ghrelin changes of fecal microbiota transplantation in rats

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**Summary.** *Background:* Fecal microbiota transplantation is a promising method to solve obesity. Our study's aim was to investigate the changes of weight and ghrelin levels in obese rats receiving fecal microbiota transplantation from lean rats. *Methodology:* Twenty-one rats were divided into three equal groups: Group 1: Obese control group; group 2: Obese recipient group; and group 3: Lean donor group. Feces which was collected from donor group was transferred to the rats in recipient group, orally by gavage, 3 times every other day. The weight and ghrelin levels were measured from each rat at the beginning and end of the study. *Results:* There was statistically significant weight gain in donor group (p:0.001), but there were no statistical significant weight chances was detected in control and recipient groups (p:0.82, p:0.12, respectively). There was an increase in donor and control groups, but a decrease was observed in the recipient group at ghrelin levels. However, there was no difference at ghrelin levels in any groups (p:0.05, p:0.2, p:0.4, respectively). There was a significant relation in control group in weight and ghrelin changes (p:0.007), but no significant relation was detected in either recipient or donor groups (p:0.29, 0.53, respectively). *Conclusion:* Metabolism changes of obese rats were observed after fecal microbiota transplantation, and it was the only group that decreasing ghrelin levels.

**Key words:** animal experimentation, body weight changes, ghrelin, microbiota, transplantation

## Introduction

Obesity is a major global public health problem that is becoming increasingly common. In 2014, the World Health Organization (WHO) reported that 38% of male and 40% of female adult individuals worldwide were overweight, with obesity ratios of 11% and 15%, respectively (1). Obesity is a multifactorial problem related to genetics, a lack of physical activity, unhealthy eating, as well as other diseases such as diabetes, hypertension, and metabolic syndrome, all of which are also major risk factors (2). There are many treatment modalities for obesity, from lifestyle changes to surgery; however, success cannot be achieved at the desired rate due to reasons arising from patients or treatment methods (1-3).

The human intestinal microbiota begins to develop from birth, depending on personal and environmental factors. The colon contains microorganisms, with a total concentration of ~$10^{11}$-$10^{12}$ CFU/g, which are in a favorable symbiotic relationship with their human hosts (4). A possible role of intestinal microbiota has been suggested in obesity pathogenesis. Human and animal studies of obesity have shown that the

gut microbiota in obese individuals was significantly changed and the bacterial diversity was reduced (5-7). Similar studies have reported that *Bacteroidetes* strains were reduced, while *Firmicutes phylum* was increased proportionately (8-11).

In recent years, fecal microbiota transplantation (FMT) has garnered significant interest and attention. In particular, several case series have been published about *Clostridium difficile* (CD) infections and inflammatory bowel disease. The success ratios for CD infections are above 90% (12-16). In studies performed on germ-free obese mice, treatment with FMT led these mice to gain weight (17).

Ghrelin was discovered after a rat gastric extraction in 1999 (18). In subsequent studies of ghrelin, it was demonstrated that ghrelin is functionally active and effective in many areas including sleep, weight control, glucose metabolism and the control of stress and anxiety (19). Ghrelin levels change depending on many factors. To date, ghrelin has been the focus of research across numerous fields, and the data concerning ghrelin levels are available for many diseases such as obesity, anorexia nervosa, cachexia, chronic obstructive pulmonary disease, chronic heart failure and after obesity surgery (19). However, from our review of the literature, we did not find any studies concerning ghrelin hormone levels or weight changes in animal models following FMT. Our aim in this study was to investigate how changes in ghrelin hormone levels after FMT impact the pathophysiology of weight control.

## Materials and Methods

This study was performed at the Experimental Animal Production and Research Laboratory and was approved by the local Animal Ethics Committee. All protocols were performed in accordance with the regulations governing the care and use of laboratory animals in the Declaration of Helsinki.

Twenty-one Wistar female albino rats {14 obese rats (mean weight was 454 g and mean age was 11 months) and 7 lean rats (mean weight was 283 g and mean age was 3 months)} were divided evenly into three groups. According to a power analysis using 0.05 accuracy and a power of 0.95, seven rats were assigned to each of the groups. The groups were defined as follows: Group 1: Obese control group (n:7); group 2: Obese recipient group (n:7); and group 3: Lean donor group (n:7). During the entire experiment, the rats were kept alive in standard laboratory mice and rat cages; the bases and sides were made of plastic, and the tops were covered with iron wire netting. The laboratory settings included a 12-hour light/12-hour dark cycle, and the rats were fed a pellet-type fabricated feed specially produced for small experimental animals.

At the beginning of the study, both the obese and lean rats (n=21) were weighed. After weight measurements, all rats were administered general anesthesia with xylazine (Rompun® Bayer Co.; 5 mg/kg body weight), and 1 ml blood samples were obtained from each subject through the intra-cardiac entry and then centrifuged at 3000 g at 10 minutes. The plasma was stored at -80°C. A fecal solution was applied to recipient rats via oral gavage, three times every other day. After the FMT application, all rats were weighed on the same day weekly. After four weeks, a 1 ml blood sample was taken from all rats under general anesthesia intracardially (Rompun® Bayer Co.; 5 mg/kg body weight). This blood sample was then centrifuged at 3000 g for 10 minutes, and the harvested plasma was stored at -80°C.

The ghrelin levels in the plasma obtained from the blood samples taken at the beginning and end of the study were measured with enzyme-linked immunosorbent assay (ELISA) kits (Sigma-Aldrich RAB0207, St. Louis, US).

### *Fecal microbiota transplantation*

Immediately after excretion by the donor lean rats, the fecal samples were put into transfer tubes (which contained pre-reduced sterile phosphate buffered saline with 0.05% cysteine-HCl, 2 mL/g). Each fecal sample was blended with the help of a mixer until homogenization. The mixtures were filtered with the help of a cloth filter, and the solid particles were separated. One milliliter of the filtered fecal solution was then given to the recipient obese rats via oral gavage.

### *Statistical analysis*

The statistical analyses were performed using IBM SPSS Ver. 21.0. In addition to the descriptive statisti-

cal methods (mean and standard deviation), we used the Paired t Test for comparing in-group variations in weight change. Additionally, we used the Wilks' Lambda for in-group variable comparisons of ghrelin changes. The correlation between the changes in weight and ghrelin, for all three groups, was analyzed with Spearman's rho test. The results were evaluated at the 95% confidence interval and $p<0.05$ significance level.

## Results

In this study, the weight changes, ghrelin levels of the groups before and after the study were compared. The weight changes in rats before and after this study are given in table 1. When the weight changes in the groups over time were compared, the mean weight of the group three was 283.28±27.63 g at the beginning of the study and 319.14±27.17 g at the end of the study and this was the significant weight changes in groups ($p<0.05$); however in group two, while there was weight reduction over time, it was not statistically significant ($p>0.05$). Also there was no weight changes in group one ($p>0.05$).

The ghrelin levels before and after the study are given in table 2. We observed an increase at ghrelin levels in the rats of groups 1 and 3, while the ghrelin values of the rats in group 2 decreased. Despite these observed trends, none of these were found to be statistically significant (p:0.2, p:0.05, p:0.4, respectively).

Next, the correlation between the weight changes and ghrelin changes of all three groups was analyzed. No significant relation was found between the changes in weight and ghrelin in groups 2 and 3 (p:0.29 and 0.53, respectively), however, there was a significant relation detected in group 1 (p:0.007).

**Table 1.** Mean weight changes in the groups over time.

| | Group 1 (g ±SD) | Group 2 (g ±SD) | Group 3 (g ±SD) |
|---|---|---|---|
| Week 0 | 447.00±39.21 | 462.57±55.26 | 283.28±27.63 |
| Week 4 | 446.71±39.36 | 454.57±55.99 | 319.14±27.17 |
| p value (Paired t test) | 0.82 | 0.12 | 0.001 |

**Table 2.** Mean ghrelin changes in the groups over time (ng: nanogram, ml: milliliter)

| | Ghrelin (ng/ml) before the study ± SD | Ghrelin (ng/ml) after the study ± SD | p values (Wilks' Lambda) |
|---|---|---|---|
| Group 1 | 55.71±18.71 | 69.34±21.46 | 0.05 |
| Group 2 | 45.91±11.38 | 38.98±9.21 | 0.2 |
| Group 3 | 47.52±18.47 | 51.87±16.93 | 0.4 |

## Discussion

FMT is one of the current treatment methods used for treating *C. Difficile* and inflammatory intestinal infections (20,21).

It is well known that the fecal microbiota of obese individuals is different from those found in non-obese individuals and that FMT in obese individuals changes their fecal microbiota. Previous studies in animal models have shown that obesity increases *Firmicutes* levels and decreases levels of *Bacteroidetes* in the respective host's fecal microbiota (22). Fecal microbiota transplantation was associated with favorable changes in gut microbiota, including greater bacterial diversity and a 2.5-fold increase in butyrate-producing bacteria (17,23-25). A double-blind randomized controlled trial of nine middle-aged patients with metabolic syndrome who experienced a fecal microbiota transfer from lean individuals via a nasoduodenal tube reported similar findings. Six weeks after GMT, the study reported a 75% increase in their insulin sensitivity, increased the diversity of gut microbiota, and increased levels of the butyrate-producing bacteria *Roseburia intestinalis* in the obese patients with metabolic syndrome post-fecal microbiota transfer (26).

There are several studies that address the effect of FMT on insulin resistance and weight change. One such study utilized germ-free rats and found that the germ-free rats gained more weight after fecal microbiota transplantation from obese rats when compared with transplantation from lean rats (17). More recently, some scientists inoculated the GF mice with gut microbes from four pairs of female twins, in which one of the twins was obese and the other had a healthy weight. The mice that received the obese humans' microbes gained more body fat, put on more weight, and

showed increased levels of markers for metabolic disorders (27). In one case report, a woman successfully treated with FMT developed new-onset obesity after receiving stool from a healthy, but overweight donor (28).

Germ-free mice do not become obese, nor do they develop insulin resistance after exposure to a high-fat diet. The transfer of gut microbiota from a laboratory-raised mouse into germ-free mouse results in an increase in body fat content and insulin resistance, despite reduced food intake (29). As shown, animal studies were generally performed on germ-free animals. On the other hand, our study evaluated the outcomes of FMT application on rats with developed microbiota. According to results of our study, despite the minor weight loss observed, there was no statistically significant difference in weight between the beginning and the end of the study. This effect could have been due to the short duration of our study as well as the multifactorial causes effecting both the development of obesity and fecal microbiota.

Some studies have accepted ghrelin as a possible weight loss mechanism, which may be a consequence of sleeve gastrectomy and gastric bypass surgery that are applied to treat obesity [30]. In the majority of the studies that investigate the ghrelin levels after sleeve gastrectomies, a decrease in ghrelin levels is observed, whereas in the studies in which the ghrelin levels were measured after gastric bypass report that there can be a decrease, an increase, or no change in the ghrelin levels (30-32). In our review of the literature, we found many studies about the changes in microbiota with FMT; however, we found no study that evaluated the relation between fecal transplantation and ghrelin levels. However, we did find two studies similar to our own in certain ways. In the first study, fecal microbiota changes and ghrelin levels were investigated in cats fed different foods and a positive relation was observed between *Bifidobacteria* and blood ghrelin levels (33). This finding supports our previous statement mentioning that the differences in microbiota can change ghrelin levels. In the second study, Oxyntomodulin, which is released from intestinal L-cells, was found to be effective in weight loss, but the iv application of its synthetic analog can be difficult. Therefore, significant weight loss was observed in obese rats fed with a probiotic, namely *Bifidobacterium*, and an integrated human Oxyntomodulin gene, compared to rats fed normal feeds without the gene transfer. The Oxyntomodulin levels in the blood were found to be high, while ghrelin levels were found to be low (34). This finding showed us that weight loss could occur with increased amounts of Oxyntomodulin, which may reverse the effect of ghrelin, and that decreased amounts of ghrelin and gut hormones, such as ghrelin, can have an effect on weight changes after transplantation.

Although it was not statistically significant, we observed a decrease in the levels of ghrelin in rats with weight loss after FMT application and an increase in ghrelin levels in lean rats who gained weight and obese rats to whom FMT was not applied. These results show that there can be changes in the metabolism of obese rats after FMT and that the metabolism of FMT applied rats can behave differently from rats in the control group. It is possible that ghrelin may play a similar role in weight loss as noted in previous studies with FMT.

There were some limitations in this study. First, fecal microbiota analysis was not controlled before or after FMT application. The reasons for this limitation were that looking for fecal microbiota is an expensive procedure and there were many studies in the literature about microbiota changes with FMT (17,23-25). And our study had a short follow-up time.

## Conclusion

Although FMT is a treatment method growing in popularity and its success has been demonstrated in many studies of certain diseases, its use in studies of obesity remains limited. Although it is a known fact that FMT can cause changes in the fecal microbiota, there was no study of its effect on intestinal hormone levels, namely on ghrelin levels, in relation to weight changes in animals and humans. Although the results of our study did not show any statistical significance, ghrelin levels decreased only for the fecal transplantation applied group and only these group lost weight. This finding made us think that there can be changes in the metabolism of obese rats after FMT. The metabolism of FMT applied rats can behave differently than

for rats in the control group, and fecal transplantation can cause weight loss via changing levels of ghrelin. As this study is the first to evaluate ghrelin levels after FMT, we think the differences can be brought to statistically significant levels with future studies that include the use of more animals, longer observation periods and different transplantation protocols.

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Original article

# A mixture of vegetable extracts (chamomile, passionflower, caraway, fennel) and enzymes (beta-galactosidase) for Irritable Bowel Syndrome (IBS): an observational study ("BIOVES")

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**Summary.** *Aims:* Irritable bowel syndrome (IBS) is a functional intestinal disorder. This syndrome may creates psychological disorders in patients who are affected and severely limits daily activities and lifestyle. *Methods:* We investigated the effect of the natural product consisting of chamomile, fennel, caraway, passionflower and melissa (*Spasmicol®)*, on IBS patients. For the study, 187 patients with IBS, enrolled by primary care doctors, were treated with *Spasmicol® (Aristeia Farmaceutici s.r.l.)*, two tablets/daily, for 30 days. At the end of the study, patients were re-evaluated to analyse the effects of therapy. *Results:* After 30 days, patients showed a marked reduction of symptoms (abdominal pain, abdominal distention, stool habit changes). *Conclusions:* Due to to the combination of chamomile, fennel, caraway, melissa, passionflower and beta-galactosidase, the product *Spasmicol®* turned out to be remarkedly effective in treating IBS symptoms.

**Key words**: irritable bowel syndrome, gastroenterology, supplements, alternative treatments

## Introduction

Irritable bowel syndrome (IBS) is a functional intestinal disorder that affects 5% to 15% of the general population, moreso women than men (ratio 2:1). IBS is defined, according to Roma IV criteria, as the presence of recurrent abdominal pain on average at least 1 day per week during the previous 3 months (1-3). Furthermore, in order to make a correct diagnosis, the pain should be associated with at least two of the following symptoms or clinical signs:
- related to defecation;
- related to a change in evacuation frequency;
- related to a change in stool consistency.

These criteria have to be satisfied for at least the previous 3 months, while the onset of symptoms has to occur at least 6 months prior to diagnosis (1,2).
According to Roma IV criteria, IBS can be classified as follows:
- IBS that manifests itself mainly with constipation (IBS-C);
- IBS that manifests itself mainly with diarrhoea (IBS-D);
- Mixed type IBS (IBS-M), in which there is an alternation of diarrhoea and constipation;
- Unclassified IBS (IBS-U).

Stool classification is based on the Bristol Stool Scale (1,4).

IBS could be also classified as:

- sporadic IBS (or not specific IBS);
- post-infectious IBS (PI-IBS);
- IBS associated with Inflammatory Bowel Diseases (IBD) (IBD-IBS) (1,3,5).

The syndrome produces psychological disorders in patients who are affected and severely limits their lifestyle, as health-care utilization, and daily activities, such as work productivity, intimacy, leisure activities, interpersonal relationships, and eating habits.

Dietary measures may include fibre supplementation in order to improve the symptoms of constipation and diarrhoea; polycarbophil compounds (eg, Citrucel, FiberCon) to produce less flatulence than psyllium compounds (eg, Metamucil); judicious water intake, especially for those patients who predominantly experience constipation; caffeine avoidance in order to limit anxiety and symptom exacerbation; legume avoidance in order to decrease abdominal bloating. Lactose, fructose, and/or FODMAPs (fermentable oligosaccharides, disaccharides, monosaccharides, and polyols) should be limited or avoided in patients with these contributing disorders. Probiotics are being studied for their use in decreasing IBS symptoms (6).

Pharmacologic agents used for the management of symptoms in IBS include the following:

- Anticholinergics (eg, dicyclomine, hyoscyamine);
- Antidiarrheals (eg, diphenoxylate, loperamide);
- Tricyclic antidepressants (eg, imipramine, amitriptyline);
- Prokinetic agents;
- Bulk-forming laxatives;
- Serotonin receptor antagonists (eg, alosetron);
- Chloride channel activators (eg, lubiprostone);
- Guanylate cyclase C (GC-C) agonists (eg, linaclotide, plecanatide);
- Antispasmodics (eg, peppermint oil, pinaverium, trimebutine, cimetropium/dicyclomine);
- Altering bacterial flora and gas formation (eg, rifaximin).

In this open-label prospective observational study we aimed to investigate the clinical efficacy of *Spasmicol*®,, a nature-derived dietary supplement which acts effectively in IBS patients, thanks to the synergistic combination of the following constituents:

– *Chamomile:* it has an anti-inflammatory effect COX–LOX dependent and antispasmodic, sedative and hypnotic effects (7);
– *Passionflower and Melissa*: they have a sedative effect mediated by the GABAergic system (8-10);
– *Melissa*: it adjusts the digestive processes thanks to its ability to moderate the frequency and the size of the slow intestinal waves, by doing so it has a myorelaxant effect (8,9);
– *Caraway and Fennel*: they reduce the mobility of the smooth intestinal muscle as a result of the release of acetylcholine (10,11);
– *Beta-galactosidase*: it increases the digestibility of lactose.

## Materials and Methods

Despite the double-masked, randomized, placebo controlled, parallel-group trial is the gold standard for testing the efficacy of new treatments, we designed an open label, prospective observational study, due to the higher placebo rates found in European randomized controlled trials (RCTs) compared with those conducted in other continents, in trials evaluating antispasmodics, and in trials using shorter duration of therapy.

This prospective observational study involved primary care physicians (11 in total); each doctor recruited a maximum of 20 consecutive patients suffering from IBS. In the present study, 183 consecutive patients were recruited.

The patients were consecutively recruited and successively classified, according to Rome IV criteria, in IBS-C, IBS-D, IBS-M and IBS-U.

We excluded patients:

– with any alarm symptoms;
– with any unexplained weight loss;
– with any uninvestigated rectal bleeding;
– with past or present disease likely to complicate the evaluation of the study;
– with abdominal pain relieved by acid-inhibiting drugs;
– with pregnancy or lactation;
– with inability to complete the questionnaire;
– with family history of bowel cancer in a first or second-degree relative;

– with a recent (12 month) history of assumption of benzodiazepines, antidepressants, barbiturates, psychotropics, analgesics, prokinetics (cisapride, metoclopramide), bulk laxatives, antispasmodics, antidiarrhoeal agents, alternative medicines as herbal compounds.

We identified, accordingly:
– 76/183 patients with IBS-M;
– 40 patients with IBS-C;
– 40 patients with IBS-D;
– 24 patients with IBS-U.

In IBS-C patients, we evaluated, with a binary response (yes/no), if they have a satisfactory relief of their IBS symptoms during the past week (with particular attention to abdominal pain and altered bowel habits, considering in this case a complete spontaneous bowel movements (CSBM) per week superior to 3/week).

In IBS-D patients, we evaluated, with a binary response (yes/no):
– an assessment of stool consistency, by using the Bristol Stool Form Scale (a type 3 or 4 score was considered as a normalized consistency with a baseline type 5, 6 or 7 score);
– if they have a satisfactory relief of the abdominal pain, during the past week.

In IBS-M and IBS-U patients, we evaluated with a binary response (yes/no):
– if they have a satisfactory relief of the abdominal pain, during the past week;
– an assessment of stool consistency, by using the Bristol Stool Form Scale (a type 3 or 4 score was considered as a normalized consistency, with a baseline type 5, 6 or 7 score);
– if they have a complete spontaneous bowel movements (CSBM) per week greater than 3/week.

All the patients were treated with a daily oral intake of *Spasmicol*® 500 mg (Aristeia farmaceutici s.r.l., Valguarnera, Italy). The chewable tablets were administered two times daily, before the main meals, for 30 days. All the patients were re-evaluated at the end of the study ($t_1$). Four patients (2.1%, 2 females) were discarded due to their scarce adherence to therapy (they did not take the drug constantly).

Finally, safety was also assessed, while quality of life was not assessed.

Table 1 and 2 shows bowel habits variations and pain relief, respectively, after 30 days of treatment with *Spasmicol*®.

Data were reported as percentages for categorical variables and as means (95% confidence intervals) for quantitative variables. The comparison between groups ($t_0$ versus $t_1$) was performed using the non-parametric Pearson chi-square test. Stata (StataCorp. 2016. Stata Statistical Software: Release 14.1. College Station, TX: StataCorp LP) was used for database management and analysis.

**Table 1.** Bowel habits variations pre and post treatment with Spasmicol®

| | **Subtypes of irritable bowel syndrome at $t_0$ (number of cases/ total of patients)** | **Responders according to bowel habits at $t_1$ (number of cases/ total of patients)** |
|---|---|---|
| IBS-M | 76/183 | 45/183 |
| IBS-C | 40/183 | 25/183 |
| IBS-D | 43/183 | 31/183 |
| IBS-U | 24/183 | 15/183 |

**Table 2.** Pain relief pre and post treatment with Spasmicol®

| | **Subtypes of irritable bowel syndrome at $t_0$ (number of cases/ total of patients)** | **Responders according to abdominal pain relief at $t_1$ (number of cases/ total of patients)** |
|---|---|---|
| IBS-M | 76/183 | 56/183 |
| IBS-C | 40/183 | 30/183 |
| IBS-D | 43/183 | 22/183 |
| IBS-U | 24/183 | 16/183 |

## Results

In the present study, 183 consecutive patient were recruited (55 male [29.4 %] and 132 female patients), ranging from 18 to 80 years (mean: 50.2 years).
After 30 days of treatment:

- 45 out of 76 IBS-M patients reported a normalized stool consistency, with a complete spontaneous bowel movements (CSBM) per week greater than 3/week, however with 20 out 76 not showing satisfactory relief of the abdominal pain;
- 25 out of 40 IBS-C patients reported a complete spontaneous bowel movements (CSBM) per week superior to 3/week, however with 10 out of 40 a satisfactory relief of the abdominal pain;
- 31out of 43 IBS-C patients reported a normalized stool consistency, however with 11 out of 43 not showing a satisfactory relief of the abdominal pain;
- 16 out of 20 IBS-U patients reported a normalized stool consistency , with a complete spontaneous bowel movements (CSBM) per week greater than 3/week, however with 9 out of 20 not showing satisfactory relief of the abdominal pain.

Significant severe, moderate or mild adverse events were not reported by the enrolled patients. The statistical analysis showed that the scores relative **to stool habits and abdominal pain resulted significantly different** ($p < 0.001$ and $p = 0.002$ respectively) between the considered observational points ($t_0$ and $t_1$).

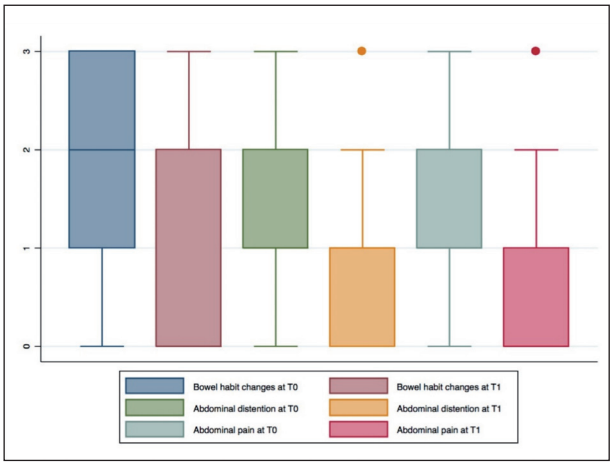


**Figure 1.** *The Box and Whisker Plot chart* highlights the difference in distribution (regarding median and interquartile distance) of the scores above $t_0$ and $t_1$, and underlines the presence of outliers in abdominal distension and abdominal pain, observed at $t_1$.

For the parameter "abdominal distension" the difference was not statistically significant ($p = 0.116$), but this consideration could be biased by the great subjectivity of this parameter.

## Discussion

Despite the costs and numerous investigations into the pathophysiology and treatment of this disorder, our understanding of IBS is still incomplete. Over the last ten years, increasing insight into the enteric nervous system and how its dysfunction may play a role in IBS pathology has emerged. Additionally our increasing understanding of the gut microbiome and how its potential disruption may lead to IBS symptoms has also been highlighted (12-17). Currently, many clinicians use a treatment approach based on the predominant symptoms of the patient: constipation (IBS-C), diarrhoea (IBS-D), or mixed symptoms (IBS-M). Medications that relax smooth muscle *via* anticholinergic mechanisms or calcium channel antagonism have been commonly utilized for the treatment of IBS. Among these are alverine, dicyclomine (with or without cimetropium), hyoscyamine, otilonium, pinaverium, scopolamine, and trimebutine. Generally, antispasmodics have been utilized for their effects on gastrointestinal motility in attempts to reduce abdominal pain associated with IBS. Some of them could improve IBS symptom scores and global assessment. Unfortunately, anticholinergic side effects of these agents often include dose-related vision disturbances, dry mouth, and dizziness. Moreover, antispasmodics can also cause constipation, thus they should be used cautiously in patients with IBS-C.

In this study we have chosen open label, prospective observational design, due to the higher placebo rates found in European randomized controlled trials (RCTs) compared with those conducted in other continents, in trials evaluating antispasmodics, and in trials using shorter duration of therapy; in spite of this factor, the double-masked , randomized, placebo controlled, parallel-group trial is the gold standard for testing the efficacy of new treatments in IBS.

The placebo effect in clinical trials has long been known, and because of the vague nature of IBS symptoms and the use of primary outcomes that are often subjective in nature, high placebo response rates have been noted in IBS trials. However, Kim et al. (18) have also described the potential for a "pre-cebo" effect in IBS, which impacts the treatment outcome even before the study begins. The pre-cebo effect describes the impact of consent language used in clinical trials on expectations of benefit from the study medication.

The obtained results showed a marked reduction of IBS symptoms, according to *Roma IV criteria*. The remission or reduction of symptoms related to the disease could be identified into the synergistic activity of the principles contained in the product. They have shown their ability to reduce abdominal pain by increasing the digestibility of lactose, thanks to an anti-inflammatory effect on the intestinal mucosa, by relaxing the intestinal smooth muscles, reducing bowel gas formation, and modulating and regulating intestinal motility.

## Conclusions

The combined action of chamomile, fennel, caraway, melissa, passionflower and beta-galactosidase, demonstrated potentially beneficial effects on all physiopathological components of IBS, improving patients' quality of life, without important side effects. *Spasmicol*® has been found to exert beneficial effects for patients with IBS. The product regularizes bowel movements, reducing or eliminating diarrhoea or constipation, reducing the formation of intestinal gases and decreasing or eliminating intestinal distention; it also reduces or even eliminates abdominal pain thanks to the triple synergistic effect of its main components (anti-inflammatory, improvement of lactose digestibility, spasmolytic).

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## Conflicts of interest

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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