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The Health Impacts of Ramadan Fasting

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ABSTRACT

Ramadan is the holy month of obligatory fasting for all the healthy Muslims who have reached puberty. In Ramadan, fasting individuals consume two meals per day, the first of which is before down to prepare for fasting (Sahur), and second meal is at sunset (Iftar). Fasting is a ritual and religious obligation with numerous beneficial health effects, especially in patients with diet-dependent diseases. Fasting could significantly reduce peptic and duodenal ulcers in combination with H2 blockers and improve the lipid profile, especially through the regulation of total cholesterol, high-density lipoprotein, and lo-density lipoprotein, which may be involved in the incidence of heart failure. Furthermore, fasting could enhance the systolic and diastolic blood pressure more significantly compared to the use of drugs such as perindopril/indapamide. Evidence suggests that ischemic stroke and cancer could also be improved by intermitted fasting via multiple pathways and factors such as BDNF, bFGF, GRP78, Hsp70, tyrosine kinase receptor B (TrkB), fibroblast growth factor receptor 1 (FGFR1), and ketosis. The present study aimed to systematically review the published literature regarding the effects of Ramadan fasting on the mentioned diseases.

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Introduction

Ramadan is the ninth month in the Islamic lunar calendar and a holy month of obligatory fasting for 29-30 days. The word Ramadan is of the Arabic root *Ramida*, which means dryness [1]. Fasting is an Islamic obligation, which involves fasting for one month from sunrise to sunset. Duration of fasting in Ramadan varies depending on the geographical location of every country; however, the average duration is 10-19 hours per day [2, 3]. Fasting is obligatory for all the healthy Muslims who have reached puberty, and the exemptions of fasting include patients with chronic diseases (e.g., diabetes, cancer, and cardiac diseases), travelling individuals, and menstruating, lactating, and pregnant women [4].

Fasting is a ritual and religious obligation with numerous beneficial health effects against diet- and lifestyle-dependent diseases, as well as the disorders with challenging therapeutic treatment, including cardiac diseases, osteoporosis, stroke, cancer, and hypertension [5]. Moreover, fasting has remarkable effects on basic homeostatic function. Previous findings have indicated that fasting could protect cells against oxidative stress, maintain the level of total cholesterol [6], significantly reduce systolic and diastolic blood pressure [7], improve peptic ulcers [8], protect the body against various cancers (especially breast cancer) [9], and improve ischemic stroke [10].

Physiology of Fasting

In healthy individuals, insulin secretion is stimulated by the dietary behaviors that enhance the storage of glucose in the muscles and liver as glycogen. Fasting leads to the decreased level of glucose, which in turn significantly reduces insulin, as well as the levels of catecholamines and glucagon [11]. In a study in this regard, Al Arouj et al. [2] claimed that after several hours of fasting, the levels of glycogen and circulating insulin decreased, thereby eliminating fatty acids from the adipocytes. As a result, the fatty acids

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were oxidized, producing ketone bodies that were used as fuel by various body organs, such as the liver, kidneys, cardiac and skeleton muscles, and adipose tissues.

A series of complex mechanisms are involved in the transition from the feeding state to prolonged fasting, which occurs in three stages, including the postabsorptive phase (6-24 hours), gluconeogenic phase (2-10 days), and protein conversion phase (after 10 days of fasting). A healthy individual use approximately seven grams of glucose per hour in overnight fasting [2, 11].

Within the past two decades, several studies have been focused on the effects of Ramadan fasting on various diseases, proposing heterogeneous findings. However, fasting has been reported to positively influence some disorders, which have been discussed in the following section.

Benefit of Fasting for Patients with Peptic and Duodenal Ulcers

Ulcers occur due to the depletion of the upper thick layer of mucus, which protect the stomach against the acidic nature of digestive fluids, as a result of which the mucus layer becomes thin, and acidic fluids or foods could readily cross the tissues and ultimately form the ulcer [12]. Fasting is considered to be a common, yet underrated solution to this issue. Fasting contributes to the balance and in healing process of the body. According to a research in this regard, almost 15 days of regular fasting (12-14 hours) is required for the proper treatment of peptic ulcers [8, 13].

Two cohort studies confirmed the role of Ramadan fasting in the treatment of peptic and duodenal ulcers, and the findings were compered between fasting and non-fasting patients. Accordingly, during the treatment of peptic ulcer (PU), the fasting and non-fasting subjects were administered with H_2 blockers at Sahur and Iftar in Ramadan, and both groups were examined before and after Ramadan. After repeated examinations, a significant improvement was observed in the fasting group, while no changes occurred in the non-fasting group. Both of the mentioned studies also proposed findings regarding the treatment of erosive duodenitis (ED). According to the first study, approximately 43.75% of the fasting patients were recovered from ED, while in the second study, all the fasting patients were reported to be healthy after Ramadan [8]. With respect to the treatment of duodenal ulcer, both fasting and non-fasting subjects were administered with omeprazole (40 mg/day), which is a proton-pump inhibitor. Both cohort studies revealed that the fasting patients with duodenal ulcer significantly improved compared to the non-fasting subjects [14].

Fasting and Lipid Profile

Attarzadeh Hosseini et al. [15] and Langsted et al. [16] have reported that after Ramadan fasting, a significant improvement was observed in the levels of total cholesterol (TC) and high-density lipoprotein (HDL), which could be associated with the reduction of coronary heart disease. Additionally, another study in this regard indicated that the level of TC significantly reduced in fasting individuals during Ramadan, which resulted in the improvement of HDL [17, 18].

The study by Shehab et al. [19] was performed on 65 individuals, and the obtained results demonstrated a significant improvement in the HDL level of the subjects. On the other hand, the findings of another study confirmed the improvement of LDL in fasting individuals during Ramadan [7]. Further investigations are required to obtain more accurate results in this regard.

The role of Fasting on Blood Pressure

Rehman et al. [7] conducted a research on 20 fasting individuals during Ramadan, reporting the significant reduction of diastolic and systolic blood pressure. Similarly, Dewanti et al. [20] examined normotensive individuals, reporting the significant reduction of systolic and diastolic blood pressure in fasting individuals, while denoting the hypotensive effects of fasting. Samad et al. [21] conducted an experiment on 40 normotensive and non-smoker males aged 18-40 years, who were fasting in Ramadan. The researchers regularly assessed the patients one week before Ramadan, as well as on days seven, 14, and 21 of Ramadan before and after Iftar. Furthermore, they reported a significant reduction in the systolic blood pressure (approximately 7.61 mmHg) before (7.61

mmHg) and after Iftar (2.72 mmHg) (P<0.005); the same reduction was observed in the diastolic blood pressure (approximately 3.19 mmHg).

In another study by Laurent (2003) [22], the correlation between fasting and low-dose administration of perindopril/indapamide was assessed, and the results indicated that after three weeks of fasting, blood pressure decreased by approximately 8/3 mmHg (mean: 9.4±14.3 mmHg), while the intake of indapamide (0.625 mg; P=0.023) and perindopril (2 mg; P=0.001) was associated with the reduction of blood pressure by only 8.0±16.5 mmHg within 12 weeks. It is notable that the mentioned study had some limitations, and the findings cannot be generalized to other patients with chronic diseases [21].

Treatment of Ischemic Stroke

A complex cascade is involved in ischemic stroke, which is stimulated spatially and temporally in the induction of damage to cerebrovascular tissues. The brain requires energy in the form of adenosine triphosphate (ATP) for proper function, and energy failure leads to ischemia and loss of ionic homeostatic, thereby activating the glutamate receptors, which play a key role in the cell death in ischemic stroke [9, 23]. Strong evidence suggests that intermittent fasting (IF) plays a pivotal role in the protection and improvement of neural survival from glutamate excitotoxicity in the rodent model of cerebral ischemia via multiple pathways through increasing neuroprotective proteins and factors such as *brain-derived neurotrophic factor* (BDNF), bFGF, GRP78, and Hsp70 [24]. BDNF and bFGF exert their effects when binding to tyrosine kinase receptor B (TrkB) and fibroblast growth factor receptor 1 (FGFR1), respectively, which activate various signaling pathways, including Akt (protein kinase B) and extracellular signal-regulated kinase, thereby resulting in the activation of the transcription factor cyclic AMP response element-binding protein (CREB). CREB has been reported to exert neuroprotective effects during an ischemic stroke in animal models [9, 25].

Another study in this regard indicated that IF may be involved in the improvement of neural survival from oxidative stress in the rodent model of cerebral ischemia either by increasing the antioxidant effects or reducing the reactive oxygen species and providing mitochondrial protection in the brain [26]. Similarly, a study demonstrated that IF could also improve neural survival from inflammation in the rodent model of cerebral ischemia through multiple pathways either by the reduction of pro-inflammatory genes or eliminating inflammatory stimuli from the brain [27].

Treatment of Cancer by Fasting

Cancerous or normal cells utilize adequate energy in the form of ATP for their multiplication, while cancer cells use more energy, which is supplied by glucose through glycolysis and electron transport chain pathways. While fasting, the total availability of glucose decreases, and the requirement of cancer cells cannot be met, and these cells receive energy through ketosis-ketosis, which is a metabolic state of the body to acquire energy from ketone bodies. Ketosis often occurs during starvation or glucose shortage while fasting for approximately 10-17 hours per day. This process weakens the tumor cells due to the unavailability of nutrition and energy [9, 28].

A study conducted in 2009 reported that fasting is preferred after chemotherapy since the procedural complications could be improved by fasting during chemotherapy. DNA damage is considered to be the major complication caused by chemotherapy, which could lead to the development of secondary tumors. Therefore, fasting is an optimal method for the reduction of oxidative stress [29].

The effects of fasting on various diseases are presented in Table 1.

Table 1. Effects of Ramadan Fasting on Various Diseases

Disease	*Number of Participants*	*Type of Fasting*	*Findings*	*References*
Blood Pressure	*40 Males*	*Regular Fasting (12-14h/day)*	*SBP before Ramadan: 124.55 mmHg SBP during Ramadan: 116.94 mmHg DBP Before Ramadan (78.8 mmHg) DBP During Ramadan (75.6 mmHg)*	*[21]*
Peptic Ulcer	*470 (Male and Female)*	*12-14 Hours of Complete Fasting/Day For 15 days*	*Higher Frequency of PUD and PPU One month after Ramadan compared to Ramadan (P>0.05 and P= 0.008, respectively)*	*[8]*
Cholesterol	*1,301 (Male and Female)*	*Regular Fasting (29-30 days)*	*Before Ramadan: 5.65±0.80 ml/dl (Mean±SD) During Ramadan: 4.90±1.07 ml/dl*	*[30]*
Triglycerides	*1,301 (Male and Female)*	*Regular Fasting (29-30 days)*	*Before Ramadan: 1.65±0.81 ml/dl (Mean±SD) During Ramadan: 1.41±0.62 ml/dl*	*[30]*
HDL	*1,301 (Male and Female)*	*Regular Fasting (29-30 days)*	*Before Ramadan: 1.42±0.29 ml/dl (Mean±SD) During Ramadan: 1.34 ±0.35 ml/dl*	*[30]*
LDL	*1,301 (Male and Female)*	*Regular Fasting (29-30 days)*	*Before Ramadan: 2.78±0.99 ml/dl (Mean±SD) During Ramadan: 2.63±0.76 ml/dl*	*[30]*
Ischemic stroke	*20 (young and middle- age mouse models)*	*IF*	*Significantly reduced the risk of Ischemic stroke via a several pathways (e.g. BDNF, bFGF, GRP78, and Hsp70)*	*[9]*

SBP: systolic blood pressure; DBP: diastolic blood pressure; PUD: peptic ulcer disease; HDL: high-density lipoprotein; LDL: low-density lipoprotein; IF: intermittent fasting

Conclusion

Ramadan is a ritual and religious obligation with numerous beneficial health effects, especially on diet- and nutrition-dependent diseases. Fasting plays a pivotal role in the reduction of peptic and duodenal ulcers, improvement of TC, HDL, and LDL levels, improvement of systolic and diastolic blood pressure (8/3 mmHg; four-fold results compared to perindopril/indapamide), improvement of ischemic stroke via multiple pathways, and reduction of the risk of breast cancer by enhancing the effect of the growth hormone and ketosis. It is recommended that further investigations be conducted in this regard in order to achieve more accurate results.

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Conflicts of Interest

None declared.

Informed Consent

No informed consent was required for this research design.

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Diets and Irritable Bowel Syndrome

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ABSTRACT

Introduction: Irritable bowel syndrome (IBS) is the most common functional gastrointestinal disorder, which is characterized by the presence of abdominal pain or discomfort associated with altered bowel habits. This systematic review aimed to assess the nutritional factors (dietary patterns and food groups) associated with IBS.

Methods: Articles were collected via searching in databases such as Medline, EMBASE, CINAHL, ProQuest, PubMed, Scopus, ScienceDirect, and Google Scholar using relevant keywords and phrases, including irritable bowel syndrome, pattern, intake, behavior, habit, meal, nutrition, food, lifestyle, and prevalence. The articles were confined to the original human studies that were published in English since 2010. Duplicates and unrelated materials were excluded.

Results: Consumption of processed meat, fast foods, and legumes has been reported to increase the risk of IBS. However, the correlation between the consumption of milk, fruits, and vegetables has not been conclusive. Moreover, the association between cereal intake and risk of IBS varies depending on the type of cereals. The results also indicated that fast food dietary patterns increased the risk of IBS, while lacto-vegetarian dietary patterns reduced the risk of IBS.

Conclusion: According to the results, the effects of some food groups on IBS are unclear. In addition, research in this regard is limited to food patterns, and further investigations are required in order to reach conclusive results.

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Introduction

Irritable bowel syndrome (IBS) is a digestive system disorder, which is characterized by chronic abdominal pain, bloating, and altered bowel habits, adversely influencing the quality of life of the patients (1). Patients with IBS often have diarrhea, constipation or intermittent diarrhea and constipation. Accordingly, these patients could be classified into three groups, including patients with predominant constipation, those with predominant diarrhea, and patients with both diarrhea and constipation (mixed type) (2).

Previous studies have indicated that the prevalence of IBS is 1-22% across the world. For instance, the prevalence of IBS has been reported to be 10.9% in Middle Eastern countries, such as Iran (3). The incidence of IBS has been reported to be higher in women compared to men. Furthermore, most IBS symptoms tend to appear in the third decade of life in individuals. Considering that IBS has no specific symptoms, if the patient is not diagnosed with other digestive tract disorders (e.g., colitis, celiac, and cancer), IBS is the next probable diagnosis (4, 5).

The etiology of IBS remains unknown. However, some of the main risk factors in this regard include the family history of IBS, dietary habits, gut microbes, neuroendocrine system disorders, mental impairment, anxiety, and stress. The main causes of IBS may be classified as central and peripheral. The central causes of IBS include inappropriate nervous system responses to neural impulses and hypothalamic-pituitary-adrenal disorders. The peripheral causes of IBS

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include increased visceral sensitivity, low-grade inflammation, and epithelial dysfunction. Reports suggest that factors such as prolonged fever, anxiety, and depression could also increase the risk of IBS (3).

Despite numerous investigations regarding the identification of the causes of IBS, the findings in this regard are inconclusive. Several studies have denoted that sleep patterns, dietary habits, and other lifestyle factors may increase the risk of IBS (1). Moreover, it is likely that lifestyle changes in terms of dietary habits and eating behaviors could effectively improve the quality of life of the patients with IBS.

The present study aimed to evaluate the correlation between nutritional factors (dietary patterns and food groups) and IBS.

Materials and Methods

This systematic review study was conducted via searching in databases such as Medline, EMBASE, CINAHL, ProQuest, PubMed, Scopus, ScienceDirect, and Google scholar for the articles published until March 2018. The articles were focused on original human studies and published in English, which were retrieved by using relevant keywords or phrases, including Irritable bowel syndrome, pattern, intake, habit, meal, nutrition, food, lifestyle, and prevalence.

The literature search resulted in a limited number of studies in this regard. Therefore, the systematic review was not confined to only one type of study design, and all types of studies were considered in the search strategy. The articles meeting the inclusion criteria were selected, and the reference lists of potentially eligible articles were also checked in order to find additional related articles. Afterwards, two reviewers, who were subject matter experts, analyzed the titles and abstracts of the initially selected articles so as to confirm the final articles for the study. The articles that only assessed single nutrients were excluded. Duplications were also checked, and unrelated articles in terms of the content were also excluded.

Various data were extracted from the selected articles, including the characteristics of the subjects (e.g., sample size, age, and gender), study design, methods of dietary assessment, various dietary data (e.g., dietary patterns and food groups), and association of dietary intakes and patterns with IBS. Moreover, the obtained data were categorized based on dietary patterns and food groups.

Results

The literature search for the related studies yielded 547 articles. In total, 11 articles were included in the study. Based on the content of the extracted articles, the results of the studies were reported in two separate categories of dietary patterns and food groups in regards to IBS. With the exception of one article, the sample size of which consisted of women only, all the retrieved studies were conducted on both men and women. The participants were aged ≥18 years, and the studies involved the measurement of IBS symptoms using the Rome III or Rome II questionnaires (2), as well as various other questionnaires, to assess dietary intakes. For instance, the food frequency questionnaire was used in three studies, while two of the studies were semi-quantitative, and one was qualitative (4-6). In addition, two studies used another type of dietary assessment, including the brief self-administered diet history questionnaire (BDHQ) (7, 8). Multi-section questionnaires were also applied in another study, one of which involved the assessment of dietary intakes. In one study, the assessment method was a list of food items that might cause the symptoms of IBS. In this assessment, patients with IBS and healthy subjects were required to record their perceptions toward the role of diet in their IBS symptoms (1, 3, 9, 10).

Table 1. Reviewed Studies Regarding Dietary Patterns and Food Groups

Reference	Participants and Their Gender	Country	Study Design	Dietary Assessment Method	Dietary Patterns/Food Groups	Results
Studies on Dietary Patterns						
Ryu Satake et al. (2015)	993 Subjects (382 men, 611 women)	Japan	Cross-sectional	Self-administered Diet History Questionnaire (BDHQ)	1. Healthy 2. Western 3. Alcohol and Accompanying	Alcohol and accompanying dietary pattern was inversely associated with the risk of IBS.
Esmaillzadeh Ahmad et al. (2015)	3,846 Subjects	Iran	Cross-sectional	Dish-based Semi-quantitative Food Frequency Questionnaire (DS-FFQ)	1. Fast Food 2. Traditional 3. Lacto-vegetarian 4. Western	Lacto-vegetarian dietary pattern was inversely associated with the risk of IBS; Fast food dietary pattern was positively associated with IBS.
Camille Buscail et al. (2017)	44,350 Subjects (9,643 men and 34,707 women)	Finland	Cross-sectional	Questionnaire Consisting of 29 Food Groups	Healthy, Western, and Traditional	Western dietary pattern was positively associated with the risk of IBS; Traditional dietary pattern was positively associated with IBS in women.
Studies on Food Groups						
Ioan Chirila et al. (2012)	193 Subjects (80 men and 113 women)	Romania	Cross - sectional	FFQ	Processed Meat, Potatoes, Cereals, Canned Fruits, Milk, and Legumes	Intake of processed meat, grain bread, pasta, cereals, canned fruits, milk, and legumes was positively associated with the risk of IBS.
Yukiko Okamiet et al. (2011)	1,768 Students (650 males and 1,118 females)	Japan	Cross-sectional	Questionnaire Consisting of 69 Items (23 items on dietary habits and frequency of food items)	Milk, Meat, Fish, Egg, Green-Yellow Vegetables, Fruit, and Processed Food Products	*Women:* Intake of fish, fruit, milk, and green-yellow vegetables was inversely associated with the risk of IBS; Intake of processed food products was positively associated with the risk of IBS; *Men:* Not significant.
Yu-Bin Guo et al. (2015)	78 IBS Patients and 79 Healthy Subjects			FFQ and Chinese Lifestyle Habits Questionnaire	Fruits, Vegetables Legumes, and Tea	Intake of fruits, vegetables, legumes, and tea was significantly higher in IBS patients than controls.

Reference	Participants and Their Gender	Country	Study Design	Dietary Assessment Method	Dietary Patterns/Food Groups	Results
		China	Case-control			
Hayes et all. 2014	135 IBS patients 111 healthy subjects	Ireland	Cross sectional	perceptions of the role of diet in their gastrointestinal symptoms	Cereal, spicy food, vegetables fatty food	Cereal, spicy food, vegetables, fatty food significantly cause gastrointestinal symptoms
Farnaz Khademolhosseini et al. (2010)	1,978 Subjects (709 men and 1,269 women)	Iran	Cross-sectional	Questionnaire with Validity and Reliability	Fruits and Vegetables	Intake of fast foods was positively associated with the risk of IBS; Intake of fruits and vegetables was inversely associated with the risk of IBS.
Zhaoqiu Zheng et al. (2015)	1,082 Subjects (837 men and 245 women)	Japan	Cross-sectional	Brief self-administered Diet History Questionnaire (BDHQ)	Rice, Bread, Chinese Noodles, AND Japanese Wheat Noodles	Intake of rice, bread, pasta, and buckwheat noodles was positively associated with the risk of IBS.
Katsuhisa Omagar et al. (2013)	245 Subjects (women only)	Japan	Cross-sectional	Semi-quantitative FFQ	Milk and Eggs	Intake of milk and eggs was inversely associated with the risk of IBS.
Marion J. Torres et al. (2018)	1,870 IBS Patients and 34,578 Healthy Subjects	Finland	Case-control	Self-administered 24-Hour Dietary Records and 24-Hour Records	Milk and Fruits	Intake of fruits and milk was significantly higher in IBS patients than controls.

The Association of Dietary Patterns and IBS

Two of the selected studies investigated the association of an overall dietary pattern with the incidence of IBS. A cross-sectional study conducted by Satake et al. (2015) on a Japanese population reported three major dietary patterns in the participants, including healthy diet, western diet, and alcohol and accompanying diet. In the mentioned research, the participants with higher adherence to the alcohol and accompanying dietary pattern were at the lower risk of IBS (53%; odds ratio [OR] =0.47; confidence interval [CI]: 0.24-0.90). This dietary pattern was characterized by the high intake of beer, oily fish, squid/octopus/shrimp/shellfish, Shochu, lean fish, chicken, and small fish with bones (7).

Another cross-sectional study in this regard explored the correlation between dietary patterns and IBS. The prevalence of IBS was measured in a general population consisting of Iranian adults, who were employed in 50 healthcare centers. In the mentioned study, four dietary patterns were identified, including the fast food dietary pattern, traditional dietary pattern, lacto-vegetarian dietary pattern, and western dietary pattern. According to the

findings, the individuals in the highest quartile of the fast food dietary pattern were at the higher risk of IBS compared to those in the lowest quartile (P=0.05). In addition, an inverse association was reported between the lacto-vegetarian dietary pattern and risk of IBS (P=0.01), while no significant associations were observed between the traditional and western dietary patterns with the risk of IBS (6).

Another cross-sectional study in this regard demonstrated that the western dietary pattern was positively associated with the incidence of IBS (P<0.0001) (11).

The Association of Food Groups and IBS

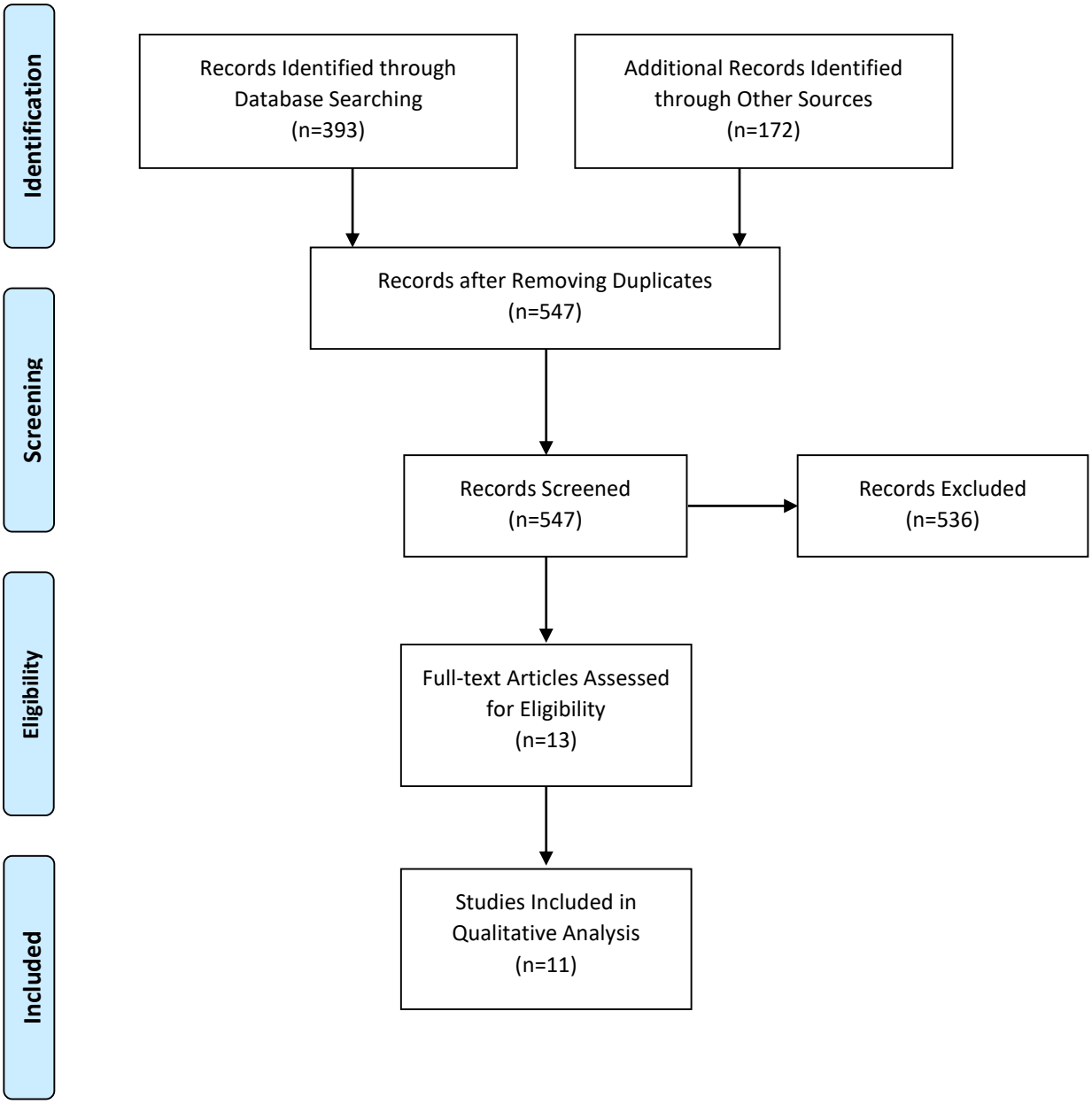
Among 11 reviewed articles, eight articles were about food groups and IBS, two of which were case-control studies, and the other six articles were cross-sectional studies.

Regarding some food groups, the mentioned studies proposed conflicting results. For instance, one study demonstrated that the prevalence of IBS in the men and women consuming high portions of fruits and vegetables was significantly lower compared to those who consumed less fruits and vegetables (P=0.027). On the other hand, the findings of some studies indicated that the intake of milk, fruits, greens, and yellow, leafy vegetables was significantly lower in the women of the IBS group compared to healthy women (P<0.05). However, another study showed a significant, positive association between the consumption of fruits and risk of IBS (P<0.001).

Another research in this regard showed that the consumption of fast foods, processed meat, canned foods, and processed food products was positively associated with the risk of IBS (3, 5, 10). Another study, in which the subjects were required to record their perception toward the role of diet in their IBS symptoms, indicated that the intake of cereals, spicy foods, vegetables, and fatty foods caused significant gastrointestinal symptoms in the patients with IBS, while no assessment was used in the study (9). According to the findings of another research, the intake of milk, yogurt, and fruits was lower in the patients with IBS compared to healthy controls (12).

The current findings regarding the association of egg and fish consumption with IBS are contradictory, so that the results of one study indicated that the intake of fish in women was significantly lower in the IBS group (8), while another study showed that the consumption of eggs and fish had no significant association with the incidence of IBS (5). Moreover, both of the studies examining the correlation of legume intake with IBS showed that the higher consumption of legumes was significantly associated with the higher risk of IBS (1, 5).

The association between various food items of the cereal food group and IBS has been investigated in two studies. In the first study, which was conducted by Zheng et al., the intake of rice, bread, pasta, and buckwheat noodles was associated with the higher prevalence of IBS. After adjustment for carbohydrate and plant protein intake, no significant association was observed between the intake of rice, bread, and pasta with the incidence of IBS. However, the association between the intakes of buckwheat noodles with IBS remained significant. Furthermore, the intake of Japanese wheat noodles had no significant correlation with IBS before and after adjustment for carbohydrate and plant protein intake. In another study, IBS was reported to be significantly associated with the higher intake of grain bread, pasta, and cereals (5, 8).



Discussion

Overall, the results of the reviewed studies indicated that the consumption of processed meat, fast food, and legumes increased the risk of IBS. However, the results of the studies investigating the correlation between the intake of various food groups (milk, fruits, and vegetables) and IBS were not consistent. The association between cereal intake and the risk of IBS depended on the type of the cereals and their gluten content. Eggs and fish have been reported to be among the foods that could decrease the risk of IBS although this finding has not been reported in all the studies in this regard.

There were some inconsistencies regarding the prevalence of IBS in various studies. For instance, one study reported the higher prevalence of IBS (35.5%), attributing the difference to the shared characteristics of the participants. The mentioned study was conducted on nurses and medical students, who had more stressful lives

compared to ordinary people. In addition, the irregular working hours of these individuals may affect their health and lead to the higher incidence of IBS (10).

IBS is a multifactorial disease, and several risk factors may play a role in its etiology. The biopsychosocial model has been most currently endorsed for IBS (13, 14). New research in this regard has demonstrated that diet affects the symptoms of IBS (5). With regard to the association between different foods and IBS, the current findings are not consistent in some food groups, while conclusive results have been yielded regarding many foods (e.g., processed meat and fast foods) (3, 5, 10).

According to the literature, allergic reactions could affect the incidence of IBS (2). In a study conducted by Zhaoqiu Zheng et al., the consumption of buckwheat noodles was associated with the higher prevalence of IBS due to the presence of allergenic proteins in buckwheat noodles, such as buckwheat 16- and 24-kDa, BW16KD, and BW24KD (8). In another research by Loan Chirila, a significant, positive association was reported between milk consumption and the risk of IBS. On the other hand, Katsuhisa Omagari claimed that milk intake was lower in the patients with IBS (4, 5). These contradictory findings may be due to the fact that in some patients, the symptoms of the disease might be caused by the consumption of certain foods (e.g., milk), which induce the production of IgG4. This antibody could be responsible for the symptoms of IBS, and in some patients, milk intolerance has also been reported. Therefore, these individuals often avoid drinking milk. Furthermore, lactose may cause IBS symptoms in some patients, and lactase deficiency must be medically confirmed by a hydrogen breath test (HBT) and not only based on the reports on the symptoms of the patient. According to a study in this regard, the prevalence of positive HBT was similar in the milk-tolerant and milk-intolerant patients with IBS (15, 16).

According to Loan Chirila et al., there was a significant association between the consumption of cereals and risk of IBS (5), which is most likely due to the gluten sensitivity of the patients with IBS. The prevalence of celiac disease has been reported to be 0-32% in the patients with IBS (17). Due to the inducement of some human leukocyte antigens (e.g., HLA-DQ2 and HLA-DQ8), many patients with celiac disease may also show signs of gluten sensitivity. The probable mechanisms explaining the role of gluten in the occurrence of IBS symptoms is through the induction of gastrointestinal motility and increased permeability of the intestinal tract (2).

In a study examining the effects of gluten-free diets on the patients with IBS and without celiac disease and wheat allergy, the patients in the gluten intervention group scored significantly higher in terms of abdominal pain, bloating, and fatigue (18). On the other hand, the findings of another research indicated no significant association between the intake of rice, bread, and pasta and the prevalence IBS after adjustment for carbohydrate and plant proteins due to gluten sensitivity. Similarly, Japanese wheat noodles were reported to have no significant association with the prevalence of IBS before and after adjustment for carbohydrate and plant proteins, which could be due to the low levels of gluten in Japanese wheat noodles compared to rice and bread (8). However, another study indicated that Biobran (a substance derived from rice bran) is effective in improving the symptoms of IBS (19).

The gastrointestinal hormones that are secreted from the gastrointestinal tract in response to foods (e.g., motilin, cholecystokinin, peptide YY, and gastrin) often fluctuate in the patients with IBS. Peptide YY secretion increases after food intake in these patients, which could lead to visceral hypersensitivity and high concentrations of ghrelin in the circulation. This chain of actions may cause gastrointestinal motility disorders. In addition, cholecystokinin (CCK) increases after eating and during fasting, thereby leading to increased visceral hypersensitivity and pain. In response to fat and protein intake, CCK could be secreted from the proximal small intestine (2).

In a study conducted by Chirila et al., a significant, positive association was observed between the consumption of processed meat and risk of IBS. Similarly, Yukiko et al. reported that the female patients consumed significantly higher portions of processed food products compared to healthy individuals (5, 10). These findings might be due

to the high fat content in processed food products and processed meat, which triggers the production of CCK. The results of the mentioned study were consistent with the previous one, in which Watson explored the effects of low-fat diets on the prevalence of IBS in an interventional study, claiming that reducing the consumed amount of fats to 42 grams could significantly improve IBS symptoms (e.g., bloating). Moreover, in another study, the consumption of liquid meals containing 60% calories from fats has been reported to reduce the threshold of discomfort and pain, while the pain scores of patients in the visual analogue scale were observed to increase after the consumption of fatty meals (20, 21).

In a study by Okami et al., female IBS patients consumed significantly lower portions of fruits compared to healthy individuals, which could be due to the low threshold of fructose tolerance in the patients. Fructose cannot be absorbed in the colon and must be converted into short-chain fatty acids by micro flora, causing flatulence and bloating (2, 10). However, Chirila reported that the intake of fruits was associated with the higher risk of IBS, which might be due to the altered dietary patterns of these patients. In the mentioned research, dietary status was assessed using the food frequency questionnaire (FFQ), which might have overestimated the intake of vegetables, while the results of another study indicated that the FFQ underestimated the intake of fiber (5, 22, 23).

Conclusion

The results of this systematic review confirmed the key role of food compositions in the onset of IBS or its symptom deterioration in the patients. Therefore, it is recommended that patients with IBS reduce their intake of processed meat and fast food and pay more attention to the recommended dietary allowance of fruits and vegetables. However, the elimination of milk and cereals from the dietary patterns of IBS patients is not recommend unless diagnostic tests confirm lactase deficiency, allergic reactions or gluten sensitivity. IBS patients must be assessed individually, and dietary restrictions should be applied based on diagnostic tests results.

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Conflict of interest

All other authors report no conflicts of interest relevant to this article.

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Anti-apoptotic Effects of Interval and Continued Training and Crocin on the Muscle Tissue of the Rats with Type II Diabetes Induced by a High-fat Diet

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ABSTRACT

Introduction: Diabetes is a metabolic disease, which is directly correlated with increased physical disabilities and muscle tissue damage. The present study aimed to investigate the anti-apoptotic effects of interval and continued training and crocin on diabetic rats.

Methods: In this study, 49 adult rats aged eight weeks with diabetes inducaed by a high-fat diet and venous injection of streptozotocin were randomly assigned to seven groups, including high-intensity interval training (HIIT), low-intensity continued training (LICT), HIIT with crocin consumption, LICT with crocin consumption, crocin consumption, sham, and control. The animals in the HIIT and LICT groups ran on a treadmill three sessions per week for eight weeks at the intensity of 80-85% and 50-55% of the maximum speed, respectively. The animals in the crocin consumption groups received 25 mg/kg of crocin weekly for eight weeks.

Results: HIIT and LICT could significantly increase Bcl-2 and decrease Bax and p53, as well as the ratio of Bax and Bcl-2 (P≤0.05). In addition, crocin consumption could significantly increase Bcl-2 and decrease Bax (P≤0.05). Training with crocin consumption had interactive effects on the increase of Bcl-2 and decrease of p53 and ratio of Bax to Bcl-2 (P≤0.05).

Conclusion: According to the results, continued and interval training along with the consumption of crocin exerted interactive anti-apoptotic effects on the rats with diabetes induced by a high- fat diet.

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Introduction

Type II diabetes is a worldwide phenomenon. According to the World Health Organization (WHO), the number of patients with type II diabetes increased from 108 million in 1980 to 422 million in 2014 (1). Type II diabetes is caused by increased insulin resistance due to obesity and sedentary lifestyle, which in turn leads to the reduction of insulin secretion from the beta cells (2). Strong evidence suggests that oxidative stress and elevated reactive oxygen species (ROS) are important factors caused by hyperglycemia in diabetic patients, which lead to the mutation of the mitochondrial genome and onset of cell death through necrosis or apoptosis (3).

Increased oxidative stress, the tumor suppressor protein (*p53*) activates the apoptosis process, thereby preventing the proliferation and repair of muscle cells and accelerating cell death. In this mechanism, *p53* activates caspase-9 (4, 5), and the activation of caspase-9 from the internal pathway increases the *Bcl-2*-associated X protein (*Bax*) gene as the precipitant of apoptosis and decreases the expression of *Bcl-2* as an anti-apoptotic agent (5,6).

Some researchers believe that physical exercise could decrease apoptosis through the reduction of oxidative stress (7). However, there is uncertainty regarding the effects of exercise on apoptotic markers. For instance, four weeks of endurance training at the speed of 15-18 m/min

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have been reported to significantly increase in the levels of soluble Fas and Fas/Fas ligand (FasL), while causing no changes in the level of *Bcl-2* in the cardiac tissue of diabetic and non-diabetic rats (8). On the other hand, high-intensity interval training has not been reported to cause a significant increase the *Bcl-2* levels, while it could increase the *Bax* levels in rats with myocardial infarction.

Several therapeutic approaches are used for the control and treatment of diabetes, such as the use of medicinal plants and sedentary lifestyle modifications, which are recommended to diabetic patients (9). Saffron (*Crocus sativus*) is a medicinal plant belonging to *Iridaceae* family, which contains a substance known as crocin. Researchers have assessed the beneficial effects of crocin on glycemic indices and lipid profiles (9).

With regard to the effects of inactive lifestyle on the progression of diabetes, as well as the reduction of motor ability and muscle tissue damage in diabetic patients, physical exercise plays a key role in decreasing the complications associated with diabetes, and medicinal plants could also be used for the improvement of diabetes owing to their antioxidant properties.

The present study aimed to investigate the simultaneous effects of crocin and endurance training on apoptotic markers in the muscle tissues of rats and assess the interactive effects of endurance training along with the consumption of crocin on the apoptosis markers in the soleus muscle of rats with type II diabetes induced by a high-fat diet.

Materials and Methods

This experimental study was conducted on 49 male Sprague-Dawley rats aged eight weeks, with the mean weight of 150±30 grams, which were purchased from the Animal Reproduction and Breeding Center of Islamic Azad University, Marvdasht Branch, Iran. The animals were transferred to the physiology laboratory and preserved in standard conditions.

Diabetes Induction

For the induction of type II diabetes, a combination of a high-fat diet and streptozotocin (STZ) was used. To this end, the animals received a fatty diet for eight weeks. The dietary pattern consisted of 45% total fat (derived from animal fats), containing 24 grams of fat, 24 grams of protein, and 41 grams of carbohydrate per 100 grams (10). After eight weeks, diabetes was induced via injecting a single dose of STZ (30 mg/kg*)* (10). For the confirmation of diabetes, the rats with higher glucose levels than 300 mg/dl at 96 hours after the injection were selected as the samples (10).

Animal Grouping

Based on the serum glucose, the rats were divided into seven groups, including high-intensity interval training (HIIT), low-intensity continued training (LICT), HIIT with crocin consumption, LICT with crocin consumption, crocin consumption, sham, and control. Groups one and three received training in three weekly sessions for eight weeks at the intensity of 80-85% of the maximum speed for two minutes, along active rest periods (one minute). From six intervals in the first week of training, HIIT reached 12 intervals in the last week.

Groups two and four also received training in three weekly sessions for eight weeks at the intensity of 50-55% of the maximum speed. LICT was initiated during the first week at 25 minutes, reaching 50 minutes in the last week. It is notable that the total volume of the exercise (intensity, duration, and repetition) was matched between the LICT and HIIT groups (10). In addition, groups three, four, and five received 25 mg/kg of crocin (dissolved in normal saline) intraperitoneally (11).

In order to control the effects of injection on the study variables, the animals in the sham group were intraperitoneally administered with soluble crocin daily (Sigmma, Cat No. 17304). The rats were anaesthetized 24 hours after the last training session. Following that, the soleus muscle was extracted by experts, placed in liquid nitrogen in microtubes, and stored at the temperature of -70°C for further analysis.

Measurement of the Research Variables

Quantitative real-time polymerase chain reaction (qRT-PCR) was used to measure the research variables and investigate gene expression. The

sequence of the primers used in the study is presented in Table 1.

Table 1. Sequence of Forward-Reverse Primers of Genes in Real-time Polymerase Chain Reaction

Gene	Forward (5'-3')	Reverse (5'-3')	Product Size (bp)
B2M	CGTGCTTGCCATTCAGAAA	ATATACATCGGTCTCGGTGG	244
Bax	CTGCAGAGGATGATTGCTGA	GATCAGCTCGGGCACTTTAG	147
Bcl-2	ATCGCTCTGTGGATGACTGAGTAC	AGAGACAGCCAGGAGAAATCAAAC	134
p53	GGCTCCGACTATACCACTATCC	GAGTCTTCCAGCGTGATGATG	104

Statistical Analyses

Data analysis was performed in SPSS version 21. Shapiro-Wilk test was used to determine the normal distribution of the data, and the changes in the weight of the animals at pretest and posttest were evaluated using paired-sample t-test. Moreover, the effects of training and crocin consumption on the study variables were assessed using two-way analysis of variance (ANOVA) and Bonferroni's post-hoc test at the significance level of P≤0.05.

Results

Table 2 shows the weight of the animals in the study groups. The levels of *Bcl-2*, *Bax*, *p53*, and ratio of *Bax* to *Bcl-2* in the rats are depicted in Figures 1-4, respectively.

Weight Changes in Study Groups

At the posttest, the weight of the rats in the control group (P=0.001) and sham group (P=0.001) significantly increased compared to the pretest. On the other hand, the weight of the rats in the LICT group (P=0.001), LICT with crocin consumption group (P=0.001), and HIIT with crocin consumption group (P=0.001) significantly reduced at the posttest compared to the pretest. However, no significant difference was observed in the pretest and posttest weight of the rats between the HIIT group (P=0.10) and crocin consumption group (P=0.09).

Effects of Training and Crocin Consumption on Bcl-2 Gene Expression

According to the obtained results, training (F=10.22; P=0.001; effect size: 0.36) and crocin consumption (F=24.76; P=0.001; effect size: 0.40) could significantly increase *Bcl-2*. Furthermore, the combination of training and crocin consumption had interactive effects on the increase of *Bcl-2* (F=5.04; P=0.01; effect size: 0.21). The obtained results also indicated that HIIT (P=0.03) and LICT (P=0.001) could significantly increase *Bcl-2*, which indicated the similar effects of HIIT and LICT on the increase of *Bcl-2* (P=0.25).

Effects of Training and Crocin Consumption on Bax Gene Expression

According to the findings, training (F=47.23; P=0.001; effect size: 0.72) and crocin consumption (F=32.01; P=0.001; effect size: 0.47) could significantly decrease *Bax*. However, training with crocin consumption had no interactive effects on the reduction of *Bax* (F=2.18; P=0.26; effect size: 0.10). Moreover, the obtained results indicated that HIIT (P=0.001) and LICT (P=0.001) could significantly reduce *Bax*, which confirmed the similar effects of HIIT and LICT on the reduction of *Bax* (P=0.99).

Effects of Training and Crocin Consumption on p53 Gene Expression

According to the findings, training (F=9.50; P=0.001; effect size: 0.34) could significantly reduce *p53*, while crocin consumption (F=2.16; P=0.15; effect size: 0.05) had no significant effect on *p53*. However, training along with crocin consumption had interactive effects on the reduction of *p53* (F=15.28; P=0.001; effect size: 0.45). Furthermore, the obtained results indicated that HIIT (P=0.001) and LICT (P=0.005) could significantly decrease *p53*,

which confirmed the similar effects of HIIT and LICT on the reduction of *p53* (P=0.99).

Effects of Training and Crocin Consumption on the Bax/Bcl-2 Ratio

According to the findings, training (F=4.08; P=0.02; size effect: 0.16) could significantly reduce the *Bax*/*Bcl-2* ratio, while crocin consumption (F=3.63; P=0.003; effect size: 0.07) had no significant effect on the reduction of the *Bax*/*Bcl-2* ratio. In addition, training along with crocin consumption had interactive effects on the reduction of the *Bax*/*Bcl-2* ratio (F=6.78; P=0.003; effect size: 0.27). The obtained results also indicated that HIIT (P=0.006) and LICT (P=0.005) could significantly decrease the *Bax*/*Bcl-2* ratio, which confirmed the similar effects of HIIT and LICT on the reduction of the *Bax*/*Bcl-2* ratio (P=0.99).

Table 2. Pretest and Posttest Weight of Rats in Study Groups

Group	Pretest (g)	Posttest (g)	Paired-sample T-test	
	Mean±SD	Mean±SD	t	P-value
HIIT	360.64±13.12	342.12±44.11	1.91	0.10
LICT	375.12±33.18	352.11±17.18	-21.71	0.001
Crocin Consumption	345.25±44.08	364.12±13.10	-1.97	0.09
HIIT with Crocin Consumption	410.47±30.87	392.41±46.52	4.24	0.005
LICT with Crocin Consumption	394.88±25.66	354.22±18.12	17.47	0.001
Sham	390.59±42.33	409.62±45.17	-21.71	0.001
Control	384.64±50.41	420.88±62.14	-10.01	0.001

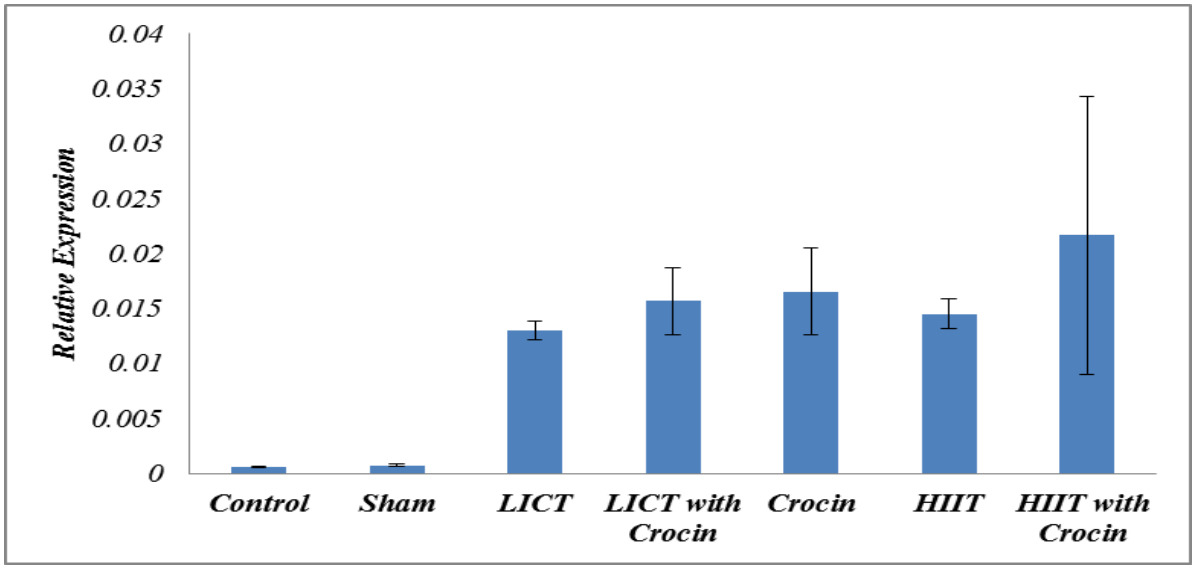

Figure 1. *Bcl-2* Gene Expression in Study Groups

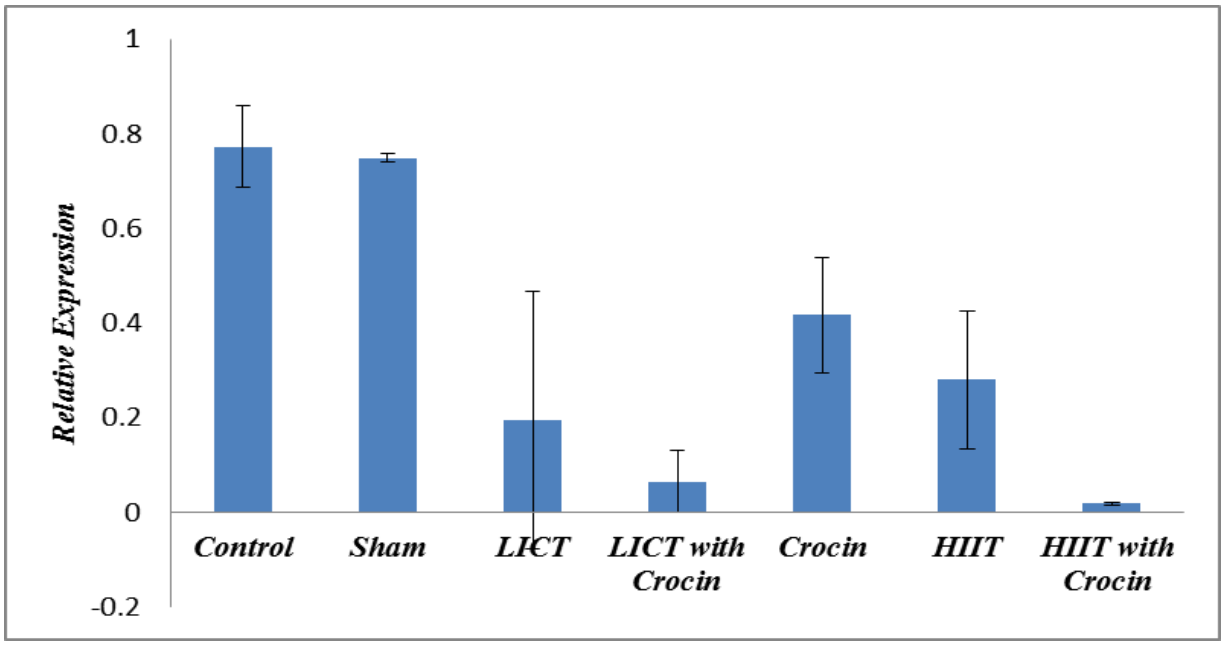

Figure 2. *Bax* Gene Expression in Study Groups

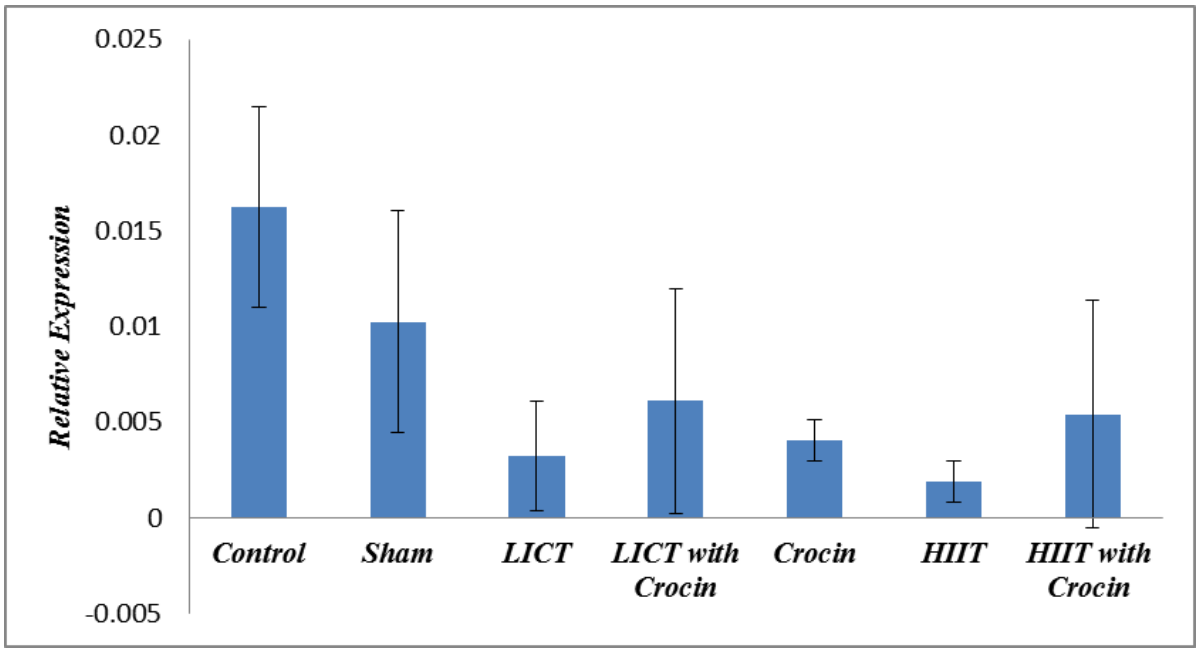

Figure 3. *P53* Gene Expression in Study Groups

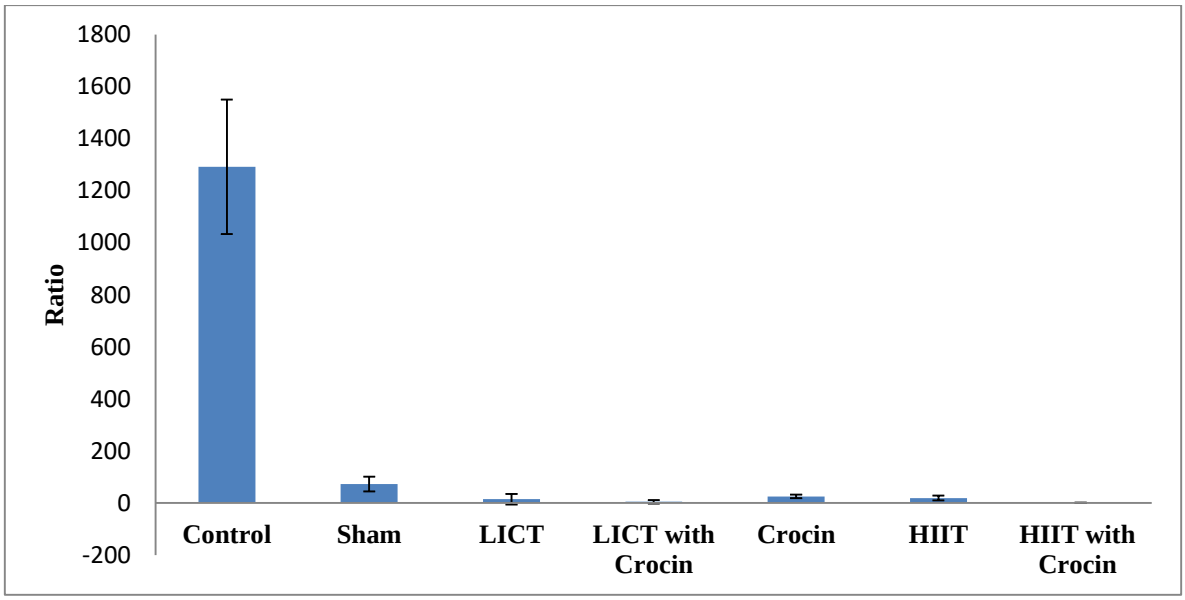

Figure 4. *Bax*/*Bcl-2* Ratio in Study Groups

Discussion

According to the results of the present study, eight weeks of HIIT and LICT could significantly increase *Bcl-2* and reduce *Bax*, *p53*, and *Bax*/*Bcl-2* ratio in the soleus muscle of the diabetic rats. Several studies have investigated the effects of physical exercise on the gene expression of *Bcl-2* and *Bax*. In line with the results of the present study, moderate aerobic training has been reported to increase *Bcl-2* and decrease *Bax* and caspase-3 in the rats with STZ-induced diabetes (12). This consistency could be attributed to the similarities in the research samples and duration and intensity of training. On the other hand, six weeks of low-intensity interval training have been reported to reduce *Bax* gene expression and increase *Bcl-2* gene expression in rats with myocardial infarction. However, HIIT has been reported to increase *Bax* gene expression, with no significant effects on the changes in *Bcl-2* gene expression in the cardiac tissues of rats with myocardial infarction (5). The discrepancies in the findings of the mentioned studies regarding increased apoptosis after HIIT could be due to the differences in the sample populations and type of the studied tissues. Correspondingly, 10 weeks of regular swimming in male rats increased the levels of anti-apoptotic proteins (Bcl-2 and Bcl-x) and decreased the levels of apoptosis-promoting proteins (e.g., BAD), followed by the phosphorylation and reduction of BAD to the *Bcl-2* ratio (13).

With respect to the association between the mechanism of the effects of HIIT and LICT, researchers have claimed that for the improvement of the mitochondrial function due to the low and high adaptation of intracellular apoptotic pathways, pre-apoptotic signal molecules, such as the pre-apoptotic proteins of the Bcl-2 family (e.g., BAK and Bax) are transmitted to the mitochondria, where they induce a series of temporary permeable pores in the external mitochondrial membrane, which inhibit the release of cytochrome C, leading to the reduction of the caspase activity (5).

According to the findings of the current research, eight weeks of crocin consumption could significantly increase *Bcl-2* and decrease *Bax* in the soleus muscle tissues of diabetic rats. However, crocin consumption had no significant effects on the reduction of *p53* and the *Bax*/*Bcl-2* ratio. A mechanism that causes crocin to decrease apoptosis may be the reduction of *p53* expression, as well as the antioxidant properties of crocin and its effects on the reduction of blood glucose and HbA1c. This mechanism depends on the anti-apoptotic effects of reducing hydrogen peroxide, H_2O_2, and caspase-3 (14).

According to the literature, crocin could inhibit oxidative stress and reduce lipid peroxidation, thereby inhibiting ROS, caspases, and *p53* and preventing apoptosis induction (15). The only study regarding the effect of crocin on *Bcl-2* and *Bax* has been conducted by Sadoughi (2017), the findings of which have indicated that the administration of 0.5 milliliter of crocin could reduce *Bax*, increase *Bcl-2* and antioxidant enzymes, and decrease lipid peroxidation (15).

According to the results of the present study, eight weeks of training along with the consumption of crocin had interactive effects on the increase of *Bcl-2*, as well as the reduction of *p53* and the *Bax*/*Bcl-2* ratio, in the soleus muscle tissues of diabetic rats. However, training with crocin consumption had no interactive effects on the reduction of *Bax*. Physical exercise could block apoptotic pathways by increasing the expression and enhancement of protein kinase B activity through the phosphorylation of the anti-apoptotic proteins of the *Bcl-2* family, inhibition of apoptotic-promoting proteins (e.g., Bax) or the direct inhibition of caspase activity (8). It seems that training and simultaneous use of crocin could also increase *Bcl-2* and anti-apoptotic processes by controlling the caspase activity and reducing the ROS (16).

Some of the limitations of the present study were failure to measure the muscle weight, muscle mass, and muscle strength in rats. Therefore, it is recommended that further investigations in this regard be conducted based on similar protocols to the present study. In such experiments, it is also suggested that factors such as muscle weight, muscle mass, and muscle strength be measured in order to confirm the positive effects of training and crocin consumption. Furthermore, it is recommended that further investigations in this regard consider the effects of resistance training and swimming along with crocin consumption on the apoptotic markers in diabetic rats.

Conclusion

According to the results, HIIT and LICT along with the consumption of crocin could exert interactive anti-apoptotic effects on the rats with diabetes induced by a high-fat diet.

Conflicts of interest:

None declared.

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JOURNAL OF NUTRITION FASTING AND HEALTH

Effects of Oregano Methanolic Extract on the Chemical, Microbial, and Sensory Properties of Yogurt

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ABSTRACT

Introduction: The demand for antimicrobial compound alternatives to replace synthetic additives is on the rise. In food commodities, use of synthetic antimicrobials based on herbal extracts is attracted attention. The present study aimed to assess the chemical, microbiological, and sensory properties of the yogurt samples treated with oregano extract (0%, 0.75%, 1%, and 1.5%) during storage for 30 days at refrigerated temperature.

Methods: The in-vitro antibacterial effect of oregano extract was evaluated using agar disk-diffusion assay. Natural yogurt was prepared using a combination mixture of whole milk and 4% skimmed milk powder. Various concentrations of the oregano extract (0%, 0.75%, 1%, and 1.5%) were added to the samples. During 30 days of refrigerated storage, various parameters were evaluated, including titratable acidity, pH, mold and yeast counts, total coliforms, and sensory attributes (overall liking, appearance, and aroma).

Results: The descending order of the in-vitro antibacterial effect of the oregano extract was as follows: Listeria monocytogenes>Staphylococcus aureus>Bacillus subtilis>Bacillus cereus>Salmonella typhimurium>Escherichia coli O157:H7. Differences were observed in the counts of mold and yeast in the samples over time. Accordingly, bioactive yogurt had lower mold and yeast counts (1-1.5 log CFU/g) after 30 days of storage compared to the control ($P<0.05$). In addition, significant differences were observed in the hedonic scores of aroma and appearance between the samples containing the oregano extract compared to the control group ($P<0.05$).

Conclusion: According to the results, oregano extract could be used as a natural compound to improve the shelf life of yogurt at refrigerated temperatures for 30 days. Furthermore, oregano extract is considered to be an effective inhibitory compound against L. monocytogenes, S. aureus, B. subtilis, B. cereus, S. typhimurium, and E. coli O157:H7.

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Introduction

In recent decades, the functional food market has grown rapidly owing to the increased interest of consumers for the purchase of fresh food products with remarkable health benefits [1, 2]. This rising trend has remarkably influenced the food industry, especially in case of milk and dairy products, setting a special goal for the functional food market, which is faced with the challenge of offering novel products with appropriate functional and organoleptic properties to satisfy consumers [3].

Yogurt is considered to be the most consumed dairy product across the world. It is obtained from the fermentation of milk through the combined action of culturing various microorganisms, including *Streptococcus salivarius* ssp. *thermophilus*, *Lactobacillus delbrueckii* ssp., and *Bulgaricus*. These bacteria consume lactose in order to obtain energy, thereby producing the lactic acid required to coagulate milk [4]. Coagulated milk preserves the fat, mineral, and vitamin contents of pure milk despite the low lactose level, resulting in the higher digestibility of the food product compared to milk [5].

A wide variety of commercial yogurts are available on the market, which vary in terms of the composition, texture, and taste [6]. Development of dairy products with new flavors and health benefits could potentially increase the market sales. Some studies have elaborated on the functional products supplemented with fruits, vegetable oils, medicinal plants, and nutrient fortification [7, 8]. Regular consumption of yogurt has positive health effects, including the reduction of cholesterol levels, improving lactose digestion, intestinal syndromes and infections, acute diarrhea, and colon cancer, reduction of the

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nasal colonization of various pathogens (e.g., *S. pneumoniae, S. aureus*, and hemolytic streptococci), and strengthening of the immune defense mechanisms [8]. Meanwhile, the natural antioxidants and antimicrobial agents found in fruits and medicinal plants have attracted the attention of researchers and consumers [6, 9]. Furthermore, reports have confirmed the health benefits of using fresh tropical fruits and plants, which represents a growing body of research [10].

Chemical synthetic preservatives have recently been replaced by natural compounds in food commodities due to their side-effects on the health of consumers [11]. The demand for antimicrobial compound alternatives to replace synthetic additives has risen, and the replacement of synthetic antimicrobials by herbal extracts in food commodities has attracted noticeable attention [12].

Oregano (*Origanum vulgare*) is a native plant that grows in tropical regions, especially in Iran, Pakistan, and Turkey [13]. Oregano is extensively cultivated in several regions in Iran. It is a tropical plant and an abundant source of anthocyanins and natural phytochemical compounds with pharmacological properties [14]. The main compounds found in oregano extract include phenols (e.g., carvacrol and thymol), monoterpene hydrocarbons, cymene, and terpinene. Carvacrol and thymol constitute the major antibacterial content in oregano, while the possible synergistic antimicrobial actions of this plant have been attributed to the presence of terpenes. Other potential antioxidant phenols have also been obtained from the herbal extract of oregano, the most abundant of which has been reported to be rosmarinic acid [15].

To the best of our knowledge, no studies have been published regarding the effects of oregano extract on the shelf life and quality of fresh yogurt. The present study aimed to assess the chemical, microbiological, and sensory properties of the yogurt samples treated with various concentrations of oregano extract (0%, 0.75%, 1%, and 1.5%) during storage for 30 days at refrigerated temperature.

Materials and Methods

Experimental Materials

Milk and oregano were obtained from the local markets in Kermanshah, Iran. The commercial starter culture of yogurt consisting of *Lactobacillus delbrueckii* ssp., *Bulgaricus*, *Streptococcus salivarius* ssp, and *Thermophilus* was also purchased. All the chemicals and microbial cultures in the present study were purchased from Merck, Germany.

Extraction of Oregano

All parts of the oregano plants were dried in a dark place at room temperature. Afterwards, 10 grams of the powdered plant was dissolved in 20 milliliters of methanol and extracted using a shaker at room temperature for 24 hours. The extract was filtered using the Whatman filter paper No. 3, concentrated in a rotary evaporator at the temperature of 40±1ºC, and preserved in chilled condition for further experimentation [16].

In-vitro Antibacterial Effect of Oregano Extract Preparation of Microorganisms

At this stage, a panel of microorganisms was used for the antibacterial examination of the methanolic extract of oregano, which contained *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633), *Bacillus cereus* (ATCC 11774), and *Listeria monocytogenes* (ATCC 19118) as gram-positive bacteria, as well as *Salmonella typhimurium* (ATCC 14028) and *Escherichia coli* O_{157}:H_7 (ATCC 10536) as gram-negative bacteria. The cultures were purchased from the culture collection of the Iranian Research Organization for Science and Technology (IROST) in Tehran, Iran and maintained on slants of brain heart infusion agar (BHI; Merck, Germany). In addition, the bacterial inoculants (8 log CFU/ml) were prepared based on our previously published approach [17].

Agar Disk-diffusion Assay

In order to perform the agar disk-diffusion assay, one milliliter of each bacterial culture (8 log CFU/ml) was uniformly spread on the BHI agar medium using sterile cotton swabs. Following that, the sterile paper discs (diameter: 6 mm), which were dipped in 10 microliters of diluted oregano extract, were placed on the surface of each BHI agar medium. The plates were incubated for at the temperature of 37±1°C for 24 hours, and the inhibition zone was calculated as πr^2 [17].

Yogurt Production

Natural yogurt was prepared using a combination of whole milk and 4% skimmed

milk powder. The mixture was incubated at the temperature of 90±1°C for five minutes and chilled to the temperature of 44±1°C for the incorporation of the commercial starter culture. After the addition of the commercial starter culture, the milk was transferred to sterile beakers aseptically, and various concentrations of the oregano extract (0%, 0.75%, 1%, and 1.5%) were added. Afterwards, all the batches were incubated at the temperature of 44±1°C until reaching the pH of 4.6. At the next stage, the designated products were sealed and stored at the temperature of 4±1°C for 30 days. The control yogurt (without added oregano extract) was also considered in the experiments [18].

Chemical Analysis

On the first day of yogurt production, the levels of protein, fat, ash, and salt in the yogurt samples were measured based on the standards of the Association of Official Analytical Chemist (AOAC) [19]. In addition, the titratable acidity of the samples during refrigerated storage was assessed based on the AOAC method [19]. Changes in the pH of the treated and untreated samples during storage were monitored using a digital pH meter [19].

Microbial Analysis

For the enumeration of molds, yeasts, and total coliforms, potato dextrose agar and violet red bile agar were used, respectively [20].

Sensory Analysis

In order to determine the sensory effects of the addition of oregano extract on the treated and untreated yogurt samples, nine panelists (aged 22-30 years; four females and five males) examined the products based on a nine-point hedonic score (Extremely Dislike=1, Neither Like nor Dislike=5, and Extremely Like=9) for the overall acceptability of the yogurt samples, as well as the acceptability of their appearance and aroma. The samples marked with three-digit random numbers were placed in small, white plastic glasses and served immediately after heat treatment at the approximate temperature of 20±1°C [4].

Statistical Analysis

Data analysis was performed in SPSS version 25, and all the experiments were performed in triplicate. One-way analysis of variance (ANOVA) was used to determine the significant differences between the samples, and the P-value of less than 0.05 was considered statistically significant.

Results and Discussion

In-vitro Antibacterial Effect of Oregano Extract

According to the information in Table 1, the descending order of the *in-vitro* antibacterial effect of the oregano extract was as follows: *L. monocytogenes>S. aureus>B. subtilis>B. cereus>S. typhimurium>E. coli* O_{157}:H_7.

Table 1. Antibacterial Effect of Methanolic Oregano Extract Based on Agar Disk-diffusion Assay

	Inhibition Zone (mm)					
	S. aureus	***B. subtilis***	***B. cereus***	***L. monocytogenes***	***S. typhimurium***	***E. coli* O_{157}:H_7**
Extract	6.32±0.03	5.12±0.05	4.32±0.01	7.06±0.09	3.14±0.01	ND

ND: Not determined

The significant difference in the sensitivity of the bacteria to natural antibacterial compounds could be due to the outer cytoplasmic membrane covering the thin peptidoglycan structure of gram-negative microorganisms, which restricts the diffusion of hydrophobic constituents through its lipopolysaccharide covering [21, 22]. Moreover, the periplasmatic space contains enzymes that could break down the foreign molecules that are introduced from the outer environment [23, 24].

In a study in this regard, Lv et al. [23] investigated the antibacterial effects of some herbal extracts on four food-borne pathogens, including *E. coli*, *S. typhimurium, S. aureus*, and *B. subtilis.* According to the findings, gram-negative bacteria were the most resistant to the evaluated natural compounds, which is consistent with the results of the present study. In another study,

Gilles et al. [24] evaluated the antimicrobial effects of three species of Australian *Eucalyptus* using the agar disc-diffusion method, reporting that gram-positive bacteria were more sensitive compared to gram-negative bacteria. In addition, *S. aureus* was reported to be the most sensitive bacteria, while *P. aeruginosa* was the most resistant strain.

Microbial Analysis of Yogurt

In the current research, the absence of coliforms and bacterial microorganisms (*Salmonella* spp., *L. monocytogenes*, *E. coli* O_{157}:H_7 and *S. aureus*) during storage confirmed the proper sanitary practices of the food production unit in the process of product elaboration in terms of the time and temperature of adequate storage [19].

The counts of molds and yeasts were observed to differ in the yogurt samples over time, and bioactive yogurt samples had lower 1-1.5 log CFU/g after 30 days of storage compared to the control group ($P<0.05$) (Figure 1). In the control group, the initial counts of molds and yeasts were below the detection limit (1 log CFU/g) and significantly reached 3.71 log CFU/g after the study period ($P<0.05$). The decreased counts of yeasts and molds in the treated samples could be attributed to the antimicrobial effect of the oregano extract. According to our previous study, this herbal extract had remarkable effect on extending the shelf life of raw beef meat, while inhibiting the growth of some foodborne pathogenic bacteria [16].

According to the literature, the antibacterial mechanism of herbal extracts is associated with their major compounds, especially polyphenolic compounds, which are able to interact with the cytoplasmic membrane of bacterial cells, thereby leading to the leakage of cellular components [26]. Although some damage in the outer membrane and loss of the cell content are tolerated by bacteria without losing their viability, the extensive loss of cell content or critical molecules and ions could lead to microbial cell death [24]. In the present study, all the treated samples had significantly lower mold and yeast counts compared to the microbiological acceptability limit of 3 log CFU/g throughout the storage period of 30 days ($P<0.05$). Moreover, a significant difference was observed between storage time and treatments ($P<0.05$).

In a research in this regard, Al-Turki et al. [28] evaluated the antimicrobial property of aqueous extracts of oregano, marjoram, sage, and licorice at the concentrations of 1% (v/v) and 5% (w/w) against *E. coli* and *B. subtilis* in milk and labneh, reporting that all the examined aqueous herbal extracts exerted antimicrobial effects against *E. coli* and *B. subtilis*. According to the current research, the addition of various concentrations of the oregano extract significantly maintained the population of *L. bulgaricus* and *S. thermophilus* during the shelf life of the samples ($P<0.05$). In another study, the values obtained by Tabasco et al. [29] were consistent with the results of the present study. In the mentioned research, the findings confirmed the sensitivity of *S. thermophilus* to the grape extracts rich in phenolic compounds, while the deleterious effects on *L. bulgaricus* were not evident.

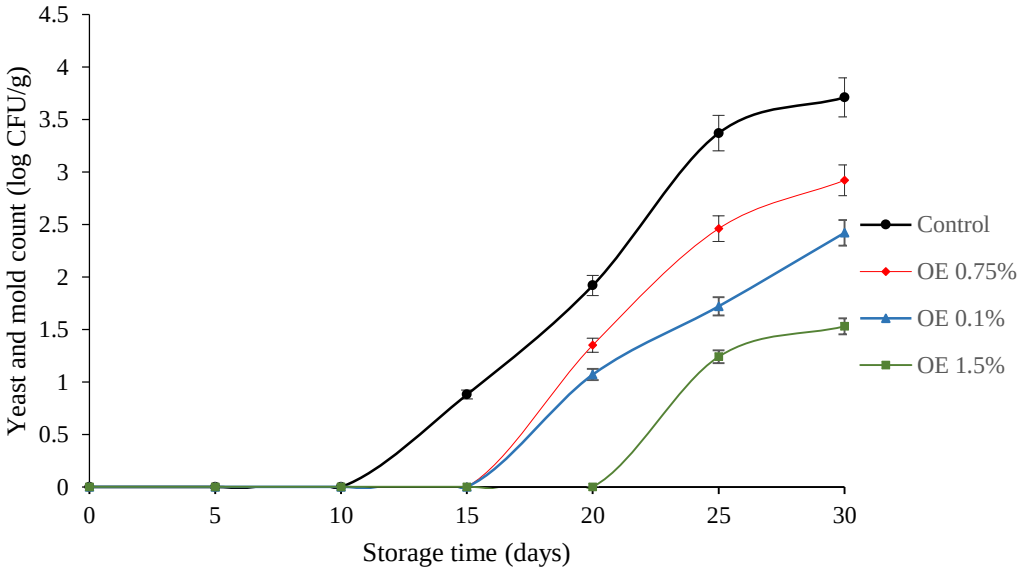

Figure 1. Changes in Yeast and Mold Counts in Yogurt Samples during Storage at Temperature of 4±1ºC for 30 Days (Data expressed as mean±standard error)

Chemical Analysis of Yogurt Samples

On the first day of yogurt production, the levels of protein, fat, ash, and salt in the fresh samples were estimated at 4.83±0.08, 4.43±0.13, 0.86±0.01, and 1.01±0.01, respectively. These findings are in line with the previous studies in this regard [3, 4, 29].

In the current research, the milk used to produce yogurt had an average pH of 6.1 and titratable acidity of 0.18 g lactic acid/100 g. During the fermentation process, pH decreased due to the production of lactic acid by the cultured *S. thermophilus* and *L. bulgaricus*. Furthermore, significant interaction were observed between treatment and storage ($P<0.05$); with increased storage time, pH decreased and acidity increased (Figures 2 & 3). The pH of the samples was within the range of 4.52-4.19. Similarly, Thabet et al. [27] reported that the incorporation of essential oils affected the pH and total volatile fatty acid values of the prepared labneh.

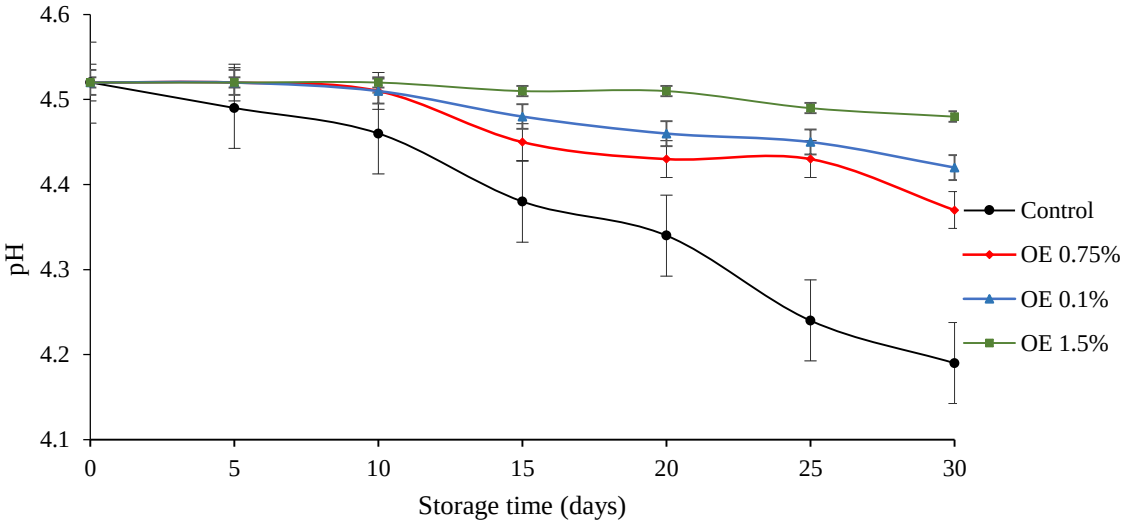

Figure 2. Changes in pH of Yogurt Samples during Storage at Temperature of 4±1ºC for 30 Days (Data expressed as mean±standard error)

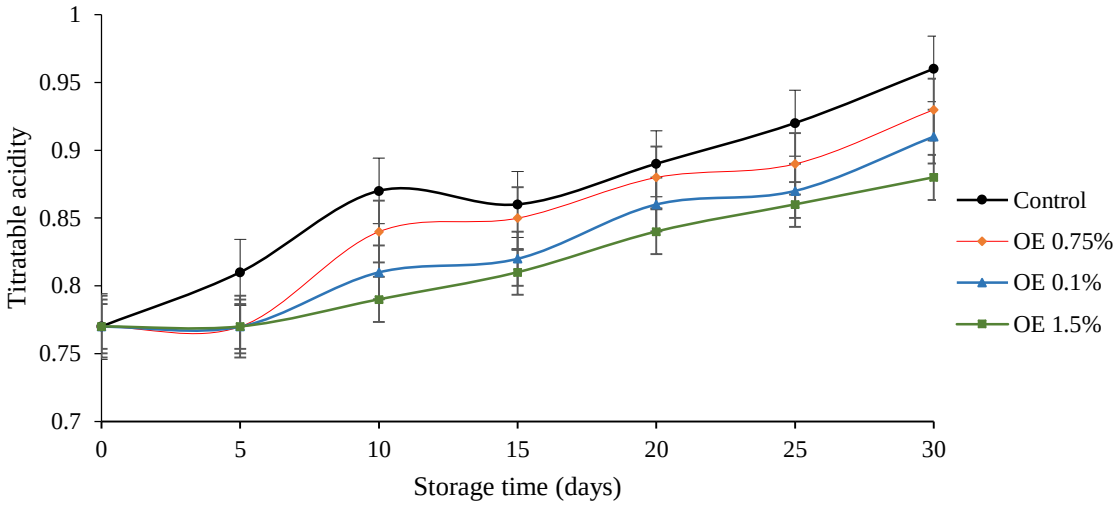

Figure 3. Changes in Titratable Acidity of Yogurt Samples during Storage at Temperature of 4±1ºC for 30 Days (Data expressed as mean±standard error)

Sensory Analysis of Yogurt Samples

The results of the sensory evaluation of the yogurt samples that were stored at refrigerated temperature indicated no significant differences between the appearance and aroma of the samples treated with the oregano extract at the concentrations of 0.75%, 1%, and 1.5% (P>0.05) (Figure 4). However, significant differences were observed in the hedonic scores of the aroma and appearance of the samples containing the oregano extract at the concentrations of 0.75%, 1%, and 1.5% compared to the control group (P<0.05).

The control and treated samples with 1% oregano extract had the lowest and highest sensory values in terms of all the studied parameters. Some studies have investigated the sensory quality of the food products treated with natural extracts, aiming to predict the applicability of the food products in terms of consumer acceptance [30-32]. The published data in this regard are conflicting in various foodstuffs, and the discrepancy could be due to the organoleptic attributes of the food products, applied concentrations of the extracts, and differences in the storage time and temperature of the products.

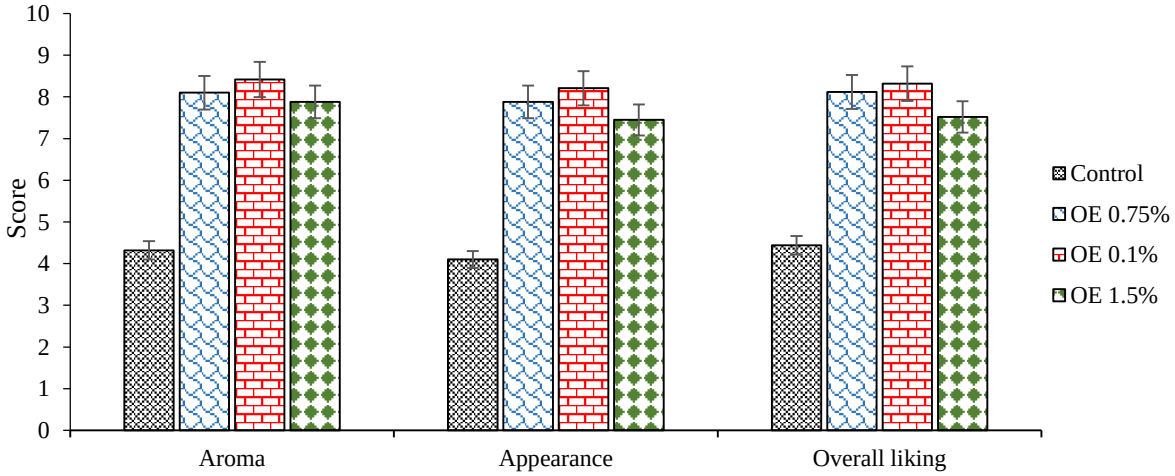

Figure 4. Sensory Attributes (aroma, appearance, and overall liking) of Yogurt Samples during Storage at Temperature of 4±1ºC for 30 Days (Data expressed as mean±standard error)

Conclusion

According to the results, oregano extract is an appropriate natural compound to extend the shelf life of yogurt during refrigerated condition for 30 days. Moreover, oregano extract has remarkable antibacterial effect against some foodborne pathogenic bacteria, including *L. monocytogenes*, *S. aureus*, *B. subtilis, B. cereus, S. typhimurium*, and *E. coli* O_{157}:H_7. Based on the literature review, this is the first study to assess the *in-vitro* antimicrobial property of oregano extract against the aforementioned pathogens and its application in the production and maintenance of fresh yogurt.

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Conflicts of interest

None declared.

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Comparison of Diet Records between Patients with Non-alcoholic Fatty Liver Disease and Controls

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ABSTRACT

Introduction: Non-alcoholic fatty liver disease (NAFLD) is considered to be a severe health threat across the world, the prevalence of which has significantly increased in recent years. Considering the role of diet in the pathogenesis of NAFLD, the present study aimed to evaluate and compare the dietary intakes of patients with NAFLD with healthy subjects.

Methods: This case-control study was conducted on 120 participants aged more than 18 years. The case group included 60 patients who were diagnosed with NAFLD based on Fibroscan assessment. The diet records of the subjects were analyzed using a three-day dietary record questionnaire.

Results: After modulation based on energy intake, the total intakes of energy, fiber, vitamin D, and vitamin E were significantly lower in the patients with NAFLD compared to the control group. In addition, the level of trans-fatty acids after energy adjustment was significantly higher in NAFLD patients compared to the controls. However, analysis after the energy adjustment indicated no significant difference between the case and control groups in terms of protein, carbohydrate, saturated fatty acid, monounsaturated fatty acid, and polyunsaturated fatty acid intakes.

Conclusion: According to the results, the diet records of the patients with NAFLD and healthy subjects differed in terms of the intakes of energy, fiber, trans-fatty acids, vitamin D, and vitamin E. Therefore, special attention must be paid to the dietary patterns of these individuals in order to improve their lifestyle and prevent the occurrence and progression of NAFLD.

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is characterized by the accumulation of fats in the liver. The prevalence of NAFLD has been reported to be on the rise across the world (1). According to the literature, 20-30% of the adult population (2, 3) and 10% of children and adolescents (1) are affected by NAFLD. NAFLD involves changes ranging from simple steatosis to non-alcoholic steatohepatitis, followed by fibrosis and cirrhosis in the absence of chronic alcohol consumption (4).

NAFLD is a multifactorial disease with several risk factors, including male gender, metabolic syndrome, obesity, prolonged starvation, total parenteral nutrition, polycystic ovarian syndrome, insulin resistance, type II diabetes mellitus, dyslipidemia, sleep apnea syndrome, and genetic susceptibility (5). The majority of the patients with NAFLD are asymptomatic, while the symptomatic patients mainly have non-specific manifestations (e.g., right upper-quadrant pain, fatigue, and malaise) (5). In addition, the laboratory results of these patients may be normal.

Liver biopsy is considered to be the 'gold standard' method for the diagnosis of NAFLD. Nevertheless, steatosis could be identified using non-invasive diagnostic methods, such as transient elastography (fibroscan) (6). Dietary

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and lifestyle modifications are widely recognized as effective therapeutic approaches to the treatment of NAFLD (7).

Considering the key role of diet in the pathogenesis of NAFLD, the present study aimed to evaluate and compare the dietary intakes of patients with NAFLD with healthy subjects.

Materials and Methods

This case-control study was conducted on 120 patients with NAFLD aged more than 18 years, who referred to a nutrition clinic. The participants were selected via simple random sampling. Written informed consent was obtained from all the selected subjects prior to participation, and the study protocol was approved by the Ethics Committee of Mashhad University of Medical Sciences, Iran.

None of the participants were pregnant, and they were all serologically negative for viral hepatitis B and C. In addition, none of the participants had autoimmune/congenital liver diseases or cancer. Anthropometric measurements were performed in suit settings with a flexible ribbon tape with the accuracy of 0.01 meter, and the body weight of the patients was measured using a digital scale (SECA 704, made in Germany) with the accuracy of 100 grams and recorded in kilograms. The height of the patients was measured in a standing position with elevated shoulders and in inspiration using a wall tape and recorded in centimeters. In addition, body mass index (BMI) was calculated by dividing the weight (kg) by the square of height (m).

After 12 hours of overnight fasting, 10 cc of blood was collected from the subjects, and the serum was separated using the Selectra autoanalyzer. The blood samples were placed in potassium-EDTA tubes and centrifuged at 3,000 rpm for 15 minutes. Liver function tests were also carried out to measure several variables, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase, fasting blood sugar (FBS), total cholesterol, and triglyceride (TG).

After three hours of fasting and removing the magnetic and electronic devices, each patient was examined for liver steatosis via fibroscan using the Echosens 504 device. Dietary data were obtained using three-day dietary records. The eligible patients were instructed to complete the three-day dietary records consecutively. The intake of various nutrients and foods were calculated based on Iranian household measures. Afterwards, each food item was converted into an energy value, and the other nutrients were analyzed using the Nutritionist IV software (N-Squared Computing, Salem, OR, USA). The calculated energy intakes that were not within the normal range (800-4,200 kcal/day) were excluded from further evaluation.

Statistical Analysis

Data analysis was performed in SPSS version 16.0 (SPSS, Inc., Chicago, IL, USA), and the normal distribution of the continuous variables was assessed using the Kolmogorov-Smirnov test. In addition, independent samples t-test was applied for the data with normal distribution, and Mann-Whitney U test was used for the data with non-normal distribution. In all the statistical analyses, P-value of less than 0.05 was considered significant.

Results

The general characteristics of the subjects are presented in Table 1. The mean age of the subjects in the case and control groups was 43.39±12.34 and 37.23±13.46 years, respectively (P<0.05). No significant differences were observed between the groups in terms of body weight, height, and BMI. According to the results of laboratory tests, only FBS was significantly higher in the patients with NAFLD compared to the controls (P=0.009).

Table 1. General Characteristics of NAFLD Patients and Control Group

Variables	Control	Case	P-value
Age (year)	37.23±13.46	43.39±12.34	0.009
Weight (kg)	80.01±26.81	79.5±13.59	0.89
Height (cm)	166.18±10.71	167.1±9.93	0.62
Body Mass Index (kg/m^2)	28.80±8.79	28.45±4.00	0.77
Fasting Blood Sugar (mg/dl)	90.02±19.11	99.48±19.11	0.009
Triglyceride (mg/dl)	130.5±82.6	128.1±82.6	0.87
Total Cholesterol (mg/dl)	182.5±52.26	176±52.26	0.54

Alanine Aminotransferase (IU/dl)	27.02 (20.6-42.87)	23.68 (19.96-44.15)	0.69
Aspartate Aminotransferase (IU/dl)	23.37 (21.4-30.56)	22.38 (21.25-30.45)	0.61
Gamma-glutamyl Transferase (IU/dl)	26.49 (19.13-37.42)	29.65 (25.48-33.98)	0.26

According to the information in Table 2, the intakes of total energy, fiber, vitamin D, and vitamin E were significantly lower in the patients with NAFLD after modulation based on the energy intake compared to the control group. Moreover, the intake of trans-fatty acids after energy adjustment was significantly higher in the patients with NAFLD compared to the control group. After energy adjustment, the obtained results indicated no significant differences between the groups in terms of proteins, carbohydrates, saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs).

Table 2. Dietary Intakes of NAFLD Patients and Control Group

Dietary Intakes	Control	Case	P-value
Energy (kcal/day)	2132±662.0	1730±571.0	0.001
Protein (g/day)	66.5±14.04	65.43±14.04	0.69
Carbohydrate (g/day)	258.0±54.0	260.0±54.0	0.82
Fat (g/day)	60.1±20.28	57.42±20.28	0.48
SFAs (g/day)	23.84±9.9	22.64±9.9	0.51
TFAs (g/day)	1.92 (1.33-2.48)	2.23 (1.85-3.3)	0.02
MUFAs (g/day)	20.2±8.3	19.36±8.3	0.56
PUFAs (g/day)	10.7±8.26	10.14±8.26	0.71
Fiber (g/day)	12.56 (10.67-15.57)	10.65 (8.57-12.5)	<0.001
Vitamin D (mg/day)	91.7 (84.33-103.3)	84 (75.5-91.6)	0.001
Vitamin E (mg/day)	6.69 (5.5-8.63)	5.4 (4.1-67.1)	0.001

MUFAs: *monounsaturated fatty acids; PUFAs: polyunsaturated fatty acids; SFAs: saturated fatty acids; TFAs: trans-fatty acids*

Discussion

Given the importance of NAFLD and its burden, the nutritional status of NAFLD is essential in the examination of the patients. According to the results of the present study, the intakes of total energy, fiber, vitamin D, and vitamin E were significantly lower in the patients with NAFLD after modulation based on energy intake compared to the control group. Moreover, the amount of energy-adjusted trans-fatty acids was significantly higher in the patients with NAFLD compared to the controls. However, the analysis after energy adjustment indicated no significant differences in the proteins, carbohydrates, SFAs, MUFAs, and PUFAs between the case and control groups.

Similar studies have investigated the nutritional status of NAFLD patients, and the findings in this regard are mainly regional. Patients with NAFLD across the world have specific dietary regimens, which mainly depend on cultural and regional nutritional habits. Therefore, accurate findings could be obtained through the comparison of similar regions, followed by the comparison of these results with other countries.

Similar to the present study, another research conducted in Mashhad (Iran) aimed to evaluate and compare the nutritional status of NAFLD patients with normal controls (8). Consistent with our findings, the patients in the mentioned study had increased levels of FBS. On the other hand, the findings of Mosallaei et al. denoted that NAFLD patients had significantly higher levels of total cholesterol, TG, and low-density lipoprotein, as well as lower level of high-density lipoprotein. In addition, the levels of AST and ALT were reported to be significantly higher in the NAFLD patients compared to the controls. The difference in this regard was not considered statistically significant in the present study. The mentioned research was conducted on a larger sample size, and the obtained results demonstrated that the levels of vitamin A, folate, and fat intake were inversely correlated with NAFLD, and these patients had lower intakes of folate and vitamin E. This finding is in congruence with the study by Mosallaei et al. and the current research (8), highlighting the importance of attention to the proper intake of micronutrients in the dietary routine of NAFLD patients.

According to the findings of Mosallaei et al., adherence to a healthy diet could reduce the risk of NAFLD by 52%, while an unhealthy diet is associated with the two-fold increase in the risk of the disease (8). Another study in this regard was conducted by Hashemi et al. in Tehran (Iran), and the findings demonstrated that NAFLD patients had increased ALT, cholesterol, and other lipids, which is inconsistent with the results of the present study as our NAFLD patients showed no elevation in the level of FBS. Moreover, the results obtained by Hashemi et al. demonstrated no differences between NAFLD patients and controls in terms of fiber and vitamin D status (9).

In another research, Zelber et al. evaluated the nutritional status of a larger sample population (10), reporting no differences between NAFLD patients and controls in terms of the intakes of calories, fats, carbohydrates, proteins, and fiber. However, the intake of calories, fats, carbohydrates, and proteins was reported to be higher in female patients with NAFLD compared to the other female participants. Moreover, male NAFLD patients had higher calorie intake from protein compared to the male subjects without NAFLD.

In the present study, the case and control groups had no significant difference in terms of BMI, while Zelber et al. reported a significant difference in the BMI of the subjects with and without NAFLD. Furthermore, the findings of the mentioned study indicated that the patients with NAFLD had higher BMI (30 kg/m^2) compared to the subjects without NAFLD (25 kg/m^2). Among the other discrepancies between the current research and the study by Zelber et al. were the differences in gender, nationality, and sample size (10).

The mentioned research also highlighted the importance of nutritional habits based on regional effects. In addition, Zelber et al. reported that NAFLD patients were more likely to consume non-alcoholic beverages and meat and less likely to consume fish (source of omega-3) (10). Similarly, Yasutake et al. has associated the consumption of such beverages with the risk of NAFLD, denoting that the consumption of non-alcoholic beverages is twice more common in patients with NAFLD compared to normal individuals. Moreover, they reported that the consumption of complex carbohydrates (especially whole grains) could prevent NAFLD progression (11). In addition, the consumption of whole grains has been associated with the improvement of obesity, FBS, and cardiovascular events. On the other hand, the excessive consumption of fats and saturated fats has been associated with increased insulin resistance and development of NAFLD (11).

In the current research, deficiencies were observed in the nutritional status of the NAFLD patients, which must be properly considered in clinical practice. Other studies in this regard have also demonstrated that these patients may be deficient in other micronutrients, such as calcium, vitamin D, and pyridoxine (12). Such deficiencies are mostly due to nutritional diets, which have been addressed in the study by Ferolla et al. Accordingly, the Brazilian population were more likely to use fruits, diaries, and meals enriched with meat, fats, sugar, and grains (12).

Conclusion

The present study aimed to evaluate the dietary status of patients with NAFLD. According to the findings, the dietary record of the NAFLD patients differed with the healthy subjects in the control group. In addition, the differences in the intake of energy, fiber, trans-fatty acids, vitamin D, and vitamin E between the case and control groups emphasized on the fact that the dietary status of these patients requires special attention in order to control the course of NAFLD. Furthermore, lifestyle improvement could prevent and control NAFLD, along with the use of other therapeutic drug regimens.

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Associations of the Dietary Diversity Score and Food Variety Score with Serum Magnesium and Ferritin Status

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ABSTRACT

Introduction: Dietary diversity score (DDS) and Food variety score (FVS) are the appropriate measures used to evaluate the overall quality of dietary patterns. However, their associations with the serum levels of micronutrients remain unclear. The present study aimed to investigate the correlations between DDS/FVS and serum ferritin and magnesium status in the non-athlete females joining the sports clubs in Tehran, Iran.

Methods: This cross-sectional survey was conducted on 397 non-athlete women, who were members of the sports clubs in Tehran, Iran in 2013. Dietary intake was assessed using a 24-hour recall questionnaire. DDS and FVS were calculated and classified into two groups based on the guidelines of the minimum dietary diversity of women (MDD-W) and median, respectively. Serum ferritin was measured using the ELISA assay, and serum magnesium was measured using atomic absorption spectrophotometry.

Results: Mean DDS and FVS was 5.7±1.4 (range: 2-9) and 15.3±4.7 (range: 5-32), respectively. After adjustment for the confounding factors, serum magnesium in the group with high FVS was significantly higher compared to the other group (P=0.01). In the group with high FVS, serum ferritin was also higher, and the difference was considered significant (P=0.058). In addition, linear regression analysis indicated a significant association between high FVS and serum magnesium level (P=0.02). However, no significant correlations were observed between the serum levels of micronutrients and DDS.

Conclusion: According to the results, higher FVS may be associated with higher serum magnesium and ferritin levels. Further investigations are required to assess the correlations between DDS, FVS, and the status of serum micronutrients.

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Introduction

Micronutrient deficiency is considered to be a severe public health concern, which affects low-income and industrialized countries [1]. Small quantities of micronutrients, such as vitamins and minerals, play a pivotal role in normal cell growth and function [2]. According to the literature, micronutrient deficiency is remarkably involved in the etiology of chronic diseases [3].

Magnesium is an important element in the human body [4], which is involved in a wide range of biological reactions [5] and is essential to the proper functioning of various systems [4]. Magnesium deficiency is associated with several chronic diseases, including type II diabetes [6, 7], hypertension, cardiovascular diseases [7], and cancer [8, 9]. Furthermore, subclinical magnesium deficiency has been reported to be

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highly prevalent in developed and developing countries [10].

Iron deficiency is considered to be the most significant nutritional problem across the world, affecting more than two billion people [11]. Women of the reproductive age are one of the high-risk populations for iron deficiency [12], and 20% of these women experience this issue *throughout their reproductive years* [13]. According to the second National Integrated Micronutrient Survey (NIMS-II), the prevalence of anemia (low hemoglobin levels) in Iranian women ranges from 12.3% in urban areas to 14% in rural areas, while the prevalence of iron deficiency anemia (low hemoglobin, ferritin, and mean corpuscular volume) has been estimated at 6% [14]. Iron deficiency anemia leads to declined work and immune functions, as well as the increased risk of premature birth and low birth weight. Moreover, severe anemia has been associated with the increased risk of maternal and neonatal mortality [11]. Iron deficiency is also associated with other complications, including fatigue [15], attention deficit [12], depression, and low quality of life [16].

The dietary nutrients that are essential to nutritional requirements cannot be obtained from only one type of food. Therefore, regular intake of various *foods* from several *food* groups at the recommended portions is of paramount importance [17]. Since deficiencies in dietary factors are associated with the increased risk of chronic diseases and malnutrition, several dietary guidelines have been proposed regarding the consumption of various nutrients within and between different food groups [18].

Dietary diversity score (DDS) and food variety score (FVS) are proper indices in the evaluation of the overall dietary quality in various aspects [19]. Some findings have demonstrated that the total nutritional quality of a diet is enhanced by increasing the intake of healthy food groups and food items, which are measured based on the DDS and FVS, respectively [20]. In the majority of the studies in this regard, low-diversity diets, in which certain food groups are excluded, have been reported to increase the mortality rate of cancer and cardiovascular diseases [21]. In addition, dietary diversity exerts protective effects against the vascular complications associated with type II diabetes [22], thereby increasing longevity and improving the overall health status [20].

Currently, diverse diets are considered to provide adequate nutrients, thereby preventing nutritional deficiencies or over-feeding [23]. The limited intake of some foods may lead to malnutrition since essential dietary nutrients are distributed among a wide variety of foods. Lack of dietary diversity is a major challenge in developing countries due to the higher rate of poverty. A major cause of malnutrition is adherence to a monotonous diet based on starchy foods, which are often deficient in essential micronutrients [21].

Although dietary diversity has recently attracted the attention of researchers regarding chronic diseases, its association with the status of serum micronutrients has not been adequately investigated, with the exception of one study conducted on elderly adults with normal and high plasma magnesium levels, in which high DDS was reported to reduce the risk of mortality [24].

The present study aimed to assess the correlations between DDS, FVS, and serum ferritin and magnesium status in healthy women.

Materials and Methods

Subjects

This cross-sectional survey was conducted on 397 healthy, non-athlete women aged 20-50 years, who joined the sports clubs in the western municipality of Tehran, Iran in 2013. Dietary diversity and its associations with sustained attention, anthropometric measurements, body composition, and blood antioxidant status were investigated [25]. The sports clubs were selected for sampling in order to have better access to adult women and attract their cooperation in the research.

The details on the applied methods in the study have been described previously [25, 26] and are briefly described in this section. In total, 14 sports clubs were selected via *random sampling* from seven districts in the west of Tehran. Approximately 30 women who started sports activities within less than a week prior to the beginning of the study were selected randomly from each sports club.

The exclusion criteria of the study were tobacco and alcohol consumption at least once a week, diagnosis of diabetes, cardiac diseases, cancer,

and renal and liver malfunction, and pregnant and lactating women. In addition, women using medications or mineral/vitamin supplements within the past month were excluded from blood sampling. In total, 87 women were randomly selected for blood sampling and biochemical tests.

The study protocol was approved by the Ethics Committee of Tehran University of Medical Sciences, and written informed consent was obtained from the participants after explaining the objectives and procedures of the study.

Dietary Diversity Score (DDS)

In the present study, DDS was determined based on the guidelines of the minimum dietary diversity of women of reproductive age (MDD-W) [27] as proposed by the Food and Agriculture Organization of the United Nations (FAO) and USAID's Food and Nutrition Technical Assistance III Project (FANTA) in 2016. MDDW is a dichotomous indicator with the threshold of five food groups (total: 10). Accordingly, the women consuming five or more food groups are more likely to meet their micronutrient requirements compared to those consuming fewer food groups [27].

Based on the mentioned guidelines, a 24-hour recall questionnaire was completed for each participant. All the food items that were consumed by the participants based on the MDD-W guidelines were categorized into 10 food groups, including grains and potato, pulses (e.g., beans, peas, and lentils), nuts and seeds, dairy products, meat, poultry, and fish, eggs, dark green leafy vegetables, other fruits and vegetables rich in vitamin A, other vegetables, and other fruits.

The participants scored one point if they consumed a minimum of half a serving of any of the mentioned food groups. In other words, each food group had a maximum of one point out of the total DDS (=10). DDS was calculated by summing up the score of the consumed food groups by the subjects and classified into two categories of high (5≤) and low (<5).

Food Variety Score (FVS)

In the present study, FVS was calculated by the simple counting of various consumed food items during the study period (24 hours). In the counting of the food items, items such as spices, beverages, sweets, and condiments were not considered in line with the method proposed by Steyn et al. [28]. FVS was classified into two categories of high (16≤) and low (16>) based on the median of the FVS.

Physical Activity

Physical activity was assessed via interviews and using self-report physical activity questionnaires, which classified physical activity based on the metabolic equivalent task (MET). The validity of the questionnaire has been confirmed previously [29].

Anthropometric Measurements

The weight and height of the participants were measured using a SECA stadiometer without shoes and with minimum clothing at the precision of 0.1 kilogram for body weight and 0.5 centimeter for height. In addition, the body mass index (BMI) was calculated as weight (kg) divided by the square of height (m^2).

Socioeconomic Status

The socioeconomic status (SES) of the participants was determined based on the number of the household living items, including the type of the house, car, carpet, furniture, side-by-side refrigerator, computer, dishwasher, washing machine, and microwave. The principal component analysis was applied to integrate the variables into a new factor indicating the SES, which was ranked into three tertiles of low, medium, and high.

Biochemical Evaluation

Due to the limited funding of the present study, 87 women were randomly selected from the participants for biochemical evaluations. After 12-14 hours of fasting, 10 milliliters of venous blood was collected before 10:00 AM in order to measure the serum levels of ferritin and magnesium. The ELISA assay was used to measure the serum ferritin (Dia Metra S.r.l Headquarter code: DKO 039), and serum magnesium was measured using atomic absorption spectrophotometry (AA-670 Shimadzu).

Statistical Analysis

Data analysis was performed in SPSS version 16 (SPSS Inc., Chicago, IL, USA) using the Kolmogorov-Smirnov test to evaluate the normality of the data. The data with non-normal distribution were logarithmically transformed for statistical analyses. The mean and geometric

mean of the data with normal distribution after log transformation were determined. Comparison of the variables between the groups with high and low scores was performed using independent t-test for continuous variables and $\chi 2$ for categorical variables. Moreover, linear regression analysis was applied to investigate the correlations between DDS, FVS, and serum levels of micronutrient. The analysis of covariance (ANCOVA) was employed to compare the mean values of the micronutrient levels in the DDS and FVS groups after adjustment for energy intake, BMI, SES, physical activity, and education level. In all the statistical analyses, P-value of less than 0.05 was considered significant.

Results

In total, 397 healthy, non-athlete women were enrolled in the study. The mean age and BMI of the participants was 34.2±8 years and 26.6±4.5 kg/m^2, respectively. According to the findings, the mean DDS was 5.7±1.4, with the minimum and maximum scores of two and nine out of 10 points, respectively. The mean FVS was estimated at 15.3±4.7, with the minimum and maximum scores of five and 32, respectively. Table 1 shows the mean age, physical activity, energy intake, BMI, SES, and dietary supplement intake of the DDS and FVS groups.

Table 1. Socio-demographic characteristics of participants (n=397)

Variables	DDS		p-value	FVS		p-value
	<5 (n=79)	≤5 (n=318)		<16 items (n=218)	16≤ Items (n=179)	
	Mean (SD)			Mean (SD)		
Age (y)	33.3 (7.5)	34.4 (8.2)	0.3	34.4 (8.2)	33.9 (7.9)	0.5
Education level (y)	11.8 (3.0)	12.7 (3.1)	0.01	12.1 (3.4)	13.1 (2.6)	0.003
BMI (kg/m^2)	29.0 (4.0)	26.0 (4.4)	<0.001	27.4 (4.5)	25.6 (4.3)	<0.001
Physical activity (Met. H/d)	32.2 (4.9)	32.4 (5.0)	0.7	30.7 (5.4)	32.8 (4.8)	0.004
Energy intake (kcal/d)	1543.6 (419.6)	1705.9 (360)	0.001	1604.1 (369.1)	1758.3 (371.1)	<0.001
BMI≥30 (kg/m^2) (%)	38	18.6	<0.001	29.4	14	<0.001
Marital status (%)						
Single	16.5	18.2	0.7	16.5	19.6	0.1
Married	74.7	75.2		74.3	76	
Widowed and Divorced	8.9	6.6		9.2	4.5	
Socio-economic status (%) *						
Low	45.6	30.2	0.005	40.8	24	0.001
Middle	35.4	33		32.1	35.2	
High	19	36.8		27.1	40.8	
Occupation (%)						
Housewife	58.2	55	0.8	54.1	57.5	0.7
Employee	27.8	29.9		30.7	27.9	
Other	13.9	15.1		15.1	14.5	
Dietary supplement intake (%)	29.1	35.2	0.3	32.1	36.3	0.3

* Based on principal component analysis method, Low: first tertile, middle: second tertile, high: third tertile

Abbreviations: FVS, Food Variety Score; DDS, Dietary Diversity Score; BMI, Body Mass Index; Met. H/d, Metabolic equivalent- Hour per day.

P< 0.05 is statistically significant.

According to the obtained results, the energy intake was higher in the groups with high DDS

and FVS, while the BMI was lower in these groups. In addition, physical activity was higher in the group with high FVS (P<0.005). Significant correlations were observed between the SES, DDS, and FVS. Accordingly, the SES was higher in the groups with high DDS and FVS. Furthermore, the groups with high FVS and DDS had higher education levels.

The findings of the current research indicated no significant associations between the occupation status, marital status, and use of dietary supplements with the DDS and FVS in the women. On the other hand, comparison of the food group intakes between the DDS and FVS groups demonstrated that food group consumption was higher in the groups with high DDS and FVS. However, the obtained results were not significant in terms of grain consumption in the FVS and DDS groups, as well as dairy product and eggs consumption in the FVS group (Table 2).

Table 2. Food group consumption across dietary diversity score (DDS) and food variety score (FVS) in healthy women (n=397)

Food groups	DDS		p-value	FVS		p-value	Total
	<5 (n=79)	≤5 (n=318)		<16 items (n=218)	16≤ items (n=179)		
Grains and potato (%)	97.5	99.4	0.13	98.6	99.4	0.41	**99**
Dairy (%)	70.9	86.2	0.001	82.6	83.8	0.74	**83.1**
Dark green leafy vegetables (%)	13.9	51.3	<0.001	29.4	61.5	<0.001	**43.8**
Other vitamin A rich vegetables and fruits (%)	8.9	43.1	<0.001	27.1	47.5	<0.001	**36.3**
Other fruits (%)	35.4	81.4	<0.001	63.3	83.2	<0.001	**72.3**
Other vegetables (%)	51.9	82.4	<0.001	72	81.6	0.02	**76.3**
Meat, poultry and fish (%)	60.8	85.8	<0.001	73.9	89.4	<0.001	**80.9**
Eggs (%)	6.3	37.7	<0.001	29.8	33.5	0.42	**31.5**
Pulses (beans, peas and lentils) (%)	13.9	28.6	0.007	21.1	31.3	0.02	**25.7**
Nuts and seeds (%)	2.5	34	<0.001	15.1	43	<0.001	**27.7**

According to the information in Table 3, the serum levels of micronutrients were not associated with the DDS, and serum magnesium was significantly higher in the group with high FVS (P=0.02). After the adjustment for energy intake, BMI, SES, physical activity, and education level, the correlation of serum magnesium with FVS remained significant (P=0.01). Furthermore, a positive correlation was observed between serum ferritin and FVS (P=0.058). The results of the linear regression analysis of the DDS and FVS are presented in Table 4. The linear regression analysis indicated a significant association between high FVS and serum magnesium levels (P=0.02).

Table 3. Serum micronutrients across dietary diversity score (DDS) and food variety score (FVS) in healthy women (n=397)
*Data are shown as the geometric mean.

Micronutrients	model	DDS		p-value	FVS		p-value
		<5 (n=79)	≤5 (n=318)		<16 items (n=218)	16≤ items (n=179)	
		Mean (SD)			Mean (SD)		
Ferritin(µg/l)*	1	12.4 (0.5)	13.0 (0.2)	0.2	12.6 (0.3)	13.3 (0.2)	0.1
	2	12.5 (0.5)	13.0 (0.2)	0.4	12.5 (0.3)	13.4 (0.3)	0.058
Magnesium (mg/l)	1	4.6 (0.1)	4.4 (0.08)	0.2	4.3 (0.1)	4.6 (0.08)	0.02
	2	4.6 (0.1)	4.4 (0.07)	0.3	4.3 (0.09)	4.6 (0.1)	0.03

Model 1: Unadjusted
Model 2: Adjusted for energy intake, BMI, socio-economic status, physical activity and the years of education.

Discussion

The present study aimed to assess the correlations between DDS, FVS, and the serum levels of some micronutrients in adult women. The obtained results indicated a positive association between serum magnesium and FVS, and serum magnesium was higher in the group with high FVS even after the adjustment of the possible confounding factors. In addition, the correlation between FVS and serum ferritin was considered significant following the adjustment for the energy intake, BMI, SES, physical activity, and education level. However, no significant correlation was observed between DDS and serum micronutrients.

Few studies have investigated the correlations between DDS, FVS, and serum levels of micronutrients. For instance, Huang et al. [24] reported that the plasma levels of magnesium increased with higher DDS in elderly subjects. Moreover, the mentioned study demonstrated that the higher consumption of vegetables, eggs, and calcium was associated with the significantly higher plasma levels of magnesium. The participants with normal and high plasma magnesium and high DDS were also reported to be at the relatively lower risk of mortality compared to those with low plasma magnesium and low DDS.

In another research in this regard, Akizawa et al. [30] observed that serum magnesium level had a positive correlation with its dietary intake. Previous reports have also indicated a significant association between DDS and dietary magnesium intake [31]. In the present study, the participants with higher FVS and DDS had significantly higher consumption of fruits, vegetables, and meat compared to those with lower FVS and DDS. Additionally, the intake of dairy products was observed to be higher in the group with high DDS. These food groups are abundant sources of magnesium [5], and their consumption could result in the increased serum levels of magnesium in the participants consuming these food groups.

The findings of the current research showed no significant correlation between serum ferritin status and DDS, while serum ferritin status was significantly correlated with FVS. Similarly, Nikuyeh et al. [32] denoted no significant correlation between serum iron and dietary iron intake in students. Moreover, the findings of Broderstad et al. [33] showed no association between serum ferritin and seafood intake or vegetarian and Mediterranean dietary patterns. Several other studies have reported no association between iron consumption and DDS [21, 34]. Meanwhile, other similar studies have denoted that iron intake is associated with the consumption of whole grains, nuts, seeds, and fruits rich in vitamin C [35].

In another study conducted on women, a positive correlation was observed between iron intake and the consumption of meat, nuts, and legumes [36]. In the present study, the participants with higher FVS and DDS consumed significantly higher portions of meat, legumes, and nuts. The vegetables and fruits that were highly consumed in the groups with high DDS and FVS were abundant sources of vitamin C, which could enhance iron absorption. Therefore, it could be inferred that food variety may affect serum ferritin levels by altering the consumption of food items to the higher intake of iron-containing foods. Some of the confounding factors in the measurement of serum ferritin include food components such as tannin, phytate, polyphenols, phosphate, and carbonate, as well as conditions such as infection, inflammation, and lack of stomach acid [11]; these factors were not taken into account in the current research.

Food variety and dietary diversity are considered to be proper indicators of dietary adequacy and quality [37]. However, it has been suggested that the association of food intake from different food groups and adequacy of micronutrient intake may result from the consumption of proper amounts of foods with no correlation with the variety of food groups [38]. In addition, the simple counting of food items or food groups does not provide accurate data on dietary adequacy. In the present study, the level of consumption was not considered for FVS, while compared to DDS, FVS had a more significant association with the serum levels of micronutrients. The measurement of FVS is a rapid, simple, and inexpensive assessment technique, while it is also easy for respondents.

According to the findings of the current research, a large number of the participants had high DDS, while less than half had high FVS; approximately 80% of the participants achieved the DDS of ≥5. According to the MDD-W analysis, the women who consumed five or more food groups were

more likely to meet their micronutrient requirements compared to those who consumed fewer food groups [27]. On the other hand, the mean DDS was lower in the present study compared to the research by Mirmiran et al. [39]. This discrepancy could be due to the differences in the methods of DDS calculation or type of the sample populations.

In the present study, the mean FVS was higher compared to the values reported by the previous research in this regard [19, 20]. This inconsistency could be due to the differences in the calculation methods. In the current research, the number of the consumed food items by participants within 24 hours of the study period was counted based on the FVS. The intake of beverages, sweets, spices, and condiments were not considered in the FVS calculations in the present study, while other studies did not consider spices and drinks and measured the intake of tea and coffee in the FVS calculations. Conversely, all these food items were included by other research groups in FVS calculations [19].

Lower DDS in various populations could be due to the inline differences in the SES, education level, nutritional knowledge, and food availability. Low-income families often do not have access to a wide variety of foods. Although access to sufficient food is of utmost importance, the knowledge of dietary guidelines may have more significant effects on dietary diversity [40].

One of the limitations of the present study was the use of one 24-hour recall to evaluate dietary intakes, which might have been insufficient to determine the usual dietary intakes of the individuals. Lack of dietary diversity on special days does not imply the absence of dietary variations on a daily basis [19]. Another limitation was the lack of standard methods and cutoff points for the calculation of FVS. In addition, the age range of our subjects was diverse, and the final outcomes may also reflect the age differences. However, no correlation was denoted between age and the serum levels of micronutrients. Another drawback of the current research was that the menopausal state was not considered in the participants, which might have affected the serum levels of micronutrients. Finally, similar to other cross-sectional studies, we were not able to determine the cause-and-effect relationships.

Conclusion

According to the results, high FVS is associated with increased serum magnesium and possibly ferritin status, while such association was not denoted in case of DDS. Therefore, public health awareness must be emphasized in order to promote the higher consumption of a wider variety of foods. It is recommended that further interventional studies be conducted in this regard so as to clarify the impact of increased FVS and DDS on the serum status of micronutrients.

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The Interactive Effects of Continuous and Interval Training with Crocin Consumption on the Levels of Interleukin-17 and Interleukin-18 in the Soleus Muscle of Rats with Type II Diabetes

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ABSTRACT

Introduction: Muscle inflammation and atrophy are among the main complications of diabetes, which lead to motor disability in the patients. Studies have shown that physical exercise with various intensities and use of herbal medicines could positively affect diabetes. The present study aimed to evaluate the interactive effects of continuous and interval training with crocin consumption on the levels of interleukin-17 (IL-17) and interleukin-18 (IL-18) in the soleus muscle of rates with type II diabetes.

Methods: This experimental study was conducted on 49 adult diabetic rats, which were randomly assigned to seven groups, including: high-intensity interval training (HIIT), low-intensity continuous training (LICT), HIIT with crocin consumption, LICT with crocin consumption, crocin consumption, sham, and control. The HIIT and LICT groups received training for eight weeks using a rodent treadmill. The crocin consumption groups were intraperitoneally administered with crocin (25 mg/kg) daily for eight weeks. The gene expression levels of IL-17 and IL-18 were measured using real-time polymerase chain reaction (RT-PCR).

Results: HIIT increased IL-17 and IL-18 in the soleus muscle tissue of the diabetic rates ($P \leq 0.05$), while LICT had no significant effects on the levels of IL-17 and IL-18 ($P \geq 0.05$). On the other hand, crocin consumption decreased the expression of IL-18 and increased the expression of IL-17 in the soleus muscle tissue ($P \leq 0.05$), and the interactive effects of LICT and crocin consumption were considered significant in increasing IL-17 and IL-18 ($P \leq 0.05$).

Conclusion: According to the results, HIIT may exert inflammatory effects on the muscle tissue of rats with type II diabetes. However, the interactive effects of LICT and crocin were significant on increasing IL-17 and IL-18 in the soleus muscle tissue of diabetic rats.

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Introduction

In recent decades, the prevalence of type II diabetes has increased drastically in humans [1]. Muscle atrophy is an uncontrolled diabetes marker, which leads to increased proteolysis and inability of the damaged skeletal muscles to regenerate through protein synthesis [2]. In addition, the metabolic disorders induced by diabetes reduce the physical ability to respond to physiological stresses, such as increased oxidative stress and inflammation. In general, insulin resistance and deficient energy production in muscle tissues play a pivotal role in the pathology of diabetes [1, 3]. Several studies have confirmed the association of diabetes and increased inflammation markers [2]. For instance, increased fat mass may cause an upsurge in insulin resistance in various tissues (e.g., muscles and liver) by increasing chronic inflammation [3].

According to the literature, physical exercise plays a key role in the management and control of diabetes. Furthermore, physical exercise could have potential effects on weight loss and the improvement of type II diabetes [4], thereby leading to changes in inflammatory and pro-inflammatory cytokines, such as interleukin-17 (IL-17) and interleukin-18 (IL-18) as the key predictors of diabetes [5]. Numerous studies have been focused on the effects of physical exercise on inflammation markers in diabetic patients, proposing conflicting results. For instance, researchers have reported that one

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session of moderate-intensity resistance training could reduce IL-18 and increase IL-6 in physically inactive, obese men [6]. Moreover, 12 weeks of high-intensity interval training has been reported to increase IL-17 levels and improve glycemic indices in obese men, while no significant changes have been denoted in IL-10 levels [7]. In another study in this regard, four weeks of no training followed by four weeks of combined training could increase the levels of IL-17 in obese men [8].

In addition to the substantial effects of regular physical exercise on the health of patients with type II diabetes, researchers believe that proper diet and use of herbal medicines, along with physical activity, could accelerate the treatment process of type II diabetes [9]. Crocin is a compound found in saffron with potent antioxidant and anti-inflammatory properties. By reducing free radicals and oxidative stress, crocin could stimulate homeostasis [10, 11]. On the other hand, some studies have examined the health benefits of saffron and its components along with physical exercise. For instance, resistance training combined with saffron supplementation has been reported to produce testosterone and cortisol [11], while aerobic exercise combined with the use of the aqueous extract of saffron has been shown to increase the antioxidants in the liver tissues of the rats with streptozotocin (STZ)-induced diabetes [12], and swimming along with saffron consumption has been reported to improve glycemic indices in diabetic rats [13].

Considering the contradictory findings regarding the effects of physical exercise on IL-17 and IL-18, as well as the scarce data on the interactive effects of crocin and physical exercise on these factors in muscle tissues, such investigations could provide beneficial data for the prevention of the muscle damage induced by diabetes.

The present study aimed to investigate the interactive effects of high-intensity interval training (HIIT) and low-intensity continuous training (LICT) along with crocin consumption on the levels of IL-17 and IL-18 in the soleus muscle tissue of rats with diabetes induced by a high-fat diet and STZ.

Materials and Methods

This experimental study was conducted on 49 male Sprague-Dawley rats aged eight weeks, with the mean weight of 150±30 grams. The animals were purchased from the reproductive center and animal house of Islamic Azad University, Marvdasht Branch, Iran. Afterwards, they were transferred to the animal sport physiology laboratory in standard conditions and kept at the temperature of 22-27°C, relative humidity of 50%, and controlled light (12-hour light/dark cycle) for the seven-day adaptation period. The animals had *ad libitum* access to water and food during this period.

In this study, type II diabetes was induced using the combination of a high-fat diet and STZ injection. To do so, the rats were exposed to a high-fat diet containing 45% of total fat (derived from animal fat), 24 grams of fat, 24 grams of protein, and 41 grams of carbohydrate per 100 grams for eight weeks (15). After eight weeks, diabetes induction was performed intraperitoneally using a single dose of STZ dissolved in sodium citrate buffer (pH=5.4 mg/kg) [14]. Approximately 96 hours after the injection, diabetes was confirmed in the rats with the glucose levels of >300 mg/dl, and these animals were selected as the study samples [14]. Based on blood glucose, the rats were divided into seven groups (n=7), including HIIT, LICT, HIIT with crocin consumption, LICT with crocin consumption, crocin consumption, sham, and control. In order to estimate the maximum running speed, the sport performance test was performed with zero gradient. To perform the test, the rats initially started running at the speed of 10 m/min, followed by the increasing of the treadmill speed to 1 m/min every one minute. This trend continued until the rats were unable to run (i.e., exhaustion) [14]. After estimating the speed of groups one and three for eight weeks, HIIT was performed three sessions per week at the intensity of 80-85% of the maximum running speed for two minutes, along with an active rest period of one minute. The frequency of training reached six sessions within the first week and 12 sessions by the last week.

In groups two and four, training was also performed three sessions per week for eight weeks at the intensity of 50-55% of the maximum running speed. In the first week, the duration of LICT was 25 minutes and reached 50 minutes by the last week. It is also notable that the HIIT and LICT groups were matched in terms of the total volume of physical activity (severity, duration, and repetition) [14]. In addition,

groups three, four, five were intraperitoneally administered with crocin (25 mg/kg) dissolved in normal saline on a daily basis [15].

In order to control the effects of the injection on the study variables, the sham group received daily crocin solvent intraperitoneally. At the end of the eight weeks and 24 hours after the last training session, the rats were surgically treated to measure the study parameters. Initially, the animals were anesthetized with 10% ketamine (50 mg/kg of body weight) and 2% xylazine (10 mg/kg of body weight). After five minutes, their soleus muscle was extracted by specialists. A cryotube was placed in liquid nitrogen and stored for further investigation at the temperature of -70°C.

RNA extraction was carried out in accordance with the instructions of the RNA extraction kit (Yektasaz Tajhiz Company, Iran) using the extraction kit solutions and based on the proposed protocols of the manufacturer. In addition, cDNA synthesis was performed in accordance with the instructions of the Fermentas kit (K1622). The sequence of the applied primers is presented in Table 1.

Table 1. Sequence of Forward-Reverse Primers of Genes in Real-time PCR Reaction

Gene	Forward (5'-3')	Reverse (5'-3')
IL-17	CTGAAAGTCCTCAACTCCCTTAG	CTCATTGCGGCTCAGAGT
IL-18	ACCGCAGTAATACGGAGCAT	GATCAGCTCGGGCACTTTAG

After completing the device activity and observation of the diagrams to increase the number of the provided pieces, several factors were assessed and measured, including the fluresence diffusion based on the calculation of ΔΔCt, changes in the expression of the genes relative to B2m, and control state without distinct environments. Following that, the expression of the genes was estimated using the $2^{-\Delta\Delta Ct}$ formula.

In data analysis, the Shapiro-Wilk test was used to determine the normal distribution of the data. The effects of the crocin solvent on the study variables were evaluated using independent sample t-test between the control and sham groups. In addition, the interactive effects of endurance training and crocin consumption were determined using the two-way analysis of variance (ANOVA), and Bonferroni's mean comparison test was applied to verify the differences between the two training programs ($P \leq 0.05$).

Results

Table 1 shows the mean values of the research variables. According to the results of independent sample t-test regarding the differences between the control and sham groups in terms of the effects of the crocin solvent, the levels of IL-17 (P=0.43) and IL-18 (P=0.09) had no significant differences between these groups.

Analysis of IL-17

According to the results of two-way ANOVA to investigate the interactive effects of endurance training and crocin consumption on IL-17, endurance training (P=0.001) and crocin consumption (P=0.001) had significant effects on the increasing of IL-17 levels in the soleus muscle tissues of the rats with diabetes induced by a high-fat diet and STZ. Moreover, the interactive effects of endurance training and crocin consumption in the increasing of IL-17 levels in the soleus muscle tissues of these rats were significant (P=0.001).

According to the results of Bonferroni's comparison of means to examine the differences between the training types (Figure 1), the IL-17 levels in the HIIT groups were significantly higher compared to the animals receiving no training (P=0.001). Furthermore, LICT had no significant effects on the increasing of the IL-17 levels in the soleus muscle tissues of the rats compared to the animals receiving no training (P=0.40). The obtained results also indicated that the IL-17 levels in the HIIT groups were significantly higher compared to the LICT groups (P=0.001).

Analysis of IL-18

The results of the two-way ANOVA are presented in Table 3. Accordingly, endurance training had a significant effect on the changes in the levels of IL-18 in the soleus muscle tissues of the diabetic rats (P=0.001). In addition, crocin consumption could significantly reduce the IL-18 levels in the soleus muscle tissues of the diabetic rats

(P=0.001). Endurance training along with crocin consumption could also significantly increase the levels of IL-18 in the soleus muscle tissues of the diabetic rats (P=0.001).

Figure 2 depicts the results of Bonferroni's comparison of means test. As can be seen, the IL-18 levels in the HIIT groups were significantly higher compared to LICT groups (P=0.001). However, HIIT training had no significant effect on the increasing of the IL-18 levels compared to the animals receiving no training (P=0.001). Moreover, LICT had no significant effect on the reduction of the IL-18 levels compared to the animals receiving no training (P=0.22).

Table 2. Mean IL-17 and IL-18 Gene Expression Levels in Soleus Muscle Tissues of Diabetic Rats in Study Groups

Group	IL-17	IL-18
Control	0.29±0.22	**2.50±0.49**
Sham	0.41±0.31	**2.04±0.44**
Crocin Consumption	0.87±0.49	**1.81±0.40**
HIIT	0.38±0.17	**1.14±0.33**
LICT	0.72±0.27	**1.72±0.63**
HIIT with Crocin Consumption	4.04±1.77	**6.32±1.26**
LICT with Crocin Consumption	1.35±0.32	**1.65±0.44**

Table 3. Results of Two-way ANOVA Regarding Effects of Endurance Training and Crocin Consumption on Research Variables

	Factor	Sum of Squares	Degree of Freedom	Mean of Squares	F	Significant	Size Effect
IL-17	Endurance Training	19.70	2	9.85	16.14	0.001	**0.47**
	Crocin Consumption	27.70	1	27.70	45.40	0.001	**0.55**
	Endurance Training with Crocin Consumption	21.77	2	10.88	17.84	0.001	**0.49**
IL-18	Endurance Training	32.08	2	16.04	35.35	0.001	**0.66**
	Crocin Consumption	22.69	1	22.69	50.02	0.001	**0.58**
	Endurance Training with Crocin Consumption	72.64	2	36.32	80.05	0.001	**0.81**

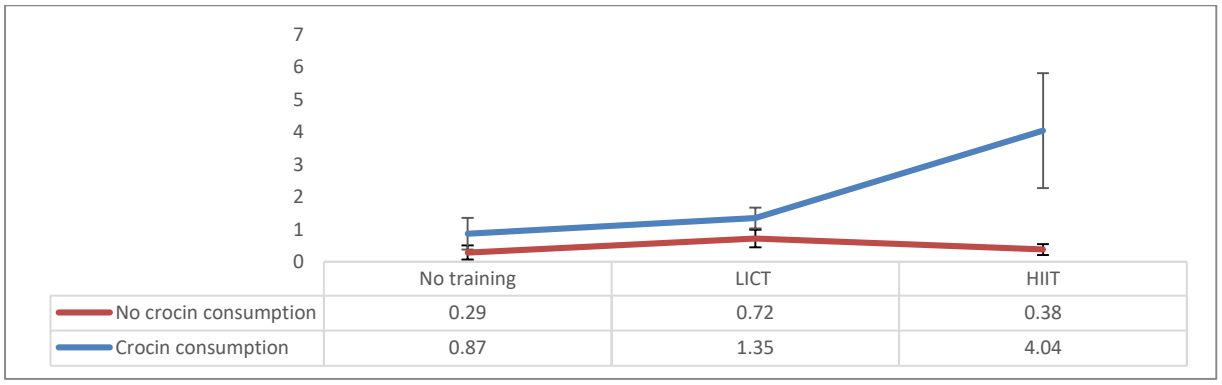

Figure 1. IL-17 Gene Expression in Soleus Muscle Tissues of Research Groups

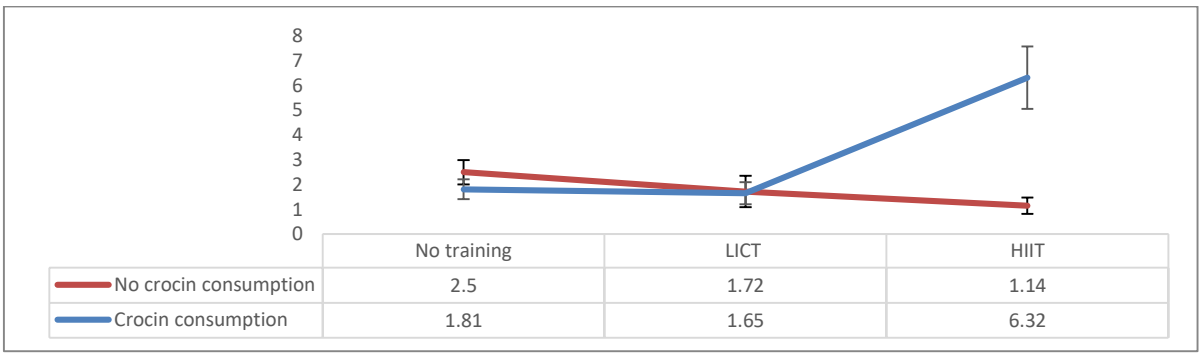

Figure 2. IL-18 Gene Expression in Soleus Muscle Tissues of Research Groups

Discussion

According to the literature, obesity and type II diabetes are associated with mild-to-severe inflammation. The risk of obesity and metabolic syndrome increases with the secretion of IL-18 from the adipose tissue and smooth muscle cells and secretion of IL-17 from macrophages, TH-17, natural killer cells, and other pro-inflammatory [16]. In the pathogenesis of type II diabetes, diabetic nephropathy is involved in the activation of the Kappa B nuclear transcription factor and the provocation of the factors that promote the pathways of cell damage [17].

Scientists believe that cytokines variably affect different tissues, and some cytokines could exert anti-inflammatory effects as well. Since IL-17 and IL-18 are both anti-inflammatory cytokines, the skeletal muscles seem to release some IL-6 into the bloodstream during physical exercise although this cytokine leads to increased insulin resistance in the liver and adipose tissue. However, the metabolic effects of this cytokine on skeletal muscles have also been confirmed. The increase in this cytokine in the skeletal muscles is associated with the higher levels of IL-17 and IL-18, which in turn increase insulin sensitivity in the skeletal muscle tissues. As such, previous studies have denoted that muscle contractions could result in the positive expression of the levels of some cytokines, such as IL-18, TNF-α, and IL-8 [5].

The changes in IL-17 seem to be influenced by the intensity of physical exercise. According to the results of the present study, HIIT could increase the secretion of the anti-inflammatory cytokines that express IL-17 in the skeletal muscles [18]. Several studies have investigated the effects of various sports with variable intensity on the expression of these cytokines. In line with the current research, previous findings have demonstrated that high-intensity training could increase the serum and protein levels of IL-17 in the skeletal muscles of rats, while moderate-intensity training has been reported to have no significant effects on the changes in IL-17 [19].

According to a study in this regard, resistance training and endurance training had no significant effects on the changes in the serum IL-17 levels in athletes [18, 20]. However, 12 weeks of resistance training was reported to significantly decrease the IL-18 levels in elderly men [21]. On the other hand, eight weeks of swimming has been reported to have no significant effects on the changes in the serum levels of IL-18, while 10 weeks of swimming has been shown to reduce the serum levels of IL-18 in rats [22]. Although the findings of most of the studies in this regard suggest that these cytokines are pro-inflammatory agents in some tissues, their pathway and mechanism of expression following physical exercise in the skeletal muscles remain unknown. Therefore, further investigations are required in this regard.

According to the results of the present study, crocin consumption could significantly increase IL-17 gene expression and reduce IL-18 gene expression in the muscle tissues of the rats with diabetes induced by a high-fat diet and STZ. It is believed that crocin consumption could also protect unsaturated fatty acids in the cell membrane by decreasing free radicals. Moreover, crocin has been reported to inhibit the activation of reactive oxygen species by inhibiting oxidative stress and reducing lipid peroxidation. Crocin consumption may also increase NF-κB and decrease lipid peroxidation, exerting lipid-lowering effects on diabetic patients. However, most of the studies in this regard have indicated that the antioxidant and anti-inflammatory effects of crocin are dose-dependent [23, 24].

In the current research, the administration of 25 mg/kg of crocin increased IL-17 and decreased IL-18 in the muscle tissues of the diabetic rats. Despite extensive research in this regard, no data is available on the effects of crocin on IL-17 and IL-18 levels, especially in the skeletal muscle tissues. Inconsistent with our findings, 60 days of crocin consumption at the concentration of 40 mg/kg was reported to significantly reduce the expression of IL-17 in an arthritic model of rats. In the mentioned study, the concentrations of 10 and 20 mg/kg of crocin had no significant effects on IL-17 changes [25]. According to another research, the administration of 500 mg/kg of the hydroalcoholic extract of saffron was reported to significantly decrease the IL-17 levels in diabetic laboratory rats [26]. Studies have shown that the increased level of IL-6 is one of the pathways that could stimulate IL-10 and IL-1ra expression, as well as TNF-α inhibition, thereby resulting in the decreased levels of IL-18 [6]. It seems that factors such as ambient temperature, level of obesity,

and mobility may influence the changes in IL-17 levels, which could justify the increased IL-17 level in the present study [22].

According to the current research, endurance training with crocin consumption had interactive effects on the increased levels of IL-17 and IL-18 in the muscle tissues of diabetic rats. However, the results of Bonferroni's comparison of means test indicated that HIIT training could significantly increase the IL-17 and IL-18 gene expression in the muscle tissues of the rats, while continuous training had no significant effects on these pro-inflammatory factors. Therefore, it could be inferred that the intensity of physical activity is a major influential factor in reducing the expression of IL-17 and IL-18 in muscle tissues [18, 22]. On the other hand, the crocin concentrations used in most studies indicates that the reductive effects of IL-17 and IL-18 are most likely associated with the administration of the higher doses of this natural compound [23, 24].

Few studies have been focused on these pro-inflammatory factors and their mechanism of action in muscle tissues. Moreover, there have been no studies to simultaneously examine the effects of training and crocin consumption on the levels of IL-17 and IL-18 in muscle tissues. Such example is the study by Hassanpour et al., which showed that endurance training and crocin consumption had interactive effects on the reduction of cell death-promoting factors and increasing anti-apoptotic proteins in the cardiac muscle cells of rats with type II diabetes [27]. In the mentioned study, HIIT and crocin consumption were reported exert interactive effects to increase hematological factors in doxorubicin-poisoned rats, while doxorubicin could diminish these factors [28]. On the other hand, moderate-intensity continuous training along with the consumption of crocin (100 mg/kg) has been reported to decrease inflammatory factors and increase antioxidants in the brain tissues of rats with Parkinson's disease [29]. This is inconsistent with the results of the present study, and the discrepancy could be due to the differences in the intensity of training and crocin concentration.

According to the results of the present study, the ineffectiveness of crocin as a potent antioxidant could be attributed to pharmacologic interference, which is specifically discussed in medical sciences. Although such interferences cause the positive effects of medications, they may occasionally induce adverse side-effects. Therefore, it is suggested that in the further investigations in this regard, the consumption of crocin not be considered before and during training, and the effects of crocin consumption be examined after the training period.

Considering the studies conducted in this respect, it is noteworthy that the levels of IL-6 expressed in the muscles may indicate increased insulin sensitivity and be considered a potent stimulant for the expression of IL-17 and IL-18. One of the limitations of the current research was the lack of the measurement of the gene expression of this cytokine in the muscle tissues. Therefore, it is recommended that further studies in this regard investigate the gene expression of this cytokine in the muscle tissues along with physical exercise. Additionally, since both these variables are known as pre-inflammatory factors, and data is scarce regarding their function in the muscle tissues, the levels of anti-inflammatory agents should be measured in further research in order to obtain accurate results.

Conclusion

According to the results, HIIT could have inflammatory effects on the skeletal muscle tissues in the rats with type II diabetes, while LICT had no significant effects on the changes in the inflammatory factors of the muscle. In addition, crocin consumption could decrease IL-18 expression and increase IL-17 expression in the skeletal muscles. However, endurance training and crocin consumption had significant interactive effects on the increment of inflammatory factors in the muscle tissues of the rats.

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3rd Pakistan International Biennial Conference on Ramadan and Health

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During recent years, there has been an emerging, upward trend in research and publication of studies on the effects of Ramadan fasting on the healthy population as well as on the populace diagnosed with various diseases and conditions. To publicize the outcomes of these research works, many awareness programs, symposia and conferences are being organized globally. International coordination groups, such as 'Diabetes and Ramadan: International Alliance' have been established to coordinate the efforts and investigations of researchers involved in this work. Periodicals like 'Journal of Nutrition, Fasting and Health of Iran' are playing a pivotal role in disseminating research outcomes related to the effects of Ramadan fasting. Guidelines for fasting are being developed for patients of various diseases, such as 'Diabetes and Ramadan: Practical Guidelines' devised by the International Diabetes Federation. 'New guidelines on diabetes management during Ramadan' has been published recently by Baqai Institute of Diabetes and Endocrinology (BIDE), Pakistan. Departments and study groups are being established in institutions to facilitate researchers in conducting studies on the effect of Ramadan fasting on different diseases.

Jinnah Sindh Medical University (JSMU), Karachi under the visionary leadership of the Honorable Vice Chancellor, Prof. Syed Muhammad Tariq Rafi, annually organizes, just before the month of Ramadan, an awareness program regarding diet and drug adjustment for conditions such as pregnancy, diabetes, renal diseases, and coronary artery disease. Furthermore, every alternate year, JSMU organizes an 'International Biennial Conference' on Ramadan and Health. These conferences are organized to disseminate studies conducted on the effects of Ramadan fasting and these studies are presented by Pakistani speakers as well as by invited foreign speakers. The third issue of the conference, titled '3rd Pakistan International Conference on Ramadan and Health' will be held from 23rd to 25th August' 2019.

Four foreign speakers: Dr. Mohsen Nematy, Mashhad University of Medical Sciences, Mashhad, Iran; Dr. Abdolreza Norouzy, Mashhad University of Medical Sciences, Mashhad, Iran; Prof. Mafauzy Mohamed, University of Sains, Malaysia and Dr. Kamran Mahmood Ahmed A.Aziz, Ministry of Health, Abha, Saudi Arabia, have consented to present their studies in this conference. Fourteen invited speakers from Pakistan will also present their work related to

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various aspects of the effects of Ramadan fasting.

The theme of the conference is 'Ramadan: Bridge between Health and Spiritual Path'. The inauguration session will be held in Marriott Hotel, Karachi on the evening of 23rd August' 2019. The honourable Governor of the province of Sindh, Mr Imran Ismail and Chairman of Hilton Pharma, Dr. Sardar Muhammad Yasin Malik, have consented to be the Chief Guest and Guest of Honor, respectively, for this ceremony. Prof. Anis Ahmed, a well-known scholar and Vice Chancellor of Riphah University, Islamabad will deliver a talk on the theme of the conference. Scientific sessions would be held on the 24th and 25th of August in Sindh Medical College campus of JSMU. There will be five plenary sessions on Ramadan and Diabetes, Ramadan and Drug Management, Ramadan and Medical Education and Ramadan and Medicine; and four sessions for contributed papers. On the evening of 24th August' 2019, dinner at a beachfront restaurant along the coastline of the Arabian Sea will be hosted for the delegates and the organizing committee. The conference will culminate with closing remarks and suggestions from the delegates in the afternoon of 25th August. We cordially invite all interested delegates to come to Karachi and be part of this educational and enlightening experience. Meanwhile, we are eagerly waiting for our guests and friends to join us.

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Body Composition Changes Following Lenten fasting: A Study in Ethiopia

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ABSTRACT

Introduction: The Ethiopian Orthodox Church (EOC) Lenten fasting (Abiy Tsom) is a seven-week period of fasting before Easter. It is a religious fasting practice, which has been less documented in the scientific literature. The present study aimed to investigate effect of EOC fasting with vegan diet practices on body composition parameters.
Methods: This prospective study was conducted on 98 subjects (40 females and 58 males) aged 18-40 years, who were willing for Lenten fasting for seven weeks. Several parameters were evaluated and measured, including body weight, height, waist circumference, hip circumference, body mass index (BMI), and waist-to-hip ratio (WHR). In addition, body fat percentage (BF %) was determined based on skinfold thickness at three sites, including the abdomen, triceps, and suprailiac using a caliper (Holtian Ltd., UK).
Results: Weight, BMI, BF%, and fat mass significantly decreased ($P< 0.05$) during eight weeks of Lenten fasting compared to eight weeks after fasting. The male subjects experienced a more significant reduction in this regard compared to the female subjects.
Conclusion: According to the results, adherence to the Lenten diet (vegan diet) decreased anthropometric and body composition parameters more significantly compared to regular mixed diets, which could be potentially associated with improved cardiometabolic health.

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Introduction

Although food is considered important in religious observance in various religions, not all the believers of a religion are able to follow the same food practices. Based on religious dietary norms and instructions, the major religions in the world that promote vegetarian diets as part of their basic teachings include Buddhism, Jainism, Hinduism, and the Seventh-Day Adventist [1]. The majority of Hindus and Buddhists consume no meat, fish, seafood, eggs, and dairy products (with the exception of milk), while the followers of Judaism are forbidden to consume pork, its products, and all carnivorous animals if not slaughtered and prepared in an appropriate manner. On the other hand, the Seventh-Day Adventists abstain from all animal products, with the exception of dairy products and eggs (lacto-ovo vegetarianism) [2].

In Greek-orthodox Christianity, the followers adhere to the diets that are completely free of animal products (with the exception of fish and seafood) for 180-200 days of fasting per year, which includes the three major fasting periods of Nativity Fast, Easter Lenten, and the Assumption [3]. In the past, the Catholic Church used prohibited the consumption of meat on Fridays. In contrast, the Ethiopian Orthodox Church recommends a number of fasting periods, during which all foods of animal origin are eliminated from the routine diet, including dairy products, eggs, poultry, and fish. Theses fasting practices are performed on Wednesdays, Fridays, and the entire Lenten season [4]. Therefore, the followers of the Ethiopian orthodox adhere to a vegan diet in these periods.

Although the term vegetarian is often used to describe a whole range of diets practiced with variable degrees of restriction, studies have generally indicated that vegetarian diets are associated with potential health benefits [5, 6]. Traditionally, vegetarianism is defined as the avoidance of meat [7], while there are various types of vegetarian diets. For instance, the lacto-

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ovo vegetarian diet is characterized by the avoidance of meat, poultry, and fish, while dairy products and eggs are allowed. Among the other subsets of vegetarian religious groups are pescatarians (those who do not consume meat but use fish) and vegans (those who abstain from all foods of animal origin, including dairy products and eggs, in addition to meat, poultry, and fish) [8].

With this background, the wide variety of dietary habits in certain religious groups and their difference with the eating habits of the general population has resulted in the challenging comparison of the health benefits of various vegetarian diets. In the studies conducted in this regard, vegetarians and non-vegetarians have been reported to gain or lose weight with variable patterns, and the health benefits associated with the vegetarian lifestyle have not been thoroughly clarified.

The present study aimed to assess effect of EOC fasting with vegan diet practices on body composition parameters.

Materials and Methods

This prospective study was conducted on 98 generally healthy orthodox Christian fasting individuals (58 males and 40 females) in Ethiopia, East Africa. A random sample of the orthodox Christians who were willing to fast during Lenten were recruited from the College of Health Sciences at Tikur-Anbesa Medical School, Addis Ababa University.

The inclusion criteria of the study were healthy adults aged ≥18 years, who were planning to fast for eight weeks of Lenten and provided their consent for participation. The individuals with acute and chronic diseases or those receiving medications were excluded from the study. The study was conducted in accordance with the Declaration of Helsinki, and all the procedures involving human subjects were approved by the Ethics Committee of the College of Health Sciences at Tikur-Anbesa Medical School, Addis Ababa University. Written informed consent was obtained from all the subjects prior to participation.

Data were collected using a questionnaire that was completed by the participants and included data on the name, age, gender, type of diet, alcohol consumption, and smoking habits of the subjects. The measurements were performed during the last week of Lenten fasting and seven weeks after Lenten fasting at 8-10 AM.

Anthropometric Measurements

Both body weight (kg) was measured without shoes and with light clothing using a beam balance (Seca, GmbH, Germany). Height was taken at standing position with heads, backs and buttocks vertically aligned to the height gauge and the result was rounded to the nearest 0.5 cm, and weight was recorded and rounded to the nearest 0.5 kg. BMI (kg/m^2) was calculated by dividing the body weight in kilograms by height in square meters. Waist circumference was measured using a measuring tape at the navel point, and hip circumference was measured at the widest portion of the buttocks in order to calculate the waist-to-hip ratio (WHR). Body fat percentage (BF%) was determined with the skinfold thickness at three sites, including the abdomen, triceps, and suprailiac, using a caliper (Holtain Ltd., UK). A trained individual carried out the skinfold measurements. The values obtained from the skinfold thickness were directly applied to predict BF% based on the formula proposed by Jackson and Pollock [10]. Based on the BF%, the fat-free mass (FFM) and fat mass (FM) were calculated, as follows:

$$FM\ (kg)=[BW\ (kg)\times BF\%)\div 100]$$
$$FFM\ (kg)=BW\ (kg)-FM\ (kg)$$

Statistical Analysis

Data analysis was performed in SPSS version 21.0 using descriptive statistics to describe the characteristics of the subjects. Two ANOVA with Post hoc pairwise comparisons were performed as necessary with Bonferroni adjustment. In all the statistical analyses, P-value of less than 0.05 was considered significant, and the data were expressed as mean and standard deviation (SD).

Results

In total, 101 subjects were approached and provided with the data checklists, and three subjects did not complete the study due to the lack willingness or unavailability. The final samples size included 98 subjects who completed the study (58 males and 40 females), with the mean age of 27.1±0.4 years.

The analysis of the results indicated that body weight (kg), BMI (kg/m^2), BF%, and FM (kg) decreased significantly at baseline compared to after Lenten fasting ($P<0.05$), while they returned to the baseline levels seven weeks after the end of fasting (Table 1). In addition, body weight (kg) and BMI (kg/m^2) significantly reduced at the end of Lenten fasting compared to

seven weeks after the end of Lenten fasting (54.69±8.92 and 57.57±8.58, respectively; P=0.018 versus 21.73±2.16 and 19.96±2.13 kg/m^2, respectively; P=0.023). With regard to the parameters of body composition, BF% (8.43±5.90% versus 16.67± 6.11%; P=0.002) and FM (9.33±3.36 versus 10.55±3.52 kg; P=0.001) significantly decreased at the end of Lenten fasting compared to seven weeks after the end of Lenten fasting (Table 1).

Comparison of the male and female subjects was indicative of significantly lower body weight, BMI, and BF% in men compared to women (Table 2).

Discussion

The Ethiopian Orthodox Church prohibits all foods of animal origin, including dairy products and eggs, poultry, and fish, during Lenten, which encompasses seven weeks of fasting before Easter [4]. However, after the break of lent, the followers eat all foods of animal origin for another eight weeks, including on Wednesdays and Fridays. This prospective study aimed to compare the changes in anthropometric and body composition parameters before, at the end, and after Lenten fasting in Ethiopian orthodox individuals.

According to the results of the present study, adherence to the Ethiopian orthodox diet (vegan diet) for Lenten fasting had beneficial health effects on the fasting men and women. Furthermore, the obtained results indicated that all the subjects experienced significant reduction in the body weight, BMI, BF%, and FM during Lenten compared to baseline. However, body weight, BMI, BF%, and FM significantly increased to higher levels seven weeks after the Lenten fasting compared to during Lenten fasting. These findings are of great significance since Lenten fasting is a key practice in the Ethiopian orthodox religion. Such vegan diets have a low fat content and mostly contain large proportions of carbohydrates and fibers [11].

In the current research, the daily activities of all the participants were considered to be minimal as they maintained a sedentary lifestyle without involvement in formal physical activities, mostly spending their time learning (e.g., university students), teaching (e.g., university lecturers), and providing health care to patients in hospitals (e.g., healthcare professionals). Therefore, we believe that the changes in the physical activity of the subjects had no impact on the obtained results.

A study in this regard investigated fasting in Seventh-Day Adventists (lacto-ovo-vegetarians) [13, 14], Greek Orthodox Christians (pesco-vegetarian) [14, 15], and Ramadan [16, 17], reporting a significant reduction in the body weight and BMI of the subjects. Other similar studies have also denoted that differences in body composition parameters decrease during Lenten fasting [19] and Ramadan [20, 21], while other findings have demonstrated no such effects [18].

Findings regarding the effects of Ramadan fasting on body weight, BMI, and body composition are not conclusive. Some studies have denoted that body weight and body composition are not affected by Ramadan fasting [22-24], while other findings have indicated that Ramadan fasting leads to the reduction of body weight and BMI [15, 16]. This inconsistency could be attributed to the variations in calorie intake during Ramadan or variations in the duration of fasting and physical activity.

In the present study, the effects of age-matched gender differences were assessed in the female (n=40) and male subjects (n=58) before, at the end, and after eight weeks of Lenten fasting. According to the obtained results, the male subjects experienced a significant reduction in the body weight, BMI, BF%, and FM during Lenten fasting compared to the baseline level. However, these values significantly increased again and became higher than the values during Lenten fasting when compared to seven weeks after Lenten in men. Despite the need for further investigation in this regard, the differences between the genders could be due to the variations in nutrient oxidation and changes in energy expenditure during the fasting period.

Table 1. Anthropometric and Body Composition Parameters before, at End, and Seven-Weeks after Lenten Fasting (n=98; values expressed as mean±SD)

Variable	Pre-Lenten	Lenten	Post-Lenten	P
Weight (kg)	55.59±7.56	54.69±8.92	57.57±8.58	0.018
HC (cm)	88.45±4.34	89.95±6.24	88.92±6.31	0.064
WC (cm)	68.12±6.0	70.72±7.16	70.69±7.175	0.095
WHR	0.74±0.02	0.78±0.05	0.79±0.07	0.101
BMI (kg/m²)	20.67±1.45	21.73±2.16	19.96±2.13	0.023
BF%	16.10±4.86	18.43±5.90	16.67±6.11	0.002
FM (kg)	6.98±2.09	9.33±3.36	10.55±3.52	0.001
FFM (kg)	47.00±6.90	49.28±8.70	49.59±8.49	0.120

Independent and paired t-test used for comparisons; HC: hip circumference; WC: waist circumference; BF%: percentage of body fat; FM: fat mass; FFM: fat-free mass; WHR: waist-to-hip ratio; P: comparison of means by time

Table 2. Anthropometric and Body Composition by sex (values expressed as mean±SD)
Independent and paired t-test used for comparison of male and female subjects; BF%: percentage of body fat; FM: fat mass; FFM: fat-free

Variable	Sex	Before Lenten	During Lenten	After Lenten	P
Weight (kg)	Male (n=58)	23.02±3.74	22.34±3.86	23.12±2.98	0.045
	Female (n=40)	24.43±7.52	23.71±7.02	24.09±8.05	
BMI (kg/m²)	Male (n=58)	21.34±3.45	20.40±2.34	21.33±1.62	0.002
	Female (n=40)	20.19±1.23	19.66±1.95	20.32±2.55	
BF%	Male (n=58)	13.96±1.42	12.76±3.12	14.97±3.64	0.032
	Female (n=40)	22.12±3.79	21.57±4.98	22.94±4.89	
FFM (kg)	Male (n=58)	57.61±4.46	54.65±5.56	53.67±5.49	0.054
	Female (n=40)	40.73±5.37	40.31±6.17	39.75±4.84	
FM (kg)	Male (n=58)	5.92±3.02	5.67±3.17	8.08±2.90	0.154
	Female (n=40)	8.45±2.33	7.45±3.43	11.00±3.06	

mass; BMI: body mass index; P_1: comparison of means before and at end of Lenten;

According to the results of the present study, the Ethiopian Orthodox Christian individuals who adhered to a vegan diet during Lenten fasting had a slight reduction in their systolic and diastolic blood pressure compared to after fasting, which is consistent with the previous findings in this regard [3]. This could be due to the fact that the Orthodox Christian Church prohibits the consumption of meat, dairy products, fats, and olive oil on almost all fasting days. Such restrictions may lead to the reduction of the total energy, total fat, and saturated fat intakes, which in turn leads to the reduction of the body weight and blood pressure. In a similar research, Chiu et al. [23] stated that vegetarian diet is high in potassium and fibers, which are known to reduce blood pressure.

One of the limitations of the present study was the limited age range of the subjects, and since the majority of the participants were city dwellers, the obtained results cannot be generalized to wider populations. Moreover, dietary intakes were not assessed in our research, while it was confirmed that the subjects adhered to a vegan diet in accordance with the doctrine of the Ethiopian Orthodox Church regarding dietary recommendations. In order to validate our findings, it is suggested that a properly designed, population-based study encompassing various socioeconomic and geographical areas be conducted in developing countries.

Conclusion

According to the results, the subjects experienced a significant reduction in their body weight, BMI, BF%, and FM with adherence to a vegan diet for Lenten fasting, and the reduction was considered to be more significant in men compared to women.

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Conflicts of interest

None declared.

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