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Assessment of Fetal Malnutrition Based on the CANSCORE Index and Anthropometric Indices

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ABSTRACT

Introduction: To prevent the adverse effects of fetal malnutrition (FM), its management and early diagnosis by reliable tools are essential. The present study aimed to identify the rate of malnutrition in a referral maternity center in Iran using different systems for evaluating the nutritional status and determining a better index for FM.

Methods: This cross-sectional study was performed on the neonates born in the maternity ward of Hajar Hospital in Shahrekord, Iran in 2020. FM was evaluated based on different parameters, including the CANSCORE index, body mass index (BMI), Ponderal index, and mid-arm circumference/head circumference (MAC/HC) index. After recording the measurements, birth weight and height were plotted on the intrauterine growth chart, and the infants were classified into groups of appropriate for gestational age (AGA), small for gestational age (SGA), and large for gestational age (LGA).

Results: Based on the fetal growth status index, 14.7% of the neonates were in the AGA group, and 95.3% were in the LGA group. Malnourishment was detected in 40.5% of the neonates based on the CANSCORE index, 8.3% based on the MAC/HC index, 6.9% based on the BMI, and 5.2% based on the Ponderal index. In addition, the CANSCORE index had a significant positive correlation with all the growth indices (except the MAC/HC index), and the Ponderal index was also significantly correlated with all the growth indices except height.

Conclusion: According to the results, the CANSCORE index could show growth status and FM more accurately compared to other anthropometric parameters that may underestimate FM. Furthermore, BMI is a highly sensitive indicator, and infants malnourished in terms of BMI should be examined based on the CANSCORE index to accurately identify FM.

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Introduction

Fetal malnutrition (FM) is a term first proposed by Scott and Usher in 1966 to describe infants with evidence of soft tissue loss at birth regardless of specific etiology (1). FM is defined as the lack of sufficient quantum fat and muscle mass during intrauterine growth (2). The importance of addressing the hidden complications associated with FM is because studies have shown that the potentially severe complications of malnutrition in various body systems are irreversible (3).

Perinatal problems or long-term consequences of the central nervous system occur mainly in infants with FM despite being appropriate for gestational age (4). FM may also occur due to poor maternal diet, inability of the mother to metabolize and transfer sufficient nutrients, impaired nutritional supply (vascular and placental) to the fetus, and increased fetal demand due to faster growth (5). Changes in the structure and physiology of the fetus may occur due to the lack of the nutrients or components needed to build high-quality organs and tissues and adaptation to nutrient depletion through reduced fetal growth or prioritizing essential organs (6).

The regulation of the endocrine system (especially hormones that regulate fetal growth and maturation) and support of the tissues are different. It has been hypothesized that metabolic changes after birth remain to increase the risk of diabetes and cardiovascular diseases

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in the future, especially in the presence of additional stressors in later life stages (e.g., obesity and sedentary lifestyle) (7, 8). Overall, an infant who is not properly nourished during the intrauterine growth period is at the risk of short-term and long-term complications after birth, which adversely affect the growth, intelligence, and overall health of the neonate. To prevent these consequences and have healthier infants and future adults, it is crucial to diagnose the symptoms of infant malnutrition properly and provide treatment to high-risk infants immediately. The management of these neonates at birth may reduce complications and improve their survival.

Congenital malnutrition is assessed using various systems, including anthropometric indices, fitness indices (e.g., Ponderal index, head-to-toe ratio, chest circumference, mid-arm circumference, mid-arm-to-head circumference), body mass index (BMI), and the CANSCORE index, which is a scoring system based on nine 'superficial' and readily detectable signs of malnutrition in newborns (9). Several studies have used these criteria, and some have suggested the CANSCORE index to be a more accurate measure of the nutritional status for infants compared to the birth weight index for gestational age. The CANSCORE index could also identify the infants who are small for gestational age (SGA) but not malnourished (10, 11). Although this score is recognized as the primary standard of diagnosis, it is a time-consuming method and requires adequate staffing and appropriate skills to perform the required examinations on all infants and provide equal grading (12). Although the CANSCORE index appears to be a more appropriate tool for the assessment of infant nutrition, studies have shown that the Ponderal index (PI) and BMI are simple and common tools for the examination of FM in infants, as well as more reliable diagnostic markers of FM compared to the CANSCORE index (13).

In this study, we intended to identify the prevalence rate of FM in newborns in a maternity ward using various systems for evaluating the nutritional status and determining a better index for FM. Our findings could contribute to the identification of an appropriate screening method to detect primary malnutrition in infants and prevent the complications of FM. The best screening method found in this study can be proposed to the governing health authorities to be implemented in national guidelines and routine maternity checklists.

The present study aimed to compare the nutritional status of the neonates born in a referral maternity ward based on the CANSCORE index and anthropometric indices.

Materials and Methods

This cross-sectional study was performed on 363 neonates born in the maternity ward of Hajar Hospital in Shahrekord, Iran in 2020. The inclusion criteria were term infants (gestational age>37 weeks based on modified Ballard date and score), age of <48 hours, and no medical complications or congenital anomalies. The exclusion criteria of the study were as follows: 1) multiple births; 2) requiring NICU care; 3) neonates of mothers with gestational diabetes and 4) neonates of mothers with unreliable estimates of gestational age.

Based on similar studies, assuming that the prevalence of malnutrition in infants is equal to 40%, and considering 95% confidence interval and 5% accuracy, the sample size of the study was determined to be 368 using the following formula (14):

$P = (40\%\ d=5\%)\ \ N = Z^2p(1-p)/d^2 = 368$

The study protocol was approved by the Ethics Committee of Shahrekord University of Medical Sciences (ethics code: IR.SKUMS.REC.1398.144). After obtaining written consent from the legal guardians, the infants were weighed at birth by an experienced nurse using a Misaki scale (made in Japan) with an accuracy of 50 grams. Neonatal height and head circumference (HC) were also measured using a standard tape, and arm circumference was measured using a non-stretch tape at the midpoint of the arm. After recording the mentioned measurements, birth weight and height was plotted on the intrauterine growth chart, and the infants were classified into groups of appropriate for gestational age (AGA), SGA, and large for gestational age (LGA).

According to the Alexander nomogram, infants weighing less than 10 percentile are SGA, and infants weighing 10-90 percentile are AGA. Based on the study by Soundarya et al., mid-arm circumference/head circumference (MAC/HC) index with the cutoff point of 27 was used to determine malnutrition (13). In addition, BMI was calculated by dividing the weight (kg) by squared height (m), and the cutoff point of <11.2

kg/m^2 was considered the malnutrition index. The PI was also determined by calculating the ratio of weight to the cubic length, and the score of <2.2 g/cm^3 was considered the malnutrition index (15).

To assess the status of clinical nutrition, the CANSCORE scoring system was employed.

In the CANSCORE system, the physical factors of nine infants were assessed during the examination, and four states (worst to best) were defined for each factor. In this regard, the worst state was scored one, and the best state was scored four. Therefore, the lowest and highest possible scores for each infant were nine and 36, respectively. An infant with the CANSCORE index of less than 25 was diagnosed with FM (16).

Nine signs for the clinical assessment of nutritional status in newborns (CANSCORE index) include hair (large amount, straight, and soft hair that is easily styled [4], less hair [some straight 'staring' hair] [3], still thinner [more straight, 'staring' hair that does not brush] [2]), cheeks (progression from full buccal pads and round face [4] to significantly reduced buccal fat, with a narrow, flat face [1]), neck and chin (double/triple chin fat fold, neck not evident [4] to thin chin, no fat fold, neck with loose wrinkled skin [very evident] [1]), arms (fullness, cannot lift the skin [4], slightly thin arms, check on the pressure of hands, accordion-like folds may form [3], thinner arm with more accordion-like folds [2], very limited fat, significant accordion-like folds [1]), legs (like arms), back (different to grasp and left skin in the interscapular area [4] to loose skin easily lifted in a thin-fold from the interscapular area [1]), buttocks (full, round gluteal fat pads [4], slightly reduced fat [3], significantly reduced fat with wrinkles [2], fat disappears, loose skin over the upper posterior thigh as well [1]), chest (full found ribs not seen (4); to progressively prominence of the ribs with an obvious loss of the intercostal tissue [1]), and abdomen (fullness, thick subcutaneous fat [4], slightly reduced fat [3], abdominal wall thinning, may form accordion-like folds [2], boat-shaped abdomen, loose skin, may form accordion-like folds [1]) (10, 17).

Data analysis was performed in SPSS version 22 (IBM Corp. Released 2013, Armonk, New York) using descriptive statistics to describe the data, including mean and standard deviation (SD) for the quantitative variables and frequency and percentages for the categorical variables. To assess the correlations between the studied parameters, the Pearson's or Spearman's correlation-coefficients were used. In all the statistical analyses, the P-value of less than 0.05 was considered significant.

Table 1. Neonatal growth indices in Hajar Hospital, Shahrekord (2020)

Index	Minimum	Maximum	Mean±SD
Weight (gr)	2000	4400	3126±426
Height (cm)	43	56.0	49.2±1.81
Head circumference (cm)	31	38.5	34.6±1.20
Chest circumference (cm)	28	37.0	32.7±1.60
Arm circumference (cm)	8	14.0	10.7±1.13
Arm to head circumference (cm)	0.24	0.38	0.31±0.029
Body mass index (kg/m^2)	9.05	16.4	12.86±1.24
Head circumference to height (cm)	0.65	0.78	0.703±0.024
Ponderal index	1.93	3.37	2.61±0.24
CANSCORE index	15	38.0	26.02±4.23

Table 2. Frequency of malnutrition based on neonatal growth indices in Hajar Hospital, Shahrekord (2020)

Index	Cutoff value	Frequency (%)
Birth weight (gr)	AGA	17 (4.7)
	LGA	342 (95.3)
CANSCORE index	<25	147 (40.5)
	≥25	216 (59.5)
Arm to head circumference	<0.27	30 (8.3)
	≥0.27	333 (91.7)
Body mass index (kg/m^2)	≤11.2	25 (6.9)
	>11.2	338 (93.1)
Ponderal index	≤2.2	19 (5.2)
	>2.2	344 (94.8)

Table 3. Spearman correlation coefficient of CANSCORE and Ponderal indices with other neonatal growth indices

Growth index	CANSCORE index		Ponderal Index	
Weight	0.728	<0.001	0.580	<0.001
Height	0.546	<0.001	-0.056	0.289
Head circumference	0.487	<0.001	0.266	<0.001
Chest circumference	0.661	<0.001	0.461	<0.001
Arm circumference	0.627	<0.001	0.431	<0.001
Arm to head circumference	0.525	<0.001	0.381	<0.001
Body mass index	0.623	<0.001	0.910	<0.001
Head circumference to height	-0.059	0.265	0.354	<0.001

Results

In total, 363 infants were studied, including 188 boys (51.8%), and the remaining were girls. Based on the fetal growth status index, 17 neonates (4.7%) were in the AGA group, 342 (95.3%) were in the LGA group. Malnourishment was detected in 40.5% of the neonates based on the CANSCORE index, 8.3% based on the MAC/HC index, 6.9% on the BMI, and 5.2% based on the PI (tables 1 & 2). Table 3 shows the correlation-coefficients of the CANSCORE index and PI with the other growth indices. Accordingly, the CANSCORE score had a significant positive correlation with all the growth indices, except the head-to-toe ratio (P<0.001). In addition, the PI had a significant positive association with all the growth indices, except height (P<0.001).

Discussion

The present study aimed to assess and compare the nutritional status of infants born in Hajar Hospital maternity ward based on the CANSCORE index and anthropometric indices in 2020. In total, 363 infants were studied, and the rate of FM was estimated at 40.5%, 8.3%, 6.9%, and 5.2% based on the CANSCORE scoring system, arm-to-head circumference ratio, BMI, and PI, respectively. Furthermore, the CANSCORE index was considered a more reliable and accurate measure of FM comparatively. In line with our findings, the study by Singh evaluated FM and its ratio between AGA and SGA infants using the CANSCORE index, reporting that the CANSCORE index may be a simple clinical indicator for the detection of FM and prediction of the associated neonatal complications without the need for advanced equipment (14).

In a study conducted by Lakkappa et al. (2018) on single-term infants born in a reference hospital, 8% of the infants were SGA, and 92% were AGA. In the mentioned study, the prevalence of malnutrition based on the CANSCORE index was estimated at 21%, while it was 4% based on the PI, and 5.6% based on the MAC/HC index (18). In another study performed by Soundarya et al. (2012) (15) on 300 full-term and single twins, 23% of the infants were SGA, and 77% were AGA or LGA. When these SGA neonates were assessed based on the care assessment need (CAN) score, 23% (n=16) were observed to be well-nourished, while 8.2% of the AGA newborns (n=19) presented with clinical signs of malnutrition, which was considered statistically significant. In the mentioned research, 26% of the malnourished neonates (n=78) based on the PI were clinically well-nourished after evaluation based on the CANSCORE index, and 39.7% (n=31) were also clinically well-nourished. The remaining infants with a normal PI (11.2%; n=25) were reported to have severe malnutrition. According to Soundarya, FM is optimally detected by the CAN Score, and BMI could also be a reliable screening tool to diagnose malnutrition when used in conjunction with the PI.

In another study conducted by Adebami et al. (2008) (19), 442 full-term infants were examined, and 44 cases (10.8%) were SGA, 381 cases (86.2%) were AGA, and 13 cases (3%) were LGA. Based on the PI index, 36 infants (8.1%) were malnourished, while based on the CANSCORE index, 83 infants (18.8%) were considered malnourished. In most of the studies in this regard, the prevalence of malnutrition has been reported to be higher based on the CANSCORE index compared to other growth indices, such as the PI, BMI, and MAC/HC. Therefore, it could be inferred that compared to other growth indices, the CANSCORE index could detect FM in a larger number of infants, especially those who have not been identified by other methods. Moreover, the CANSCORE index could identify the infants who are SGA but not malnourished. In this regard, Singhal reported that based on the CANSCORE index, 8% of AGA infants and 23.2% of SGA infants were

malnourished. In the mentioned study, the CANSCORE index also detected malnutrition in 65% of the neonates with the PI of less than 2.2 (17).

Our findings are inconsistent with the study by Ezenwa, which aimed to determine the incidence of FM based on the CAN score and compare nutritional assessment with anthropometry. According to the results of the mentioned study, FM was highly prevalent in premature infants, and the BMI and PI were considered simple and easy tools for the assessment of FM in premature infants. These indices were also reported to be a better benchmark for the detection of FM in premature infants compared to the CANSCORE index (13).

In the present study, the CANSCORE index was most significantly correlated with birth weight, chest circumference, arm circumference, and BMI. In a study by Adebami et al. (19), the mean weight, MAC, and PI of CANSCORE-malnourished infants were significantly lower compared to those without malnutrition. Although the mean head circumference and height of the malnourished infants were lower in the mentioned study, the differences were not considered statistically significant.

In the current research, the PI had the most significant correlation with BMI, which is not unexpected considering that the weight-to-height ratio is measured in both scales. On the other hand, the correlation of the PI with the other growth indices was less significant compared to the CANSCORE index, which implied that the CANSCORE index could indicated the growth status and malnutrition of the fetus more accurately.

In general, FM is highly prevalent at birth (especially in developing countries) and could be detected regardless of natural anthropometric indices. The CANSCORE index could be used as an alternative method to identify and manage FM in low-resource areas, especially in developing countries. The CANSCORE index is a simple and easy approach to assessing malnutrition compared to other complex methods. Moreover, it is easy to understand and perform by using Figural rating scales for measurement. (16). Since the rate of FM at birth is high and regardless of the natural anthropometric indices of neonates, the accuracy of the CANSCORE index could be enhanced with time and by using specialized techniques to diagnose the nutritional complications of infants and recommend the necessary treatment.

Limitations of the Study

The most important limitation of this study was a lack of cooperation on behalf of some of the parents despite the tact of the research team and their specialized opinions. Consequently, the implementation of the plan in all the phases was minimized.

Conclusion

According to the results, the CANSCORE index was significantly correlated with growth indices and could identify a larger number of malnourished infants. Furthermore, it is considered more accurate in showing the growth status and malnutrition of the fetus compared to other anthropometric indices, which may underestimate FM. The CANSCORE index is also a simple and appropriate clinical indicator for the detection of FM and preventing its complications. It may also have the potential to predict the complications caused by FM without the need for complex equipment. Therefore, it is recommended that such an advantageous technique be employed in developing counties such as Iran.

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Role of Naturopathic Diet and Treatment Modalities in the Successful Management of Psoriasis Vulgaris in an Adolescent Girl: A Case Report

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ABSTRACT

Introduction: Psoriasis Vulgaris (PV) is a chronic inflammatory dermatological disorder that affects skin, nails, and joints and has various systemic associations. The current case report is the first of its type to highlight the observations on manifestations of Psoriasis Vulgaris in an adolescent girl with Naturopathic diet, treatment, and Yoga Intervention.

Method: An eighteen-year-old female who was diagnosed with Psoriasis Vulgaris at 9 years of age was administered Nature cure and Yoga for 16 weeks as an individualized diet and treatment protocol in an inpatient Nature Cure and Yoga Hospital in South India. Improvement in Psoriasis Area Severity Index (PASI) score and reduction in body mass index (BMI) were observed.

Conclusion: The changes observed are found to be consistent over 1 year. This case report also demonstrates the beneficial effects of Naturopathic diet, treatment, and Yoga as an effective treatment modality in the management of Psoriasis Vulgaris for both physical and psychological levels.

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Introduction

Psoriasis (PV) is a chronic inflammatory dermatological disorder that affects skin, nails, and joints and has various systemic associations. In India, hospital-based epidemiological studies show that the prevalence of psoriasis varies from 0.8 to 2.8%, with peak onset in girls is between 11–15 years age group[1]. The etiology of psoriasis remains unknown and it is believed to be multifactorial with numerous key components including genetic susceptibility, environmental triggers in combination with skin barrier disruption, and immune dysfunction. Psoriasis confers significant physical and psychological distress and impairment usually resulting in a detrimental impact on patient quality of life and psoriasis patients are often stigmatized by the disease[2]. As the cause of psoriasis is still unknown, conventional treatment is available to control symptoms through topical and systemic therapies as well as phototherapy. Care for patients also involves Psychosocial interventions because psoriasis patients are more likely to suffer from depression and anxiety disorders and have an increased rate of suicidal ideation[3]. The unconventional approach was also efficacious in the treatment of psoriasis which includes herbal use, dietary modification, meditation, and acupuncture[4]. Naturopathy is a rational and evidence-based system of medicine imparting treatments with natural elements based on the Theory of Vitality, Theory of Toxemia, Theory of Self-Healing Capacity of the body, and the principles of health. In India Naturopathy is combined with Yoga and with the ancient concept of Panchamahabuthas (Five great elements) for the prevention and treatment of diseases. The current case report highlights the observations on manifestations of psoriasis Vulgaris in an adolescent girl with Naturopathy treatment modalities.

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Case Presentation

An eighteen-year-old girl was admitted to a Nature Cure Hospital in South India, with Psoriasis Vulgaris with onset at 9 years of age. She presented with complaints of itching and scaling of chronic plaque-type patches on her scalp, neck, hip, and both thighs. The dermatological examination showed that it is Psoriasis Vulgaris (PV) with no family history of psoriasis. She has been managing her condition with the Indian system of Medicines (Siddha) till one year after onset and later with Homeopathy medicines for 4 years from 13 years to 17 years of age, with temporary relief with those medications.

Clinical Findings

A detailed case history was taken when she was admitted to the hospital. On initial examination, the resting blood pressure was 110/ 70 mm Hg, the pulse rate was 72 beats per minute, height 158 cm, body weight 78.5 kgs, and body mass index (BMI) was calculated to be 31.4 kg/m^2. Weight for Age Percentiles is 93.94 and z score is 1.55 which is considered as overweight. On physical examination, plaque-type patches with scaling were observed on the scalp, neck, hip, and both thighs. Since the patient had already been diagnosed with psoriasis, on initial assessment the diagnosis of psoriasis is primarily clinical. The hallmark of classic plaque psoriasis is well-demarcated, symmetric, and erythematous plaques with overlying silvery scales. The severity of psoriasis was assessed using the PASI (Psoriasis Area Severity Index) score which is recommended for the scientific evaluation of the severity of plaque psoriasis[5]. PASI scores were obtained during the day of admission (day 1) and the day of discharge (at the end of 16 weeks of treatment) and follow-up was done at 6th month and 1-year post intervention Table-1.

Table 1. Timeline of events

Date	Day of the event	Assessment (PASI) score	General observation		
			Blood pressure (mm. Hg)	Weight (Kgs)	BMI
08/11/2016	Before treatment	33	110/ 70	78.5	31.4
08/03/2017	After 16 weeks of treatment	3.2	110/70	63.5	25.4
08/09/2017	Follow-up after 6 months	1.6	108/66	60	24.03
08/03/2018	Follow-up after 1 year	0.8	110/68	65	26.04

Table 2. Details of Naturopathy and Yoga intervention:

	Intervention	Frequency
Diet	Intake of fresh fruit juice/ vegetable juice/ buttermilk/ plantain pith juice/ tender coconut water (300 ml/ serve)	Twice/ day (Breakfast & Dinner)
	Intake of Mixed fruits (1 bowl 250 gms) of and raw vegetable salad (100 gms)	Once/ day (Dinner)
	Vegetarian meal high in fiber (Vegetables & Millet based diet)	Once/ day (Lunch)
Hydrotherapy	Mud therapy:	Once/ 3 days
	1. Mud application to whole body	Daily
	2. Mud pack application to abdomen and eyes	
	Hydrotherapy:	Daily
	1. Cold hip bath	Once/ 3 days
	2. Steam bath	Once/ 3 days
	3. Colon hydrotherapy	
	Heliotherapy:	Once/ 4 days
	1. Plantain leaf bath	
	Herbal therapy:	
	1. Fresh neem leaves, aloevera and turmeric paste application over the plaques	Once/ 3 days
	2. Drained water of boiled brown rice and turmeric application	
	3. Poppy seeds and turmeric application	Once/ 3 days
	4. Coconut oil application	Once/ 3 days
		Alternate days for initial 4 weeks
Yoga	Yogic cleansing procedures:	Twice/ week
	1. Vaman dhauti	Six days/ week (Regularly practiced after the intervention duration)
	2. Yoga practices including Asanas, Meditation and Pranayama	

Therapeutic Focus

Following a detailed history, initial counseling, and signed informed consent, naturopathy treatments were planned for 4 months initially. As per Naturopathic understanding the root cause of psoriasis, upon disturbance in the homeostasis of the body, due to improper elimination of toxins leading to its accumulation leading to disturbance in the functioning of the immune system which is expressed as skin lesions and other manifestations. Hence, the holistic therapeutic focus is to detoxify the body and improve the functioning of the alimentary system, and also to improve the body-mind

stability through Yoga. Treatments were administered for 16 weeks which comprised of Naturopathic diet – juice fasting, intermittent fasting, fruit diet, followed by boiled vegetarian diet; mud therapy; hydrotherapy; heliotherapy; topical herbal applications and Yogic cleansing procedure which is presented in Table-2. She was advised to follow the satvik diet (soothing diet) consisting of boiled vegetables, fruits, and millet-based food, and strictly advised to avoid junk and fried food, to limit the consumption of non-vegetarian diet to monthly once if needed, topical herbal application.

Results

During the process of treatment the clinically visible skin lesions were reducing and the patient reported a better quality of life. After 16 weeks of treatment, we observed a reduction in the severity of plaques and a reduction in PASI score from 33 at the beginning to 3.2 at the end of the intervention. During the follow-up visits, the PASI score was 1.6 and 0.8 at 6th month and 12th month respectively post-intervention. There was also a reduction in dryness and redness of the skin and fading off of the psoriatic patches. Other changes observed were a 15 kgs reduction in weight and BMI reduced to 25.4 kg/m^2 from 31.4 kg/m^2. The changes observed are persistent over four years. During discharge, the patient was advised to continue with a vegetarian diet (approx. 1200-1500 Kcal/day) and practice Yoga (1 hour/ day), coconut oil application thrice a week. The patient reported having good compliance with the follow-up advice. The patient visited for follow-up at 6th and 12th months and assessment was done using PASI score (Table 1).

Discussion

The present case is an eighteen-year-old girl, presented with symptoms of Psoriasis Vulgaris at 9 years of age. As indicated in many studies she had the appearance of psoriatic lesions during the early year of puberty, which can be related to the hormonal changes which occur at puberty, which might have triggered or worsened her condition[6]. The treatment was planned at addressing several factors observed in Psoriasis Vulgaris patients like incomplete protein digestion, bowel toxemia, excessive consumption of animal fats, which are considered to be morbid matter in Naturopathy, and impaired liver function, bile deficiency, nutritional deficiencies and stress, which may be due to lack of vitality[7]. A low-fiber diet is associated with an increase in levels of gut-derived toxins like cyclic guanosine monophosphate (GMP) within skin cells, thereby increasing the rate of proliferation of skin cells dramatically[8]. Polyamines are shown to be higher in psoriatic individuals due to incomplete protein digestion or poor intestinal absorption of protein breakdown products [9]. Fasting encourages effective detoxification by the autophagy mechanism is adequately studied. The significant improvement in the patient's condition may have occurred due to decreased levels of gut-derived toxins and polyamines, which can be attributed to the prescribed low protein diet, raw diet intake, and light vegetarian gluten-free diet. Intake of non-vegetarian diet which is the source of Arachidonic acid is restricted to reduce the production of inflammatory leukotrienes from arachidonic acid which is many times greater than normal in the skin of psoriatic individuals. Steam bath^s can act as detoxification therapy causing increased oxidation and destruction of nitrogen-containing wastes and toxins and eliminating them through the skin. Colon hydrotherapy aids in the detoxification function of the liver by removing large quantities of decomposing stuff and toxins from the intestine. A cold hip bath with friction is one of the best of all measures for the relief of constipation and to strengthen the smooth muscles of the abdominal and pelvic organs. All these above-mentioned physiological effects of hydrotherapy treatments may have brought about significant changes in the patient's condition. Along with diet and hydrotherapy, topical herbal and mud applications are known to modulate autoimmune mechanisms by reducing stress markers. Mud therapy decreases pro-inflammatory factors like interleukin I, TNF-α, and radical-mediated per oxidations like myeloperoxidase and glutathione peroxidase[10]. Plantain leaf bath is regarded as one of the detoxification processes by inducing profuse sweating. We observed that starch fortified turmeric baths produced a significant reduction in skin lesions, by acting as an anti-inflammatory agent[11]. Aloe vera application may exert anti-inflammatory actions by blocking the generation of inflammatory mediators and reducing neutrophil infiltration. Neem application has a role of free radical scavenging properties due to rich source of antioxidants,

nimbolide, azadirachtin, and ascorbate, and anti-inflammatory properties via regulation of proinflammatory enzyme activities including cyclooxygenase (COX), and lipoxygenase (LOX) enzymes and it suppresses the activity of nuclear factor-kappa B[12]. Stress is a major factor observed in many psoriasis patients, stress triggers the pro-inflammatory cytokines which contribute to the progression and maintenance of psoriatic lesions. Yoga in reducing stress is evident in this case[13], [14]. Vaman dhauti a yogic cleansing procedure, prescribed for the elimination of toxins from the upper gastrointestinal tract might help in aiding the detoxification process of the liver and intestine. The patient gained confidence by observing the changes in herself and that her quality of life in terms of physical and social aspects had been improved. This encouraged her to continue with the follow-up of the Naturopathic lifestyle.

Conclusion: Apart from physical symptoms psoriasis is also associated with a multitude of psychological impairments. From this case, Naturopathy and yoga which focuses on the whole system approach can be an effective choice of treatment for patients with Psoriasis Vulgaris focusing on both physical as well as the psychological aspect of patients. Diet modifications, topical herbal application, and yoga seem to be effective in immune modulation and its skin manifestations. We propose to further large-scale studies to generate evidence through clinical trials to assess the efficacy and effectiveness of this potential treatment protocol.

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Conflict of Interest

We author of this study wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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Effect of Ramadan Fasting on the Quality of Life of Elderly Muslims

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ABSTRACT

Introduction: Ramadan fasting may affect the quality of life of elderly Muslims and cause some challenges. The present study aimed to assess the effect of Ramadan fasting on the quality of life of elderly Muslims.

Methods: This observational-correlational study was conducted throughout the natural intervention of Ramadan fasting in 2016 when the length of daily fasting was about 16 hours. In total, 100 individuals aged more than 60 years were selected from the patients referring to the health centers in Rafsanjan, Iran via convenience sampling. Data were collected using the 12-item short-form survey of quality of life (SF-12) at three measurement time points before (T1), one day after (T2), and one month after Ramadan (T3). Data analysis was performed in SPSS version 18 using two-way repeated measures analysis of variance (ANOVA), and the P-value of less than 0.05 was considered significant.

Results: A significant difference was observed in the total score of quality of life between the three measurement time points (T1: 47.02±14.15, T2: 50.76±17.06, T3: 51.53±16.79; P<0.0001).

Conclusion: According to the results, the quality of life of elderly Muslims improved through Ramadan fasting.

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Introduction

Muslims constitute a wide cultural group, and their population increases by 35% each year (1). With 1.2 billion followers, Islam is the second largest religion in the world after Christianity (2, 3). Ramadan is the ninth month of the lunar calendar and a holy month for Muslims around the world. Fasting during Ramadan is one of the five pillars of Islam (2). Fasting is an important religious belief and ritual of Muslims (4). All healthy adult Muslims must fast each year during the month of Ramadan (4, 5). In Ramadan fasting, Muslims take one meal before sunrise and keep their fast until sunset. During the day, they refrain from eating and drinking (6), as well as some other activities that break their fast (7). Such a lifestyle during Ramadan differs from the usual lifestyle of Muslims in the other months of the year (8). On average, the length of fasting is 10-19 hours per day depending on the seasons and geographical region (6).

Ramadan fasting is not mandatory for elders and patients if it is likely to adversely affect their health (1). Another exemption from fasting is fasting intolerance by the elderly (9). Nevertheless, the elderly mostly insist on fasting during Ramadan for a number of reasons, such as the habit of fasting from an early age, strong religious beliefs, the fear of being labeled as non-believers, and self-satisfaction and self-esteem by performing a religious duty (10). Healthcare providers should decide whether these individuals are able to fast and offer advice on safe fasting. Therefore, they must have adequate information in this regard (9).

The quality of life (QOL) of the elderly population is one of the challenges of the current century, which has attracted the attention of health policymakers. Geriatric care is considered optimum and proper only when it results in the prolongation of life, along with the improved QOL of the care receivers (11). Socioeconomic factors,

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demographic characteristics (12), dietary habits, literacy, and cultural beliefs could affect the QOL of the elderly (11).

Conflicting data have been proposed regarding the effects of Ramadan fasting on QOL. For instance, Teng et al. (2011) reported the adverse effects of limited calorie intake on QOL, health status, and mood (13), while Guillaume Fond (2013) observed that fasting could improve mood and QOL (14). In addition, Nugraha et al. (2017) reported that fasting improves fatigue, drowsiness, and QOL (15). These discrepancies imply the inadequate knowledge of healthcare professionals on the effects of Ramadan fasting (7). Moreover, previous studies have not investigated the effects of fasting in old age (16). Considering the Islamic culture and beliefs, the elderly often opt for fasting for the positive spiritual outcomes of this religious rite.

The present study aimed to evaluate the effect of Ramadan fasting on the QOL of elderly Muslims.

Materials and Methods

Research Design

This observational-correlational study was conducted at the healthcare centers located in Rafsanjan, Iran, and Patients were selected from the general population of Rafsanjan city. The study was performed at three measurement time points before, one day after, and one month after Ramadan in 2016. During this period, the length of fasting was about 16 hours per day.

Sample Population

The sample population included 100 patients who referred to the healthcare centers of Rafsanjan. The inclusion criteria were as follows: 1) Islamic religion (Shiite); 2) age of 60-80 years (both males and females); 3) ability to fast during Ramadan (minimum of three days); 4) Iranian or Afghan nationality and 5) ability to understand and speak Persian. The exclusion criteria of the study were a history of known psychosis (e.g., paraphrenia or schizophrenia), chronic obstructive pulmonary disease, cardiovascular diseases, and active gastrointestinal diseases (e.g., peptic ulcer).

Variable Measurement

The demographic data of the participants were collected and recorded at baseline, such as age, gender, nationality, and education level. QOL was measured using the 12-item short-form survey of quality of life (SF-12) at three measurement time points before (T1), one day after (T2), and one month after Ramadan (T3). SF-12 consists of eight domains, including physical functioning (two items), physical role functioning (two items), emotional role functioning (two items), vitality (one item), mental health (two items), social functioning (one item), bodily pain (one item), and general health perceptions (one item). The items in this scale are scored based on three-, five- or six-point Likert scales. The raw scores are converted into the scale of 0-100 to compare the domains. Scores zero and 100 are indicative of the highest and lowest possible QOL, respectively. In addition to the scores of the SF-12 domains, the total QOL score was calculated based on the standard mean score of SF-12 (17). Montazeri et al. (2009) have previously assessed and confirmed the validity of the Persian version of the SF-12 using known-groups comparison and convergent validity, as well as its reliability based on internal consistency (18). In the mentioned study, the two-factor structure (physical and mental health) jointly explained 57.8% of the variance, confirming the validity and reliability of SF-12 for the measurement of health-related QOL in the Iranian population.

One day after Ramadan, we enquired about the number of the fasting days. At each measurement time points, the participants who had experienced severe issues or conditions affecting their health were excluded from the study. From 100 elderlies who were enrolled in the study, 92 cases completed the questionnaires at the measurement time points, and eight cases were excluded due to failure in referral to the study setting or our inability to make phone contact.

Statistical Analysis

Data analysis was performed in SPSS version 18 using descriptive statistics (mean, standard deviation, frequency, and percentage). In addition, two-way repeated measures analysis of variance (ANOVA) was used for the comparison of the QOL scores at each measurement time point between the categories of demographic characteristics. Within-subject comparison of the QOL scores across the three measurement time points was also performed using repeated measures ANOVA. In all the statistical analyses, the significance level was set at $P<0.05$.

Ethical Considerations

The study protocol was approved by the Ethics Committee of Rafsanjan University of Medical

Sciences (IR.RUMS.REC.1394.111), and written informed consent was obtained from the subjects prior to enrollment.

Results

In total, 92 elderly subjects participated in the study, including 33 males (35.9%) and 59 females (64.1%). The mean age of the subjects was 67.14±9.86 years. In terms of education level, the majority (n=53; 57.6%) were illiterate, seven subjects (7.6%) had primary education, 11 subjects (12%) had secondary education, 13 (14.1%) had a high school diploma, and seven (8.7%) had an academic degree. In addition, 52 participants (56.5%) were Iranian, and 40 (43.5%) were Afghan. The majority of the participants (62%) had fasted 5-30 times before, while the others had not experienced fasting.

Table 1. Comparing elderly people's QOL at the three measurement time points in different categories of their demographic characteristics

Variables	Time points	Categories	Mean±SD	*P value
Gender	Before	Female	41.46± 17.17	Measurement time points: P value < 0.0001
		Male	42.50 15.54	
	Immediately after	Female	46.90 ± 20.08	
		Male	47.98 ± 16.12	
	One month after	Female	46.81± 22.86	Gender: P value = 0.877
		Male	46.81 ± 18.45	
Nationality	Before	Iranian	49.65±14.92	Measurement time points: P value < 0.0001
		Afghan	38.40 ± 16.11	
	Immediately after	Iranian	53.80 ± 15.76	
		Afghan	44.42 ± 19.22	
	One month after	Iranian	55.51±16.33	Nationality: P value = 0.018
		Afghan	42.98 ± 22.14	
Ramadan Fasting	Before	Yes	47.02 ± 14.15	Measurement time points: P value < 0.0001
		No	39.40 ± 17.09	
	Immediately after	Yes	50.76±17.06	
		No	45.66±19.29	
	One month after	Yes	51.53±16.79	Fasting: P value = 0.159
		No	44.59 ± 22.85	
Educational Status	Before	Illiterate	38.81± 17.00	Measurement time points: P value < 0.0001 Educational status: P value = 0.347
		Primary	47.08 ±15.82	
		Secondary	45.31 ± 14.82	
		Diploma	55.55 ± 11.79	
		University	46.66 ± 0.000	
	Immediately after	Illiterate	44.71 ± 19.94	
		Primary	57.50 ± 22.02	
		Secondary	48.75 ± 15.74	
		Diploma	62.50 ± 13.22	
		University	46.88 ± 0.000	
	One month after	Illiterate	43.19 ± 22.03	
		Primary	65.13 ± 31.07	
		Secondary	49.27 ± 17.75	
		Diploma	63.88± 19.15	
		University	57.08± 0.00	

* The two-way repeated measures analysis of variance (ANOVA)

According to the statistical analysis, the mean QOL score of the Iranian participants had a significant difference with the mean score of the Afghan participants (P=0.018). In addition, significant differences were observed between the three measurement time points regarding the mean score of QOL with the demographic characteristics of the subjects (P<0.0001) (Table 1).

The results of repeated measures ANOVA indicated significant differences between the three measurement time points regarding the mean scores of physical functioning, physical role functioning, emotional role functioning, mental health, general health perceptions, and the total score of QOL (P<0.05) (Table 2).

Discussion

In the current research, the QOL of the elderly increased one day after Ramadan compared to before Ramadan. Compared to before and one day after Ramadan, the QOL of the subjects was higher and lower at T3 (i.e., one month after Ramadan), respectively. This is inconsistent with the findings of Dube (2014), who evaluated the

correlation between the religious attitude and QOL of elderly individuals, reporting an inverse correlation between the variables (19). The discrepancy could be because in the study by Dube, subjects with a lower economic status had better religious attitudes.

Table 2. Comparing the mean scores of different domains of QOL across the measurement time points

Domains	Time points	Mean±SD	*P value
Physical functioning	Before	34.82±22.96	0.0001
	Immediately after	47.91 ±34.75	
	One month after	44.94± 35.30	
Physical role functioning	Before	42.46 ± 24.45	0.004
	Immediately after	46.38 ± 29.25	
	One month after	40.15 ± 31.02	
Emotional role functioning	Before	48.10 ± 28.52	0.001
	Immediately after	55.85 ± 22.27	
	One month after	48.89±29.43	
Vitality	Before	25.91 ± 23.71	0.06
	Immediately after	29.26 ± 23.17	
	One month after	28.35 ± 23.49	
Mental status	Before	47.32 ± 24.31	0.003
	Immediately after	48.80±23.81	
	One month after	51.93±23.24	
Social functioning	Before	47.91 ± 28.04	0.767
	Immediately after	47.32 ± 26.89	
	One month after	48.21 ± 24.78	
Bodily pain	Before	58.75 ± 22.35	0.819
	Immediately after	58.25 ± 21 .86	
	One month after	58.25± 20.91	
General health perceptions	Before	19.76± 24.28	0.0001
	Immediately after	33.43± 22.88	
	One month after	35.17± 23.75	
Total SF-12 score	Before	41.84± 16.50	0.0001
	Immediately after	47.29± 18.64	
	One month after	46.81 ± 21.24 2	

* The repeated measures analysis of variance (ANOVA)

Gender has been reported to be a determinant of QOL (20). According to a study conducted in Iran, women had lower QOL compared to men (21). Consistently, our findings indicated that the QOL of the female participants was lower compared to the males at the three measurement time points although the differences were not considered significant. On the other hand, previous studies have shown that factors such as age, gender, education level, and diseases may significantly contribute to the QOL of the elderly (22, 23). Lee Kyung Hee et al. (2020) also reported that the QOL scores of men were significantly higher compared to the scores of women (24).

According to our findings, the Iranian participants had higher QOL compared to their Afghan counterparts. A similar study indicated that Afghan immigrants had limited access to social services, modest income, and poor residential status in Iran, and elderly Afghans had to work hard due to these problems (25). Evidently, our findings did not undermine the effects of Ramadan fasting on the QOL of the Afghan participant, and the obtained results in this regard could be attributed to the better living conditions of Iranians compared to Afghan immigrants.

Before Ramadan, the QOL of the participants who could successfully keep their fast was higher compared to those who did not fast in the present study. This could be due to the fact that the elderly who kept fasting had more profound religious beliefs. In this regard, previous findings have indicated that the power resulting from religious beliefs could positively influence health and wellbeing (26), and spiritual wellbeing could improve QOL (27).

An interesting finding of the present study was that Ramadan fasting had similar effects on the QOL of the elderly who kept fasting and those who did not fast. In other words, the QOL of these groups did not differ significantly. This is consistent with the results obtained by Nugraha (2017), who evaluated the impact of fasting on mood, fatigue, and health-related QOL in non-fasting and fasting groups and reported no

significant differences in these variables between the study groups (15). Therefore, it could be inferred that the spiritual atmosphere of Ramadan affects even the individuals who do not fast. Previous studies have indicated that the social climate of all Islamic communities becomes more spiritual during the holy month of Ramadan, and performing religious duties (e.g., abstaining from immoral acts, soul purification) affects the mental health of the community members (28).

According to the literature, the mean QOL score of 50±10 is considered as the accepted norm for the elderly population (29). However, the QOL of our participants was lower than the QOL score reported by the previous study (11). Other studies have also indicated that the QOL of the elderly is often below the norm, and immediate interventions are required to improve their QOL (30). Several sociodemographic factors may affect the QOL of the elderly, such as education level, income status, nutritional status, government support, living condition, access to welfare facilities, personality, beliefs, shortages, failures, and former experiences (12).

With the exception of the social functioning and physical pain domains, the mean scores of the other domains of QOL differed significantly across the three measurement time points in the present study. Although the QOL of the participants one month after Ramadan was slightly lower compared to one day after Ramadan, the value remained higher than the baseline QOL. Moreover, the score of the physical functioning domain was higher than the mental health. These findings could be due to the fact that despite the significant correlation between age and the prevalence of chronic diseases (31), high-quality healthcare services could decrease physical problems in the elderly and increase their coping abilities (29, 32). Consequently, our participants experienced fewer physical functioning problems as opposed to mental problems. Inconsistently, previous studies have indicated that the score of mental health was higher than physical functioning (11, 32), which could be attributed to the better social status and greater respect for the elderly in their families in the past (33, 34). Notably, the elderlies who experience loneliness and isolation less tend to have higher QOL (35). Our findings in this regard could be due to the inadequate family support of the elderly, which might have intensified their sense of isolation and loneliness (36).

Although the effects of Ramadan fasting on the vitality domain of QOL were rather long-lasting, the score of this domain was significantly lower than the other domains of QOL, denoting the need for immediate interventions in this regard. Furthermore, the relatively low score of the vitality domain could be due to difference in life priorities at different ages. For instance, while the youth mostly value vitality, happiness, work, and income, the elderly may prioritize their health status (37). However, lack of recreational facilities for the elderly, as well as their loneliness, might undermine the mental health of these individuals and decrease their vitality. Previous findings in this regard have indicated that due to the significant correlation between the mental health and QOL of the elderly (38), more constructive interventions are essential to the improvement of their QOL (39, 40).

The strengths of our study were the use of real world data and a population-based design, and the main limitation was not recording the dietary habits and physical activity of the subjects, which should be addressed in the further investigations in this regard.

Conclusion

This study aimed to evaluate the effects of Ramadan fasting on the QOL of elderly Muslims. According to the results, fasting was associated with improved QOL in the elderly Muslims.

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Conflicts of Interest

None declared.

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Malnutrition Screening and Prevalence in a Population of Inpatients with Non Communicable Diseases: A Cross-Sectional Study (Algeria, 2020)

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ABSTRACT

Introduction: Hospitalization is a stressful event that affects the nutritional status of adults and elderly. Inpatients with non-communicable diseases (NCDs) represent a vulnerable group facing an increased risk of malnutrition. The aim of this study was to estimate the prevalence of malnutrition in Algerian inpatients with non-communicable diseases in a public hospital in Skikda (Algeria).

Methods: This was a cross-sectional, descriptive survey carried out during one month from the 15th November 2020 to the 10th December 2020, with inpatients with at least one NCD (cardiovascular diseases, cancers, diabetes, and chronic respiratory diseases). The assessment of their nutritional status was carried out using the Subjective Global Assessment tool (SGA).

Results: One hundred and five patients were included. One of two patients had at least one non communicable disease. In our study, the SGA allowed us to estimate the malnutrition prevalence at 44.76% (ranks B and C: moderate and severe malnutrition). Our study showed a length of stay (LOS) of 4.14±7.69days.

Conclusion: The considerable prevalence of malnutrition was, in part, due to reduced food intake and short LOS. In order to detect and prevent malnutrition, the nutritional status assessment must be included in the routine of health care in Algeria.

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Introduction

Non-communicable diseases (NCDs) are the leading cause of death worldwide. In Algeria, the four major NCDs (cardiovascular diseases, cancers, diabetes, and chronic respiratory diseases) accounted for 76% of all deaths in 2016 [1]. NCDs represent an economic and health burden on individuals and populations because they require repeated interactions with the health system, with recurring and continuous medical expenditures. Hospitalization constitutes one of the principal healthcare-related costs of NCDs and it is a stressful event that affects the nutritional status of adults and elderly by causing malnutrition (under-nutrition), called hidden hunger, which is frequent in the hospital setting leading to poor hospitalization outcomes and survival due to the lack of basic knowledge concerning dietary requirements and practical aspects of the hospital's food provision. Hence, the relationship between malnutrition and hospitalization is reciprocal [2-8].

Thus, malnutrition represents a double burden within populations in low and middle-income countries like north African countries, including Algeria, that has experienced significant demographic, social, economic change, and real nutritional, epidemiological, and lifestyle transitions (9,10). These countries are characterized by nutritional inequalities, low-income levels, and insufficient health expenditure and face a shift in disease burden from communicable to non-communicable

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diseases (11–13). This heavy burden of malnutrition on health-care systems is to be taken seriously because the experience with the global pandemic shows that health care systems can be in an unexpected way challenged and exhausted (14,15). For example, a study in Wuhan, China, confirms that malnutrition contributed to prolonged hospitalization of inpatients with COVID-19 (16). On the other side, it is now well established that NCDs, particularly among the elderly, increase the susceptibility to COVID-19 disease (17).

Despite the importance of this topic, research on it is few and modest. In Algeria, no official reports on the nutritional status of inpatients with NCDs were available. Except for some studies about the nutritional status assessment of some vulnerable groups of the population (18–21), there is no considerable research about NCDs patients. Provided data on malnutrition status would help to adopt control strategies to avoid malnutrition among NCDs inpatients. Different tools exist to assess the nutritional status of NCDs inpatients. Subjective Global Assessment (SGA) is the most validated standardized assessment of malnutrition, (22,23). It considers all aspects of the nutritional diagnoses, such as the quantity and type of food intake, weight loss, symptoms related to nutritional disorders, and associated functional difficulties. SGA is widely used for hospitalized patients with good sensitivity and specificity. Besides, the capacity of malnutrition assessment tools is usually measured against it (24,25).

This research aims to study the prevalence of malnutrition among inpatients with NCDs, related risk factors such as comorbidities, and hospitalization length.

Patients and Methods

Study Design

This study was a cross-sectional study of a representative sample of hospitalized adults in a public hospital in Skikda (east of Algeria), from the 15th November 2020 to the 10th December 2020. A questionnaire was filled using information collected from interviewing patients face to face.

Setting and Population

Abdul Razzaq Bouhara is the second public hospital in Skikda, with 240 beds and eight wards (26). Our study was conducted among adult inpatients in two medical departments we were allowed in: general medicine and oncology. We excluded from the target population pregnant women and patients who could not be interviewed for diverse reasons: end of life, impaired health status, deaf and voiceless person.

Ethics

The conduction of this research was approved in coordination between the University of Batna and the Directorate of Health of Skikda. Abdul Razzaq Bouhara Hospital was the only hospital not receiving cases of Coronavirus in Skikda, for this reason it has approved our admission to conduct the research. The study was conducted according to the Declaration of Helsinki. Informed consent was obtained from all individual participants included in the study.

Sampling

Our study sample is a stratified random sample of 105 patients from an initial population of 167 patients. After excluding records with incomplete information, the population was stratified into four strata representing the four principal NCDs.

Measurements

A questionnaire was used to document general information of participants, including the year of birth, gender, date of admission, name of ward, and type of diseases. Bodyweight was measured using Seca® mechanic scale to the nearest 100g, and body height was measured using a stadiometer to the nearest 0.1cm. The BMI is defined as the body mass divided by the square of the body height, and is expressed in units of kg/m^2.

The nutritional status of participants was assessed using the Subjective Global Assessment tool (SGA), which considers nutrient intake, weight loss, symptoms, functional capacity, and physical exam. It classifies nutrition status as well-nourished (A), mild to moderately malnourished (B), or severely malnourished (C). A patient is rated as SGA class B if there was at least 5% weight loss without any recent stabilization or regain, dietary intake reduction, and mild loss of subcutaneous tissue. A patient is ranked as SGA class C if he/she had severe loss of subcutaneous tissue, muscle wasting, and edema (27,28). SGA does not use an explicit numerical weighting scheme. Instead, a rank is assigned based on subjective weighting. For example, If the patient had considerable edema, ascites, or

tumor mass, the amount of weight loss has less influence, if the patient had a recent weight gain that did not appear to be merely fluid retention, he or she is ranked A, even if the net loss was between 5% and 10 % (29).
To better understand the assessment method, we suggest three examples:

Case 1: A 63-yr-old man was admitted to the hospital for complications of his chronic heart disease. He had suffered from vomiting, but this problem was resolved. He had lost 10.25% of his usual body weight (78 kg); however, his weight had been stable for the previous three months. He reported he had been working with his usual energy up to the time of admission. On physical examination, no evidence of loss of fat, muscle, edema or ascites. This patient was classified as "A," well-nourished. Although his net weight loss was moderate, it had stabilized recently.

Case 2: A 68-yr-old woman with breast cancer and heart disease was admitted to the hospital for chemotherapy. Her body weight was stable for more than six months. She reported inadequate nutrient intake for more than six months without any improvement, and vomiting, nausea, and diarrhea that we considered chemotherapy side. The patient felt slightly weak but was able to ambulate. There was no fever. On physical examination, she had a small amount of loss of subcutaneous fat and muscle. There was no edema or ascites. This patient was classified as "B," moderately malnourished. The ranking was influenced by the continuing limitation of nutritional intake to a suboptimal solid diet and mild loss of fat and muscle.

Case 3: An 82-yr-old woman was admitted to the hospital for chemotherapy. She had breast cancer and diabetes. She had improved gastrointestinal symptoms (diarrhea, pain on eating, and anorexia), affecting her nutritional intake. These symptoms led to a severe body weight loss in the previous six months. She has no dysfunction. On physical examination, there was subcutaneous tissue loss and muscle wasting. There was trace edema in the ankles and no ascites. This woman was classified as "C," severe malnutrition. The ranking was influenced most by the continuing large weight loss, change in dietary intake, and acute physical findings.

Statistical methods

Epi-Info 3.5.3 was used for data entry, processing, and analysis. Descriptive statistics were used to characterize the study population. It was checked that obtained values were normally distributed. The test of analysis of variance (ANOVA) was used to compare between the SGA rating and the hospitalization conditions (NCDs, comorbidities, length of stay (LOS), cause of hospitalization). Pearson's correlation was applied for the relationship between SGA rating and test features (nutritional intake, weight loss, nutritional impact symptoms, functional capacity, and physical exam). A P-value of < 0.05 was considered significant.

Table 1. Population characteristics (Skikda-Algeria, 2020)

Characteristics		N=105 (100%)
Age (years)		58.21±13.69 [18.00-90.00]
Gender [N(%)]	Female	79 (75.20)
	Male	26 (24.80)
NCD [N(%)]	Cancer	66 (62.86)
	CVD	46 (43.81)
	Diabetes	31 (29.50)
	CRD	13 (12.38)
Number of NCDs [N(%)]	One NCD	62 (59.05)
	Two NCDs	33 (31.43)
	Three NCDs	9 (8.57)
	Four NCDs	1 (0.95)
Co-morbidity [N(%)]	Yes	42 (40.00)
	No	63 (60.00)
LOS		4.14±7.69 [1-60]
Hospitalization related to NCD [N(%)]	Yes	96 (91.40)
	No	9 (8.60)

Qualitative data as shown as percentage of subjects [N(%)]and quantitative data are means(SD) [minimum-maximum]
N: Population size; NCD: Non Communicable Disease; CVD: Cardio Vascular Disease; CRD: Chronic Respiratory Disease; LOS: Length of Stay

Results

Population characteristics

The characteristics of the surveyed population are summarized in Table 1. The age of patients was between 18 and 90 years old with an average of 58.21±13.69 years. The majority of the population reported NCD as cause of hospitalization (91.40%). Figure 1 represents comorbidity in details.

Subjective global assessment results

Prevalence of malnutrition according to SGA

According to the SGA, 55.24% of inpatients were well-nourished, 19.05% were classified as "B," moderately malnourished and 25.71% were classified "C", severely malnourished (Figure 2). The SGA rating was significantly related to the BMI (r=-0.28; p<0.01). However, the SGA rating is not significantly related to the BMI ranks of malnutrition (malnourished if BMI<18.5kg/m^2 (30)).

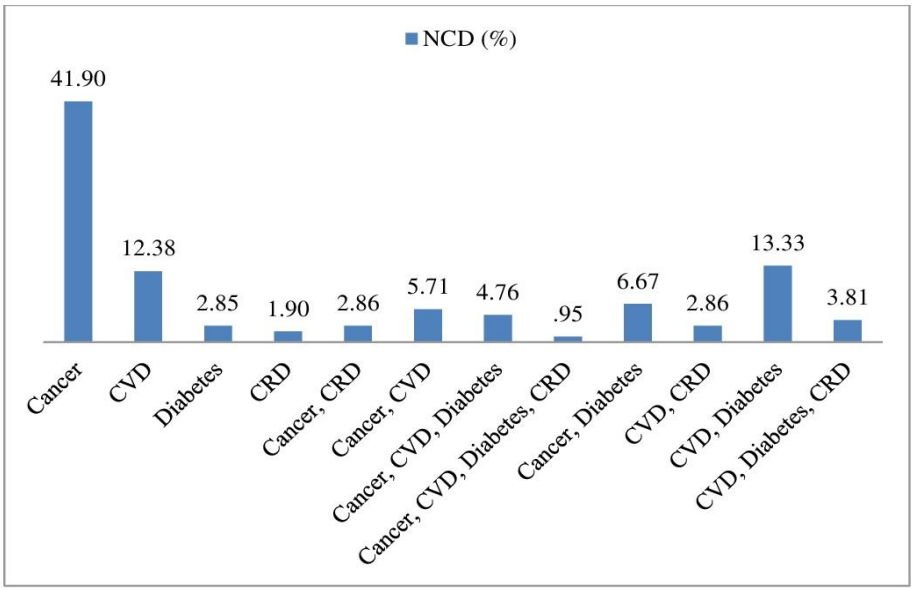

Figure 1. Comorbidities among study population (Skikda-Algeria, 2020)

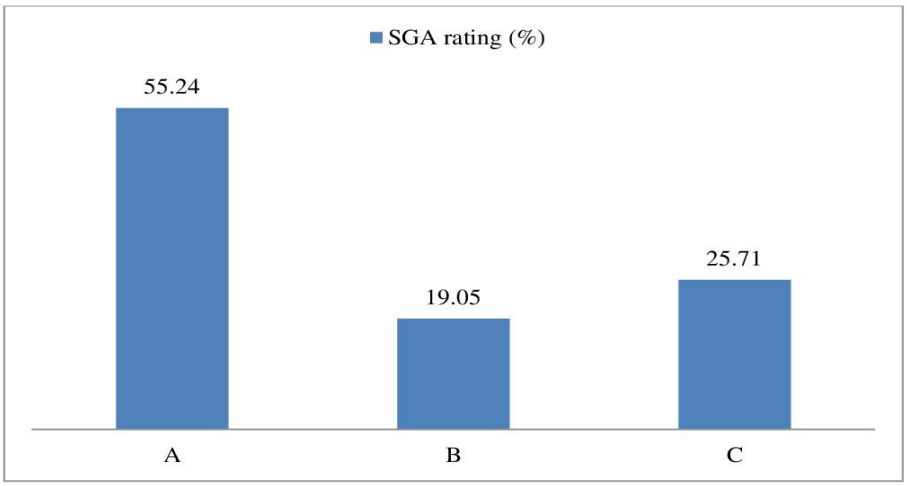

Figure 2. SGA rating results among study population (Skikda-Algeria, 2020)

SGA and hospitalization

The ANOVA test showed that there had not been found a significant difference between the SGA ranks and four hospitalization conditions (NCD type, number of NCDs, comorbidities and cause of hospitalization) (p>0.05). However there was a significant difference between the SGA ranks and the LOS (p=0.013). The post-hoc analysis using Scheffe's method showed that the group "C" (severely malnourished inpatients) was significantly different from the other two groups of patients. The test of correlation Pearson showed a significant positive correlation between the SGA rating and the LOS (p=0.05).

SGA rating and assessment criteria

The Pearson's test presented a significant correlation between the SGA rating and the assessment criteria (nutrient intake, weight loss, functional capacity, physical exam and number of symptoms) (p=0.01). The ANOVA test showed that there had been found a significant difference between SGA ranks and the assessment criteria. However, the nutritional intake related-symptoms show no differences. The post hoc analysis showed that the well-nourished group was different in nutrient intake and weight loss, and the severely malnourished group was different in functional capacity and physical exam (p=0.05).

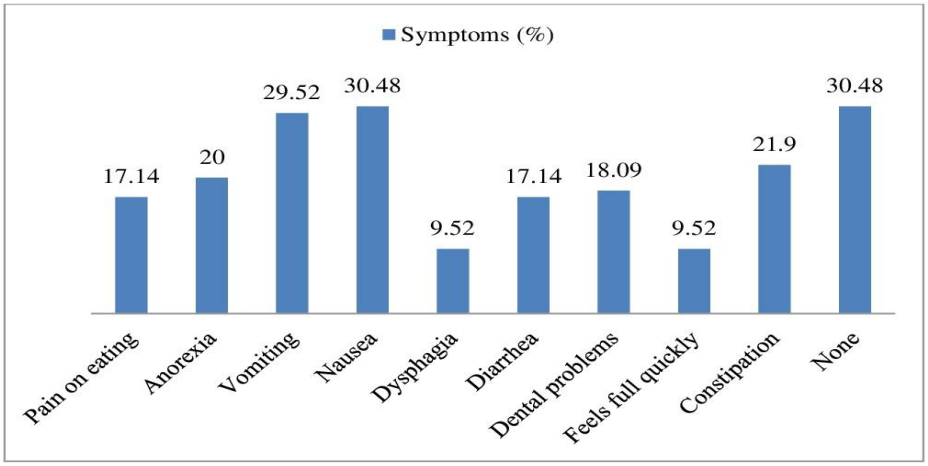

Figure 3. Prevalence of nutritional intake related symptoms among study population (Skikda-Algeria, 2020)

Prevalence of nutritional intake symptoms

The most common nutritional impact symptoms leading to reduced food intake were nausea (30.48%), vomiting (29.52%), constipation (21.90%), and anorexia (20%) (Figure 3). Sixty nine point five percent of patients might have at least one nutritional symptom. More than one of three (38.1%) patients had severe symptoms. More than one-third (33.4%) reported three to seven symptoms. The nutritional impact symptoms "pain on eating, anorexia, vomiting, nausea, feels full quickly" were significantly and negatively related to the nutrient intake.

Discussion

This cross-sectional study allowed us to assess the nutritional status and to estimate the prevalence of malnutrition of NCDs' inpatients at the public hospital Abd errazaq Bouhara in the city of Skikda (Algeria).

The average age of studied inpatients puts them at risk of early death, as more than 14 million people dying annually between the ages of 30 and 70 due to NCDs (31). NCDs share common lifestyle risk factors, thus the coexistence of two or more diseases in the same person, especially diabetes and heart diseases, which represents metabolic syndrome (32,33). As we mentioned earlier, NCDs are among the main reasons for hospitalization. An increased number of NCDs is associated with a higher utilization of primary and secondary heath care services (34) which explains that the majority of studied patients were hospitalized for a direct reason with the NCDs they suffer from.

The SGA allowed us to estimate the malnutrition prevalence at 44.76% (ranks B and C: moderate

and severe malnutrition). Malnutrition effects on average 20 to 50% of hospitalized patients. Its negative repercussions in terms of morbidity and mortality have been well-demonstrated (35). In Spain, 50% of inpatients in a tertiary hospital (*n*= 197) were malnourished, according to the SGA (36). In Vietnam, Tran *et al.* found that the prevalence of malnutrition in patients on hospital (n=883) was at 48.1% using the SGA or the BMI<18.5kg/m^2 (37). As for our research, we have reservations about using BMI as an indicator of malnutrition. The results we obtained proved that a BMI of more than 18.5kg/m^2 does not necessarily mean the absence of malnutrition. For values below 18.5kg/m^2, different grades of under-nutrition exist. The lower limit of normality of the BMI is higher in the elderly due to the physiological weight gain associated with age. So, beyond the age of 75, there is under-nutrition or risk of malnutrition when the BMI is less than 21kg/m^2 (35). BMI is an imperfect indicator of percentage fat, but also, it doesn't take into consideration fluid retention that falsely increases body weight measurements (29,38).

Our research indicates that severe malnutrition is associated with a longer LOS, confirming previous studies about malnutrition independently associated with higher mortality risk, longer LOS, and increased hospitalization costs (39). The LOS is a crucial indicator of the efficiency of hospital management. According to the Organization for Economic Co-operation and Development (OECD), the average length of stay in hospitals for all causes across OECD countries was about eight days (40,41). Our study showed a shorter LOS (4.14±7.69days). Despite the fact that a reduced stay decreased the infection risk and medication side-effects, improved the quality of treatment, and increased hospital profit with more efficient bed management and reduced costs; in our study, this shortest LOS indicates probably an early discharge that could worsen patients' outcomes and promoted chances of readmission. Especially in the absence of effective diagnosis and treatment of hospital malnutrition (40–42).

Our results showed that food intake and weight loss are the most worthy criteria to place our judgment on to assess the nutritional status. Functional capacity and physical exam helped to describe and confirm the degree of the severity of malnutrition. Detsky *et al.* instructed their clinical raters to place most of their judgment on the variables weight loss, reduced dietary intake, and physical exam (29). Our results confirmed the relationship between nutritional symptoms and reduced nutrient intake. These symptoms could be a result of low quality of life on admission and also side-effects of medications like chemotherapy (43,44).

This study derives its importance from several aspects. Firstly, it is the first to assess the nutritional status of inpatients with NCDs in Algeria. Secondly, despite the presence of some studies that dealt with a specific NCD or a vulnerable group of patients, our research represents a less expensive option. NCDs share many risk factors and are among the most important causes of hospitalization, as we mentioned earlier. Therefore, comprehensive studies provide us an estimated value of the prevalence of malnutrition in hospitals in general. However, more work is needed. Conducting grander studies on larger samples and researches about assessment criteria, mainly nutrient intake and related symptoms, by studying the nature, quality, and quantity of inpatients' food intake is primordial.

The circumstances associated with the Coronavirus spread represented several obstacles that accompanied our research, as we were unable to reach many institutions, which seemed to affect the sampling method, the sample size and its composition. Which explains many of the results, principally the evident difference in the nature of studied NCDs, as cancer patients represented the largest group, and respiratory disease patients the least group, because of the presence of cancer patients in a special ward compared to the other three diseases in the general medicine ward, as well as the transfer of most of patients with respiratory infections to the ancient hospital designated for Coronavirus cases, as they are considered suspected and vulnerable cases. However, our approach aimed to overcome these obstacles, as we chose to conduct a simple stratified random sampling to ensure the representation of every patient group, which allowed us to get a representative sample (45,46)

Conclusion

Our study assessed the nutritional status of a sample of NCDs inpatients. The prevalence of malnutrition among less than half of the patients

was due to reduced food intake and some nutritional impact symptoms; the absence of monitoring by specialists and the LOS considered less than necessary given the nutritional status of patients that require specialized intervention. Accordingly, the nutritional status assessment must be included in the routine of health care in Algeria to understand more the causes of hospital malnutrition and adopt strategic solutions.

Conflicts of interest

None

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Effects of Ramadan Fasting, Physical Activity, and Dietary Patterns on Diabetic and Hypertensive Patients

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ABSTRACT

Introduction: The present study aimed to compare the effects of Ramadan fasting and dietary patterns on the blood pressure, fasting glucose level, and body mass index (BMI) of the patients with diabetes mellitus (DM), hypertension (HTN), DM and HTN, and healthy subjects.

Methods: This prospective, cross-sectional study was conducted in Shaban, Ramadan, and Shawwal months in 2020 on 155 subjects who were divided into groups of HTN (G1; n=42), DM (G2; n=32), DM and HTN (G3; n=41), and healthy (G4; n=40). The subjects were interviewed three times during the study period to collect data on demographics, dietary habits, and physical activity. In addition, physical parameters (height and weight) and clinical parameters (systolic and diastolic blood pressure and fasting blood sugar) were measured in each visit. Data were collected three times in the last ten days of each month. Data analysis was performed using the repeated measures ANOVA.

Results: Mean weight and BMI reduced significantly from Shaban (V1) to Ramadan (V2). Systolic and diastolic blood pressure also reduced significantly from V1 to V2. Moreover, the mean sleeping hours significantly decreased from V1 to V2. Considering Tarawih prayer as physical activity, a significant increase was observed in the mean metabolic energy turnover value from V1 to V2. Mean calorie, carbohydrate, and sodium intake also increased significantly from V1 to V2, while they reversed significantly to almost the same values in V3. Protein and cholesterol consumption decreased significantly from V1 to V2, while the value reversed significantly in V3.

Conclusion: The study showed that the Ramadan fasting could effectively control the blood pressure and glucose levels of the patients. Furthermore, physical activity increased significantly due to Tarawih prayers, and carbohydrate, sodium, and calorie intake increased as well. On the other hand, a reduction was denoted in protein and fat consumption. Glucose levels significantly decreased in the diabetic patients, and blood pressure significantly reduced in the hypertensive patients.

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Introduction

Ramadan is the ninth month of the lunar calendar and is considered a prominent period for Muslims across the world due to mandatory religious fasting. Approximately 1.254 billion out of 1.9 billion Muslims aged more than 15 years fasted in the month of Ramadan of 2020 (1). It is obligatory to all adult Muslims to fast in this month, and exceptions are those who are ill or traveling as commanded by the Holy Quran (1:185).

During Ramadan, fasting Muslims abstain from eating, drinking, smoking, and sexual intercourse from dawn to sunset. In the late evening, most of the fasting muslims Tarawih and wake up early before dawn to eat *Sahur* as the first meal of the day. Tarawih is a special voluntary prayer performed in early night in Ramadan. During Ramadan, drastic changes in eating, sleeping, adjustment of medication doses, extra physical activity due to Tarawih, and fasting/re-feeding for 29-30 consecutive days might affect the biochemical markers of diabetic and hypertensive patients. Several studies have investigated the effects of Ramadan fasting on diabetic patients (2-7), hypertensive patients (8-12), and healthy adults (13-18). To the best of our knowledge, no studies have compared the effects of Ramadan fasting on diabetic patients, hypertensive patients, and healthy subjects.

The present study aimed to compare the effects of Ramadan fasting and dietary patterns on the blood pressure, fasting glucose level, and body mass index (BMI) of patients with diabetes mellitus (DM), hypertension (HTN), DM and HTN, and healthy subjects. Dietary habits and physical activity may vary in different countries (especially during Ramadan) depending on the

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geographical location and cultural background (19). Therefore, dietary changes, extra prayers, and lifestyle changes may interfere with the effects of Ramadan fasting on the body mass index (BMI), blood pressure, and glucose levels.

Material and Methods

This prospective, cohort study was conducted during the months of Shaban, Ramadan, and Shawaal of 1441 Hijri (2020 C.E.) on subjects who were selected via convenience sampling among the family members, friends, and neighbors of the researchers. Since this study compared diabetic and hypertensive patients with healthy subjects, three studies conducted by Khan, Samad, and Farag (2, 10, 15) were taken into account to determine the sample size. With a 95% confidence interval and 80% power, the mean maximum sample size was determined to be 32 patients per group. Since diabetes and hypertension complications often begin after the age of 30 years in most of the cases, we had to considered a limited age group in this study.

Initially, 163 patients were enrolled in the study and divided into four groups of diagnosed hypertensive (G1), diagnosed diabetic (G2), diagnosed hypertensive and diabetic (G3), and healthy control (non-diagnosed with HTN or DM) (G4). In total, 155 subjects completed the three visits, including 42 hypertensive patients, 32 diabetic patients, 41 hypertensive and diabetic patients, and 40 healthy controls; the response rate was 95.1% (155/163). The healthy subjects were also examined in terms of previously undiagnosed HTN and DM based on the 2020 ISH Global Hypertension Practice Guidelines for the diagnosis of HTN and the diagnostic criteria for Type 2 DM by the American Diabetes Association (ADA), respectively.

The inclusion criteria of the study were adult patients aged more than 30 years, consent to participate, and confirmed HTN and/or DM diagnosis (except for the healthy controls). The exclusion criteria were as follows: 1) Type 1 DM; 2) pregnant woman; 3) severe diseases; 4) severe hepatic impairment or renal failure; 5) active involvement in weight loss programs and 6) fasting for less than 20 days.

The research objectives were explained to the subjects at the initial visit, along with the benefits of free testing during the study period, and written informed consent was obtained from each subject. The field investigators were trained on the proper and safe use of the calibrated instruments for the measurement of fasting blood sugar, blood pressure, weight, and height before the first visit.

The field investigators visited the subjects on three occasions, which were during the last 10 days of Shaban (1st visit), the last 10 days of Ramadan (2nd visit), and the last 10 days of Shawwal (3rd visit). On all the three visits, physical measurements (height and weight) and clinical measurements (systolic and diastolic blood pressure and fasting blood sugar) were carried out, along with an interview on a pre-designed Performa with each patient. The questionnaire enquired about the subjects' demographics, family history, disease history (duration and treatment), comorbidities, physical activity (type, frequency, duration, and intensity), smoking habits (past/current smoking and quantity), sleeping patterns (hours of nighttime and daytime sleep and longest run of sleep), dietary macronutrients and micronutrients (calories, proteins, fat, carbohydrates, cholesterol, and sodium), and two-day food items consumed for breakfast (Sahur), lunch (Iftar), and diner.

Values of micronutrients were calculated by MyFitnessPal, and blood pressure was measured twice (at least three minutes apart) in a sitting position using calibrated aneroid sphygmomanometers after ensuring that the subjects were calm and had not just had a meal or engaged in strenuous physical activity immediately before the measurement. Fasting blood sugar was quantified after at least 10 hours of fasting using calibrated glucometers. Weight was measured in kilograms without shoes using calibrated weighing machines, and height was measured only on the first visit in centimeters without shoes.

Data were collected in the morning before breakfast in the first and third visits and before *Iftar* in the second visit. Furthermore, details of physical activity and Tarawih (night prayers) were recorded. Physical activities during Ramadan were measured based on the metabolic energy turnover (MET; kcal/kg/hour) as mentioned by Khan (3). Physical activity in Ramadan was calculated with and without Tarawih prayers using the following formula:

Physical activity = MET value x duration of activity (minutes/session) x frequency of the activity/week

The MET value for the Salah (Islamic prayers) was also determined as 1.5* kcal/kg/hour. (Personal communication with Dr. Stephen Herrmann, Stanford Research; Developer of MET values for different activities).

The study protocol was approved by the Institutional Review Committee of Jinnah Sindh Medical University in Karachi, Pakistan (Ref: JSMU/IRB/2020/-329). Data analysis was performed in SPSS version 16 using the repeated measures ANOVA, with the visits considered as the within factor, groups as the between factor, and age as a covariate to clarify the effects of the groups, visits (groups and visits), and age. If the effect of groups and visits was significant with a P-value of less than 0.1, each group was analyzed by the repeated measures ANOVA with visits considered as the within factor. Chi-square and the Friedman nonparametric ANOVA were also utilized to evaluate the associations between the nominal and ordinal scale variables. In addition, the normality of each continuous variable was assessed, and if it was significant, nonparametric ANOVA was used for the categorical variables.

Table 1. Descriptive statistics of weight. BMI, SBP, DBP and FBS in Shaban, Ramadan and Shawwal

Items	Shaban	Ramadan	Shawwal	Mean	p-value[1]
Weight					
Healthy	68.5±15.7	67.9±15.1	69.0±15.5	68.4±15.3	0.212
Hypertensive (HTS)	73.2±11.0	72.2±11.1	72.6±11.8	72.6±11.2	(group)
Diabetes (DM)	67.9±12.7	67.4±12.7	67.5±12.5	66.6±12.6	0.199
HTN + DM	72.0±15.4	71.1±15.2	71.7±14.7	71.6±15.0	(age)
Mean (Effect of visits)[2]	70.5[a]±13.9	69.8[a]±13.7	70.4[a]±13.8	70.3±13.7	
P-value for the combined effect of visits and groups.[3]	0.789(V1xV2)	0.112 (V1xV3)	0.216(V2xV3)		
BMI					
Healthy	25.0[a]±5.2	24.8[a]±5.3	25.2[a]±5.2	25.0±5.2	0.561
Hypertensive (HTS)	26.7[a]±3.9	26.4[b]±4.1	26.5[ab]±4.1	26.6±4.0	(group)
Diabetes (DM)	25.7[a]±4.1	25.5[b]±4.1	25.5[ab]±4.1	25.6±4.1	0.151
HTN + DM	27.5[a]±5.1	27.1[b]±5.0	27.4[a]±4.9	27.3±5.0	(age)
Mean (Effect of visits)[2]	26.2[a]±4.7	26.0[a]±4.7	26.2[a]±4.6	26.2±.7	
P-value for the combined effect of visits and groups.[3]	0.132 (V1 x V2)	0.530 (V2 x V3)	0.102 (V1 x V3)		
Systolic BP					
Healthy	118.5[a]±11.5	115.9[a]±12.6	117.4[a]±9.7	117.3±8.9	< 0.0001
Hypertensive (HTN)	135.2[a]±17.8	129.6[b]±14.3	128.9[b]±12.7	131.2±13.2	(group)
Diabetes (DM)	117.1[a]±8.4	117.4[a]±8.5	119.2[a]±10.5	117.9±7.6	0.001
HTN + DM	134.6[a]±13.1	131.2[a]±15.2	133.2[a]±14.8	132.9±11.4	(age)
Mean (Effect of the visits)[2]	127.0[a]±15.7	124.0[a]±14.7	125.03[a]±13.7	125.3±12.8	
P-value for the combined effect of visits and groups.[3]	0.251 (V1 x V2)	0.824 (V2 x V3)	0.057 (V1 x V3)		
Diastolic BP					
Healthy	77.6±8.1	75.7±8.6	77.4±7.8	76.9±6.7	< 0.0001
Hypertensive (HTN)	85.0±10.1	82.2±8.1	83.3±7.6	83.5±7.0	(group)
Diabetes (DM)	75.8±5.3	75.2±7.8	77.1±6.9	76.03±5.7	0.001
HTN + DM	84.3±10.7	81.6±8.9	83.7±12.0	83.2±8.2	(age)
Mean (Effect of the visits)[2]	81.0[a]±9.1	78.9[a]±8.9	80.6[a]±9.4	80.2±7.8	
P-value for the combined effect of visits and groups.[3]	0.796 (V1 x V2)	0.631 (V1 x V3)	0.965 (V2 x V3)		
Fasting Blood Sugar					
Healthy	97.2[a]±12.4	93.9[a]±11.9	97.4[a]±12.3	96.2±10.05	< 0.0001
Hypertensive (HTN)	109.0[a]±25.9	102.0[b]±21.4	110.1[a]±19.3	107.0±20.5	(group)
Diabetes (DM)	124.2a±34.6	126.3[ab]±36.4	135.0[b]±44.2	128.5±34.4	0.023
HTN + DM	136.4a±46.5	133.9a±44.04	132.6a±34.3	134.3±37.9	(age)
Mean (Effect of the visits)[2]	116.3[a]±35.3	113.4[a]±34.8	117.9[a]±32.9	115.9±31.5	
P-value for the combined effect of visits and groups.[3]	0.356 (V1 x V2)	0.067 (V1 x V3)	0.249 (V2 x V3)		

*Different alphabets indicate statistical significance.

1 This column shows the significance level (p-value) due the effect of group and age as a covariate.

2. This row give the mean value and significant differences due to the visits

3. This row contains the significance level (p-value) due the combined effect of visits and groups.

Results

In total, 155 subjects (healthy and patients) completed the three visits, including 67 (43.2%) males. About 41% of the respondents were aged 51-60 years, and the majority of the participants (78.9%) were migrants from India. The mean age of the healthy subjects, DM patients, HTN patients, and patients with both HTN and DM was 43.3±9.1 (R: 32-72), 54.3±9.0 (R: 34-69), 56.5±9.4 (R: 38-75), and 59.2±8.4 years (R: 46-77), respectively. The mean age of the healthy subjects was significantly lower than the patients with HTN and HTN with DM. However, no significant differences were observed in the mean height, weight, and BMI between the four study groups in the first visit (Figure 1). Moreover, 65% of the participants stated that they performed Tarawih.

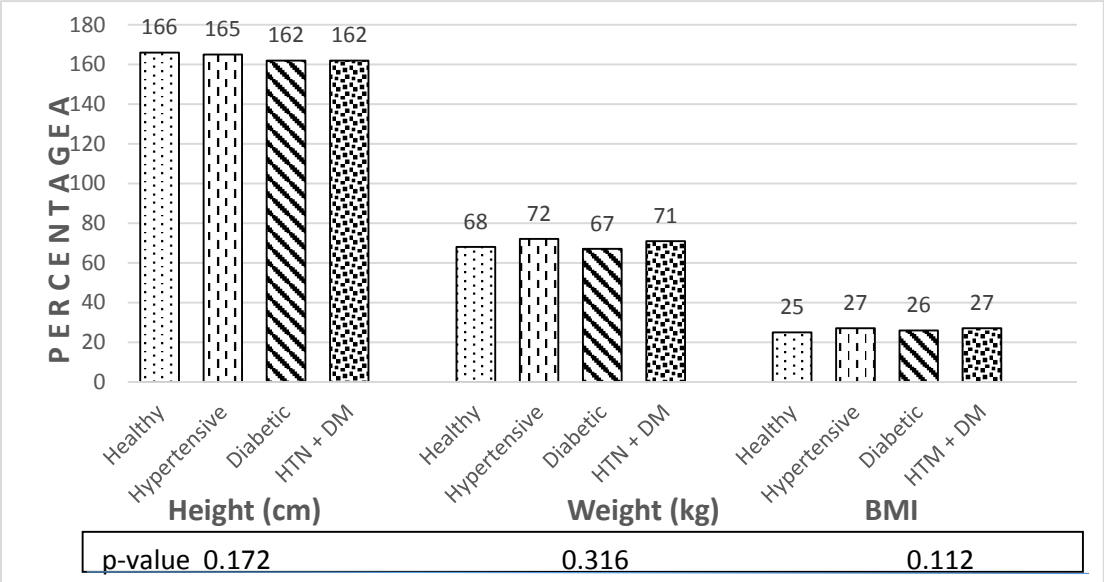

Figure 1. Descriptive statistics of height, weight and BMI

Table 1 shows the descriptive statistics regarding weight, BMI, systolic blood pressure (SBP), diastolic blood pressures (DBP), and fasting blood sugar (FBS), as well as the comparison of the means of Shaban (V1), Ramadan (V2), and Shawwal (V3) between the four groups (visits and groups), with age considered as a covariate. Accordingly, mean weight reduced from V1 to V2 and reversed to almost the same value as V1; however, the difference in this regard was not considered significant. The data showed no significant combined effect of the visits and groups, and the changes in BMI were almost the same as the changes in weight.

According to the findings, SBP reduced from V1 to V2 and increased in V3. The mean values of SBP in the patients with HTN and those with HTN and DM were significantly higher as compared to the healthy subjects and DM patients (P<0.0001). The combined effect of the visits and groups also indicated a significant effect from V1 to V3, and the repeated measures ANOVA showed a significant difference between the visits in the HTN group. In the HTN group, SBP significantly decreased from V1 to V2 and V3. However, the mean DBP decreased in V2, but reversed to almost the same value to V3.

The obtained results demonstrated that the mean FBS increased significantly from V2 to V3 (P<0.05). In addition, the mean FBS values of the healthy subjects and HTN patients were significantly lower compared to the other groups (P<0.0001). A significant difference was also observed in the combined effect of the visits and groups between V1 and V3 (P<0.1). The repeated measures ANOVA also showed a significant reduction in the mean FBS from V1 to V2, as well as an increase in this parameter from V2 to V3 in the patients with HTN (P<0.05). Furthermore, the mean FBS significantly increased from V1 to V3 in the patients with DM.

Table 2. Descriptive statistics of sleeping hours and MET values in Shaban, Ramadan and Shawwal

Items		Shaban	Ramadan	Shawwal	p-value
Total sleeping hours					
	Healthy	7.2a (7.0-8.0)	7.0b(6.0-8.0)	8.0a (7.0-8.0)	0.011
	Hypertensive (HTS)	8.0 (6.0-8.0)	7.0 (6.0-9.0)	8.0 (6.0-8.0)	0.717
	Diabetes (DM)	8.0a (7.0-9.0)	7.5b (6.0-8.8)	8.0a (7.0-9.0)	0.001
	HTN + DM	8.0 (7.0-8.0)	8.0 (6.5-9.0)	8.0 (7.0-8.0)	0.621
Mean (Effect of visits)		8.0a (7.0-8.0)	7.5b (6.0-8.0)	8.0a (7.0-8.0)	0.005
Sleeping hours day time					
	Healthy	0.5 (0.0-1.5)	1.0 (0.0-2.8)	0.5 (0.0-1.9)	0.001
	Hypertensive (HTS)	0.5 (0.0-1.6)	1.2 (0.0-3.0)	1.0 (0.0-2.0)	0.002
	Diabetes (DM)	0.8 (0.0-1.9)	1.2 (0.5-3.0)	0.8 (0.0-2.0)	0.031
	HTN + DM	1.0 (0.0-2.0)	1.5 (0.3-2.0)	1.0 (0.0-2.0)	0.236
Mean (Effect of visits)		1.0a (0.0–2.0)	1.0b(0.0-3.0)	1.0a (0.0-2.0)	<0.0001
MET value without Tarawih kcal/kg/hour					
	Healthy	226.4±559.0	228.0±567.7	201.2±381.2	0.321
	Hypertensive (HTS)	235.0±578.2	168.2±357.9	181.0±338.7	0.181
	Diabetes (DM)	391.1±702.6	231.8±476.1	310.2456.2	0.043
	HTN + DM	273.3±419.5	202.6±416.1	281.4±452.0	0.001
Mean (Effect of the visits)		274.9^{a}±563	206.2^{b}±456	239.2^{a}±406	<0.0001
MET value with Tarawih kcal/kg/hour					
	Healthy	226.4±559.0	610.0±714.4	201.2±381.2	<0.0001
	Hypertensive (HTS)	235.0±578.2	641.8±561.5	181.0±338.7	0.003
	Diabetes (DM)	391.1±702.6	672.1±573.7	310.2±456.2	0.038
	HTN + DM	273.3±419.5	704.1±646.5	281.4±452.0	<0.0001
Mean (Effect of the visits)		274.9^{a}±563	655.8^{b}±635	239.2^{a}±406	<0.0001

*Different alphabets indicate statistical significance.

Table 2 shows the total sleeping hours, daytime sleeping hours, MET values with and without Tarawih prayers in Shaban, Ramadan, and Shawwal, as well as the comparison of the visits. Since sleeping hours were limited in scores and did not fulfill the normality assumption, therefore median and interquartile range were computed. Furthermore, MET values also did not meet the normality assumption, therefore, the nonparametric Friedman test was used for each group to assess the changes in this regard. The obtained results indicated that the sleeping hours of the healthy subjects and DM patients reduced significantly in Ramadan, and total sleeping hours also reduced significantly during Ramadan. In terms of daytime sleeping hours, the healthy subjects and patients with DM and HTN had longer daytime sleeping hours in Ramadan compared to the other groups. The percentage of the participants who had a daytime nap in Shaban, Ramadan, and Shawwal was estimated at 61%, 68%, and 62%, respectively. According to the Friedman two-way nonparametric test, the changes in these percentages were statistically significant (P=0.004).

According to the findings, the mean MET values reduced significantly from V1 to V2 without considering Tarawih prayers. This reduction was attributed to the decreased MET values of the patients with DM and those with HTN and DM. If Tarawih prayers were considered in the calculation of the MET values, a significant increase was observed in the mean values from Shaban to Ramadan (274.9 and 655.8, respectively).

Table 3 shows the summary statistics regarding calorie, protein, carbohydrate, fat, cholesterol, and sodium intake during the months of Shaban, Ramadan, and Shawwal, as well as the comparison of the visits, groups, age, and visits and groups. Accordingly, the mean differences in the calorie intake of the four groups were insignificant (P=0.099), while the age factor showed a significant effect (P=0.019). Notably, the calorie intake of the HTN patients was significantly higher than the other two patient groups. The mean calorie intake increased insignificantly from V1 to V2 (P>0.05) reversed significantly to almost the same value in V3. Furthermore, age had a significant effect on the protein intake of the subjects (P=0.004).

In contrast to calorie intake, the major contributor of the mean protein reduction was observed in the healthy subjects and the patients with HTN and DM together compared to the other groups. The mean carbohydrate intake was also significant among the four groups (P=0.019), and age influenced the effect as a covariate (P=0.002). The mean consumption increased in Ramadan and decreased in Shawwal in the four groups, and the mean intake levels increased by

approximately 40 points in V2 without significance.

Table 3. Descriptive statistics of consumption of calories, protein, carbohydrate, fat, cholesterol and sodium per day in the month pf Shaban, Ramadan and Shawwal

Items	Shaban	Ramadan	Shawwal	Mean	p-value[1]
Calories					
Healthy	1414±517 (21.5)4	1496±610 (23.3)	1408±489 (21.3)	1439±538	0.099
Hypertensive (HTS)	1421±513 (19.7)	1626±702 (23.0)	1497±584 (20.7)	1515±606	(group)
Diabetes (DM)	1276±444 (19.4)	1524±650 (23.4)	1246±445 (19.0)	1349±531	0.019
HTN + DM	1316±476 (18.6)	1417±528 (20.4)	1328±365 (18.9)	1355±460	(age)
Mean (Effect of visits)[2]	1361[a]±490 (19.8)	1516[a]±623 (22.4)	1377[a]±484(20.1)	1418±540	
P-value for the combined effect of visits and groups.[3]	0.659 (V1 x V2)	0.375 (V1 x V3)	0.390 (V2 x V3)		
Protein					
Healthy	72.3[a]±56.6 (1.1)	53.3[b]±25.7 (0.8)	63.6[a]±26.7 (1.0)	63.1±39.5	
Hypertensive (HTS)	57.9[a]±22.7(0.8)	62.9[a]±49.9 (0.9)	65.0[a]±27.4 (0.9)	61.9±35.2	0.204
Diabetes (DM)	59.2[a]±21.1(0.9)	56.4[a]±19.8 (0.9)	58.3[a]±23.9 (0.9)	58.0±21.5	(group)
HTN + DM	71.3[a]±55.2 (1.0)	52.1[b]±24.0 (0.8)	60.3[a]±24.0 (0.9)	61.3±37.9	0.004
Mean (Effect of visits)[2]	65.4[a]±43.3 (0.9)	56.2[a]±32.8 (0.8)	62.0[a]±25.5(0.9)	61.2±34.8	(age)
P-value for the combined effect of visits and groups.[3]	0.061 (V1 x V2)	0.245 (V1 x V3)	0.573 (V2xV3)		
Carbohydrate					
Healthy	201.0±125 (3.0)	224.1±122 (3.5)	193.3±96.1(2.9)	206.2±115.3	
Hypertensive (HTS)	195.4±74.5 (2.7)	259.3±200 (3.7)	191.6±94.7(2.7)	215.4±137.3	0.019
Diabetes (DM)	167.2±75.5 (2.5)	221.4±94.8 (3.4)	160±62.3 (2.4)	182.9±82.6	(group)
HTN + DM	176.5±73.5 (2.5)	194.9±71.4 (2.8)	181.9±75.6(2.6)	184.4±73.3	0.002
Mean (Effect of visits)[2]	186.0[a]±91 (2.7)	225.4[a]±134(3.3)	183[a]±84.6(2.7)	198.1[a]±107.3	(age)
P-value for the combined effect of visits and groups.[3]	0.352 (V1 x V2)	0.944 (V1 x V3)	0.207 (V2 x V3)		
Fat					
Healthy	56.5±57.6 (0.8)	41.8±20.9 (0.7)	71.5±164.9 (0.7)	56.6±101.4	0.209
Hypertensive (HTS)	74.6±126.1(1.0)	61.0±81.7 (0.9)	68.6±78.3 (0.9)	68.1±97.2	(group)
Diabetes (DM)	45.2±17.6 (0.7)	52.1 ±41.5 (0.8)	44.4±17.8 (0.7)	47.2±27.9	0.506
HTN + DM	68.8±167.6 (0.6)	40.5±17.7(o,6)	50.7±24.4 (0.7)	44.7±20	(age)
Mean (Effect of visits)[2]	62.3[a]±112 (9.8)	48.8[a]±48.9 (0.7)	48.9[a]±94.2 (0.8)	54.6±74.4	
P-value for the combined effect of visits and groups.	0.292 (V1 x V2)	0.892 (V1 x V3)	0.275 (V2 x V3)		
Cholesterol					
Healthy	201.5±125 (3.0)	153.7±170 (2.3)	220.6±203 (3.2)	191.9±170.4	0.537
Hypertensive (HTS)	167.6±117 (2.3)	143.3±145 (2.0)	189±111 (2.6)	166.7±126	(group)
Diabetes (DM)	180.5±135 (2.7)	132.1±95 (2.1)	191.7±130 (2.9)	168.1±123.3	0.618
HTN + DM	186.8±121 (2.6)	118.5±113 (1.7)	207.7±124 (3.0)	171±124.9	(age)
Mean (Effect of visits)	184.1[a]±124(2.7)	137.1[b]±135(2.0)	202.7[a]±146(2.9)	174.6±138	
P-value for the combined effect of visits and groups.[3]	0.420 (V1 x V2)	0.984 (V1 x V3)	0.411 (V2 x V3)		
Sodium					
Healthy	2033±760 (30.9)	2265±1234(34.9	1997±585 (30.7)	2098±903.1	0.080
Hypertensive (HTS)	2013±689(27 .9)	2194±878 (31.2)	2177±1025(29.5	2128.3±872.4	(group)
Diabetes (DM)	1881±760 (28,9)	2259±927 (34.7)	1930±722 (29.5)	2023.6±817.9	0.128
HTN + DM	1819±634 (25.9)	1982±705 (28.6)	1942±615 (27.8)	1915±651.1	(age)
Mean (Effect of visits)[2]	1940[a]±708 (28.3)	2170[a]±952 (32.2)	2017[a]±761 (29.3)	2024.6±818.5	
P-value for the combined effect of visits and groups.[3]	0.823 (V1 x V2)	0.917 (V1 x V3)	0.754 (V2 x V3)		

*Different alphabets indicate statistical significance.

1 This column shows the significance level (p-value) due the effect of group and age as a covariate.

2. This row give the mean value and significant differences due to the visits

3. This row contains the significance level (p-value) due the combined effect of visits and groups.

4. Values in the parenthesis are the macronutrient adjust by the weight.

According to the obtained results, fat intake was significantly higher in the HTN patients, while the means of the study groups had no significance in this regard due to the high standard deviations (P=0.209). The mean cholesterol intake of the healthy subjects was significantly higher than the other groups, while it could not reach a significant level due to the high standard deviation. The mean sodium intake was also insignificant in the four groups (P=0.080). Surprisingly, the mean sodium intake was higher in the hypertensive patients compared to the diabetic patients, and the consumption increased significantly by more than 10% in V2 (P<0.05). However, it decreased significantly in the Shawwal visit (V3) (P<0.05). Notably, the visits and groups had no significant effect between any of the visits.

Discussion

As mentioned earlier, no study has been found regarding the simultaneous comparison of the effect of Ramadan fasting on diabetic patients, hypertensive patients, and healthy subjects. Therefore, the present four-arm study was conducted with this objective. The study groups included healthy subjects, only hypertensive patients, only diabetic patients, and both diabetic and hypertensive patients. The patients were selected via convenience sampling for the ease of data collection in 2020, corresponding to 1441 Hijri.

The mean age of the healthy subjects was lowest and significantly different from the patient groups. With increasing age, the risk of diabetes or hypertension also increases (20, 21), therefore, the significantly lower age of the healthy subjects was considered normal. To compensate, age was also added as a covariate to the analysis in the current research. Our findings indicated no significant differences in the mean height, weight, and BMI between the four study groups in V1. Therefore, the initial values weight and BMI had no effects on the changes of the subjects due to other possible co-factors.

Although the difference in the mean BMI from V1 to V2 was only 0.7 kg/m^2 and clinically quite insignificant, the results of the repeated measures ANOVA indicated a statistical significance in this regard. Furthermore, the mean weight and BMI of the subjects decreased in V2. This is consistent with the previous findings regarding the reduction of body weight and BMI from V1 to V2 (2, 5, 6, 10, 11, 14, 16). However, the reduced weight and BMI in Ramadan reversed in Shawwal in our study (V3). This is in line with the results obtained by Jehangir et al. (6). This study demonstrated that BMI decreased significantly in V2 in three patient groups (except the healthy group), while it increased significantly afterwards only in the patients with HTN and DM in the present study. Therefore, it could be concluded that the patients with both HTN and DM were more careful in controlling their diet and experienced more weight loss and BMI reduction compared to the other groups.

As expected, the hypertensive patients (with or without DM) had a higher mean SBP and DBP compared to the other two groups. With the exception of the DM patients, the mean SBP of the other groups reduced in V2, and statistical significance was only observed in the HTN patients in this regard. The significant reduction of SBP and DBP in the HTN patients in V2 is consistent with previous studies (5, 9, 10), and the insignificant change in V3 is in line with the study by Aslan et al. (11).

As expected, the mean FBS was significantly higher in the DM patients (with and without HTN). In contrast to the SBP and DBP, FBS reduced from V1 to V2 in all the groups, except the DM patients, and only the HTN patients showed a significant reduction in this regard. These findings are consistent with the results obtained by Khan (2), Khan (3), Tiboura (4), Khan (8), and Darzabi (16).

In the current research, a significant difference was denoted in the total sleeping hours of the subjects from V1 to V2. However, an individual group analysis indicated that only the sleeping hours of the healthy subjects and DM patients decreased significantly due to Ramadan activities. In a similar study, BaHammam (23) and Roky (24) also reported that the sleeping hours of healthy fasting subjects reduced in Ramadan. Furthermore, Alghamdi (25) stated that the total sleeping hours reduced during Ramadan in DM patients. In the present study, daytime nap significantly increased during Ramadan to compensate for reduced night's sleep, which is in line with the findings of Alghamdi (25).

In the present study, physical activity assessment based on the MET value showed that physical exercise and other strenuous physical activities

significantly decreased in the fasting subjects to avoid hunger or thirst while fasting. The least significant reduction in this regard was observed in the healthy subjects since ill individuals are often more careful and believe that physical exercise will make it difficult to complete the number of their fasting days. This is consistent with the studies conducted by Khan (3), Khan (8), and Prastya (14). On the other hand, Harder-Lauridsen (18) and Alghamdi (25) reported no significant reduction in physical exercises, while strenuous physical activities showed a downward trend in the mentioned studies.

To the best of our knowledge, Tarawih as a physical activity in numerical terms has not been discussed in the literature. In addition, the MET value of the Islamic congregation prayers has not been listed in the "2011 Compendium of Physical Activities: a second update of codes and MET values". This institution assigned the MET value of more than 800 physical activities. After discussing with the institution, we decided that the MET value of Tarawih prayers should be 1.5. According to our findings, physical activities would increase significantly in Ramadan if Tarawih prayers were included in the calculations. In the Muslim community, it is rather customary for individuals to attend Tarawih prayers even if they do not adhere to obligatory prayers regularly. Tarawih prayers takes more than one hour and is often performed with enthusiasm. As a result, it significantly increases the mean MET value of physical activity in Ramadan. The MET value has been reported to reach more than 600 points, which is the cutoff point for an active individual (8).

According to the results of the present study, calorie intake increased during Ramadan and reversed to almost the same level in Shawwal. Although the frequency of meals decreases for most fasting individuals during Ramadan, high-calorie diets are also common for *Sahur* and *Iftar* meals most of the time, which increase the total consumed calories in Ramadan. This trend of changes is in line with the findings of Zaroouk et al. (22) and inconsistent with the results obtained by Prasetya and Sapwarobol (14). However, most of the studies in this regard have not shown any significant change in calorie intake (5, 18, 22). In the current research, the trend of calorie intake during the three-month period was the same in all the groups. Therefore, it could be inferred that although diabetic patients avoid sweets and hypertensive patients avoid salts, energy intake in the form of calories followed the same trend in all the three months. Due to the significant age effect, the increase of approximately 150 points in calorie intake had no significant difference.

According to the current research, the mean protein intake reduced by more than nine points in Ramadan and reversed to almost the same amount as that of Shaban in Shawwal. The healthy subjects and the patients with both HTN and DM consumed significantly less quantities of protein in Ramadan compared to Shaban. In the study by Sadiya (7), the participants had metabolic syndrome, and a significant reduction was reported in protein intake from Shaban to Ramadan. Furthermore, studies on healthy subjects have shown the reduction of protein intake during Ramadan.

According to the results of the present study, carbohydrate intake reduced significantly in Ramadan and reversed to almost the same amount as Shaban in Shawwal in the four groups. This is inconsistent with the earlier findings in this regard (5, 7, 14, 17, 22). Similarly, fat intake was observed to decrease insignificantly in Ramadan. However, the mean value in Shawwal remained almost the same as that of Ramadan. Most of the studies in this regard (22) have reported no significant change in fat intake during the study period. However, Sadiya et al. (7) observed a significant increase in fat intake in the last week of Ramadan compared to the first week of the month in patients with metabolic syndrome. In the present study, the mean cholesterol intake in Ramadan was significantly lower compared to that of Shaban.

Dietary habits are diverse in different cultures and countries (9). Therefore, the similar trend of decrease or increase in any particular dietary component is not necessarily comparable. Since dietary habits were self-reported only for the last two days of the visits in our study, they may have differed from the regular food items consumed by the participants. Furthermore, recall bias might have affected the results. In addition, each community of Pakistan prepares its *Iftar* and *Sahur* meals according to their ethical customs and ancestral traditions. As mentioned earlier, most of our participants were immigrants and follow their traditional and familial dietary habits for *Sehur* and *Iftar* meals. Therefore, our findings should be generalized with caution.

Conclusion

According to the results, Ramadan fasting could be able to control blood pressure and blood glucose levels. Furthermore, physical activity increased significantly due to Tarawih prayers. Carbohydrate, sodium, and calorie intake showed increasing trend, while protein and fat intake showed decreasing trend, FBS levels significantly decreased in the diabetic patients, while blood pressure significantly reduced in the hypertensive patients.

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The Effect of Consume Leucine Supplement Before and After Resistance Exercise on Protein Metabolism Indices in Fasting Male Athletes

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ABSTRACT

Introduction: Fasting is one of the religious practices of Muslims during Ramadan. The aim of this study was to investigate the effect of consume Leucine supplement before and after resistance exercise on protein metabolism indices in fasting male athletes.

Methods: In this study, 33 male bodybuilders were selected and randomly divided into three groups: resistance training (N=11), resistance training and supplementation group (N=11) and control group (N=11). Subjects received 0.1 g of leucine or placebo each day. Leucine intake was 0.1 $g.kg^{-1}.d^{-1}$ of body weight. Subjects poured leucine tablets in powder form into 1 g empty capsules and dextrose as a placebo was in the form of 1 g capsules, the same shape, size and color of 1 g leucine tablets. The training protocol of the two training groups was performed for eight weeks in 3sessions per week. Each training session lasted 40 minutes. 24hour before the first training session and 24hours after the last training session, 10 ml of blood was taken from the subjects' brachial vein. To analyze the data, the statistical method of analysis of covariance was used and to determine the differences between the groups, Bonferroni post hoc test and at a significant level in all tests, $P \leq 0.05$ was considered.

Results: The findings showed that leucine supplementation before and after resistance exercise on uric acid levels in fasting male athletes was not significantly different between the two groups ($F=6.22$, $p=0.133$). However, in the amount of urea ($F=8.074$, $p=0.000$), creatinine ($F=6.106$, $p=0.001$), amount of Hypoxanthine ($F=11.511$, $p=0.002$) and Xanthine oxidase ($F=14.231$, $p=0.000$) There was a significant difference between the three groups.

Conclusion: Finally, it can be said that resistance training combined with leucine supplementation in fasting athletes can reduce protein catabolism due to exercise.

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Introduction

One of the most important goals of participating in sports activities is to promote the health of people in the community. Accordingly, maintaining the health of athletes during competitions and training has always been a topic of concern for coaches and doctors of sports teams. Recently, however, there have been concerns about some of the side effects of certain types of exercise combined with calorie restriction or weight loss on antioxidant defense systems. However, in Islamic countries, it is possible for Muslim athletes to exercise in fasting conditions (lack of access to water and food) due to the coincidence of competitions or sports camps with the holy month of Ramadan. Therefore, there is always the concern whether water and food restrictions in conditions such as Ramadan can cause a decline in performance of these athletes (1).

At rest, the energy used by the human body is predominantly derived from the oxidation of carbohydrates and fats. Blood glucose, plasma-free fatty acids, muscle glycogen, and intramuscular triglycerides are major substrate sources for energy production in skeletal muscles. The contribution of proteins to the pool of usable energy is very limited, as amino acid oxidation is usually strictly adjusted to the intake of amino acids (2).

Studies show that protein catabolism is increased in high-intensity exercise. In other words, there is a direct relationship between the intensity of exercise and the breakdown of body proteins. On the other hand, by increasing the training time below the maximum and reducing

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the body's carbohydrate reserves, the share of protein energy in exercise increases (3). Fasting with prolonged starvation (usually more than 24 hours), carbohydrate-free diet, and lowering blood glucose cause proteins to be forcibly broken down for energy and to maintain plasma glucose concentrations, and their carbon components to be used by muscles. Excretion of urea, uric acid and creatinine through urine as well as their accumulation in the blood are the reasons for the catabolism of proteins to produce energy (4).

In recent years, the improvement of adaptive responses to exercise through dietary interventions, especially the use of sports supplements, has been considered. It has also been observed that the stimulation of protein synthesis after eating certain foods is greatly influenced by the amino acid content of the diet, especially the amino acid leucine (5, 6). Leucine, isoleucine and valine are called branched chain amino acids (BCAAs). BCAAs make up about one-third of muscle protein, with leucine accounting for about 5 to 10 percent of the body's total protein. Leucine oxidation during exercise is significantly higher than isoleucine and valine. In addition, among BCAAs, leucine activates key signaling pathways for protein synthesis, such as mTOR, after exercise (7). Resistance training also stimulates protein synthesis by increasing mTOR signaling, which is a key component in regulating protein synthesis in skeletal muscle (8, 9, 10).

Regardless of age and sex, leucine resistance training and amino acid activation of mTOR leads to an increase in 4E-BP1 and S6K1 and binding of mRNA to ribosomes, thus increasing protein synthesis (11, 12).

Gil and Kim (2015) also investigated the interactive effect of leucine supplementation and resistance training on protein synthesis in rats. The results of their research showed that their perception that taking leucine with exercise could increase muscle mass was incorrect (12). New research shows that some purine derivatives, especially plasma hypoxanthine, can be considered as an indicator of intensity, and hence some limitations seen in classical indicators, such as the level of elite athletes, the level of activity intensity in training, Usability in all training courses (general, specific, competition and transfer) can be eliminated by using hypoxanthine. Hypoxanthine can be used as an indicator to estimate muscle metabolism, training level, age of athletes in various competitive and non-competitive sports, anaerobic exercise, and adaptation to their training status (13, 14, 15).

Hypoxanthine is a sign of degradation of adenine nucleotide in muscle and is an indicator of energy stress in exercise. Hypoxanthine can also be used as an indicator of the intensity and level of exercise activity (14, 15).

Fasting during Ramadan is a unique metabolic pattern in which a person abstains from eating and drinking from sunrise to sunset. It seems that changes in the number and timing of meals as well as sleep patterns during Ramadan can have different metabolic and hormonal effects. The effect of fasting on metabolism is very complex, many of the body's mechanisms work to maintain balance during the fasting period. Because in this period, in addition to physical activity, meal and bedtime are changed (16, 17) subsequently, the body's metabolism is individually altered. For this reason, it is difficult to determine the effect of each of these factors alone on metabolism during fasting.

Due to the importance of fatigue, maintaining and enhancing performance during fasting and reducing protein catabolism in athletes, using authorized sports supplements and replacing them with prohibited and illegal substances can help athletes achieve healthier as much as possible. Help achieve sports goals and success during fasting and Considering that no research was found on the effect of leucine supplementation before and after resistance activity on post-Ramadan protein breakdown indices during the fasting period of athletes, Therefore, the researcher decided to answer the question whether taking leucine supplement before and after resistance activity in fasting athletes can affect protein breakdown or not?

Material and Methods

The present study is a quasi-experimental study with pre-test and post-test design. In this study, three groups including resistance training group, resistance training and supplementation group and control group participated who have a history of regular exercise 3 times a week for one year. The subjects of the study who participated in this study voluntarily were randomly simple and equally divided into three groups and were informed of all stages of the research and the risks and possible consequences of the research

and their consent was obtained. In this study, it was tried to influence the factors and variables in the field of research and in different stages of project implementation such as nutrition, temperature, body mass index, location, age, gender, absence of diseases, status and health history, sleep schedule before Examine the test carefully. A few days before the test and before fasting, the subjects were given the necessary explanations for scheduling sleep and breakfast and iftar. Food and supplements before the test. Subjects poured leucine tablets in powder form into 1 g empty capsules and dextrose as a placebo was in the form of 1 g capsules, the same shape, size and color of 1 g leucine tablets. Leucine intake will be 0.1 g per kg of body weight. The method of use was that the number of capsules on fasting days before resistance training and after training. To standardize the test, the tests were performed at a specific time of day. Initial assessments including height, weight, body fat, BMI, and $VO2_{max}$ were performed two weeks before exercise. 24 hours before the first training session and 24 hours after the last session, urine samples were taken from the forearm of the subjects of all three groups in the fasting state (8:30 am) at a rate of 10 cc. Blood samples are centrifuged at 3000 rpm for 10 minutes and the level of the desired variables is measured with the appropriate kits purchased in the laboratory. To measure body mass index, the formula of weight to weight ratio (kg) to height (m) square two was used.

The protocol of the present study will include resistance training, each session of which consisted of three stages. The first stage was warming up for five minutes, the second stage was performing resistance training for 30 minutes. In designing resistance exercises, it was tried that the movements were multi-jointed and also included the large muscles of the lower torso, upper torso and middle limbs of the body. These exercises consisted of three sets with ten maximum repetitions, including: 1) Bench Press, 2) crunch with a bent knee, 3) leg press, 4 Back Extention, 5) Knee Flexion, 6) lateral puls, And 7) Overhead Press. In the last step, cool down for five minutes was considered. The total time of each training session was 40 minutes on average. Data collection tools and equipment include a personal information questionnaire to access the basic information of the subjects, a stadiometer made by Satrap company to measure the height of the subjects, a medical scale for measuring hand weight made in China with an accuracy of 0.01 kg to measure the weight of the subjects, Laboratory kits for measuring subjects' urine samples were (urea, uric acid and creatinine, hypoxanthine and xanthine oxidase).

In order to analyze the statistical data of this study, analysis of covariance was used to compare the mean of pre-test and post-test between groups at a significant level ($p \leq 0.05$) using SPSS 25 software.

Table 1. Mean and standard deviation of height, weight, age and BMI of subjects in groups

Groups	Age (years) M±SD	Height (cm) M±SD	Weight (kg) M±SD	BMI M±SD
Resistance training group	26.5 ± 3.12	177.1 ± 4.11	76.21 ± 4.74	24.53 ± 0.19
Resistance and supplementation training group	27.1 ± 2.81	177.6 ± 3.85	78.93 ± 4.14	25.04 ± 0.29
control group	26.7 ± 1.9	176.7 ± 3.90	76.58 ± 5.11	24.50 ± 0.54

Findings

The Kolmogorov-Smirnov test was used to determine the normal distribution of data. The results of this test showed that the data distribution was normal. For inferential analysis of data, we used parametric statistics and analysis of covariance for differences between pretest and posttest. Table 1 shows the mean and standard deviation of height, weight and age and BMI of the subjects in the groups. The results show that the distribution of subjects in both groups is almost the same.

The results of analysis of covariance did not show a significant difference between the three groups in the amount of uric acid but there was a significant difference in the levels of urea, creatinine, and hypoxanthine and xanthine oxidase between the three groups (Table 2). Bonferroni post hoc test was used for differences between groups.

According to the results of Bonferroni post hoc test, differences in the amount of all variables were observed between the resistance training group and the control group. Also, a significant difference was observed between the resistance training group and supplementation and the resistance training group (Table 3).

Table 2. Results of analysis of covariance to compare variables between groups

variable	group	Pre-test	Post-test	F	P
Uric acid content (mg / dL)	Resistance training group	4.74 ± 0.88	4.56 ± 1.07	6.22	0.133
	Resistance training and supplementation group	4.83 ± 1.41	4.69 ± 1.11		
	control group	4.48 ± 1.45	4.42 ± 1.66		
Urea level (mg / dL)	Resistance training group	40.11 ± 3.85	33.21 ± 5.44	8.074	0.000*
	Resistance training and supplementation group	36.48 ± 6.21	34.89 ± 3.98		
	control group	38.69 ± 4.74	37.25 ± 2.19		
Creatinine level (mg / dL)	Resistance training group	0.97 ± 0.12	0.76 ± 0.08	6.106	0.001*
	Resistance training and supplementation group	1.05 ± 0.09	1.02 ± 0.14		
	control group	1.11 ± 0.10	1.09 ± 0.11		
Hypoxanthine content (ng / µl)	Resistance training group	14.01 ± 2.23	16.87 ± 3.65	11.511	0.002*
	Resistance training and supplementation group	12.74 ± 3.09	13.52 ± 2.96		
	control group	11.64 ± 3.21	11.01 ± 2.11		
Xanthine oxidase content (ng / µl)	Resistance training group	25.14 ± 7.11	28.66 ± 7.23	14.231	0.000*
	Resistance training and supplementation group	27.15 ± 3.88	25.96 ± 4.47		
	control group	26.23 ± 2.89	27.24 ± 3.08		

Table 3. Results of Bonferroni post hoc test of variables in three groups

Variable	group	Resistance training group	control group
Urea (mg / dL)	Resistance training group	----------	M=10.221,P=0.000*
	Resistance training and supplementation group	M=8.63,P=0.000*	M=8.71,P=0.079
Creatinine (mg / dL)	Resistance training group	----------	M=5.095,P=0.000*
	Resistance training and supplementation group	M=5.141,P=0.000*	M=0.214,P=0123
Hypoxanthine (ng / µl)	Resistance training group	----------	M=2.857,P=0.003*
	Resistance training and supplementation group	M=4.325,P=0.006*	M=0.114,P=0.078
Xanthine oxidase (ng / µl)	Resistance training group	----------	M=5.158,P=0.009*
	Resistance training and supplementation group	M=3.325,P=0.001*	M=1.544,P=0.101

Discussion

Statistical analysis did not show a significant difference between groups in uric acid variable. Also, the results showed that resistance training significantly decreased urea, creatinine and increased hypoxanthine and xanthine oxidase. Resistance training with supplementation significantly increased urea, creatinine and significantly decreased hypoxanthine and xanthine oxidase compared to the resistance training group.

The importance of protein for athletes has long been known and the use of protein supplements in many athletes, especially strength athletes to increase their performance and performance seems to be necessary (18). The effectiveness of dietary proteins or protein supplements in athletes is such that supplementation, in addition to increasing muscle mass and preventing protein catabolism during intense or prolonged exercise, also increases glycogen synthesis after exercise and prevents sports anemia. It is associated with increased synthesis of hemoglobin, myoglobin, oxidative enzymes and mitochondria during exercise (19). Adequate protein uptake is also essential for accelerating synthesis and increasing muscle mass under these conditions. Resistance training simultaneously increases both the synthesis and breakdown of muscle proteins. But under these

conditions, muscle protein synthesis overcomes its failure, which ultimately leads to an increase in pure protein (20). Therefore, the need for protein and positive energy balance increases in those who participate in intense resistance training sessions.

There are different results about the effect of fasting on uric acid and urea. In a study, Azwany et al. examined the effect of one month of fasting on 43 Muslims. They fasted after 4 weeks, although the amount of water absorption was normal; reported a significant increase in urinary osmolality. Blood urea levels did not change significantly during 4 weeks (21). Comparing the blood samples of 19 fasting men during the first days and 23 months of Ramadan, Indral and colleagues found that serum urea, triglyceride, total cholesterol and LDL-C levels were significantly reduced (22). Azizi stated in a review study that serum uric acid levels increase abnormally during long-term starvation, possibly due to decreased glomerular filtration rate (GRF) and uric acid release. However, in Islamic fasting, there is only a slight increase in uric acid; this condition can be due to the nature of short-term and intermittent fasting. No change in uric acid may be attributed to the small number of samples studied or the high dispersion of scores (23, 24).

In the study of Bizheh et al. (2012), 12 weeks of aerobic exercise with 3 sessions per week was associated with increased aerobic capacity and decreased body mass index along with decreased uric acid (25). In this study, although aerobic exercise was used as an exercise intervention, exercise intervention led to a decrease in uric acid. This discrepancy can perhaps be attributed to weight loss and body mass index in response to aerobic exercise in the study.

The increase in urea concentration in the blood may be due to increased protein catabolism and may be due to resistance activity or decreased renal blood flow. Some studies suggest an increase in the concentration of urea in the blood, which may be due to exercise and resistance, which stimulates energy consumption and reduces energy intake. In this regard, when causing physical stress, albumin and urea excretion also increases in individuals. Exercise is one of the factors that can alter these biochemical factors. Other factors that can increase blood urea levels include increased protein in the diet, gastrointestinal bleeding and dehydration, or inadequate fluid intake, especially during fasting (26).

Shahdoost reports show similar results by examining the effect of selected aerobic exercise on protein catabolism in 15-year-old running students. However, in high-intensity training, due to the ratio of respiratory exchange, carbohydrates are the main source of energy production. Involvement of proteins in the energization of high-intensity activities also depends on training time. This means that proteins are burned more in high-intensity workouts that last longer (27). Ghanbari Niaki by studying the effect of 2 consecutive anaerobic tests with rest on female students of physical education (28), Ramezan pour by studying the effect of short-term physical activity with maximum ability on uric acid, blood urea and creatinine and urine of professional wrestlers (29), Savucu et al., By studying the effect of long-term training on blood and physical variables of adolescent female handball players (30) and Singh Bal et al., By studying the effect of plyometric training on physical and biochemical fitness parameters of high jump athletes (31)they report similar results to the present study.

The effect of aerobic activity on changes in blood urea and 24-hour urine urea with excreted urea through sweating, which indicates protein catabolism, may be due to: B) Under natural conditions and as a result of "amino acid amination", the ammonia produced is transported to the liver, where it is converted to urea by the Chris cycle during certain reactions. In exercise, this cycle also increases under active aerobic conditions and urea production (27); C) Urea produced in the body is excreted through the kidneys and urine. When glycogen stores are depleted, the muscles and kidneys use protein to provide energy during activity in the non-food environment (28).

Creatinine is produced mainly as muscle excretion and is a good measure of kidney function, because if the kidneys do not remove it from the blood, its concentration in plasma will increase. Sometimes long-term fasting, thirst, and dehydration transiently increase creatinine levels, which can be relieved by compensating for dehydration (32). Trappe et al. In the study designed a training session to evaluate the effect of exercise on net protein catabolism of excreted urea, cranitine and 3-methyl histidine. In this

study, eight healthy men rode a bicycle for 90 minutes with about 45% of their maximum oxygen consumption. During exercise, total urinary urea increased by 100% compared to before exercise, and excreted creatinine increased by 50%. Also, although the amount of excreted 3-methyl histidine tended to increase relative to creatinine, which is an indicator of protein catabolism, it did not change (33). In the present study, resistance training in the resistance training group reduced the amount of urea and creatinine, but the resistance training group with the supplementation of this reduction in urea and creatinine was less than the resistance training group, and this was probably the effect of leucine supplement on protein catabolism and it has reduced. Based on the information we have, the present study is the first to investigate the effect of leucine supplementation and fasting on protein catabolism.

Hypoxanthine is the final product in the recycling pathway of purine adenines, so that if it is converted to xanthine in the oxidation pathway by xanthine oxidase enzyme, purine is lost and finally in human it is converted back to uric acid by xanthine oxidase activity and excreted from the body. Therefore, the amount of hypoxanthine is important and can be considered as an indicator of severity (34) there is a correlation between an increase in hypoxanthine and a decrease in blood pH, and it has been shown that a critical point at 107-115% of maximum oxygen consumption is a critical point for hypoxanthine (15).

In a study by Chung Liu et al. (2005), it was reported that xanthine oxidase is the main source of free radical production in intense and tedious activities. They suggested that mitochondria play a lesser role in this type of activity. Activation of xanthine oxidase enzyme has been shown to be one of the important reasons in the production of free radicals. Xanthine oxidase is a metaflavone protein (35) that produces large amounts of free radicals by consuming oxygen, and as a result, this enzyme is one of the most important sources of O2 and H2O2 production in the body.

In the present study, resistance training in the resistance training group increased the amount of hypoxanthine and xanthine oxidase, but in the resistance training group with supplementation, the amount of hypoxanthine in the post-test increased compared to the pre-test and this increase was less than the official training group. And the amount of xanthine oxidase was observed in the resistance training group with supplementation, and this is probably the effect of leucine supplementation on protein catabolism and has reduced its amount. Based on the information we have, the present study is the first to examine the effects of leucine supplementation and fasting on hypoxanthine and xanthine oxidase levels.

Conclusion

Finally, it can be argued that resistance training combined with leucine supplementation in fasting athletes can reduce protein-induced protein catabolism. Also, it can be said that coaches and athletes can use leucine supplementation to increase performance and reduce injury to reduce catabolism caused by sports activities during Ramadan. Combining resistance training with leucine supplementation can reduce the catabolism of exercise-induced protein in fasting athletes with relative effects on urea, creatinine, hypoxanthine, and xanthine oxidase. Therefore, this solution can be considered by coaches and athletes. It seems that coaches and athletes, using knowledge-based factors affecting exercise such as nutrition, can increase performance and reduce injury.

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Interactive Effects of Endurance Training With Royal Jelly Consumption on Motor Balance in an Experimental Encephalomyelitis Model

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ABSTRACT

Introduction: Multiple sclerosis (MS) is a chronic inflammatory demyelinating factor of the central nervous system that leads to decreased balance and increased risk of falls. The aim of this study was to investigate the interactive effects of endurance training (T) with royal jelly (RJ) consumption on the motor balance of rats with MS.

Methods: In this experimental study, 56 rats with MS (using complete Freund's adjuvant) were divided in 7 groups of eight animals, including: 1) control (MS), 2) Sham (royal jelly solvent), 3) 50 mg/kg RJ, 4) 100 mg/kg RJ, 5) T, 6) T+RJ50, 7) T+RJ100. Rats in the royal jelly consumption groups received the prescribed doses of royal jelly peritoneally each day for 5 weeks. Also, rats in the endurance training groups performed endurance training on a rat treadmill for five weeks, five sessions per week, each session 30 minutes at a speed of 11 m/min. At the end of 48 hours after the last training session and royal jelly consumption, the motor balance of rats was measured using a rotarod device. Also, the weight of brain cerebellum tissue was measured by a digital scale. The one-way analysis of variance with Tukey's *post hoc* test were used to analyze the findings ($P \leq 0.05$).

Results: The duration of motor balance in the T, T + RJ50 and T + RJ100 groups was significantly higher than the MS group ($P = 0.001$); also, in the T + RJ50 and T + RJ100 groups, it was significantly higher than the RJ50 and RJ100 groups ($P = 0.001$)

Conclusion: It appears that training and royal jelly consumption have an interactive effect on improving motor balance, and improving motor balance is training-dependent. Given the existence of effective physiological mechanisms, it seems necessary to conduct further studies by examining the pathological and physiological aspects.

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Introduction

Multiple sclerosis (MS) is a chronic inflammatory demyelinating factor of the central nervous system that is associated with inflammation of tissues and apoptosis of nerve cells . The prevalence of this disease in women is 2 to 3 times that of men and it often occurs in the age group of 20 to 40 years (1). MS is the most common neurodegenerative autoimmune neurological condition in young adults and affects more than 2.3 million people worldwide. Due to the high probability of disability and decreased nerve function, there are concerns about the role of exercise in MS patients with a fear of increased injury (2). MS is a multifocal inflammatory disease of the brain and spinal cord. The underlying cause of MS is unknown, although genetic and environmental factors have been shown to play a role (3). It is a neurodegenerative disease characterized by the formation of a lesion in the nerve center, inflammation, and destruction of myelin sheaths. Given that patients with MS have poorer balance in most field balance tests than healthy individuals, it seems that maintaining balance in patients with MS is of great importance (4). Although balance is defined as the ability to maintain a position to perform voluntary activities and deal with disturbances (internal and external) and, biomechanically, to maintain the body mass center within the level of reliance, having a natural balance requires coordination between different bodily systems such as the musculoskeletal, visual, atrial, nervous and

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cardiovascular systems. Balance disorder causes a person to reduce their social participation for fear of falling (5). Prescribing exercise is currently considered as a useful treatment strategy to minimize the lack of functional ability in chronic diseases. In this regard, it has been reported that both endurance and resistance training involve balance systems in patients with MS. Both types of training are effective in preventing falls, stimulating deep receptors, increasing muscle strength, flexibility and movement control (6).

Studies have been conducted in this regard, for example, a study by Hosseini et al. (2020) showed that induction of Alzheimer's with trimethyltin disturbed the motor balance of rats. However, performing eight-weeks of regular exercise improved the motor balance time of Alzheimer's rats (7); another study showed that rehabilitation exercise training improved brain function and improved balance and quality of life in patients with MS (Chen, 2021). In addition, the results of another study showed that balance exercises at home increased the quality of gait test, increased balance and improved the quality of life of men and women with MS (8).

Still again, in another study it was shown that aerobic training and Pilates resulted in a significant increase in serum BDNF in patients with MS (9). Studies have shown that proper nutrition along with exercise can have beneficial effects on disorders caused by MS. Royal jelly is a viscous substance secreted by young worker bees, containing 12-15% protein, 10-12% carbohydrates, 7-3% lipids (including sterols and fatty acids), minerals and vitamins. Royal jelly has been shown to have a variety of biological activities in various cells and tissues of the body, especially the nervous system. Royal jelly contains phosphorous compounds, especially acetylcholine, which is one of the carriers of neural messages from one cell to the next. Royal jelly increases oxygen delivery to brain tissue. Another component of royal jelly is 10-hydroxy-trans-2-decanoic acid (HDEA), an unsaturated fatty acid that facilitates neurogenesis by NSCs (Neural stem / progenitor cells). Royal jelly has also been shown to increase oxygen delivery to brain tissue (10).

Studies show that royal jelly also plays a role in improving neuronal cell functions by mechanisms similar to insulin-like growth hormone (IGF-1), growth hormone (GH), and improving neurotrophins (7). In this regard, the study of Hosseini et al. showed that consumption of 100 mg / kg RJ for eight weeks improved the motor balance of rats with Alzheimer's disease (7); royal jelly consumption also reduced muscle atrophy during aging and improved the quality of life of the elderly with MS (11).

Studies have shown that the use of antioxidants in different doses along with exercise training in conditions of neurological disorders can be a good way to improve the patients' health.

It also appears that keeping balance and muscle strength in people with diseases of the nervous system is an important factor in preventing reduced movement and physical activity. Therefore, the use of antioxidants along with exercise training can be a novel idea in the prevention of muscle atrophy and nervous system that needs to be investigated in more details.

In this vein, it seems that a combination of voluntary exercise with royal jelly supplementation can improve functional and behavioral disorders in MS patients (12). Therefore, the present study seeks to investigate the interactive effects of endurance training with royal jelly consumption on motor balance in an experimental encephalomyelitis model.

Material and Method

In this experimental study, fifty-six Sprague-Dawley female rats with an age range of 8-10 weeks and a weight range of 220-2200 g were provided from the Laboratory Animal Breeding Center of Marvdasht Islamic Azad University and kept in the laboratory for one week for adaptation.

During the research period, the animals were kept in standard conditions of light (12:12 hour dark-light cycle), temperature (22-24 ° C), and humidity (55-60%). Also, in this study, ethical considerations according to the ethical principles of working with animals of Marvdasht University and the Helsinki Agreement were observed.

On the eighth day, 20 guinea pigs were prepared from the Iranian Pasteur Institute to induce EAE. After anesthesia, the spinal cord of guinea pigs was extracted for use as antigen and immediately immersed in nitrogen tank and crushed. For homogenization, spinal cord tissue was mixed with an equal amount of normal saline and placed in a shaker at 5° C.

The homogenized solution was then converted to emulsion solution in a 1: 1 ratio with complete Freund`s adjuvant (CFA). To prepare this suspension, two glass syringes were connected by a stainless steel interface and equal amounts of adjuvant and homogenized spinal cord solution were used in two syringes.

After complete anesthesia of rats with ketamine and xylazine, 400 µl of the antigen and adjuvant mixture was injected subcutaneously in the dorsal and 100 µl into the cushion area of each animal with needle number 25 (1,13).

To diagnose induction of the disease, the daily morbidity process was evaluated and the morbidity scale was assessed based on the following: zero: no disease, 1: tail movement disorder, 2: tail paralysis, 3: gait disorder, 4: one-leg paralysis, 5: two-leg paralysis, 6: paralysis of all legs and hands, and 7: death (Mousavi, 2018; Abedi, 2017).

After ensuring EAE induction, 49 rats with EAE were divided into seven groups, including: (1) MS control, (2) sham, (3) 50 mg / kg royal jelly (RJ50), (4) RJ100, (5) aerobic training (T), (6) T + RJ50, and (7) T + RJ100. Also, 8 healthy rats were included in the healthy control (HC) group to investigate the effects of EAE induction on the research variables. Rats in the RJ groups, received peritoneally royal jelly from the Marvdasht Agricultural Jihad Center for 5 weeks daily at doses of 100 mg / kg and 50 dissolved in normal saline (14).

Also, rats in the training groups performed endurance training at a speed of 11 meters per minute on a special treadmill for five weeks, five sessions per week and each session for 30 minutes. The endurance training protocol started approximately 10 days after induction of the MS experimental model. To perform endurance training, first the rats were introduced to the treadmill every day for 5 to 25 minutes at a speed of 6 meters per minute and an inclination of 11 degrees for a week, then they performed endurance training at a speed 11 meters per minute every day for 30 minutes, for 5 weeks. One of the reasons for choosing this training protocol was the neuroprotective effects of this type of training on rats and mice with cognitive impairments in the experimental model of Parkinson's and encephalomyelitis (EAE) (15,16).

At the end of 48 hours after the last training session and royal jelly consumption, the motor balance of rats was measured using a rotarod device. Also, 24 hours later, rats were anesthetized with ketamine and xylazine and the cerebellar tissue of rats was extracted and measured by digital scales. In order to measure the motor balance, rats were first placed on the rotarod device for 3 minutes for familiarization and were trained to move on it according to the main protocol (at a rotation speed of 10 rpm with acceleration of 7 rpm^2) and 30 minutes later, the balance test was performed (at a speed of 10 rpm with acceleration of 7 rpm^2). The duration of the animal resistance to maintain balance on the wheel was recorded in seconds. The total duration of this test was considered to be 300 seconds. This test was measured three times for each rat with repeated intervals of approximately 30 minutes and their mean was calculated for analysis.

Statistical Analysis Procedure

The Kolmogorov-Smirnov test and one-way analysis of variance with Tukey's *post hoc* test were used to analyze the findings ($P \leq 0.05$).

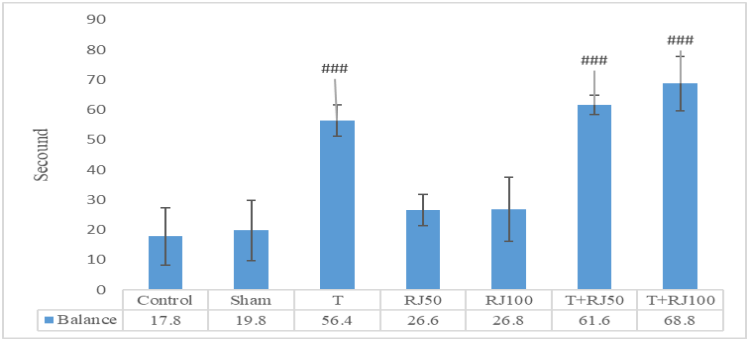

Figure 1. Balance levels in the research groups
($P \leq 0.001$) Significant increase compared to the control, sham, 50 mg / kg royal jelly consumption, and 100 mg/kg royal jelly consumption groups

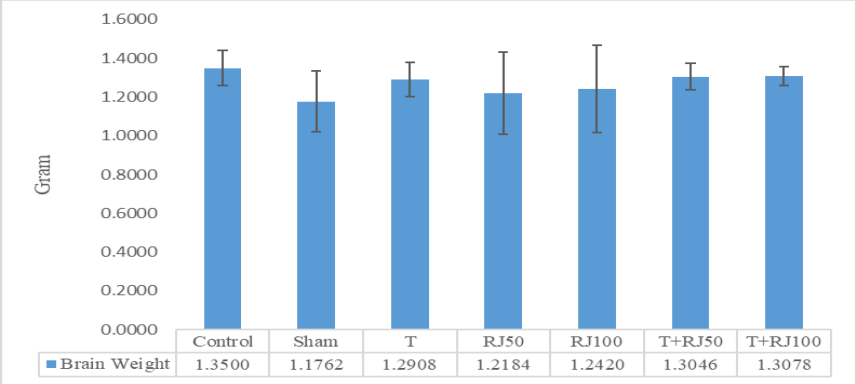

Figure 2. Brain weight levels in the research groups

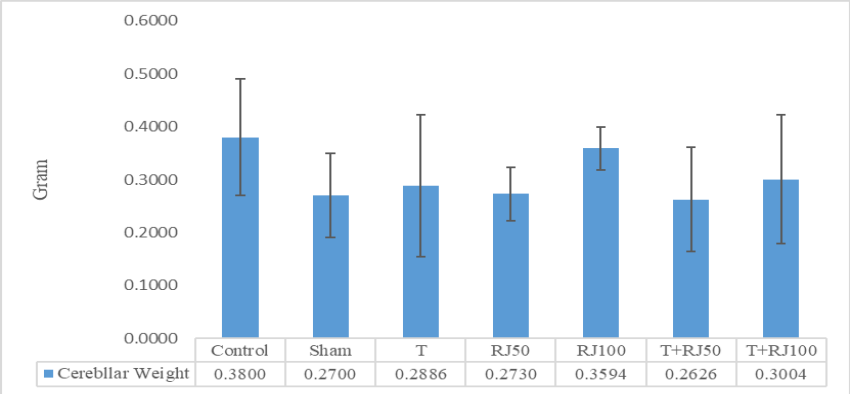

Figure 3. Cerebellum weight levels in the research groups

Results

The mean and standard deviation of motor balance, brain and cerebellum weight are reported in Figures 1 to 3, respectively. The results of one-way analysis of variance showed that there was a significant difference in the amount of motor balance in the 7 groups of the study (P = 0.001). However, there was no significant difference in the weight of the brain (P = 0.50) and cerebellum (P = 0.37) in the 7 research groups. The results of Tukey's *post hoc* test showed that the balance levels in the training, training + 50 mg / kg royal jelly consumption and training + 100 mg / kg royal jelly consumption groups were significantly higher than the control, sham, 50 mg / kg royal jelly consumption, and 100mg / kg royal jelly consumption groups (P = 0.001) (Figure 1).

Discussion

The results of the present study showed that 5 weeks of training improved balance in rats with MS, however it had no significant effect on the brain and cerebellum weight of rats with MS.

Studies show that with increasing inflammatory and oxidative factors, damage occurs to various parts of the central nervous system, so that these factors with damage to regions such as the motor cortex and pre-motor decrease motor power, decline the ability to perform more subtle actions, and decrease motor balance in patients with neurological disorders (7); in addition, aging and impaired physical fitness, weight gain, fat gain, decreased aerobic capacity, systemic inflammation, and nerve cell impairment can cause damage to motor neurons; also, systemic inflammatory factors even on the motor plate can disrupt the function of neurotransmitters and affect the patient's balance (7,17). On the other hand, using neurotoxins and modeling neurological disorders with toxins such as trimethyltin, adjuvant and myelin oligodendrocyte glycoprotein with increased oxidative stress, increased inflammatory factors, decreased neurotrophins, degradation of myelin and finally degradation of neurotransmitters in animal model may bring about disorders of the

central and peripheral nervous system, cause chronic pain and decrease balance (7,17).

Regarding the effects of exercise on MS patients, Perese Kila *et al.,* (2017) showed that regular moderate to high intensity exercise consistently attenuates the progression and pathological symptoms of EAE, thus indicating an important non-pharmacological intervention for the improvement of immune diseases such as MS (18). Also, the findings of the studies of Kargarfard and Shariat (2017) and Divasahaiam *et al.,* (2021) indicate the favorable effects of exercise on improving balance in patients with MS (9,19). In fact, sports activities improve the balance in these patients by improving the nervous system and prevent the occurrence of most disorders as well as problems of this disease such as falls. In this regard, Papalia *et al.,* (2020) showed that physical exercise is an effective treatment to improve balance and reduce falls in the elderly (20). Khani and Kazemi (2021) also reported that regular exercise balances the activity of the sympathetic nerves (21). In other words, it modulates the hypothalamic-pituitary-adrenal (APH) axis response and leads to increased adaptation mechanisms. Smith *et al.,* (2020) found that in MS, there is an imbalance between the levels of proinflammatory and anti-inflammatory cytokines, which is associated with higher levels of proinflammatory cytokines associated with demyelination (22). Physiological benefits of exercise include reduced levels of reactive oxygen species (ROS), modulation of cellular redox, reduction of neuroinflammation, reduction of inflammatory factors such as tumor necrosis factor alpha (TNF-α), interleukin-1 alpha (IL-1α), as well as increased brain-derived neurotrophic factor (BDNF). Also, the results of the present study showed that 5 weeks of 50 and 100 mg/kg royal jelly consumption had no significant effect on improving motor balance, brain and cerebellum weight of rats with MS. Regarding the therapeutic effects of RJ, it has been reported that royal jelly consumption can improve the liver enzymes of patients with MS (23). In addition, Deh Bozorgi *et al.,* (2020) showed that royal jelly consumption has a significant effect on increasing NGF gene expression in the hippocampal tissue of rats with Alzheimer's disease (24).

Royal jelly is mainly composed of proteins, sugars, fats (including sterols and fatty acids) and small amounts of mineral salts and vitamins. These substances have been shown to exhibit different pharmacological activities such as antitumor, antimicrobial, vasodilator, antihypertensive as well as growth stimulant, infections resisting, anti-hypercholesterolemic and anti-inflammatory activities. 10-Hydroxy-trans-2-decanoic acid (an unsaturated fatty acid) has been shown to be one of the fats in royal jelly, mimicking the effects of BDNF and possibly stimulating neurogenesis in the adult brain. Royal jelly is said to contain 10-hydroxy-2-decanoic acid (10-HDA). Because 10-HDA is an unsaturated fatty acid, it can cross the blood-brain barrier. 10-HDA has been reported to mimic the effects of BDNF and possibly stimulate neurogenesis in the brain (24).

In this regard, a study by Hosseini et al. (2020) showed that eight weeks of royal jelly consumption increased the motor balance of neurotoxin trimethyltin-induced Alzheimer's rats (7). Neurotoxin-induced damage and varying effectiveness of antioxidant supplements seem to be the reasons for the discrepancies in the results. Because adjuvant, with the mechanism of inflammatory factors, leads to the destruction of myelin, but trimethyltin has a role in neurological disorders by increasing the oxidative stress. However, this should be given more attention by future researchers. In addition, due to the insignificance of brain and cerebellum weight following the interventions of the present study, it seems that the period of this study was not sufficient for general and structural changes. None the less, regarding the interactive effects, the present study showed that although training + 50 mg/kg royal jelly consumption as well as training + 100 mg / kg royal jelly consumption had no significant effect on the brain and cerebellum of rats with MS, they had a significant effect on improving balance in rats with MS. Also, training + 50 mg / kg royal jelly consumption as well as training + 100 mg/kg royal jelly consumption had a greater effect on improving balance in MS rats than consuming 50 and 100 mg / kg royal jelly. Therefore, it seems that training with royal jelly consumption has interactive effects on improving motor balance in MS patients. Research limitations include the lack of ability to control physical activity outside of training time and failure to calculate calorie intake and consumption in rats during the study period;

therefore, it is suggested that in future studies, in addition to examining the effects of sports activities and royal jelly consumption, the amount of calories received and consumed during the research period should also be calculated.

Given the role of inflammatory factors in myelin destruction in the cerebellum and brain and the affectability of balance on these regions, it appears that the lack of measurement of these factors in the present study is one of the limitations of this study. Therefore, it is suggested that in future studies, in addition to motor balance, inflammatory factors should also be evaluated. Also, considering the role of brain volume and brain tissue weight, as well as due to the pathological and physiological reasons for increasing or decreasing the weight of the nervous system, it seems that lack of pathological measurments such as the percentage of healthy cells in different areas, lack of microscopic examination of oxidative damage, lack of examination of nucleus specificity as well as nerve cell size are some other limitations of this study. Therefore, it is suggested that in future studies, pathological studies be placed next to the field data.

Conclusion

According to the results of the present study, it seems that although training, training + 50 mg / kg royal jelly consumption and training + 100 mg / kg royal jelly consumption lead to improved balance in MS patients, training with royal jelly consumption has interactive effects on improving motor balance in MS and has more favorable effects than using royal jelly alone.

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The Occurrence and Toxicity of Dioxins and Dioxin-Like Polychlorinated Biphenyls in Foodstuffs Collected From Different Cities of Iran: A Systematic Review

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ABSTRACT

Dioxins compounds are persistent organic contaminants that adversely affect human health and the environmental system. Although the occurrence and toxicity of dioxins congeners are reported worldwide, their status in Iran is very scarce, and no study has been conducted to understand the evidence for occurrence and toxicity of dioxins compounds in Iran yet. Therefore, this systematic review provided a comprehensive report on the levels, occurrence, and health outcome effects of dioxins and dioxin-like Polychlorinated biphenyls (DL-PCBs) in foodstuffs collected from different cities of Iran. We used The Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) to design this review, and six databases (Scopus, Scientific Information Database (SID), PubMed, Web of Knowledge, Scopus, and Google Scholar) were searched from inception until January 2022. A total of 9 articles with data on 396 food samples were considered for the final report. The total toxicity equivalency quantity (TEQ) was ranged from 0.06 ± 0.02 to 15.72 ±16.38 and the mean total dioxin congeners levels in most of the samples were below the standard limits and the measured intake levels of dioxin congeners were in the safe level. Although most of the samples did not exceed the maximum allowable thresholds, 44% of studies reported that the concentration of dioxin compounds in most of their samples was above the thresholds defined by world health organization (WHO) (3 pg TEQ/g fat). Therefore, suitable policy actions and more attention are needed to control and reduce the emission of dioxin congeners and their associated risk factors.

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Introduction

Dioxins and dioxin-like Polychlorinated biphenyls (DL-PCBs) are the main groups of persistent organic pollutants (POPs) including 135 polychlorinated dibenzofurans (PCDF), 75 polychlorinated dibenzo-p-dioxins (PCDDs), and 12 polychlorinated biphenyls congeners, of which 30 have significant toxicity effect on human health [1, 2]. These compounds have a long half-life in the different media environments, and dioxins are highly persistent because of their high lipophilicity[3, 4]. Dioxins compounds are mainly generated by various anthropogenic activities such as industrial processes (manufacturing of chlorine bleaching of paper pulp, smelting, pesticides, and herbicides), waste incinerators (hospital and solid waste). Likewise, small quantities of dioxins can be generated by natural processes, including volcanic eruptions and forest fires [3, 5].

Decreasing dioxin exposure is the main public health target for reducing the type of disease. World Health Organization's International Agency for Research on Cancer (IARC) classified dioxin as a "known human carcinogen" according to human epidemiology data and animal data[4]. Long-term exposure of humans to the high concentration of dioxin is associated with an impairment of the endocrine system, nervous system, immune system, and reproductive functions [5]. Short-term exposure may be linked to skin lesions and altered liver function. Dioxin compounds formation is local, but their distribution in the world is global. The highest concentration of dioxins is found in the sediment, soil, and food supply (fish, meat, and shellfish), especially dairy products, while the lowest concentration is found in air, water, and plants [4, 6]. Several developed countries monitor the level of dioxin compounds in their food supply because early detection of pollutants led to preventing adverse effects on a larger scale [1, 7, 8]. It was evidenced that more than 90% of the exposure of humans to dioxins is through the

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food supply, mainly seafood, dairy products, and meat[6, 9]. Thus, it is critical to protect the food supply to reduce dioxin emissions. Appropriate practices and controls during the production of food supply, their processing, sale, and distribution are all necessary to produce safe food [3, 6].

In low- and middle-income countries like Iran, the available data related to the occurrence and toxicity of dioxins is very scarce yet, and we are not aware of any systematic research on the evidence for occurrence and toxicity of dioxins and DL-PCBs in the food supply[3, 10]. In Iran, several studies monitored the occurrence and toxicity of dioxins and DL-PCBs in environmental samples and their risk that influence human health. However, these studies are disconnected because no general view and systematic investigation on the situation of dioxins and DL-PCBs pollution in Iran. In consequence of adverse health effects posed by dioxins and DL-PCBs pollutants in the food supply, examining the exact concentration of these compounds and their possible health risks is essential to reduce their emission into the food supply and food chain, especially, seafood, meat, and, dairy products [3, 8]. Therefore, conducting a systematic review with the target to provide comprehensive data on dioxins and DL-PCBs' pollution levels and their distribution could be practical to improve food safety programs. This review was performed as a comprehensive database on the levels, occurrence, and health outcome effects of dioxins compounds in foodstuffs collected from different cities of Iran.

Materials and Methods

Study Design

The Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) was used as a framework to design this study[11]. The main outcome for this systematic review is the occurrence and concentration of dioxins (PCDF and PCDDs) congeners and DL-PCBs compounds in food supply (Table 1) and the level of health risk originating from the effects of dioxins compound. The main question in this study is what is the dioxin level and status in foodstuffs collected from Iranian market?

Search Strategy

We searched five databases were searched, including the scientific information Database (SID), PubMed, Web of Knowledge, Scopus, and Google Scholar were searched from inception until January 2022, from inception until January 2022. Each database was independently searched, and the references list of all studies was hand-searched to find relevant literature, which may be ignored during the search process. The main keywords related to the term "dioxins", "DL-PCBs", and "dioxin-like Polychlorinated biphenyls" were used. Then, these keywords were combined with the terms "foodstuffs", "meat", "dairy products", "fish", "meat", "vegetable", "health risk", and "Iran.

Table 1. Congener's distribution of dioxins, furans and dioxin-like PCBs

Congener	WHO_{2005}-TEF	Congener	*WHO_{2005}-TEF
Polychlorinated dioxins **(PCDDs)**		**Non-*ortho*** polychlorinated biphenyls (PCBs)	
2,3,7,8-TCDD	1	PCB-77	0.0001
1,2,3,7,8-PeCDD	1	PCB-81	0.0003
1,2,3,4,7,8-HxCDD	0.1	PCB-126	0.1
1,2,3,6,7,8-HxCDD	0.1	PCB-169	0.03
1,2,3,7,8,9-HxCDD	0.1	**Mono-*ortho*** polychlorinated biphenyls (PCBs)	
1,2,3,4,6,7,8-HpCDD	0.01	PCB-105	0.00003
1,2,3,4,6,7,8,9-OCDD	0.0003	PCB-114	0.00003
Polychlorinated dibenzofurans **(PCDFs)**		PCB-118	0.00003
2,3,7,8-TCDF	0.1	PCB-123	0.00003
1,2,3,7,8-PeCDF	0.03	PCB-156	0.00003
2,3,4,7,8-PeCDF	0.3	PCB-157	0.00003
1,2,3,4,7,8-HxCDF	0.1	PCB-167	0.00003
1,2,3,6,7,8-HxCDF	0.1	PCB-189	0.00003
2,3,4,6,7,8-HxCDF	0.1	PCB-180	0.00003
1,2,3,7,8,9-HxCDF	0.1		
1,2,3,4,6,7,8-HpCDF	0.01		
1,2,3,4,7,8,9-HpCDF	0.01		
1,2,3,4,6,7,8,9-OCD	0.0003		

*Toxic equivalency factors (TEF) established by WHO in 2005

Inclusion and Exclusion Criteria

In this systematic review, studies were included conducted on detecting the concentration of dioxin compounds in foodstuffs of Iran, publishing in the Persian or English language, reporting average concentration of dioxins and their health risk level, and focused the exposure to dioxins compounds and their adverse effects on human health in Iran. The eligibility of studies and their selection were conducted by monitoring the abstracts, titles, and full texts of manuscripts. All duplicate studies were excluded. There was no time limit in this review.

Data extraction and quality assessment sources

The relevant data were extracted based on the publication author and year, study aim, sample size, study location, time of sampling, analytical technique, type(s) of samples, type of dioxin concentration, and potential contamination. In this study, was used to examine the quality of the selected studies[12]. This checklist includes 6 questions that were designed based on a "yes" or "no" response to estimate a general quality score for each study (Table 2).

Table 2. Quality assessments of the included studies based on Newcastle–Ottawa scale

Code	Authors (years)	Standard sampling protocol indicated	Period of sampling indicated	QA/QC conducted	All objectives achieved	Report on Dioxin sources	Report on mean Dioxin levels	Overall quality score
1	Zeynab Samadi Jirdeh, 2013[17]	No	Yes	Yes	Yes	No	Yes	4/6
2	Mohsen Rezaei, et al, 2012[18]	Yes	Yes	Yes	Yes	No	Yes	5/6
3	Reza Ahmadkhaniha et al, 2017[13]	Yes	Yes	Yes	Yes	No	Yes	5/6
4	Zeinab Samadi Jirdeh et al, 2013[18]	No	Yes	Yes	Yes	No	Yes	4/6
5	Azadeh Nakisa et al, 2012[19]	Yes	Yes	Yes	Yes	No	Yes	5/6
6	Ayub Ebadi Fathabad et al , 2019[20]	Yes	No	Yes	Yes	No	No	3/6
7	Seyede Pegah Azarchehry et al, 2021[14]	Yes	Yes	Yes	Yes	Yes	Yes	6/6
8	Sara Bayat et al., 2011[15]	Yes	No	Yes	Yes	No	Yes	4/6
9	Shakoorzadeh, A. et al., 2017[16]	No	Yes	No	Yes	Yes	Yes	4/6

Results

Search Outcome and Quality Assessment

In this study, we selected 47 published articles for quality assessment from which 9 articles with data on 396 food samples were considered for the final report because these articles met inclusion criteria (Figure 1, Table 2). All data were extracted from 7 different provinces of Iran as follows: Tehran (n= 4 studies)[13-16], Qazvin (n= 2)[17], Khuzestan Province (n=2)[18, 19], provinces of North of Iran (n=2)[14, 20], and provinces of Central Iran (n=1)[14]. Our results showed that the highest number of studies was conducted in Tehran (capital of Iran) [13-16] (Table 3). According to the quality assessment information, 44% of the eligible studies (n=4) were in good-quality categories and rated from 5 to 6 out of 6[13, 14, 18, 19]. These studies used valid protocol and methods for sampling and measurement of dioxin congeners provided reliable results and all objectives were achieved in their studies. Likewise, 44% of the eligible studies (n=4) were in moderate-quality categories with an average score of 4[15-17], and about 12% of the studies showed low quality (from 2 to 3) [20] because non-indication of average concentration of dioxin compounds, as well as period of sampling and, were not clear in their researches. The first studies related to evaluating dioxin in foodstuffs were published in 2011[15] (Table 2).

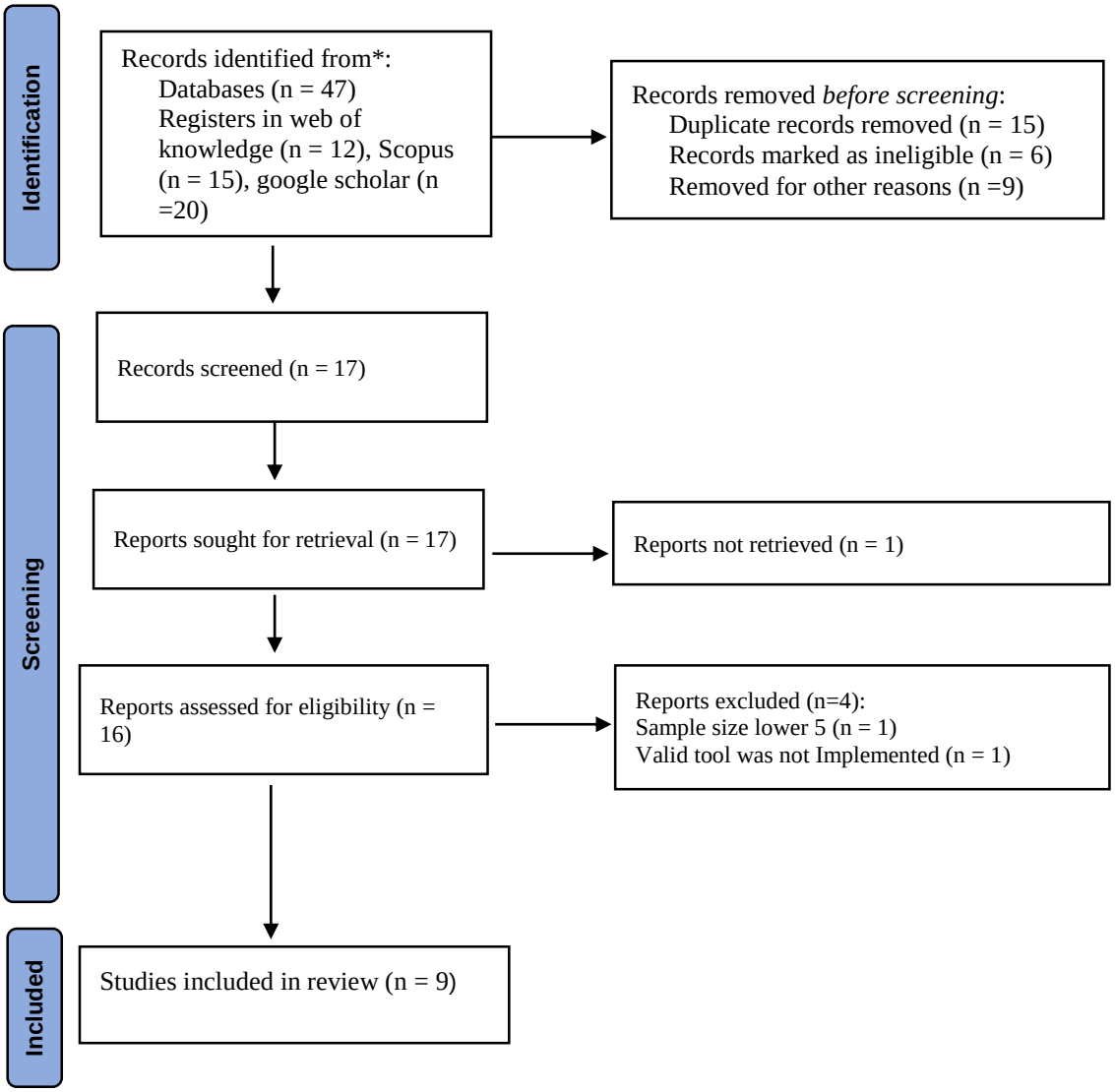

Figure 1. PRISMA flowchart for studies selection

Study Samples Characteristics

Of 396 food samples included studies, 245 (62%) samples included raw or fresh milk (28%) [14, 17, 19] and pasteurized milk (34%) [13-15, 17, 18], 125 (32 %) samples included fishes of Caspian Sea [20], and 26 (6%) samples included pinto beans [16](Table 3). Most of the studies (67%) have measured Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) as dioxin compounds[13, 14, 16-19] and 5 studies examined DL- PCBs[13-15, 17, 18, 20]. In this review, 6 (67 %) studies measured dioxin compounds using Gas Chromatography-mass spectrometry (GC–MS) or by High-Resolution Gas Chromatography coupled with High-Resolution Mass Spectrometer (HRGC/HRMS)[13, 14, 16, 17, 20] . Likewise, 3 studies measured dioxin compounds by using liquid chromatography (HPLC)[15, 17-19]. Based on our findings, HRGC/HRMS is the most common valid method, which was recently implemented to measure dioxin congeners in Iran, while other methods were rarely used.

Table 3. Characteristics of included studies

Code	Study area	Sample/ sample size	Analytical Methods	Concentration (pg TEQ/g fat)			Outcomes
				PCDD/F	DL-PCBs	Total TEQ	
1	Qazvin	Row milk (n=19)	GC-MS/ Kawashiro *Methods*	15.3 ±16.18 (2.6–73.7)	0.40± 0.304 (0-0.92)	15.72 ±16.38 (2.5–74.57)	Mean of total TEQ is above the thresholds defined by WHO/2,3,7,8 -TCDD, 1,2,3,7,8 –PeCDD and 1,2,3,4,7,8 –HxCDD were the dominant congeners
2	Khuzestan Province	Pasteurized milk (n=45)	HPLC/ A liquid-liquid extraction	4.02 ±0.21 (0.59-3.17)		4.02 ±0.21	Concentrations of dioxins in summer were more than other seasons/2,3,7,8 -TCDD and 1,2,3,4,7,8 –HxCDD were the dominant congeners/Mean of total TEQ is above the thresholds in all samples.
3	Local market in Tehran	10 brand Pasteurized milk (n=20)	GC-MS-Ms		0.49 ± 0.12	0.49 ± 0.12	Mono-ortho congeners were more abundant than the non-ortho ones and congener 118 and 180 was the dominant chemicals (over 80%)/Mean of total TEQ is lower the thresholds in all samples/ There is no significant difference between seasons /Non-carcinogenic risk was in safe level (HQ < 1).
4	Qazvin	Pasteurized full-fat milk (n=7)	*HPLC/ HRMS	0.74±0.30 (0.34-1.10);	0.137±0.040	0.88±0.34 (0.36-1.14)	Mean of total TEQ was lower than the thresholds/ 2,3,7,8-TCDD and 1,2,3,4,7,8-HxCDD were dominant
5	Khuzestan Province 2012	Fresh milk (n= 60)	HPLC/ Kawashiro	1.77 (0.35 - 3.03)		1.77 (0.35 - 3.03)	The mean total content was below the recommended threshold /2,3,7,8-TCDD and 1,2,3,4,7,8-HxCDD were dominant /There were statistically significant between differences seasons with highest concentration in summer and the lowest in winter.
6	Caspian sea	Fish (n=125)	HRGC/HRMS/ USEPA 1668		0.59 – 0.82	0.59 – 0.82	The mean total content was below the recommended threshold/ Mono-ortho congeners were more abundant and congener 118 and 180 was the dominant chemicals (over 80%)/Non-carcinogenic risk was in safe level (HQ < 1)/ Cancer risk exceeded the value of 1×10^{-6}
7	Central Iran	Raw milk (n= 12)	HRGC_HRMS/EC/252/2012	2.42 ± 0.97 0.63-4.08		2.42 ± 0.97 0.63-4.08	The mean concentration is higher in some samples
7	North of Iran	Raw milk (n= 9)	HRGC_HRMS/EC/252/2012	1.13 ± 0.4 0.63-1.89		1.13 ± 0.4 0.63-1.89	The mean total content was below the recommended threshold in all samples/2,3,7,8-TCDD and 1,2,3,4,7,8-HxCDD were the dominant chemicals
7	Tehran	Pasteurized milk (n= 15)	HRGC_HRMS/EC/252/2012	0.06 ± 0.02 0.03 to 0.1		0.06 ± 0.02 0.03 to 0.1	The mean total content was below the recommended threshold in all samples
8	Tehran	Pasteurize milk (n=58)	AOAC method		0.75± 0.24	0.75± 0.24	The mean total content was below the recommended threshold in all samples
9	Tehran	Pinto beans (n= 26)	GC/ MRLS METHOD	2.13 ± 0.02		2.13 ± 0.02 0.62 to 3.67	Samples are cultivated near factories and are exposed to their wastewater were made toxic by dioxins; while dioxins was not observed in the other samples, which were located far from factories

PCDD/Fs: Polychlorinated dibenzo-p-dioxins and dibenzofurans; and DL- PCBs: dioxin-like polychlorinated biphenyls; TEQ: Toxic equivalent/ HRGC/HRMS: High Resolution Gas Chromatography coupled with High Resolution Mass Spectrometer; *the national standard of Iran with no. 8262/2005; HPLC: High Performance Liquid Chromatography.

Likewise, 3 studies measured dioxin compounds by using liquid chromatography (HPLC)[15, 17-19]. Based on our findings, HRGC/HRMS is the most common valid method, which was recently

implemented to measure dioxin congeners in Iran, while other methods were rarely used. Three (33%) and two studies (22%) extracted dioxin compounds using the method of Commission Regulation (EU) No 252/2012, and Kawashiro et al., 2008 method, respectively [13, 14, 16, 17, 20]. Other studies used different protocols for the extraction process [15, 17-19]. Our finding showed that 44% of the surveys were performed in Tehran[13-16] and the sample size was ranged from 7 to 125 (Table 3).

Dioxin Concentration in Samples

According to our findings in this review, the concentration of PCDD/F and DL-PCBs ranged from 0.06 ± 0.02 to 15.3 ±16.18 and from 0.137±0.040 to 0.75± 0.24 pg TEQ/g fat, respectively. The total TEQ was also ranged from 0.06 ± 0.02 to 15.72 ±16.38. Of the 9 studies that measured concentration of dioxin in foodstuffs collected from different cities of Iran, 4 studies (44%) reported that the concentration of dioxin compounds in most of their samples were above the thresholds defined by WHO (3 pg TEQ/g fat)[15-18]. Most implicated dioxin included 2,3,7,8 -TCDD, 1,2,3,7,8 –PeCDD and 1,2,3,4,7,8 –HxCDD were the dominant congeners[14, 17-19] (Table 3).

Risk Assessment of Dioxin Congeners

In this systematic review, only two studies examined carcinogenic and non-carcinogenic risks (CR) in foodstuffs samples[13, 20]. This study showed reported acceptable or negligible non-carcinogenic risk or hazard quotient (HQ < 1) through consumption of fish in Caspian Sea sample and pasteurized milk. However, carcinogenic risk exceeded the threshold value of 1×10-6 in fish samples (Table 3).

Discussion

The primary aim of this systematic review was to evaluate the levels, occurrence, and health outcome effects of dioxins and DL-PCBs in foodstuffs collected from different cities of Iran. In fact, we tried to conduct systematic research to verify whether the level of dioxins and DL-PCBs in the foodstuffs samples comply with WHO and European Union (EU) maximum allowable thresholds for milk (3 pg TEQ/g fat) and other foodstuffs (0.492 pg/g of fat)[2, 4]. This is the main question that must be replied to before deciding which measures are practical to reduce and control dioxins congeners in the food chain and environment.

In the present study, 9 published articles met inclusion criteria identified that measured dioxins and DL-PCBs in foodstuffs marketed Iran; among them, only two studies estimated the carcinogenic and non-carcinogenic risk effect of dioxins and DL-PCBs exposure on human health [13, 20]. Our finding showed that dioxins and DL-PCBs concentration in most of foodstuffs samples (56%) were below the thresholds defined by WHO and EC legislation [13-15, 17, 18, 20]. These studies examined dioxin congeners in pasteurized milk and fresh milk, and fish from 2011 to 2019. Overall dioxins level in these samples ranged from 0.06 to 0.88 pg TEQ/g fat. Therefore, it seems that the consumption of these samples which are commercially available in Iranian markets is not a threat for consumers in Iran. The mean of the sum of PCDD/F and DL-PCBs in milk products (n= 5640) which were collected in different European countries ranged from 0.07 to 5.68 pg TEQ/g fat[2]. According to our findings, the concentration of PCDD/F and DL-PCBs in most of the foodstuffs samples in Iran is significantly lower than the EU samples[2, 4]. Likewise, contamination levels observed in this review are similar to those reported by some other researchers: 0.65 in Catalonia, 0.43 in the United Kingdom, 0.31 in Sweden, 0.18 in Greece, and 0.16 pg/g of fat in Finland [1, 2, 13].

Although most of the samples did not exceed the maximum allowable thresholds provided by WHO and EU Regulation, 4 studies (44%) on pasteurized milk, raw milk, and pinto beans reported that the concentration of total dioxin compounds in most of their samples were above the standard thresholds for milk [15-18]. Dioxin levels were ranged from 2.42 to 15.72 pg TEQ/g fat. The high concentration of dioxin congeners in these samples may occur via different pathways. The main pathways of dioxin compounds are related to animal feed and emission of industrial wastewater in environmental media (air, soil, and water)[7, 14]. All of these studies on milk were conducted in industrial cities in Iran (Qazvin and Khuzestan) where the main oil gas and petrochemical industries were

developed[17, 18]. Likewise, other industries related to the production of ferrous and nonferrous metal, mineral products, open burning processes, and chemicals are more abundant in these cities compared to other cities in Iran [5, 18]. It was evident that from 1990 to 2010, ferrous/nonferrous metal production and open burning processes significantly contributed to 24.4% to 32.2% and 45.8% to 35.7% of the total PCDD/PCDFs emissions into the environment of Iran[5]. Likewise, the study on pinto beans samples showed that samples are cultivated in farms where located near factories were significantly toxic by dioxins while dioxins were not observed in the other samples, which were located far from factories[16]. Therefore, the observations confirm that industrial wastewater and animal feed have a significant contribution to food contamination. This result is in agreement with findings from other studies on dioxin compounds that were conducted in other countries such as China, the Brazilian citrus pulp, and the Belgian dioxin crisis, which show, small industries are able to cause severe health consequences if a high concentration of pollutants in foodstuff is detected quite late in processed food of animal origin on the market[1, 2]. Considering the high amount of this product on the national or international market, it is essential to pay more attention to the contamination of dioxin compounds in feed, particularly in industrial cities of Iran.

Health risk assessment examines the effect of hazard, risk level, and exposure, according to the potential toxicity of dioxin pollutants, the severity of exposure, and risk characterization[10]. Based on our results on risk evaluation for dioxin compounds, 2 studies reported safe risk levels via consumption of pasteurized milk and fish in the Caspian Sea[20]. However, studies on dioxin risk and its effect on human health are limited in Iran; therefore, conducting more studies is important to better understand a potential health risk related to dioxin contamination in foods products.

The highest contribution to the total concentration of dioxin compounds is from 2,3,7,8-TCDD , 1,2,3,4,7,8-HxCDD, and 1,2,3,7,8 – PeCDD [14, 17-19]. Among DL-PCBs, Mono-ortho congeners were more abundant than the non-ortho ones and congener 118 and 180 showed the highest concentration (over 80%)[13, 20]. A similar dioxin profile was observed in dairy products examined in Italy [1, 8]. Likewise, several studies on food samples reported that the mono-ortho congeners were more abundant than the non-ortho congeners in dairy products[2, 9]. This same profile of the dioxin pattern could be due to the same sources of emissions into the environment and food chain including the presence of similar factories and industries in different countries and the use of the same source of crop and silage for cow feeding [2, 9, 20].

In addition, seasonal variations were reported for dioxin levels in tested samples with the highest concentration in summer and the lowest in winter[18, 19]. However, one study reported an insignificant difference between seasons[13]. These differences between seasons could be due to differences in climate conditions (atmospheric deposition, dust storm, and dust particles), air pollution conditions, the status of soil contamination, and the amount of fertilizers that are used in agricultural activities[6, 12]. However, seasonal trends need repeated tests throughout the year.

Conclusion

This present study tried for the first time systematically reviewed the levels, occurrence, and health outcome effects of dioxins and DL-PCBs in the Iranian market, which is important to better understand the situation of dioxin pollution in a national context. This present review showed that the mean total dioxin congeners (PCDD/F and DL-PCBs) levels in pasteurized milk and fresh milk, and fish in most of the samples were below the standard limits defined by the WHO or EU legislation and the measured intake levels of PCDD/F and DL-PCBs were in the safe level. However, our findings highlighted samples were collected from industrial cities of Iran are significantly toxic by dioxin congeners because the concentration of total dioxin compounds in their samples was above the standard limitation. Considering the existence of several sources for dioxin pollution, their adverse effects on human health and the

ecosystem, and their bio-accumulative properties, suitable policy actions, and more attention are needed to control and reduce the emission of dioxin congeners and their associated risk factors. Likewise, we recommended continuous monitoring of dioxin compounds in foodstuffs and environment media especially in industrial reigns in Iran. This residual monitoring is useful in controlling emission points for ensuring the safety of food. Further studies on other foodstuff-based repeated analyses in different seasons are suggested to find out the spatial and temporal emission trends of dioxin congeners.

Abbreviations

Dioxin-like Polychlorinated biphenyls (DL-PCBs); Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA); Scientific Information Database (SID); toxicity equivalency quantity (TEQ); world health organization (WHO); persistent organic pollutants (POPs); polychlorinated dibenzofurans (PCDF); polychlorinated dibenzo-p-dioxins (PCDDs); Cancer Risk (CR); hazard quotient (HQ); European Union (EU).

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Conflict of Interest

There is no conflicts of interest in this work.

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JOURNAL OF NUTRITION FASTING AND HEALTH

Estimating the Years of Life Lost and Mortality Caused By COVID-19 in Mashhad, the Second-Largest City in Iran

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ABSTRACT

Introduction: The disability-adjusted life years (DALY) may provide a better indicator of the burden of disease than mortality. This study provides the estimates of both Years of Life Lost (YLL) and COVID-19 mortality in Mashhad.

Methods: This cross-sectional study was carried out in Mashhad, the second-most populous city in Iran. The mortality data in the population served by Mashhad University of Medical Sciences (MUMS) were extracted from the national mortality data system. In this system, all deaths in the whole of the country are recorded based on the International Classification of Diseases, tenth edition (ICD10). The data in the current study comprised all cases of death recorded in the population under the auspices of MUMS from January 21st to April 19th 2019 and 2020 which were transferred to SPSS software after sorting. The method recommended by the World Health Organization (WHO) was used for the estimation of YLL.

Results: The YLL per 1000 caused by COVID-19 was 1.2 years and increased with age, and was higher in men (1.6 years) compared to women (0.9 years). The incidence of COVID-19 was higher in the urban area (9.8%) compared to rural areas (4.8%). During the study period, COVID-19 was the fourth most prevalent cause of death in Mashhad after cardiovascular disease (35.4%), cancer (12.9%) and respiratory disease (10.6%).

Conclusions: The YLL per 1000 caused by COVID-19 was 1.2 years, increased with age and was greater in males than in females.

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Introduction

The spread of coronavirus disease 2019 (COVID-19) in Wuhan, China, has swiftly grown into a worldwide public health crisis, which was declared as a pandemic by the WHO. COVID-19 is caused by the SARS-CoV-2 virus, from the same family as the coronaviruses that previously caused severe acute respiratory syndrome (SARS-CoV) and Middle East respiratory syndrome (MERS-CoV). COVID-19 is a large virus (120 nm) and is enveloped, with a positive-sense single-stranded RNA. It is a highly transmittable

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virus that has spread quickly and efficiently. The majority of patients with the COVID-19 virus will endure minor to moderate breathing problems that improve without any specific treatment. However, the senile citizens and people with underlying diseases like chronic respiratory disease, diabetes, cardiovascular disease and cancer are more likely to develop serious illnesses. More than six million cases have been confirmed globally, and the number of deaths now exceeds 391,000. The pandemic has caused severe strain to health systems, and in some countries, there have been deficiencies in critical supplies. At present, there is no specific treatment for the disease, although steroid treatment and antiviral therapies have been shown to reduce mortality, and research teams around the world have joined forces to develop an effective vaccine (1).

The World Health Organization (WHO) initially estimated the mortality rate from COVID-19 to be 2% at a press conference on Wednesday, January 29, and then revised this to, about 3.4% of reported COVID-19 cases (2). However, the reported case mortality rates for COVID-19 vary from 0.27% to 10%, possibly dependent on the testing strategy, demographic differences and the presence of underlying co-morbidities (3).

Researchers at Johns Hopkins University have estimated the rate of death of COVID-19, or the known deaths divided by confirmed cases, was more than ten percent in Belgium, France, Italy, United Kingdom, Hungary, Netherlands, Sweden, Spain and Mexico (4).

Iran is a developing country in the Middle East, at the intersection between Europe and Asia (5). In the Iranian population, the main causes of death include motor vehicle accidents, cardiovascular diseases, intentional and unintentional injuries and cancers. Globally, cardiovascular disease (CVDs), cancer, diabetes and chronic lung diseases are the major causes of death (6).

According to a report of WHO from Feb 19 to 3 Oct 2020, more than 464,596 confirmed cases of COVID-19 with 26,567 deaths were recorded in the Islamic Republic of Iran (7). In Iran, the first case of coronavirus was diagnosed on 19 February 2020, in the city of Qom (8). The virus may have been brought into the country by a trader from Qom who had journeyed to China (9). By March 22, 2020, Tehran had the highest rate of infection among Iranian cities with 5,098 confirmed cases, followed by Isfahan with 1,979, Mazandaran with 1700, Gilan with 1191, Qom with 1178, and Alborz with 1177 cases (10).

According to WHO, mortality does not give the best overall estimate of the disease burden of individuals in various demographic groups. The overall disease burden is measured by the disability-adjusted life year (DALY). A time-based indicator, it merges years of life lost due to early death (YLLs) and years of life lost due to ill-health or disability (YLDs) (11). It developed in the 1990s by the Harvard School of Public Health, the WHO and the World Bank, it is assessed as the DALYs (12).

This study seeks to present an estimate of the years of life lost and mortality induced by COVID-19 to date in Mashhad, as the second-most populated Iranian city and the capital of Khorasan Razavi province in the northeast of Iran with a population of approximately 5085000 people.

Material and Methods

This cross-sectional study was confirmed by the Research Council of the Mashhad University of Medical Sciences, Mashhad, Iran (ID: 990171). Moreover, the Ethical Committee of Mashhad University of Medical Sciences endorsed the study (IR.MUMS.REC.1399.239)

Study Population

Mortality data in the population served by the Mashhad University of Medical Sciences (MUMS) were extracted from the national mortality data system. In this system, all deaths in the whole of the country will be recorded after completing the death registration form by trained physicians and determining the primary, secondary and final cause based on the International Classification of Diseases, tenth edition (ICD10) by medical record experts. The data of this study included all cases of death recorded in the population under the auspices of MUMS from January 21th to April 19th in 2019 and 2020 which was extracted from the database as an Excel file.

DALY Calculation

The methods of the World Health Organization for estimation of Years of Life Lost (YLL) were used in the current study (13, 14). As a summary indicator of premature mortality, YLL evaluates the potential years of life lost as a result of premature mortalities. This measure also accounts for the age of death, assigning further weight to deaths at a younger age (15). In other

words, YLL is obtained by multiplying the number of deaths by normal life expectancy at the age of death. The normal life expectancy used of YLL for different age groups is similar to deaths in all parts of the world, which is also adopted to estimate Disability Adjusted Life Years (DALY). Additionally, for the latter, non-uniform age weights (lower weight assigned to years lived at young and old ages) and 3% time discounting were used. In this way, the death of a newborn is identical to 33 YLL, and deaths at ages 5 to 20 corresponds to approximately 36 YLL (16). The formula used for estimation of the YLL for a given cause, sex or age, is:

YLL = N x L

Where: N is number of deaths and L is standard life expectancy at age of death (in years)

The number of deaths due to COVID 19 in the target population served by MUMS were extracted from the national mortality data system. The L values used in the above formula were available in disease burden studies as standard for the whole of the world (16) which is also used in the current study.

The indicators were calculated after cleaning and modifying the obtained data and having all the items using Excel software (version 2017). WHO declares that the Global Burden of Disease (GBD) methods could be utilized at national or sub-national scales and relative to the health values of that country (17). So the standard DALY calculation template was obtained from WHO (18) which considers the standard life expectancy for five-year age groups suggested by WHO, standard age weights (beta=0.04), standard discount rate (0.03), and standard age weights (C=0.1658).

SPSS16.0 software was used for statistical analyses (SPSS Inc., Chicago, Ill., USA). Descriptive analysis involved percentage and frequency for qualitative variables and standard deviation and mean for the quantitative variable. For the comparison of the death rates in 2019-2020 period, Chi-square test was used. A p-value< 0.05 was considered significant.

Table 1. The years of life lost (YLL) due to Coronavirus disease 2019 in Khorasan Razavi Province

	Males		Females		Total	
Age Groups (years)	**YLL (years)**	**YLL per 1000**	**YLL (years)**	**YLL per 1000**	**YLL (years)**	**YLL per 1000**
0-4	30	0.1	31	0.1	61	0.1
5-14	-	-	-	-	-	-
15-29	108	0.2	113	0.2	221	0.2
30-44	737	1.0	125	0.2	861	0.6
45-59	1,042	2.9	531	1.5	1,573	2.2
60-69	1,089	8.5	752	5.5	1,841	7.0
70-79	643	11.2	476	7.7	1,119	9.4
80+	436	11.8	253	7.2	690	9.6
Total	4,086	1.6	2,280	0.9	6,365	1.2

YLL: years of life lost

Table 2. Comparison of the causes of death in 2019 and 2020 in Khorasan Razavi Province

The reasons for death		**January 21th to April 19th in 2019**	**January 21th to April 19th in 2020**	**P-Value***
Total	**Other reasons**	5446(100.0)	5399(91.2)	<0.001
	Covid-19	0(0.0)	518(8.8)	
Rural areas	**Other reasons**	1200(100.0)	1144(95.2)	<0.001
	Covid-19	0(0.0)	58(4.8)	
Urban areas	**Other reasons**	4214(100.0)	4164(90.2)	<0.001
	Covid-19	0(0.0)	454(9.8)	
Male	**Other reasons**	3102(100.0)	3066(90.0)	<0.001
	Covid-19	0(0.0)	340(10.0)	
Female	**Other reasons**	2342(100.0)	2330(92.9)	<0.001
	Covid-19	0(0.0)	178(7.1)	

Data represented as Frequency (percentage); Statistical comparison was done with Chi-square test

Table 3. Ranking of major causes of death in Khorasan Razavi Province

The reasons for death	January 21th to April 19th in 2019		January 21th to April 19th in 2020	
	Frequency	Percentage	Frequency	Percentage
Cancer	822	15.1	764	12.9
Endocrine, nutritional and metabolic diseases	318	5.8	263	4.4
Diseases of the circulatory system	1992	36.6	2093	35.4
Diseases of the respiratory system	575	10.6	629	10.6
Certain conditions originating in the perinatal period	345	6.3	269	4.5
COVID-19	-	-	518	8.8
Other diseases	1394	25.6	1381	23.4
Total	5446	100	5917	100

Results

We found that the overall YLL per 1000 caused by COVID-19 was 1.2 years. This increased with age and was higher in men (1.6 years) compared to women (0.9 years). However, the YLL was the same in males and females aged <30 years old, whereas it was higher in men than women >30 years old (Table 1).

A total of 5917 people deceased in the target population under MUMS supervision from January 21st to April 19th, 2020. Of this figure, 8.8% (n=518) was attributable to COVID-19. The results showed that the COVID-19 incidence was higher in the urban area (9.8%) than rural ones (4.8%) (Table 2). During the same period in 2019, the mortality was 5,446 individuals in Mashhad.

As shown in Table 3, COVID-19 was the fourth most common cause of death in Mashhad after cardiovascular disease (35.4%), cancer (12.9%) and respiratory disease (10.6%).

Discussion

A total of 5917 subjects have died in Mashhad from January 21th to April 19th in 2020 out of whom 8.8% (n=518) was due to COVID-19. In the current study, COVID-19 was the fourth major cause of death in Mashhad after cardiovascular disease, cancer and respiratory disease. The results showed that the COVID-19 incidence was greater in the urban area (9.8%) than in rural areas (4.8%). The results showed that YLL per 1000 caused by COVID-19 was 1.2 years. In addition, it has been augmented by aging. YLL per 1000 caused by COVID-19 was higher in men (1.6 years) compared to women (0.9 years).

Since the start of the COVID-19 pandemic, there have been limited data regarding its' disease burden. Among the few published studies, Nurchis et al., have assessed the socio-economic effect of COVID-19 in Italy using the estimation of DALYs. The total DALYs was 2.01 per 1000 persons. These results, similar to our findings, reported the highest DALY rate in subjects in the age group of 80-89 years (19). In Italy in the first quarter of 2020, 54% (13,710) of additional deaths were attributed to COVID-19 diagnosed deaths (20). In Italy, about 85% of all COVID-19-caused deaths were in subjects aged ≥ 70 years. Only 1.1% of the deaths were reported in at ages < 50 years. The potential years of life lost (PYLLs) was more significant in the age group of 60–69 years.

The PYLLs were 5 times higher in the US than in Italy. In Germany, the PYLLs were 4 times higher than in Italy. In Germany, the older age groups had the greatest mortality rate too. The total PYLL in Germany was also lower than in Italy and the USA (21). The standardized PYLL rates suggested that the PYLL rates were approximately 4 times higher in Italy. In the US, they were 23, 25, and 18 times higher at age 70, 75, and 80 years, respectively, as opposed to Germany (21).

The use of DALYs to assess the burden of disease may be used to support Governments to determine resource allocation and health policy planning made to deter emergency incidents of such a grand scale [19].

As far as the authors' are concerned, this is the first study regarding the COVID-19 burden of disease from eastern of Iran. Although the results can provide a clear picture of the existing condition, they are highly prone to variations due to incidence peaks of COVID-19 in society. Therefore, calculating these indices at the anniversary of COVID emergence is highly suggested.

Conclusions

The YLL per 1000 attributed to COVID-19 in Mashhad was 1.2 years which increased with age and was higher in men than women.

Conflicts of Interest

The authors confirm no conflict of interest.

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Effects of High Intensity Interval Training and Intermittent Fasting on *VEGF* Gene Expression in the Cardiac Muscle Tissue of Obese Male Rats

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ABSTRACT

Introduction: The present study aim to investigate the combined effect of high intensity interval training (HIIT) and intermittent fasting (IF) on the expression vascular endothelial grow factor (VEGF) gene in the cardiac muscle tissue of obese male rats.

Methods: This study was conducted on 22 male Wistar rats that were kept on high fat -diet for 12 weeks. Afterwards the animals were randomly divided into four groups, including control, HIIT (three session for six week, 80 - 95% MRT), IF (three days for six week, 12h ours) and HIIT with IF (both protocols for six week). Real-time polymerase chain reaction (RT- PCR) was used to measure *VEGF* gene expression. Data analysis was performed using one –way analysis of variance ANOVA least significance test ($p<0.05$).

Results: no significant difference was observed between the groups *VEGF* expression ($p>0.52$).

Conclusion: According to the results HIIT with IF and HIIT or IF alone had no significant effects on not significant on *VEGF* expression in the cardiac muscle tissue of the obese male rats.

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Introduction

The American Obesity Association defines obesity as a chronic metabolic disease caused by fat accumulation in the body. The World Health Organization (WHO) announced obesity as a global epidemic in 1997 (1). Recently, the prevalence of obesity has increased in different countries and the number of obese patientsis is projected to reach 1.12 billion by 2030 globally (1). Evidence suggests that obesity is a risk factor for cardiovascular diseases, diabetes and other metabolic disorders.

Today, the benefits of exercise are well-established, and physical activity is known to enhance health and prevent disease. Physiological responses following by exercise reduce the incidence of chronic diseases, (including cardiovascular diseases) and may also improve athletic performance. Increased blood flow is a form of adaptation to exercise, which is associated with improved capillary density and maximum oxygen consumption (2). In this context, the vascular endothelial grow factor (VEGF) pathway plays a pivotal role in the process of restructuring blood vessels reconstruction and the blood flow (2).

VEGF is a key factor in the growth of endothelial cells and is significantly involved in cell proliferation and regeneration. Furthermore VEGF signals are expressed through Tyrosine Kinase VEGF1 and VEGF2 receptors in the arteries (3). Among endothelial receptors, VEGF-2 plays a key role in angiogenesis (3).

High -intensity interval training (HIIT), consists of high intensity training sessions with a period rest between the workouts, which are performed at the intensity of 80 – 100% of the maximum heart rate or maximum aerobic capacity, for 60 - 240 seconds depending on the maximum capacity of the individual (4). Recent studies suggest that HIIT is an effective intervention in increasing cardiovascular fitness. For instance, Ramos et al. (2015) reported that HIIT has far greater effects on the cardiovascular function compared to aerobic training (5). In addition, HIIT is reported to be involved in reducing oxidative stress, inflammation, and insulin sensitivity, which may cause cardiovascular diseases (6). On the other hand, different types of

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training such as traditional training with moderate intensity and high volume have proven effective in weight loss and fat oxidation, while research suggests that HIIT is more beneficial than other a weight-loss training protocols in terms of improving fat oxidation within a short period (6). Furthermore, Studies have shown that exercise plays a key role in activating the VEGF pathway (7). In this regard, Karbalaeifar et al. (2016) reported that six weeks of HIIT could increase angiogenesis and improve myocardial function in male Wister rats after myocardial infarction (8). In another study, Mohamed Tah et al. (2016) stated that HIIT increases VEGF concentration (9).

Recent findings indicate that fasting has beneficial effects on health and may be considered as a therapeutic intervention (10). Intermittent fasting (IF) includes short- term or long-term dietary periods with severe energy restriction (75% - 100% reduction in calorie intake on fasting days) which increases free access to water and food on non-fasting day (11). The Metabolic benefits from IF of could increase endothelial growth factors and stimulate the process of angiogenesis and vascular healing (10).

Previous studies have proposed conflicting results about the effect of HIIT and fasting on the VEGF pathway of the cardiac tissue. The heart tissue has the highest mitochondrial volume and the highest capillary density in the body. A comparison of different training methods indicates that compared to traditional training, HIIT yields similar responses to skeletal muscle metabolic adaptations, HIIT also saving time and periodic exercise has been observed to induce more efficient. Studies have also highlighted the importance of fasting interventions in improving vascular function and activating the anti-obesity angiogenesis process. In a review study, Peter et al. (12) reported that despite the obvious fact that cells need o_2 for aerobic metabolism blood vessels also play a pivotal role in providing o_2 during metabolism and angiogenesis (12). Evidence in this regard suggests that training interventions increase oxygen consumption and the body tissue's demand for more blood flow increases during training. Today research points to the key role of fasting in angiogenesis and improving the blood flow to various body tissues. The present study, aimed to investigate the combined effect of HIIT and IF on VEGF gene expression in the cardiac tissue of obese male rats.

Materials and Methods

This laboratory study was approved by the ethics committee of Baqiyatallah University of Medical Sciences Tehran Iran (ethics code BMSU.REC.1398.022). The study was conducted on 22 male Wistar rats aged four weeks and weighing120-130garams. The animals were obtained from the animal Home Center of Baqiyatallah University of Medical Sciences, Initially, the rats were kept on a high-fat diet for 12 weeks. Following that, they were randomly divided into four groups including control (n=4), HIIT (n=6), intermittent fasting (n=6), HIIT with intermittent fasting (n=6). The rats adapted to their new environment for one week and maintained in four polyethylene cages with metal mesh lids within a 12-hour light/dark cycle (7- 19 hours) at the mean temperature of 2.22°C and C and 50% relative humidity they had free access to food and water as well.

High-fat Diet

The rodents received a standard pelletized diet (Behparvar Company Tehran, Iran). The high-fat diet was maintained after adaptation for weight gain and was composed of 5% rodents base food and 30% sheep's oil (5+30%=35). For the first 12 weeks, all the rats had free access to the high-fat diet and sufficient water. After the rats reached the mean weight of 320±20 grams, we evaluated the rats in the HIIT, IF and HIIT with IF groups.

HIIT Protocol

The HIIT protocol was designed based on previous studies (13). At the first stage, the rats were trained for one week (3-5 sessions five minutes) at the rate of 10 meters and 0o slop. Following that, a moderately adjusted periodic HIIT protocol was implemented including five minutes of warm-up and cool-down 40% maximum running speed one minute of intense alternations with 80 - 95%maximum running speed an active rest for one minute and 55% maximum running speed for six weeks (three sessions per week).The subjects performed the maximum running speed test before the first week and at the end of the third week based on the new meanscores obtained to continue the protocol. Notably, the maximum running speed

test was performed based on the Study by Machdo et al (14) - (Table 1).

IF Protocol

The IF protocol was designed based on previous studies (15). The Subjects underwent a fasting protocol for six weeks (three alternative days in fasting mode at 7pm - 7am). While fasting, the rats only had access to water and they had the rats had free access to water and food on the other days.

Table 1. Intense periodic exercise protocol

Exercise weeks	Intense rotation the number of sets	Speed	slow rotation the number of sets	Speed
first week	5	34	4	23
second week	6	36	5	23
third week	7	38	6	23
fourth week	8	43	7	27
fifth week	9	46	8	27
sixth week	10	46	9	27

Tissue Removal

About 48 hours after the last training session and the day of implementing the research protocols, the rats fasted for 10 - 12 hours. Afterwards, they were, anesthetized by the combined injection of ketamine (10 g) and xylazine (100 g).To prevent the slightest harassment of the animals, blood was initially taken from the cardiac muscle tissue using a 3 cc syringe. Following that, the cardiac muscle tissue was removed and kept frozen at the temperature of 80 °C for tissue analysis.

Table 2. Primer Sequence of VEGF-A and GAPDH genes in cardiac muscle tissue

Primer	Primer sequence
VEGF-A	F: 5´-TGAGACCCTGGTGGACATCTT-3 R: 5 - GTAGACGTCCATGAACTTCAC-3
GAPDH	F:CAAGTTCAACGGCACAGTCA R:CCCCATTTGATGTTAGCGGG

VEGF-A Gene Expression Analysis

Real-time polymerase chain reaction (RT – PCR) was used to analyze the expression of the VEGF-A gene. To design the primers, the sequence of VEGF-A encoding genes was initially extracted from the National Center for Biotechnology Information (NCBI). Afterwards, oligo version 7 was used to design the desired primer.After optimizing the RT- PCR device, relative were made in the VEGF-A and GAPDH genes (internal control gene) using the table primers listed in Table 2.

RNA Extraction, cDNA Synthesis, PCR Reaction

After extracting the entire RNA from the cardiac tissue, a SuperplusRNA extractionKit was used for analysis.CDNA was also provided in two stages using a Max First Strand c DNA Synthesis Kit.The PCR was performed using the device (Corbett, Easy Plex Analyzer device, Australia) and Master Mix Ampliqon.Each reaction was performed twice for each sample. The 2-DDCT method was also used for the Relative Quantification of VEGF-A GeneExpression.All the analyses were performed separately one each sample.

Relative fold change in gene expression $=2^{-\Delta\Delta CT}$

$\Delta CT = CT\ target\ gene - CT\ reference\ gene$

$\Delta\Delta CT = \Delta CT\ test\ sample - \Delta CT\ Control\ sample$

Statistical Analysis

Data analysis was performed in SPSS version 25 using descriptive statistics to calculate the mean and standard deviation, Then, one-way analysis of variance (ANOVA) was also applied to compare the study groups at the significance level of $p < 0.05$.

Table 3. Shows the weight (g) of the study groups. HIIT high intensity interval training, IF, Intermittent Fasting, CON control.

Group	Mean	SD
HIIT	315	34.76
IF	333.40	16.21
HIIT& IF	325.25	39.06
Control	362	19.42

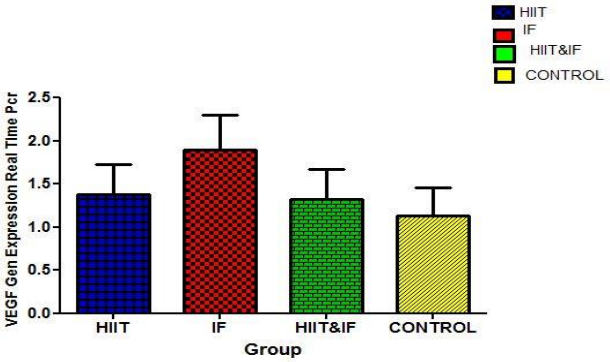

Figure 1. Shows the VEGF-A gene expression in the heart muscle tissue of obese male rats. HIIT high intensity interval training, IF, Intermittent Fasting, CON control

Results

Table 3 shows the mean weight of the rats in different of study groups. Figure 1 the results of one way ANOVA indicated no significant difference between the groups in this regard (p>0.52).

Discussion

The present study aimed to evaluate the combined effect of HIIT and IF on the expression of VEGF gene in cardiac muscle tissue. The obtained results indicated significant difference between the study groups in this regard (p>0.52). According to Bayati et al. (16) HIIT had a significant effect on the rate of change in VEGF protein in human skeletal muscles. In the mentioned study, the VEGF pathway was reported to be activated by PGC-1α to adapt angiogenesis to HIIT. Therefore, it could be inferred that PGC-1α induction is dependent on beta-adrenergic induction mediated by ERRa, other activation pathways for VEGF gene expression include the such as the HIF pathway which increases gene expression of VEGF due to the lack of oxygen and the activity of AMPK metabolic sensors, which are sensitive to metabolic insufficiency (17), In another study, Chinsombo et al.(18) showed that after training metabolic factors such as PGC-1α play a key role in activating VEGFas an angiogenesis factor (18). According to the findings of Arani et al. (19) the PGC-1α pathway effects the in the angiogenesis process by activating ERRα. Based on the aforementioned findings, it could be stated that the PGC-1α pathway and AMPK sensors significantly affect alternative angiogenesis signalin depending on the tissue type and training protocol. According to the studies by Bayti et al. (16) Chinsomio et al. (18), and Arani et al. (19), the most important variables to be considered in this regard are the tissue type, alternative angiogenesis signaling training protocol.

Previous studies have highlighted the importance of various,training parameters (e.g.. intensity, duration and volume) in training interventions in terms of activating the angiogenesis pathway and improving vascular function. Our findings in this regard indicated, there was no significant increase in VEGF gene expression in the cardiac muscle tissue following HIIT. Nevertheless, several studies confirm the effectiveness of HIIT in facilitating the angiogenesis process in the heart tissue. For instance Shabani et al. (20) reported that due to the nature of the cardiovascular system, HIIT maximizes the oxidative capacity of the cardiovascular system thereby leading to the accumulation of lactic acid, which inhibits the angiogenesis process (20). Therefore, HIIT with the intensity of 80> could be an influential factor in the inhibition of angiogenesis activation and decreased vascular function in the cardiac muscle tissue.

Studies regarding endurance training (e.g.. running, prolonged swimming) have demonstrated that such interventions could stimulate a significant increase in the oxygen and nutrient demand for the formation of new capillaries in the myocardium (21) In addition, changes in the blood flow, muscle concentration, and oxygen levels are associated with the mechanical occurrence of hemodynamics, which

is a key signal in activating vascular wall regeneration and proliferation the formation of endothelial cells and angiogenesis. In other words, there is a reciprocal response between the skeletal and cardiac muscles in order to respond synergistically to the stimuli of training (21).

In a study conducted by Bellaforeet al. (21) endurance training was reported to an increase the VEGFR1 receptor in the cardiac muscle tissue thereby activating the angiogenesis signal pathways (21). In the present study, the HIIT training protocol and the associated training stimulation might have decreased the activation of angiogenesis. The volume and duration of training interventions are of great importance for create better adaptations in different body tissues after training in different tissues of the body. Morland et al. (22), observed a significant increase in VEGF in a study following the effect of seven weeks of HIIT (five sessions per week) (22). As a result, the angiogenesis pathway was activated and the vascular function was reported to improve. In the current research, HIIT, was performed for six weeks (three sessions per week), and the volume and duration of the HIIT training protocol may have decreased sufficient stimulus to upset the balance of angiogenesis process and improve vascular function from the rest time to the training time.

Previous studies have highlighted the correlation between fasting and signaling pathways such as PPARs, which consists of PPARsα, PPARsβ, PPARsγ isoforms with different roles (12). Two of these isoforms are involved in inhibiting angiogenesis, while one promotes cell growth (12). The findings of the current research indicated the effects of IF on signaling pathways (e.g. PPARs) and the inhibiting of VEGF after six week IF notably.

Factors such as, duration (12hours), volume and type fasting (every other day) should be considered when it comes to fasting protocols. It could be stated that our IF protocol (12 hours) could sufficiently stimulate angiogenesis and improve blood flow. In addition, the type (intermittent) and volume of the fasting protocol (six weeks) in the present study might have been effective in reducing angiogenesis and the blood flow.

Conclusion

According to the result HIIT with IF and HIIT and IF alone had no significant effect on the pathway signaling of the VEGF in the cardiac muscle tissue of the obsess rats, for the activation of this cellular pathway in the heart tissue of obese rats further research is required with other types of training (e.g.. aerobic exercise) and long-term fasting protocols.

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Acute Response of Oxidative Stress and Muscle Damage Biomarkers to a Single Bout of Combined Training (Resistance-Aerobic) with Different Intensities Following Q10 Supplementation

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ABSTRACT

Introduction: Nutritional supplements and controlling exercise intensity are essential to increasing muscle mass. However, the effects of such manipulations on the health of athletes may not be apparent. The present study aimed to compare the effects of combined training (resistance-aerobic) with different intensities and the short-term supplementation of coenzyme Q10 on oxidative stress and muscle damage biomarkers.

Methods: This quasi-experimental study was conducted on 45 male bodybuilders who were divided into three groups of low-, moderate-, and high-intensity combined training (15 per each). Research stages included initial blood sampling, 14 days of Q10 supplementation (400 mg/day), a second blood sampling, a single bout of combined physical activity, and the final blood sampling. In each group, resistance training was initially performed with different intensities (55%, 70%, and 85% 1-RM), followed by aerobic training to consume 300 kilocalories at speeds of 8, 9.6, and 11.2 km/h.

Results: Q10 supplementation had no significant effect on the baseline levels of malondialdehyde (MDA), superoxide dismutase (SOD), lactate dehydrogenase (LDH), and creatine kinase (CK) ($P \geq 0.05$). After performing low-, moderate-, and high-intensity combined activities, a significant increase was observed in the levels of MDA, SOD, LDH, and CK ($P \leq 0.05$).

Conclusion: According to the results, oxidative stress and muscle damage biomarkers increased in response to training intensity, while the increase was not significant at different intensities and fixed times. The performance of the participants may have influenced the obtained results. Given the lower aerobic capacity of male bodybuilders, exercise volume is the primary factor to increase exercise pressure**.** Supplementation may also be effective over long periods.

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Introduction

Today, there is a growing tendency to incorporate physical activity into one's lifestyle to achieve health or championship goals. Meanwhile, the public chooses bodybuilding for several reasons. Achieving well-separated muscles is one of the ultimate goals of bodybuilding. Changing training intensity and volume and combining various training patterns are crucial in this regard. Some optimal approaches are combining training in the form of positive and negative muscles, the combined training of the upper and lower body extremes, and combining resistance and aerobic training to increase the training volume (1). Combined training refers to the combination of several energy-generating pathways and the simultaneous execution of several training types (e.g., resistance and aerobic training) (1). Combined training improves body composition and cardiovascular health factors more effectively compared to aerobic and resistance training alone. Combining aerobic and resistance training may also enhance fitness, body composition, and metabolic health more significantly than each method alone (2). However, the acute response of oxidative stress and muscle damage resulting from such training must be investigated.

The extent of muscle damage and a significant increase in the production of free radicals could

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be determined indirectly by measuring creatine kinase (CK) and lactate dehydrogenase (LDH) (3). Serum CK is a marker of muscle damage, while LDH plays a pivotal role in the metabolism of substances in the body, and fast-twitch fibers catalyze the conversion of pyruvic acid into lactic acid (3). The involvement of these fibers increases the level of lactic acid in the body, and the threshold stage of lactate occurs in the body. Oxidative pressure is induced during the production of free radicals, exceeding the resistance ability of the endogenous antioxidant defense system (3). Oxidative damage yields products such as malondialdehyde (MDA) and superoxide dismutase (SOD) (3, 4).

MDA is an active and highly reactive aldehyde compound produced in the human body through the peroxidation of unsaturated fatty acids, and SOD is the most crucial enzyme that degrades superoxide radicals in the human body (3, 4). During exercise, the body consumes 10-15 times more oxygen, and the oxygen flux in the active muscles increases by up to 100 times (3). Chronic oxidative stress induced by exercise may exceed the protective capacity of the antioxidant defense system. In support of this hypothesis, several studies have shown that aerobic/resistance training significantly increases the production of free radicals (5-7). It has also been reported that after resistance training activities with increased reactive oxygen species and lipid peroxidation markers (including MDA), the imbalance between oxidative pressure and antioxidant defense decreases the total oxidative capacity. After short and intense training, levels of plasma MDA and erythrocytes have also been reported to increase as indicators of lipid peroxidation in red blood cell membranes (8).

In addition to the influential factors in training, using antioxidant supplements reduces the muscle damage and fatigue caused by physical activity. Coenzyme Q10 is a fat-soluble vitamin-like substance and an essential carrier of electrons in the mitochondria (9, 10). Q10 supplementation is an essential electron transporter in the respiratory chain of the inner surface of the mitochondrial membrane for ATP production, as well as an essential antioxidant in the body. This substance may affect maximal oxygen consumption and counteract free radicals, thereby reducing muscle damage (11). Coenzyme Q10 could prevent delayed onset muscle soreness by counteracting free radicals. Therefore, researchers believe that coenzyme Q10 supplementation may also prevent stress or adverse changes in some biochemical parameters due to energy loss during various sports activities. As an antioxidant and anti-fatigue supplement, coenzyme Q10 supplementation is reported to prevent adverse changes in lactate and CK after relatively strenuous activity (12, 13). On the other hand, the findings of Cook et al. (2008) showed that acute coenzyme Q10 supplementation increased coenzyme Q10 concentration without affecting MDA and SOD levels during and after exercise (14).

Objectives

Studies regarding the effects of exercise (especially combined exercise) have proposed contradictory results. Furthermore, exercise programs are generally prescribed so that continuous exercises would be used while performing combined exercises, while also increasing the intensity of exercises and adding more variety. Notably, athletes need to progress quickly and achieve results in today's world, and it is essential to assess the safety of their methods. On the other hand, researchers have mainly investigated the effects of one-step speed training protocols, high resistance, and aerobic intervals on the levels of total oxidative capacity and lipid peroxidation.

Given the importance of maintaining athletes' health and designing training programs to achieve better results, the present study aimed to compare the effects of combined training (resistance-aerobic) with different intensities, along with short-term coenzyme Q10 supplementation, on oxidative stress and muscle damage biomarkers in male bodybuilders.

Materials and Methods

The experimental protocol of the study (based on an MSc thesis) was approved by the Ethics Committee of the Islamic Azad University of Mahallat Branch, Iran (No. 20021404971003). The Ethics Committee initially approved the experimental procedures, and the study protocols were thoroughly explained to all the participants. In addition, a written informed consent form was signed after reading and understanding the details of the experiments. The research was conducted in compliance with the Declaration of Helsinki.

This quasi-experimental study was performed based on predetermined objectives. The research design involved repeated measures ANOVA with three experimental groups, including low-intensity combined training (n=15), moderate-intensity combined training (n=15), and high-intensity combined training (n=15). The sample size was determined based on previous studies. We compared the effects of different intensities of combined training along with short-term coenzyme Q10 supplementation on oxidative stress and muscle damage biomarkers in male bodybuilders. The participants included 45 male bodybuilders aged 19-25 years (Table 1) who volunteered to partake in the study. The independent variables of the research were different intensities of combined training and short-term coenzyme Q10 supplementation, and the dependent variables were oxidative stress biomarkers (MDA and SOD) and muscle damage biomarkers (CK and LDH).

Examinations confirmed the health of all the participants. In addition to signing a written informed consent form, the participants also completed the PAR-Q questionnaires and the daily food registration note. The exclusion criteria of the study were as follows: 1) recent surgery/illness; 2) skeletal and neuromuscular disorders; 3) medical conditions such as cardiovascular diseases, diabetes, hypertension, hepatic diseases, renal disorders, and respiratory disorders; 4) history of food allergies and 5) using tobacco products, alcohol, and medicines, dietary supplements such as antioxidant supplements (e.g., vitamin C and E), and anti-inflammatory drugs (e.g., aspirin subgroups) within three months before the study. None of the participants reported using exogenous anabolic-androgenic steroids, drugs, medications, or dietary supplements with potential effects on redox and inflammatory responses during the study. The subjects were not allowed to participate in the study in case of the contraindications defined and outlined by the American College of Sports Medicine (ACSM) nor if they had ingested any supplements claimed to have ergogenic properties within three months before the study.

Table 1. Mean and standard deviation of the participant's demographic characteristics

Group	Number	Age	Height (cm)	Weight (kg)	BMI (kg/m²)	Vo_2max	Chest press 1-RM
Low intensity	15	22.33±2.3	183.58±4.07	82.01±3.49	24.34±0.26	35.83±2.79	114.75±5.46
Moderate intensity	15	22.58±1.37	183.25±3.04	82.08±3.52	24.44±0.38	36.66±2.14	111.83±10.85
High intensity	15	23.14±1.76	180.89±3.78	81.25±3.54	24.83±0.67	35.75±2.56	115.42±11.27

Based on the records, the health of all the participants was confirmed. The research stages included initial blood sampling, 14 days of Q10 supplementation (400 mg/kg), a second blood sampling, a single bout of combined physical activity (resistance-aerobic), and the final blood sampling. In each group, resistance training was initially implemented with different intensities (55%, 70%, and 85% 1-RM), followed by aerobic training to consume 300 kilocalories at speeds of 8, 9.6, and 11.2 km/h.

A combined training session with different intensities was implemented to compare the intensities of the resistance training session and measure a combined activity of 55% (low), 70% (moderate), and 85% weights (high), as well as the maximum repetition of the resistance training using the following formula:

Amount of weight × number of repetitions × number of sets

The workload of the subjects was the same. Resistance training was performed as a combination of the upper and lower body movements (chest press, leg press, forearm with a barbell, knee bends, lat pulldown, and knee extension) of the upper and lower torso muscles. The number of the training sets of each movement was three repetitions. After each turn in each exercise, the rest time was determined to be 90 seconds to measure the maximum strength in the chest press. A standard formula was used to calculate the 1-RM of the subjects (15).

*1-RM=Lifted weight (kg)/ [1.0278/ (0.0278*re)]*

Aerobic training was implemented based on energy expenditure and consumption of 300 kilocalories for each subject. To compare different intensities of aerobic training in the combined activity, aerobic training was performed on a treadmill (Technogym Run Race HC 1200, Italy) at speeds of 5 mph (8 km/h) for low-intensity training, 6 mph (9.6 km/h) for moderate-intensity training, and 7 mph (11.2 km/h) for high-intensity training. The subjects were trained to 300 kilocalories of energy at the desired speed depending on their body weight.

As such, the training load in the aerobic training was equal for all the subjects.

The Q10 supplement was prepared in the form of two jelly capsules and consumed for 14 days (200 mg each, a total of 400 mg per day; safe dose equivalent to 5 mg/kg/day for a person weighing 80 kg; QSpeed Fast-Melt CoQ10; 200 mg dosage strength, made in Switzerland, manufactured in a cGMP certified facility) (14, 16).

The height and weight of the participants were measured using a Seca device (Seca 714, seca Vogel and Halk Gmbh). One day before the first blood sampling (pretest), the maximum oxygen consumption (VO_{2mx}) of the participants was determined using the Bruce test, and one-repetition maximum (1-RM) was determined based on the chest press. Blood samples were collected in three stages, including the pretest (first stage), 90 minutes after the Q10 supplementation period (second stage), and immediately after combined training (third stage) at the rate of 10 cc of subjects' forearm vein to prepare the serum. All the stages of blood collection from the forearm vein (antibiotic) were performed while the subjects were fasting (8 AM). The blood samples were immediately drained into tubes containing anticoagulants (EDTA). Following that, the samples were centrifuged at 3,000 rpm for five minutes at a temperature of 4°C. The obtained plasma was stored at the temperature of -80°C for subsequent measurements. At the next stage, the samples were preserved at the laboratory temperature for 30 minutes to separate the serum from the plasma and centrifuged (Dlasent, UK model) for 5-10 minutes at 2,000 rpm. A laboratory expert who was unaware of the subjects' condition performed the measurements. The respective kits were used to measure SOD, MDA, CK, and LDH.

MDA was measured using the USA, MI, Cayman Chemical kit. The sensitivity of the method was 0.08 µmol, and the coefficient of variation within the test was 5.9%. The unit of measurement was nanomol per milliliter. SOD was measured using the ELISA method (440 to 460 nm) and the Chemical Cayman kit with a sensitivity of four units per liter and a coefficient of change of 1.8% in liters. CK was measured using the chemical colorimetric method based on the Jaffe reaction with a sensitivity of one unit per liter and a coefficient of change of 1.6% (creatine kinase colorimetric kit, Pars Azmoun Co., Tehran, Iran); the unit of measurement was unit per liter. LDH was also measured using the enzymatic colorimetric method with a sensitivity of five units per liter and a coefficient of change of 2.1% (LDH colorimetric kit, Pars Azmoun Co., Tehran, Iran); the unit of measurement was one unit per liter.

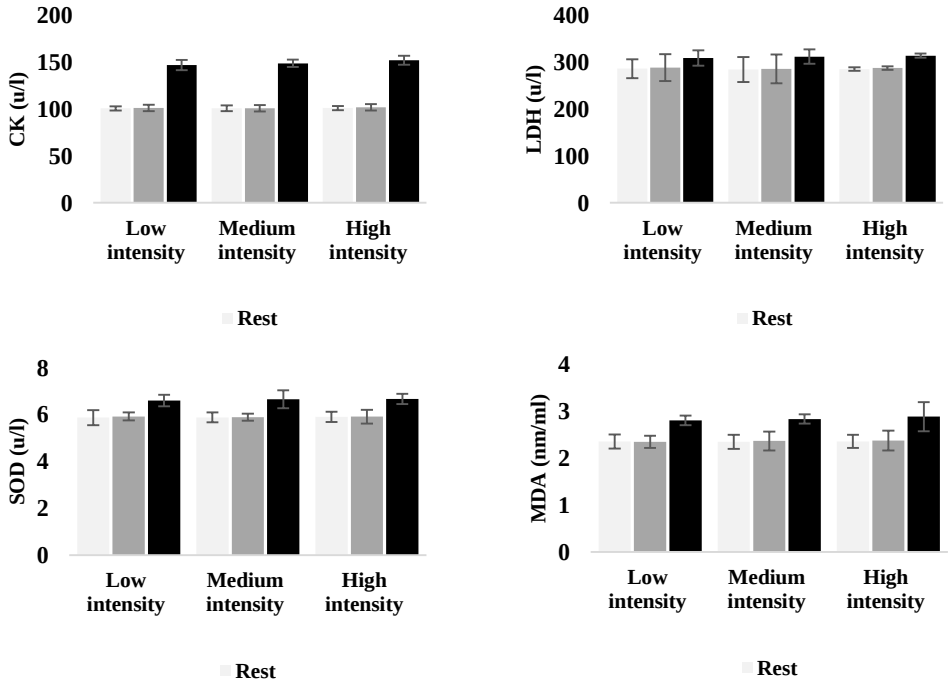

Figure 1. The changes of oxidative stress and muscle damage indices

Data analysis was performed in SPSS version 22 using the Shapiro-Wilk test to assess the normality of the data. Repeated measures analysis of variance (ANOVA) was also applied to evaluate the differences between the study groups. To compare the groups and reduce the effect of the pretest, the covariance option was used.

Results

According to the obtained results, Q10 supplementation had no significant effects on the baseline levels of MDA, SOD, LDH, and CK (P≥0.05). After performing low-, moderate-, and high-intensity combined activities, the levels of MDA, SOD, LDH, and CK increased significantly (F=205.070; P=0001; F=53.158, P=0001; F=6.540, P=0.004; F=50.401, P=0001, respectively). Furthermore, oxidative stress and muscle damage biomarkers increased in response to training intensity, while the increase had no significant difference at different intensities and fixed times. The post-hoc power calculation for each variable was estimated at >0.85 (Figure 1).

Discussion

The present study aimed to compare the intensity of combined training with short-term coenzyme Q10 supplementation in terms of oxidative stress and muscle damage biomarkers in male bodybuilders. The obtained results indicated that Q10 supplementation had no significant effects on the baseline levels of MDA, SOD, LDH, and CK, while after performing low-, moderate-, and high-intensity combined activities, the levels of MDA, SOD, LDH, and CK increased significantly. Oxidative stress and muscle damage biomarkers also increased in response to training intensity, while the increment showed no significant difference at different intensities and fixed times. Differences in research methods play a key role in conflicting and matching results. For instance, various protocols have been implemented in some studies, such as differences in participants' gender, water exercises, or different recovery methods to prevent an increase in oxidative stress indices (7). In the present study, different intensities of combined training were used in young trained men.

A contusion is a reflection of muscle damage in the physiological adaptation of the muscle to strenuous exercise. One of the mechanical causes of muscle contusion is probably damage to the sarcomeres in the muscle structure, which leads to the rupture of the z-plates (17). Current reports indicate that contusions often occur after performing strenuous and unusual activities accompanied by extroverted contractions. Delayed muscle soreness begins 8-12 hours after exercise, peaks within 24-48 hours after exercise, and will eventually resolve within 5-7 days after exercise (17). The etiology of muscle injury and muscle contusion has been extensively discussed in medical and sports communities. This mechanism has also proposed numerous hypotheses, including lactic acid accumulation, muscle spasms, connective tissue damage, and inflammation (17).

Significant oxidative damages initially and mainly occur in the mitochondrial membranes and other tissue membranes. Previous studies suggest that coenzyme Q10 may elicit different antioxidant responses depending on the type, duration, and intensity of exercise at different times after exercise (9, 10, 14). Therefore, it could be inferred that coenzyme Q10 may help athletes by affecting energy storage in the form of ATP, neutralizing free radicals, and reducing the active form of vitamin E by decreasing alpha-tocopherol radicals and increasing strength and endurance (11, 18).

Bagdanis et al. (2013) reported a significant difference in MDA levels in physically healthy men after performing three weeks of resistance training (19). Some studies show that individuals who practice resistance and aerobic training have lower muscle destruction and stress levels compared to non-trained subjects (2). In terms of oxidative stress biomarkers at rest or after the adaptation by exercise training, considered the possibility of improving the antioxidant system in individuals who exercise regularly over a long period, which maintains the redox (reduction-oxidation) system. Long-term anaerobic exercise has also been reported to increase the capacity for anaerobic energy production in the muscle and improve the antioxidant status of the muscle (7).

According to the literature, the concentration of non-enzymatic antioxidants increases with anaerobic exercise. Frequent production of free radicals due to ischemia and blood redistribution on the muscle surface resulting from such exercises seems to enhance the antioxidant

profile (20). In contrast, Gaini et al. (2011) reported that 36 weeks of interval training did not affect MDA levels (21). This lack of change seems to be due to the oxidative defense induced by regular anaerobic interval activity performance. However, studies have shown that intense and irregular physical activity affects the oxidative function of cells and the cell membrane structure by increasing hormones such as catecholamines and macrophage activity, as well as increasing oxidative stress and lipid peroxidation. Reducing local blood flow at the outset of physical activity in organs such as active muscles, kidneys, and liver could also increase lipid peroxidation (20). Regular and continuous exercise probably reduces lipid and protein peroxidation through adaptation and improving the antioxidant defense.

The results of the present study indicated that coenzyme Q10 supplementation did not affect the baseline serum levels of total CK. However, CK levels increased significantly after low-, moderate-, and high-intensity combined activity. This is consistent with the study by Sumida (2007), which indicated a significant increase in the serum levels of CK in non-athlete women after exhausting activity on the treadmill (22). Milias (2005) also investigated the effects of muscle damage caused by extroverted activity on CK levels, reporting that CK levels increased significantly after the activity (23). Our findings are also consistent with the study by Kon et al. (2007 & 2008) in which six weeks of coenzyme Q10 supplementation prevented the elevation of CK levels in male athletes post-exercise (12, 13). In addition, Wang et al. (2004) stated that taking two grams of a Q10 supplement per kilogram of the bodyweight for three months was associated with a significant reduction in CK levels in monkeys (18). Therefore, it could be concluded that the short-term supplementation of Q10 in the present study may not be beneficial.

The possible mechanism of action of coenzyme Q10 is as an antioxidant in reducing CK levels. Q10 also reduces the peroxidation of membrane cells and damages phospholipid membranes by removing free bases and increasing the body's antioxidant capacity. Therefore, it prevents the leakage and penetration of this intracellular enzyme into extracellular fluids (24). Zuliani et al. (2009) conducted research in which coenzyme Q10 supplementation was implemented for four weeks on non-athletes without affecting changes in the serum levels of total CK post-exercise (25). In another study, Kon et al. (2007 & 2008) observed that supplementation with 300 milligrams of coenzyme Q10 per day for four weeks did not increase CK levels (12, 13).

According to the current research, coenzyme Q10 supplementation at baseline did not affect LDH, while the levels of LDH increased significantly after low-, moderate-, and high-intensity combined activity. The study by Deminice et al. (2010 & 2013) consisted of two parts; the findings of the first part regarding intense anaerobic exercise and LDH are in line with the present study. However, the authors also stated that creatine supplementation reduced LDH, which is inconsistent with our findings mainly due to the effect of creatine supplementation (5, 26).

Our findings indicated that short-term coenzyme Q10 supplementation did not affect LDH which is in line with previous studies (4). Meanwhile, this is inconsistent with the findings of Cook et al. (2008) (14). The contradiction may be due to differences in the method of supplementation (type of supplement, purity, amount, and timing of consumption) and the combination of other antioxidant vitamins with coenzyme Q10. In the present study, coenzyme Q10 was administered at the dose of 5 mg/kg/day and alone. Another reason for the contradiction between the results of our study and some other studies may be the duration of the supplementation period (14). It has been reported that the pharmacological properties of coenzyme Q10 (cellular uptake and accumulation) are similar to those of creatine monohydrate is a uniform maximum or a downward trend (in some cases). It has also been observed that it may lead to flattening (uniformity) or even a decrease in muscle concentration during the supplementation period. Although the present study was performed during a 14-day supplementation period, most heterogeneous studies have examined the effect of coenzyme Q10 supplementation in a shorter period.

According to the present study, coenzyme Q10 supplementation had no effect on SOD at baseline, while SOD increased significantly after low-, moderate-, and high-intensity combined activity. In a study on the elderly, Bouzid et al. (2014) reported that during an exhausting activity on an ergometer cycle, SOD and MDA

levels increased significantly, which is consistent with our study (3). Gene Fang Liu et al. (2015) also investigated muscle damage and lipid peroxidation in 36 female weightlifters during one week of strenuous activity, stating that after one week of strenuous activity, MDA, TBARS, and CK levels increased significantly, while SOD levels decreased (27). The results of the mentioned study are not in line with our research, which could be explained by the difference in the gender of the subjects and their experience. In a study of oxidative rats, Hovanloo et al. (2012) reported that after six and nine weeks, no changes were observed in the levels of SOD enzyme in the rats, while continued training for 12 weeks caused a significant decrease in SOD levels (28). This is inconsistent with our research, which could be due to endurance training or research on rats.

As an antioxidant in the body of living organisms, Q10 has not been well elucidated in other studies. In addition, data are scarce regarding the interaction of coenzyme Q10 with other antioxidants during exercise. Kaikkonen et al. (2018) reported that three weeks of Q10 supplementation (90 mg/day) increased coenzyme Q10 concentration and the total antioxidant capacity (29). In the mentioned study, coenzyme Q10 was considered an antioxidant supplement to assess its inhibitory effects on reactive oxygen species and the activity of antioxidant enzymes. The researchers confirmed the antioxidant effects of coenzyme Q10 on inactivating free radicals, while these effects often depend on the amount and duration of the substance, and environmental conditions are also influential.

Conclusion

According to the results, coenzyme Q10 supplementation at baseline did not affect oxidative stress and muscle damage biomarkers. However, muscle injury and oxidative stress rates increased significantly after low-, moderate-, and high-intensity combined training. On the other hand, no significant difference was observed between different intensities of combined training, the level of muscle injury, and oxidative stress biomarkers. Considering the ineffectiveness of short-term coenzyme Q10 supplementation in reducing lipid peroxidation and the cell damage caused by the implemented physical activity, it is recommended that bodybuilders prevent the oxidative stress caused by strenuous exercise and use different antioxidant supplements or long-term supplementation.

Conflicts of Interest

All the authors equally contributed to the reading and approval of the final version submitted. The contents of this manuscript have not been copyrighted or published previously. Currently, the contents of this manuscript are not under consideration for publication elsewhere. The authors declare no conflicts of interest, and there was no funding for this study.

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Comparison of the Effect of Leucine and L-Arginine Supplementation Before and After Resistance Training On Athletes' Protein Catabolism Indices in Ramadan

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ABSTRACT

Introduction: Improving adaptive responses to exercise through dietary interventions, especially sports supplements, has been considered. The aim of the present study was to compare the effect of Leucine and L-arginine supplementation before and after resistance training on athletes' protein catabolism indices during Ramadan.

Methods: In this study, 40 male bodybuilders were selected and randomly divided into four groups of resistance training (n=10) and resistance training group and Leucine supplementation (n=10), resistance training group and L-arginine supplementation (n=10) and the control group (n=10). Leucine and L-arginine tablets were poured as powder into 1g empty capsules and placebo was in the form of 1g capsules, the same shape, size and color of 1g Leucine and L-arginine tablets. The amount of supplements was 0.1g per kg of body weight. The training protocol of the two training groups was performed for eight weeks in three sessions per week.To analyze the data, the statistical method of analysis of covariance was used and to determine the differences between the groups, Bonferroni post hoc test and at a significant level in all tests, P≤0.05 was considered.

Results: Statistical analysis did not show a significant difference between groups in uric acid variable (p=0.097). Also, the results showed that resistance training significantly decreased urea (p=0.001), creatinine (p=0.001) and increased hypoxanthine (p=0.000) and xanthine oxidase (p=0.000). Resistance training with Leucine and L-arginine supplementation increased there was a significant urea, creatinine and a significant decrease in hypoxanthine and xanthine oxidase compared to the resistance training group. There was a significant difference between the two groups of supplements only in the amount of xanthine oxidase. In the resistance training group and Leucine supplementation, the amount of xanthine oxidase was significantly greater than in the resistance training group and L-arginine supplementation.

Conclusion: Finally,resistance training with Leucine and L-arginine supplementation in fasting athletes could provide a platform for reducing protein catabolism due to exercise and it can be said that coaches and athletes to reduce catabolism due to exercise. During Ramadan, they can take Leucine and L-arginine supplements to increase performance and reduce damage.

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Introduction

Millions of Muslims abstain from eating and drinking for an average of 14 hours from morning to evening during the holy month of Ramadan. During Ramadan, food intake occurs more during the night, and the amount and frequency of food intake, the amount of sleep at night, and the amount of physical activity decrease (1). Different physiological responses during Ramadan are most likely the result of a disturbed sleep-wake cycle and altered eating and drinking cycles, or most likely a combination of these factors (2). In addition, eating food at unusual times can have different metabolic effects, and lifestyle changes can have significant effects on metabolism (3).

One of the goals of sports professionals and coaches is to develop physical and mental abilities, improve sports performance and delay athletes' fatigue. Most athletes use medications and supplements to improve strength, power, speed, and endurance in order to increase

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performance and achieve success (4). Therefore, in order to gain more knowledge about the effects of taking antioxidant supplements to reduce the oxidative stress caused by intense exercise, it is necessary to conduct studies to find the best type of supplement. So far, research has been done on the effect of taking these supplements on athletes' performance and contradictory results have been obtained (5).

Studies show that protein catabolism is increased in high-intensity activities. In other words, there is a direct relationship between the intensity of exercise and the catabolism of body proteins. On the other hand, by increasing the training time below the maximum and reducing the body's carbohydrate reserves, the share of protein energy in exercise increases (6). Fasting with prolonged starvation (usually more than 24 hours), carbohydrate-free diet, and lowering blood glucose cause proteins to be forcibly broken down for energy and to maintain plasma glucose concentrations, and their carbon components to be used by muscles (7). Excretion of urea, uric acid and creatinine in the urine as well as their accumulation in the blood are the reasons for the catabolism of proteins to produce energy (8).

In recent years, the improvement of adaptive responses to exercise through dietary interventions, especially the use of sports supplements, has been considered. It has also been observed that the stimulation of protein synthesis after eating certain foods is greatly influenced by the amino acid content of the diet, especially the amino acid Leucine (9, 10). Leucine, isoLeucine and valine are called branched chain amino acids (BCAAs). BCAAs make up about one-third of muscle protein, with Leucine accounting for about 5 to 10 percent of the body's total protein. Leucine oxidation rate during exercise is significantly higher than isoLeucine and valine. In addition, among BCAAs, Leucine activates key protein synthesis pathways such as mTOR after exercise (11). Resistance training also increases mTOR signaling, which is a key component in regulating protein synthesis in skeletal muscle. Stimulates protein synthesis (12). Regardless of age and sex, resistance training and the amino acid Leucine by activating mTOR increase 4E-BP1 and S6K1 and bind mRNA to ribosomes, thus increasing protein synthesis (13, 14).

Another supplement that has caught the attention of most athletes today is L-arginine, which was discovered in 1895 by Hadin et al. In mammalian protein. L-Argentine (2-amino-5-guanidinovaleric acid) is an unnecessary amino acid because it can be synthesized from the kidneys and liver (15). Under stressful conditions (severe burns, injuries, infections, etc.) it is placed in the conditional essential amino acid class. L-Arginine has many functions in the body, including protein synthesis and detoxification of ammonia waste from nitrogen catabolism (16). L-Arginine is also a precursor to the formation of nitric oxide (NO), creatine and L-glutamine. L-arginine supplementation can also increase nitric oxide levels (17). L. Argentina is found in relatively high concentrations in seafood, fruit juices, nuts, seeds, algae, meat, rice, and soy protein, and in low concentrations in the milk of many mammals (including humans, cattle, pigs). (18, 19).

Gil and Kim (2015) also investigated the interactive effect of Leucine supplementation and resistance training on protein synthesis in rats. The results of their research showed that their perception that taking Leucine with exercise could increase muscle mass was incorrect (14). New research shows that some purine derivatives, especially plasma hypoxanthine, can be considered as an indicator of intensity, and hence some limitations seen in classical indicators, such as the level of elite athletes, the level of intensity of activity in training. , Usability in all training courses (general, specific, competition and transfer) can be eliminated by using hypoxanthine. Hypoxanthine can be used as an indicator to estimate muscle metabolism, training level, age of athletes in various competitive and non-competitive sports, anaerobic exercise, and adaptation to their training status training. Hypoxanthine is a sign of degradation of adenine nucleonide in muscle and is an indicator of energy stress in exercise. Hypoxanthine can also be used as an indicator of the intensity and level of exercise activity (20, 21).

Due to the importance of fatigue, maintaining and enhancing performance during fasting and reducing protein catabolism in athletes, the use of authorized sports supplements and replacing them with prohibited and illegal substances can help athletes achieve healthier as much as possible. Aims and help achieve sports success

during fasting, and given that a study on the comparison and effect of taking Leucine and L-arginine supplements before and after found resistance activity on protein catabolism indices during Ramadan and during fasting athletes Therefore, the researcher decided to answer the question that taking Leucine and L-arginine supplements before and after resistance training in fasting athletes can affect protein catabolism, and which supplement will have the best effect in reducing protein catabolism?

Materials & Methods

The present study is a quasi-experimental study with pre-test and post-test design. In this study, four groups including resistance training group, resistance training group and Leucine supplementation, resistance training group and taking L-arginine supplement, and control group participated who have a history of regular exercise 3 times a week for one year. The subjects of the study who participated in this study voluntarily were randomly and equally divided into four groups and were informed of all stages of the research and the risks and possible consequences of the research and their consent was obtained. In this study, it was tried to influence the factors and variables in the field of research and in different stages of project implementation such as nutrition, temperature, body mass index, location, age, gender, absence of diseases, status and health history, sleep schedule before Examine the test carefully. A few days before the test and before fasting, the subjects were given the necessary explanations for scheduling sleep and breakfast and iftar food and supplements before the test. Leucine and L-arginine tablets were poured as powder into empty 1-gram capsules, and placebo was supplemented as 1-gram capsules, both in shape, size, and color, in 1-gram tablets. Consumption of Leucine and L-arginine will be 0.1 g per kg of body weight. The method of consumption was that the number of capsules on fasting days before resistance training and after training. To standardize the test, the tests were performed at a specific time of day. Initial assessments including height, weight, body fat, BMI and VO2MAX were performed two weeks before the start of training. Half an hour before the first training session and 24 hours after the last session, urine samples were taken from the forearm of the subjects in all three groups on an empty stomach (8:30 am) at a rate of 10 cc. Blood samples are centrifuged at 3000 rpm for 10 minutes and the level of the desired variables is measured with the appropriate kits purchased in the laboratory. To measure body mass index, the formula of weight ratio (kg) to height (meters) to the power of two was used.

Results

Kolmogorov-Smirnov test was used to determine the normal distribution of data. The results of this test showed that the data distribution was normal. For inferential analysis of data, we used parametric statistics and analysis of covariance for differences between pretest and posttest. Table 1 shows the mean and standard deviation of height, weight and age and BMI of the subjects in the groups. Table 1 shows that the distribution of subjects in both groups is almost the same.

The results of analysis of covariance did not show a significant difference between the four groups in the amount of uric acid, but there was a significant difference in the levels of urea, creatinine, hypoxanthine and xanthine oxidase between the four groups (Table 2). Bonferroni post hoc test was used for differences between groups.

According to the results of Bonferroni post hoc test, differences in the amount of all variables were observed between the resistance training group and all three groups. Also, a significant difference was observed between the two groups of supplements only in the amount of xanthine oxidase. In the resistance training group and Leucine supplementation, the amount of xanthine oxidase was significantly greater than in the resistance training group and L-arginine supplementation.

Table 1. Mean and standard deviation of height, weight, age and BMI of subjects in groups

Groups	Age (years)	Height (cm)	Weight (kg)	BMI
	M±SD	M±SD	M±SD	M±SD
Resistance training group	26.5 ± 3.12	177.1 ± 4.11	76.21 ± 4.74	24.53 ± 0.19
Resistance training +Leucine supplement group	27.1 ± 2.81	177.6 ± 3.85	78.93 ± 4.14	25.04 ± 0.29
Resistance training + L-arginine supplement group	27.7 ± 3.11	178.8 ± 3.59	77.39 ± 4.36	24.18 ± 0.95
control group	26.7 ± 1.9	176.7 ± 3.90	76.58 ± 5.11	24.50 ± 0.54

Table 2. Results of analysis of covariance to compare variables between groups

variable	group	Pre-test	Post-test	F	P
Uric acid content (mg / dL)	Resistance training group	4.74 ± 0.88	4.56 ± 1.07	9.885	0.097
	Resistance training +Leucine supplement group	4.83 ± 1.41	4.69 ± 1.11		
	Resistance training + L-arginine supplement group	4.59 ± 0.99	4.51 ± 1.10		
	control group	4.48 ± 1.45	4.42 ± 1.66		
Urea level (mg / dL)	Resistance training group	40.11 ± 3.85	33.21 ± 5.44	10.102	0.001*
	Resistance training +Leucine supplement group	36.48 ± 6.21	34.89 ± 3.98		
	Resistance training + L-arginine supplement group	37.85 ± 5.14	35.17 ± 4.44		
	control group	38.69 ± 4.74	37.25 ± 2.19		
Creatinine level (mg / dL)	Resistance training group	0.97 ± 0.12	0.76 ± 0.08	6.847	0.001*
	Resistance training +Leucine supplement group	1.05 ± 0.09	1.02 ± 0.14		
	Resistance training + L-arginine supplement group	1.18 ± 0.11	1.16 ± 0.08		
	control group	1.11 ± 0.10	1.09 ± 0.11		
Hypoxanthine content (ng / µl)	Resistance training group	14.01 ± 2.23	16.87 ± 3.65	15.474	0.000*
	Resistance training +Leucine supplement group	12.74 ± 3.09	13.52 ± 2.96		
	Resistance training + L-arginine supplement group	13.01 ± 2.21	14.12 ± 2.84		
	control group	11.64 ± 3.21	11.01 ± 2.11		
Xanthine oxidase content (ng / µl)	Resistance training group	25.14 ± 7.11	28.66 ± 7.23	14.769	0.001*
	Resistance training +Leucine supplement group	27.15 ± 3.88	25.96 ± 4.47		
	Resistance training + L-arginine supplement group	28.21 ± 4.24	27.48 ± 5.74		
	control group	26.23 ± 2.89	27.24 ± 3.08		

Table 3. Results of Bonferroni post hoc test of variables in three groups

Variable	group	Resistance training + L-arginine supplement group	Resistance training +Leucine supplement group	control group
Urea (mg / dL)	Resistance training group	M=6.01,P=0.000*	M=8.63,P=0.000*	M=12.020,P=0.002*
	Resistance training + L-arginine supplement group	----------	M=9.251,P=0.124	M=8.11,P=0.085
	Resistance training + Leucine supplement group	----------	----------	M=8.71,P=0.079
Creatinine (mg / dL)	Resistance training group	M=8.23,P=0.000*	M=5.141,P=0.000*	M=10.101,P=0.000*
	Resistance training + L-arginine supplement group	----------	M=8.845,P=0.089	M=0.214,P=0.123
	Resistance training + Leucine supplement group	----------	----------	M=2.268,P=0.158
Hypoxanthine (ng / µl)	Resistance training group	M=5.44,P=0.000*	M=4.325,P=0.006*	M=3.222,P=0.000*
	Resistance training + L-arginine supplement group	----------	M=6.251,P=0.102	M=0.114,P=0.078
	Resistance training + Leucine supplement group	----------	----------	M=3.71,P=0.117
Xanthine oxidase (ng / µl)	Resistance training group	M=4.141,P=0.000*	M=3.325,P=0.001*	M=4.417,P=0.000*
	Resistance training + L-arginine supplement group	----------	M=6.058,P=0.025*	M=2.144,P=0.100
	Resistance training + Leucine supplement group	----------	----------	M=1.544,P=0.101

Discussion and Conclusion

Statistical analysis did not show a significant difference between the groups in the variable of uric acid. Also, the results showed that resistance training significantly decreased urea, creatinine and increased hypoxanthine and xanthine oxidase. Creatinine and significant reduction of hypoxanthine and xanthine oxidase were compared to the resistance training group. There was a significant difference between the two groups of supplements only in the amount of xanthine oxidase. In the resistance training group and Leucine supplementation, the amount of xanthine oxidase was significantly greater than in the resistance training group and L-arginine supplementation.

Different physiological responses during Ramadan are most likely the result of a disturbed sleep-wake cycle and altered eating and drinking cycles, or most likely a combination of these factors (22). In addition, eating food at unusual times can have different metabolic effects, and lifestyle changes can have significant effects on metabolism (23).

The importance of protein for athletes has long been known and the use of protein supplements in many athletes, especially strength athletes to increase their performance and performance seems to be necessary (24). The effectiveness of dietary protein or protein supplements in athletes is such that in addition to increasing muscle mass and preventing protein catabolism during intense or prolonged exercise, it also increases glycogen synthesis after exercise and prevents anemia. Exercise is associated with increased synthesis of hemoglobin, myoglobin, oxidative enzymes and mitochondria during exercise (25). Adequate protein uptake is also essential for accelerating synthesis and increasing muscle mass under these conditions. Resistance training simultaneously increases both the synthesis and breakdown of muscle proteins, but under these conditions, the synthesis of muscle protein overcomes its breakdown, which ultimately leads to an increase in pure protein (26). Therefore, the need for protein and positive energy balance increases in those who participate in intense resistance training sessions.

There are different results about the effect of fasting on uric acid and urea. In a study, Azwany et al. examined the effect of one month of fasting on 43 Muslims. They fasted after 4 weeks, although the amount of water absorption was normal; reported a significant increase in urinary osmolarity. Blood urea levels did not change significantly during 4 weeks (27). Comparing the blood samples of 19 fasting men during the first days and 23 months of Ramadan, Indra and colleagues found that serum urea, triglyceride, total cholesterol and LDL-C levels were significantly reduced (28). Azizi stated in a review study that serum uric acid levels increase abnormally during long-term starvation, possibly due to decreased glomerular filtration rate (GRF) and uric acid release. However, in Islamic fasting, there is only a slight increase in uric acid; this condition can be due to the nature of short and intermittent fasting. No change in uric acid may be attributed to the small number of samples studied or the high dispersion of scores. In the study of Bijeh et al. (2012), 12 weeks of aerobic exercise with 3 sessions per week was associated with increased aerobic capacity and decreased body mass index along with decreased uric acid (29). In this study, although aerobic exercise was used as an exercise intervention, but exercise intervention led to a decrease in uric acid. This discrepancy can perhaps be attributed to weight loss and body mass index in response to aerobic exercise in the study.

The increase in blood urea concentration may be due to increased protein catabolism and may be due to resistance activity or decreased renal blood flow. Some studies suggest an increase in the concentration of urea in the blood, which may be due to exercise and resistance, which stimulates energy consumption and reduces energy intake. In this regard, when causing physical stress, albumin and urea excretion also increases in individuals. Exercise is one of the factors that can alter these biochemical factors. Other factors that can increase blood urea levels include increased protein in the diet, gastrointestinal bleeding and dehydration, or inadequate fluid intake, especially during fasting (30).

Creatinine is produced mainly as muscle excretion and is a good measure of kidney function, because if the kidneys do not remove it from the blood, its concentration in plasma will increase. Sometimes long-term fasting, thirst, and dehydration transiently increase creatinine levels, which can be relieved by compensating for dehydration (31). In a study to evaluate the effect of exercise on net protein catabolism, Kals et al. (2000) designed an exercise session for excreted levels of urea, cranitine, and trimethyl histidine. In this study, eight healthy men rode a bicycle for 90 minutes with about 45% of their maximum oxygen consumption. During exercise, total urinary urea increased by 100% compared to before exercise, and excreted creatinine increased by 50%. Also, although the amount of excreted trimethylhistidine tended to increase, it did not change compared to creatinine, which is an indicator of protein catabolism (32).

In the present study, resistance training in the resistance training group reduced the amount of urea and creatinine, but the resistance training group with Leucine and L-arginine

supplementation, this reduction in urea and creatinine was less than the resistance training group, and this is probably the effect of supplements on catabolism. It is a protein and has reduced its amount. Based on the information we have, the present study is the first to compare the effects of Leucine and L-arginine supplements and fasting on protein catabolism.

Hypoxanthine is the final product in the recycling pathway of purine adenines, so that if it is converted to xanthine in the oxidation pathway by xanthine oxidase enzyme, purine is lost and finally in human it is converted to uric acid again by xanthine oxidase activity and excreted from the body. Therefore, the amount of hypoxanthine is important and can be considered as an indicator of severity (33). Oxygen consumption is a critical point for hypoxanthine (34). On the other hand, hypoxanthine, because it represents ATP depletion, can be considered as an indicator of energy depletion and cellular metabolic stress (35).

In a study by Chung Liu et al. (2005), it was reported that xanthine oxidase is the main source of free radical production in intense and tedious activities. They suggested that mitochondria play a lesser role in this type of activity. Activation of xanthine oxidase enzyme has been shown to be one of the important reasons in the production of free radicals. Xanthine oxidase is a metaflavone protein (36) that produces large amounts of free radicals by consuming oxygen, and as a result, this enzyme is one of the most important sources of O2 and H2O2 production in the body (37).

In the present study, resistance training in the resistance training group increased the amount of hypoxanthine and xanthine oxidase, but in the resistance training group with Leucine and L-arginine supplementation, the amount of hypoxanthine increased in the post-test compared to the pre-test. There was a slight increase and the amount of xanthine oxidase in the resistance training group was observed along with taking both supplements, and this is probably the effect of the supplements on protein catabolism and has reduced its amount.

Finally, resistance training combined with Leucine and L-arginine supplementation in fasting athletes could provide a platform for reducing protein catabolism due to exercise, and it can be said that coaches and athletes to reduce catabolism due to exercise during Ramadan. They can use Leucine and L-arginine supplements to increase performance and reduce damage. Combining resistance training with Leucine and L-arginine supplementation can reduce the protein catabolism induced by exercise in fasting athletes with relative effects on urea, creatinine, hypoxanthine and xanthine oxidase factors, so this approach can be considered by trainers. And athletes. It seems that coaches and athletes, using knowledge-based factors affecting exercise such as nutrition, can increase performance and reduce injury.

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