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COVID-19 Pandemic Challenges for Nutrition Research

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The coronavirus disease 2019 (Covid-19) became a global health concern and dramatically affected many aspects of life on earth. The effects of COVID-19 and its preventive strategies during 2019-2020 have been studied in the fields of psychology, sociology, economy, and politics (1-4). Although the world health organization and regional health authorities responded promptly to the pandemic, but most of the recommendations especially in the prevention and treatment of the disease were based on animal studies and studies conducted on other coronavirus diseases or influenza (5). One example was the recommendation for vitamin D supplementation for prevention and treatment of COVID-19, which was suggested in a primary guideline in China (6). This recommendation was based on the findings of previous studies regarding the relationship between low serum vitamin d levels and severity and mortality of infectious respiratory diseases and preliminary observational studies that indicated low serum vitamin D among COVID-19 patients (7, 8). Findings of further observational studies also strengthened the role of vitamin D in prevention and treatment of the disease (9-11). Later on, other observational, cohort and randomized controlled trials (RCT) indicated that vitamin D has a role but this role might not be as miraculously as expected and vitamin D supplementation in COVID-19 patients might improve the condition but may not affect the disease outcome (12-14). The following nutritional recommendations suggested vitamins and minerals supplementation based on recommended dietary allowance mainly from natural sources not supplements and the treatment of vitamin deficiencies if detected in laboratory evaluations. Majority of the recent guidelines do not recommend high dose vitamin D supplementation in COVID-19 patients as there is no sufficient evidence in this regard (15).

These findings indicate a gap of knowledge and the need for conducting studies that produce high level of evidence, including cohort studies, RCTs, and meta-analysis. Enormous number of systematic reviews and Meta analyses were conducted from 2020 and the problem in many of these reviews were conducting the study on non-COVID-19 studies as the other sources of high-level evidence were lacking. Therefore, it might be hypothesized that a vicious cycle was forming in terms of developing scientific evidence. Only 88 RCTs have been registered in the clinical trials website from the emergence of the disease till the time this editorial was prepared among which two were suspended due to adverse outcomes or changes in the blood profile of the recruited subjects, four were terminated due to adverse events, stopping public PCR testing, initiation of COVID-19 vaccination, or the unlikely beneficial findings based on previous studies (https://clinicaltrials.gov/ct2/results?term=vitamin+D&cond=COVID-19&age_v=&age=1&age=2&gndr=&type=Intr&rslt=&Search=Apply). This data indicates a high risk for publication bias, wrong hypotheses, and insufficient data regarding nutritional recommendations for prevention and treatment intervention of different levels of COVID-19 nearly three years after the emergence of the disease, which can be considered normal at the time of unprecedented events.

On the other hand, the large number of retracted papers in the pandemic might be an indicator of loosened peer review standards to disseminate knowledge and the courage of researchers in presenting their hypotheses and findings (16). However, the pandemic opened door for the

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integration of new science, data science, into medicine and health sciences and provide a good match for prompt situation assessment and decision making. Nevertheless, journal review process and peer review protocols should be updated to be effective in time of emerging health and epidemiologic events.

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Health Risk Assessment and Evaluation of Nitrate and Nitrite in Salad Vegetables of Mashhad City

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A R T I C L E I N F O	A B S T R A C T
	Introduction: Nitrate and nitrite are parts of the nitrogen cycle and can enter human body through eating and drinking. In spite of the fact that nitrate is characterized as a safe compound, it may turn into nitrite form in the body and react with amines to form dangerous compounds like nitrosamines, preventive cautions; therefore, the intake of nitrate should be controlled.
	The aim of this research was to analyze data and risk assessment of nitrate/nitrite content in four commonly used salad vegetables (onion, cucumber, lettuce, and, tomato) that were sold in the Mashhad central vegetable market.
	Methods: In this study, data from 1008 samples between 21st March 2019 to 20th March 2022 that were tested based on spectrophotometric method were analyzed by SPSS version 22 and compared with the Iranian National Standard and WHO limits. **Results**: Nitrate and Nitrite mean values of four analyzed vegetables were: onion = 76±6 mg/ kg; 1.45±0.99 mg/kg, tomato = 95±15 Mg/kg; 2.35±1.28 Mg/kg cucumber 216±30 Mg/kg, 2.50 ±1.30 mg/kg, lettuce 1050±234 mg/kg;2.89±1.54 respectively. There was an increasing trend in nitrate levels over the 3 surveyed years. The Mean increase was significantly different between vegetables in 3 years (p<0.01). Mean daily intake of nitrates from vegetables was within acceptable daily intake (ADI) limit and the Noncarcinogenic index was less than EPA limits for children and adults. Target hazard quotient (THQ) and estimated daily intake (EDI) of nitrite and nitrate were less than the EDI value (1 mg/kg bw/day) and 3.70 mg/kg bw/day, respectively. But lettuce consumption should be controlled among children (THQ=0.95). **Conclusion**: Nitric compounds intake from salad vegetables is considered safe for Iranian consumers. However, the EDI of nitrate from other sources, including processed meat products and drinking water, should be regarded.

Introduction

In recent years the "Covid 19" breakout, induced the increasing consumption of vegetables as a good source of antioxidants(1). Meanwhile, vegetables are the major origins of nitrate and nitrite and nitrate entrance through comestibles can lead to human body damage(2, 3). Also, today's one great ecological issue is nitrate pollution. Accumulation of nitrate and nitrite in the body threatens human health, and problems such as cancers, digestion and absorption disorders, and even death in children may occur (4). Nitrogen cycle is the source of nitrate and its derivatives and they can infare in water and food via preservatives added to food, fossil fuels, and fertilizers (5)

In spite of the fact that nitrate is characterized as a safe compound, since it is converted to nitrite form in the body, which may react with amines to form dangerous compounds like nitrosamines, preventive cautions, the intake of nitrates should be controlled(6, 7). These compounds can cause methemoglobinemia in newborns and gastrointestinal cancer in adults and further make mutagenic and teratogenic deteriorations (8). Confrontation to nitrate derivatives also may occur by consumption of drinking water, processed meat, or vegetables (9), on the other hand, nitrate can enter drinking water through human resources. The most important sources of nitrate in the environment and drinking water are agricultural and non-agricultural

sources(10). But around 80% of nitrates uptake is from fruits and vegetables(11).

To calculate the mean intake of nitrite and nitrate, the use of each current salad vegetable per day (kg/day) is multiplied by the median value of nitrate (mg/kg fresh weight) in that type of vegetable, and the results are expressed in mg/kg body weight/day(12).

Target hazard quotient (THQ) is computed as the ratio of confronting to the injurious stuff and the reference dose which is the largest superficies at which

no antagonistic wellbeing impacts are anticipated. THQ > 1 means that Noncarcinogenic risk is not acceptable (13).

This research was established to evaluate nitrate and nitrite contents in 4 commonly selected vegetables and analyzed their health risk effects on the population of Mashhad city.

Material and Methods

Sampling

In this study 1008 fresh salad vegetable samples, including onions, tomatoes, cucumbers, and lettuce, were randomly collected between March 2019 and March 2022 from the main fruit and vegetable market located in Mashhad province of Iran based on the following sampling plan; Monthly, each month 7 samples were randomly selected from the sellers (2 kg of each sample) and totally 1008 vegetable samples were collected during 3 years.

After recording each sample's information in one predesigned form, including the sampling date and harvest time, samples were placed in clean and inert containers (i.e. plastic bags and containers to protect them from cross-contamination, damage, and leakage). They were analyzed immediately and if it was not possible to test quickly, they were packed in plastic wrap and stored in the refrigerator.

Chemicals

Nitrate Measurement

Extraction of the sample with hot water was performed following the precipitation of proteins by solutions of potassium hexacyanoferrate and zinc acetate. The obtained solution was filtered and the nitrate was reduced to nitrite by metal cadmium, sulfanilamide chloride, and N - 1 Ethylene diamide

dihydrochloride then filtered and the red (purple-pink) complex formed by nitrite was measured at 538 nm by spectrophotometer(14, 15).

Nitrite Measurement

Potassium hexacyanoferrate and zinc acetate were used for the extraction and precipitation of proteins of the sample with hot water. After filtration of the obtained solution, sulfanilamide chloride and N- (1-naphthyl) ethylenediamine dihydrochloride was added to the filtered solution and a red complex was generated by nitrite at a wavelength of 538 nm by spectrophotometer was measured(14, 15).

Risk Assessment Method

The health risks related to the consumption of vegetables were displayed in Tables 3 and 4.

To assess the risk, the concentration of these contaminants in each type of vegetable and the bulk of vegetable intake by each person per day were calculated as a base. Then, the risk potential and risk index for health risk assessment were determined. Risk of noncancerous effects was also assessed by gaining the non-cancer risk factor (NHQ). The health risk of exposure to nitrate and nitrite for an adult person[1] and children[2] due to the consumption of vegetables was determined by the following Equation1 (3).

$$EDI = \frac{EF*ED*DC*MC}{bw*AT} \quad \text{(Equation: 1)}$$

EDI stands for "estimated daily intake" (mg/kg bw/day); EF: "exposure frequency" (365 days/year); ED: exposure duration for an adult (70 years for adults and 6 years for children); DC: Daily Consumption. Based on references, per capita consumption of cucumber, tomato, onion, and lettuce, was 109, 109, 39, and 58, g/day for Iranian adult consumers and half of these for children (3, 12), MC: Mean Concentration of nitrite and nitrate (mg/kg wet weight); bw: Mean body Weight of adult consumers aged 16–70 years (70 kg); and children aged 6 years (20 kg). AT: Average Time 25550 days for adults and 2190 days for children (16, 17).

Hazard Quotient

Noncarcinogenic risk was calculated via the Target Hazard Quotient (THQ) used by previous researchers (18).

[1] 70years old and 70 kg body weight

[2] 6 years old and 20 kg body weight

Total Hazard Quotient (THQ) was obtained by Equation 2.

$$THQ = \frac{EDI}{Rfd} \qquad \textbf{(Equation: 2)}$$

In this formula, Rfd 1.6 is the reference dose of nitrate (mg/kg bw/day), and 0.1 is used for nitrite according to the risk information system proposed by the United States Environmental Protection Agency(19).

If THQ was calculated less than 1, it shows that it is in the acceptable range of risk level for chronic health risk, and if THQ was higher than 1, it indicates that the non-carcinogenesis risk is not at a suitable level(20), there is no carcinogenic effect defined for nitrate and nitrite defined yet(21, 22).

Total Non-carcinogenic risk is calculated based on the following equation:

Total THQ (HI) = ∑THQ (Equation: 3)

Statistical Analysis

The outputs were analyzed by the SPSS software (version 22.0). At first, to examine the normality of data, a Kolmogorov–Smirnov test was used. Then a k- independent sample test was used to examine the significant relationship between nitrite/nitrate levels of vegetables. Plots were illustrated by Excel 2016. Comparisons between years and seasons were done by statistical Kruskal – Wallis test and $P<0.05$ was considered as statistical significant difference.
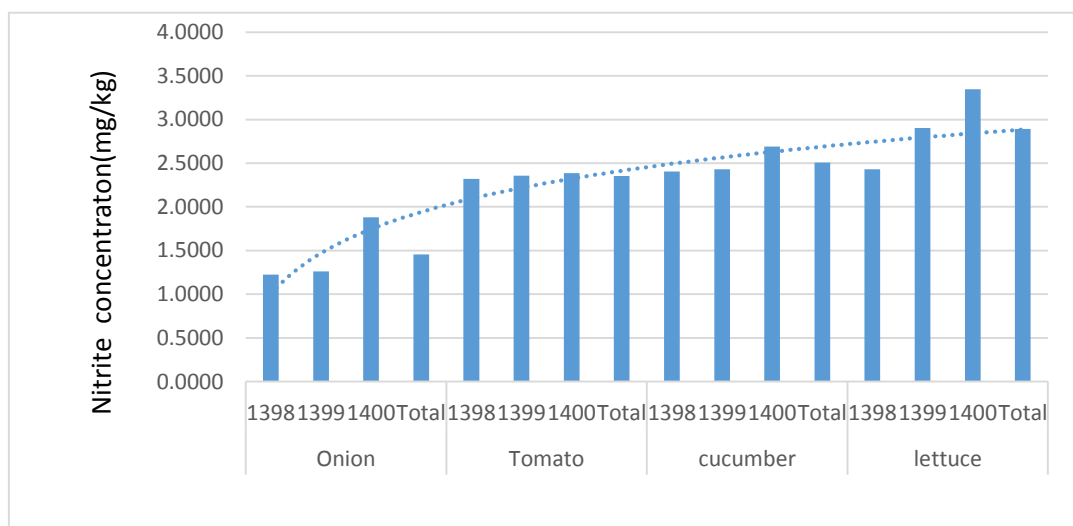

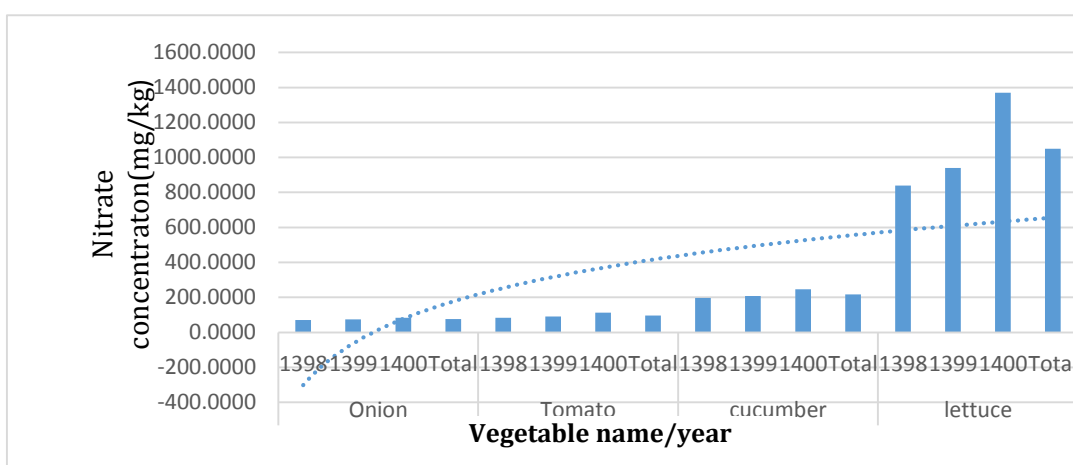**Figure 1.** Nitrite concentration (mg/kg) comparison in 4 salad vegetables in Mashhad, Iran

Figure 2. Nitrate concentration (mg/kg) comparison in 4 salad vegetables in 3 years in Mashhad, Iran

Results

Nitrite

As can be seen in figure 1, a relatively high difference in the concentration of nitrite was recorded between and within the different types of vegetables. The mean concentration of nitrite in different vegetables in 3 years data, can be summarized as followed; onion (1.45 ± 0.99mgkg_1), tomato (2.35 ± 1.28mgkg_1, cucumber (2.50 ± 1.30mgkg_1), and lettuce (2.89 ±1.54mgkg_1), respectively.

Nitrate

As can be seen in figure 2, relatively high variability was recorded considering the concentration of nitrite between different types of crops (p value<0.05). In this regard, the mean concentrations of nitrate in different groups of vegetables, can be summarized as onion (76.15 ± 6.83mgkg_1), tomato (95.90 ± 15.65mgkg_1), cucumber (216.93 ± 30.24mgkg_1), and lettuce (1050.06 ±234.56mgkg_1), respectively.

As is shown in figure 2 the nitrate mean in lettuce was the highest compared to other vegetables.

Risk Assessment

Results of risk assessments are shown in tables 1 and 2.

Table 1. Nitrite Estimated daily intake and THQ of nitrates through 4 vegetables consumed in Mashhad (3 years average concentration)

Vegetable	Mean nitrite content (mg/kg)	Per capita consumption (g/day)	Mean nitrite intake (mg/day/person)	EDI Child(mg/kg bw/day d)	EDI Adult(mg/kg bw/day)	RfD (mg/kg bw/day)	THQ CHILD	THQ ADULT
Onion	1.45	39	0.92	0.001	0.005	0.1	0.01	0.05
Tomato	2.35	109	2.56	0.005	0.003	0.1	0.05	0.03
Cucumber	2.50	109	2.72	0.007	0.006	0.1	0.07	0.06
Lettuce	2.89	58	1.67	0.004	0.002	0.1	0.04	0.02

Table 2. Nitrate Estimated daily intake and THQ of nitrates through 4 vegetables consumed in Mashhad (3 years average concentration)

Vegetable /standard limit	Mean nitrate content (mg/kg	Per capita consumption (g/day)	Mean nitrate intake (mg/day/person)	EDI Child(mg/kg bw/day	EDI Adult(mg/kg bw/day)	RfD (mg/kg bw/day)	THQ CHILD	THQ ADULT
Onion/90	76.15	39	2.97	0.07	0.04	1.6	0.04	0.02
Tomato/120	95.90	109	10.45	0.26	0.15	1.6	0.02	0.09
Cucumber/300	217.06	109	23.65	0.06	0.03	1.6	0.04	0.02
Lettuce/1500	1050.06	58	60.94	1.52	0.87	1.6	0.95	0.54

Discussion

This study intended to evaluate the nitrate/nitrate concentration level mean in the 4 most commonly used salad vegetables sold in Mashhad central fruit and vegetable. Accordingly, nitrite and nitrate levels of 1008 samples of vegetables including onion, lettuce, tomato, and cucumber, was determined.

Nitrite and Nitrate Concentration

2.50 ± 1.30 mg/kg, lettuce 2.89 ± 1.54 mg/kg; respectively. Currently, there is no defined limit for nitrite content in these 4 salad vegetables in existent national or international standards. The mean nitrite levels in these crops ranged; onion 1.45 ± 0.99 mg/kg, tomato, 2.35 ± 1.28mg/kg cucumber

Nitrite level in onion (1.45mg/kg) was more than 0.49 mg/kg.in Bahadoran et.al results (3) and the mean nitrite content in cucumber samples was 2.5mg/kg which is higher than 2.1 mg/kg in Roshana et al 2021 study (20) and more than 0.57 mg/k in Bahadoran et al (3) study.

Our result indicated that nitrite level in lettuce (2.89mg/kg) was more than 0.54mg/kg reported in the study by Bahadoran et.al (3) and was approximately similar to the reported 2.6 mg/kg in studies by Correia et al (2010) and Roshana et al (2021) (20, 23).

Our results indicated that nitrite concentration in all vegetables was very low and was directly related to their nitrate content. Mean Nitrite levels in lettuce was greater than other vegetables. Comparison of the samples obtained from three years of evaluation showed that the nitrate level increased during the three years. Our findings also showed no significant difference in pollutants between different months and seasons of the year, and the difference in the amount of pollutants in three years was not significant because of a wide range

of crop cultivation (45 different geographical regions).

Nitrate content of onion in this study (76mg/kg) was lower than the standards limits (90 mg/kg), but comparison between 3 years showed a significant increase in nitrate content (p value<0.05). Anyway, it might be due to the increased use of nitrate fertilizers during the 3 years.

Nitrate mean concentration in other samples including onion (82.25 mg kg-1) and tomato (110.93 mg kg-1) was greater compared to similar studies, such as the study by Mehri et. al(24).

In our study Lettuce with mean nitrate concentration of 1050.06 mg/ kg had the highest level of nitrate among other vegetables. But these results were lower than the studies performed in Taiwan (1520 mg/ kg) (25); in UK (2330 mg/kg) (26); and in Iran (3637 mg/kg)(27).

Mean nitrate concentration was not significantly different between seasons and months in all 4 vegetables (p>0.05).

Nitrate content in vegetables can be affected by various factors like vegetation time, temperature, soil type, the density of vegetables in the field, crop maturity, harvest interval, humidity, and fertilizers (7).

Risk Assessment

The risk for nitrate and nitrite ingestion via vegetables depends on the type of vegetables, environmental condition, farming methods, soil nature as well as the status and period of product storage and/or food processing (28).

The nitrite THQ WAS very lower than index and there is no worry about this in these vegetables.

The largest nitrate THQ was related to lettuce and the lowest amount was shown in tomatoes in children, but in adults it was lettuce> tomato> cucumber= onion.

THQ of lettuce for children (0.95) was near the standard limit (1). Therefore, it is recommended to control lettuce consumption in children less than 6 years old.

Hence, local people are not at carcinogenic risk due to the ingestion of these four vegetable crops. The hazard index caused by both contaminants was less than 1, and no adverse effect in terms of health risk was considered.

Total exposures resulting from these 4 crops and hazard index values of children aged 6 years were found significantly higher than adults (p-value < 0. 05).

Nitrite EDI values for children decreased in the subsequent order: lettuce> > tomato > cucumber >onion. The lettuce sample had the highest EDI value (1.52 mg/kg bw/day) and while the onion sample had the lowest EDI value (0.07 mg/kg bw/day). Onion EDI was lower, and lettuce was higher in comparison with Mehri et al(24).

Some factors are associated with the health risk of nitrate and nitrite, like consumption rate, the intrinsic risk caused by nitrates and nitrite, and body weight. Our results implied that THQ values for each of the samples in this study were less than 1.

Conclusion

Our results showed that the mean nitrate concentration was less than the permissible limit in all vegetable samples.

Comparison of nitrate levels between 3 years showed an increasing trend in 4 vegetables (p<0.01) that might be due to the increasing use of fertilizers.

However, no significant difference was seen between seasons and months, vegetable type, and pollutants (p>0.05).

Health risks associated with both nitrate and nitrite were less than 1, and no adverse effect in terms of health risk was considered.

Therefore, the intake of nitrate through such food could be considered safe for Iranian consumers.

However, it would be noted that other sources such as water and processed meat products must be regarded.

Acknowledgment

Conflict of Interest

None

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Evaluation of Pre-Sarcopenia and Sarcopenia in a Well-Nourished Late-Middle-Aged Population: A Feasibility Study of a Registry

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A R T I C L E I N F O	**A B S T R A C T**
	Introduction : Sarcopenia is characterized by weakness of the skeletal muscles. This study aimed to evaluate the prevalence of sarcopenia and its relationship with dietary intake, socioeconomic status, depression, lifestyle, and physical activity for the first time in a well-nourished over 55 year's old population.
	Methods: This study was conducted on a well-nourished above 55 years old population. The European Working Group on Sarcopenia in Older People-2 (EWGSOP-2) guideline was used to determine the prevalence of sarcopenia. Muscle mass was assessed using bioelectrical impedance analysis. Muscle strength and performance were evaluated using handgrip and gait speed, respectively. The dietary intake, socioeconomic status, psychological health, lifestyle, clinical factors, and physical activity were examined using valid tools.
	Results: A total of 766 well-nourished individuals (mean age=65.14±6.84 years old; male:female ratio= 1:1.99) were evaluated for sarcopenia. The prevalence of pre-, confirmed-, and severe sarcopenia were 23.9%, 1.8%, and 1.3%, respectively. Age was significantly related to sarcopenia (OR=1.096 (95% CI: 1.069-1.124), p<0.001). Females had an increased risk of pre-sarcopenia (OR=2.189 [95% CI: 1.48-3.239], p=0.002), while males were at higher risk of confirmed and severe sarcopenia (OR=15.102 [95% CI: 4.461-51.131], p<0.001). The decision tree model of sarcopenia indicated age as the main predictor for sarcopenia.
	Conclusion: According to EWGSOP-2, the overall prevalence of sarcopenia was 27% among well-nourished almost healthy elderly population. There was a relationship between age and sarcopenia. Females and males were at higher risk of pre-sarcopenia and confirmed and severe sarcopenia; respectively. Further research is strongly suggested to asses pre-sarcopenia.

Introduction

The term *sarcopenia* combined from *Sarco* (flesh) and *Penia* (low) presents losing muscle mass (1, 2). Sarcopenia was defined in the 1980s as a progressive and generalized skeletal muscle disorder and classified as a disease since 2016 by World Health Organization (WHO) (1, 2). According to the descriptions, sarcopenia is a complex disorder, which decreases muscle strength, power, mass, and performance (1-4). The primary outcomes of sarcopenia are a higher risk of falling, physical disability, higher healthcare expenses, dependency, and mortality

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in the elderly (1-4). The prevalence of this disease varies worldwide, ranging from 4 to 36%, with a mean of 10% (5-7). The prevalence of sarcopenia is likely to be higher than reported because it is often undiagnosed (1).

The global elderly population is rising, especially in Iran, and sarcopenia is a common age-related complex disorder influenced by many factors still being explored. The leading cause of sarcopenia is aging (usually more than 65 years old), and lifestyle, nutritional patterns, financial and social statements, psychological health, and physical performance are sarcopenia's possible acquired factors (2, 3, 8). While studies are still ongoing, the evidence suggests that more sarcopenia studies are needed worldwide (9). Furthermore, the risk factors and characteristics of sarcopenia should be evaluated, as well as instruments and cut-offs compared using the principles of action research (9).

An evidence-based study should be conducted in a population with the lowest probability of bias to address gaps, determine major lifestyle risk factors, including lifestyle, dietary patterns, socioeconomic, psychological health, and physical activity, and compare data across nations and regions to provide a more qualified guideline for this disease (2, 3, 8). Disease and malnutrition are already considered as the most decisive risk factors for sarcopenia. Therefore, selecting a healthy population without these risk factors may be critical in assessing the impact of lifestyle components on sarcopenia risk and understand the goal of adapting clinical and community-based lifestyle interventions in the future (1-4, 8-10).

Despite research being conducted worldwide on this subject, there are still some gaps in the field that need to be considered. Considering the population aging and the lack of studies on lifestyle, nutritional pattern, financial, socioeconomic, psychological health, and physical performance in a population with the least bias risk, this study aimed to evaluate a well-nourished population without any severe disease as a feasibility study.

Material and Method

Ethics Approval and Consent to Participate
The Ethics Committee approval was obtained from Mashhad University of Medical Science, Iran: IR.MUMS.REC.1398.229 (http://ethics.research.ac.ir). According to the Ethical Principles and Declaration of Helsinki, informed written consent was obtained individually from all participants. According to our population's age and vast content of assessment, the short form of each questionnaire was used, and online registration was established to prevent people from long attendance at clinics. Every participant was given 15-30 minutes free of charge nutrition consolation by a Registered Dietitian and Nutritionist (RDN) with a full-color report of their status. According to medical ethics, if a specific health-related issue was recognized during any assessment stage, the individuals had been proposed to visit a specialist.

Design
This study was conducted in collaboration with the Nutrition Department of Varastegan Institute for Medical Sciences, the Welfare Organization of Khorasan Razavi (Central Khorasan) Province, and the Khorasan Razavi (Central Khorasan) Retirement Association. The data were collected from November 2019 to May 2021.

Sample Size
The sample size was estimated by assuming the prevalence of sarcopenia and pre-sarcopenia (50%) among the retired aged population (n≈275000), the confidence level (95%), and the margin of error (3.5%) using the n=$\frac{z^2 * \hat{p}(1-\hat{p})}{\varepsilon^2}$ formula. The final calculated sample size was 782 individuals.

Inclusion and Exclusion Criteria
The inclusion criteria were the age of 55 or more, currently living in one of the municipal areas of Mashhad province (city and covering villages) for the last ten years, lack of severe disease leading to catabolism (advanced diabetes, cardiovascular diseases, rheumatism, osteoporosis, cancer, and chronic kidney disease), having proper nutrition, no confirmed mental disabilities, Alzheimer, or psychological disorders. The exclusion criteria were being at the risk of malnutrition using Mini Nutrition Assessment-short-form (MNA-SF), being hospitalized in the previous full year, physical disabilities, unreported catabolic disease history, and having metal in the body. Nevertheless, obesity and overweight were not considered as the exclusion criteria.

Study Participants
The population was selected on two levels in nursing houses and the population using the

convenient method in different areas of Mashhad city (Figure 1). The sampling continued in the case of individual exclusion until the sample size was reached. All eligible individuals in nursing houses were included due to the limited population. According to the Mashhad municipality report, the population of all 13 municipal areas was equal, which provides an equal contribution to municipal regions. The living region of individuals was recorded in the nursing houses.
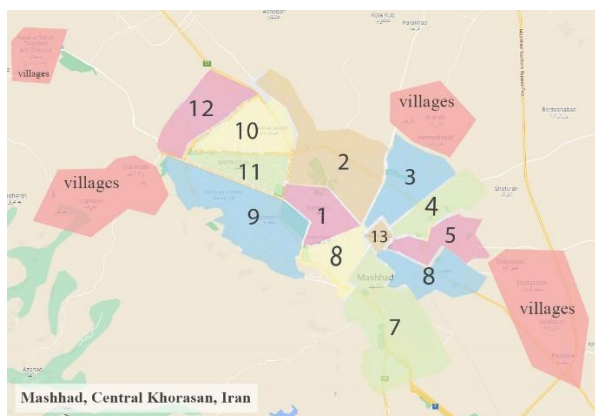

Figure 1. The 13-municipal areas of Mashhad and covering villages.

Data Collecting Progress
Two assessments were conducted on the same day. The primary assessments included demographic and social-economic records, anthropometric tests, blood pressure, nutritional, depression, and sarcopenia assessments. The secondary tests included lifestyle, anxiety, and depression tests using validated questionnaires. Expert dietitians clinically trained for three months asked all the questions in a face-to-face interview. All the data were recorded in the registration system, and no paper was used for data collection. Each assessment was performed in separate rooms.

General Information
Demographic data, working and socioeconomic status, smoking and alcohol use, family medical history, and drug consumption history were documented using a questionnaire. The financial status of each individual was determined based on their access to welfare amenities and income. The monthly income of individuals was compared with the country's average expenditures.

Malnutrition Assessment
The Mini Nutrition Assessment–short-form (MNA-SF) (2001) was used as an efficient way to diagnose wasting and protein/energy malnutrition in the aged population (11). In this questionnaire, the overall score of under seven is malnourished, 8-11 is at risk of malnutrition, and ≥12 is normal. The confounding effect of malnutrition on muscle mass and dietary intake was removed by excluding both malnourished and at-risk malnutrition populations.

Skeletal Muscle Mass Index
The bioelectric impedance analyzer (BIA) method with InBody-270 version 2018 with serial number: F1800027, InBody, Korea was used to measure skeletal muscle mass (SMM). BIA is easy to use, inexpensive, convenient, and requires minimal time for measurements with no specific difference from the dual-energy X-ray absorptiometry (DEXA) method (12,13). However, the presence of metal in the body, dehydration, and overhydration are essential factors, which may bias BIA measurements (12,14). Each person was asked to drink water and urinate before their test two hours before their test, to decrease the risk of overhydration. Dehydrated participants were asked to drink water and rest for 15-20 minutes if they were suspected of being dehydrated. Each individual was asked to stand on the BIA with the lightest available clothes and without any external objects during the assessment, including rings, necklaces, glasses, and belts, as instructed by Maughan et al. (15). SMM was divided by the square of the height in meters ($SMMI = \frac{Smm}{height^2}$)

to calculate the skeletal muscle mass index (SMMI) (3).

Muscle Strength

Muscle strength was measured using a hydraulic hand dynamometer (Hydraulic Hand Dynamometer 08-010113, Saehan, Korea) based on the Roberts et al. recommended methodology (16). Each individual sat on a chair with a bare right hand without external objects. The dominant hand upper limb was placed alongside the body with the elbow at a 90° angle, and the contralateral limb was relaxed on the thigh. The participants were instructed to provide maximum strength without shaking their hand

or feeling uncomfortable. Muscle strength was measured three times for each individual, and the mean of performances was calculated with an accuracy of 1 kg/m².

Muscle Performance

The 4-m gait speed test was applied to evaluate muscular performance. The subject was instructed to walk as fast as possible without running (3,4). The time to complete the process was recorded accurately at 0.1 seconds. The test was performed twice with 30 seconds intervals between tests, and the mean of performances was recorded.
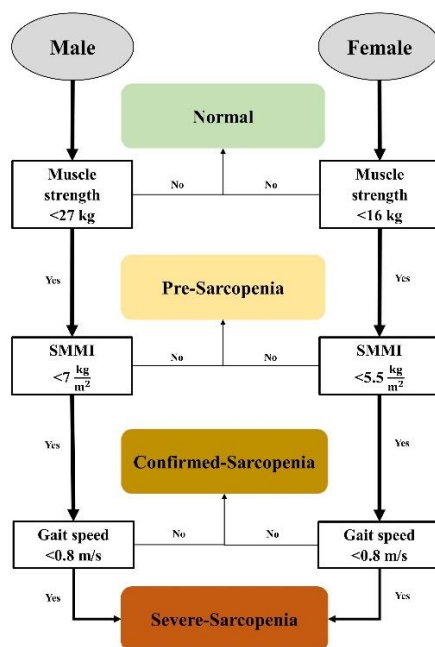
Figure 2. This figure includes cut-offs of muscle strength using handgrip, gait speed, SMMI (skeletal muscle mass index) and diagnosing definition for pre-sarcopenia, sarcopenia and severe sarcopenia according to the EWGSOP-2 (2018) guidelines.

Operational Definition and Diagnosis of Sarcopenia

The European Working Group on Sarcopenia in Older People 2 (EWGSOP-2) method was used to diagnose sarcopenia (3,4). The full description for cut-offs and classification can be found in Figure 2. In this study, people without sarcopenia are named the normal group, and three clinical stages including pre-, confirmed- and severe-sarcopenia, are categorized as the sarcopenia group.

Other Anthropometric and Clinical Assessments

Body composition data, including fat mass, muscle mass, fat-free mass, body mass index (BMI), and waist-to-hip ratio (WHR), were collected using BIA. Bodyweight and height were measured once more using a digital scale with a height measure (Body Scale Height and Weight Scale BS 703, serial number: 7030109, Body scale, Iran). The Mid-upper arm circumference (MUAC) was measured by an accuracy of 1cm as one of the critical, common, and efficient

methods for in-hospital malnutrition screenings, hospital wasting, cachexia, and mortality risks (17). A digital sphygmomanometer measured the blood pressure (OMRON 5 Series® Upper Arm Blood Pressure Monitor, Model: BP7200, Omron, Japan, and ALPK2 Sphygmomanometer A103833-8, ALPK, Japan) to evaluate the relation between blood pressure and sarcopenia as a related factor (18).

Physical Activity Assessment

The physical activity of each individual was assessed at the current age and when they were between 30-45 years as a long-term memory-based recall. According to the instruction, a valid form of the International Physical Activity Questionnaire (IPAQ) was used to classify the population into four groups (19). Then, the changes between the two physical activities were reported as increasing, decreasing, or staying the same. In addition, any specific sports or routine physical activity, including walking, swimming, and aerobics, were recorded based on the duration and frequency.

Lifestyle Assessment

The Medical Outcomes Study Health Survey Questionnaire 36-Item Short Form (SF-36) was used (20). This tool measures the quality of life based on eight domains; 1. Physical functioning, 2. Physical limitations, 3. Body pain, 4. General health, 5. Vitality, 6. Social functioning, 7. Social limitations, and 8. Mental health. This questionnaire was used for construct validity of SarQoL as a specific health-related quality-of-life questionnaire for sarcopenia in 2016 (21).

Psychological Assessment

Two tools were used for psychological assessments, and the short form of the Beck Depression Inventory (BDI-13) was utilized to validate the Iranian population (22). Additionally, Depression Anxiety Stress Scales 21-item (DASS-21) was used to assess anxiety, stress, and depression in the population (23). These questionnaires are the most common psychological assessment tools in different populations.

Nutrition Assessment

A 16-item semi-Food Frequency Questionnaire (FFQ) was developed to determine the kind and frequency of food group intake (24, 25). Then, the nutritional intake of each individual was recorded using a one-day dietary recall. Based on the evidence, one-day recall is a standard method to evaluate the nutritional intake of significant sample-sized healthy populations (26, 27). The difference between the recall and questionnaire was considered in a calculator file designed for this method to cover this method's biases and the food intake variety during a year. The data were reviewed by a registered nutritionist experienced in recall and dietary intake calculating before any changes were made. Changes included reflecting food intake from previous years and adjusting the reported intake as closely as possible to the diet pattern. The data were analyzed using the last update (December 2021) of the *United States Department of Agriculture (USDA)* food composite database (https://fdc.nal.usda.gov) with the potential to report the intake of 138 micro- and macro-nutrients with high precision. To analyze the mixed traditional foods that were not included in USDA's database, the foods were uniform to their main ingredients based on the "*Iranian Traditional Foods Recipes*," which were edited but not yet published by the "Iran Ministry of Health" and "Medical Education Nutrition Improvement Office." Cooking coefficient, weight, and containing water changes were considered in recalculating new foods during food processing.

Statistical Analysis

All statistical analyses were performed using the IBM SPSS Statistics software for Windows version 20.0. Results were presented as percentages (%) and means (M±SD) according to EWGSOP-2 sarcopenia outcome. Shapiro-Wilk, Levene, KMO, and Bartlett's tests were used to evaluate the data normality, quality, reliability, and homogeneity. One-Way analysis of variance (ANOVA) test and Tukey's test was used for continuous variables. Mann-Whitney U Test was considered for data with non-normal distribution. The qualitative variables were evaluated using the Chi-square test or Fisher's exact test, and the odds ratios (OR) for having one of three stages of sarcopenia (pre-, confirmed- and severe-sarcopenia) were obtained using binary logistic regression and scoring method. The Chi-Square Automatic Interaction Detector decision tree was applied using SPSS (P-value<0.05). However, the current study focuses only on primary findings and settings, and does not include all the analysis.

Results

There were 1074 individuals introduced for assessment (n=39 (5.1%) nursing houses vs. n=727 (94.9%) population), of which 292 were excluded, and 782 completed assessments (Figure 3). A total of 16 out of 782 data were removed because of missing data, and finally, the data of 766 individuals (mean age=65.14±6.84 years old; male:female ratio= 1:1.99) were assessed for sarcopenia (Figure 3). There was no significant difference between the 13 municipal areas of Mashhad and covering villages for the participants (p=0.952), the prevalence (p=0.271), and other factors indicating a similar distribution of this disease in the city. The number of people in the nursing house did not significantly affect the overall prevalence (p=0.859). The power of this study was 0.998 based on the leading indicator of sarcopenia.
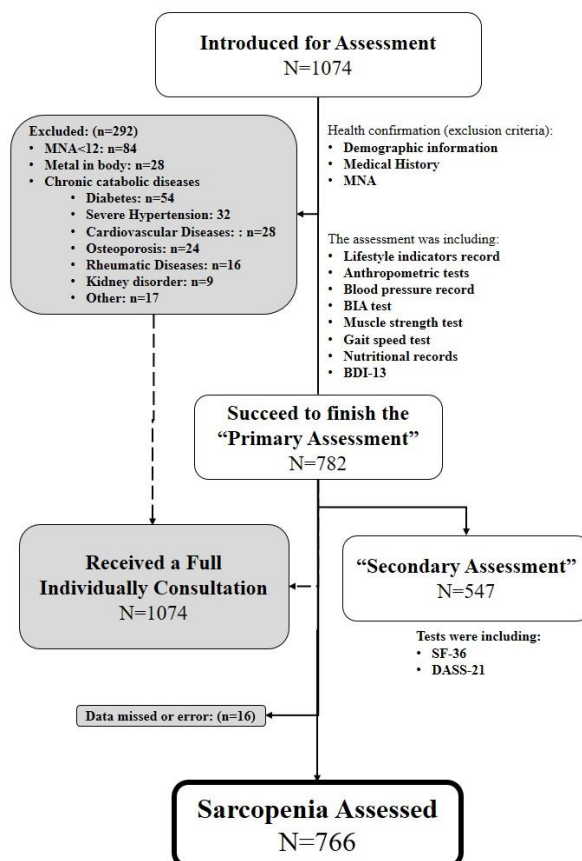

Figure 3. This flowchart includes population participant information for 1074 eligible individuals according to our registration system, the excluding path, and assessments with details. MNA: Mini Nutritional assessment, BIA: bioelectric impedance analyser, BDI-13: short form of the Beck Depression Inventory, SF-36: Medical Outcomes Study Health Survey Questionnaire 36-Item Short

The overall prevalence of normal, pre-, confirmed- and severe-sarcopenia was 73% (n=559), 23.9% (n=183), 1.8% (n=14), and 1.3% (n=10), respectively (Figure 1). There was a significant difference between all sarcopenia diagnosing criteria except the muscle performance in women, which was not significant (p=0.369), which can be related to the confirmed- and severe-sarcopenia sample size or population speed (Table 1).

Age was significantly related to sarcopenia in males (OR=1.128 (95% CI: 1.078-1.18), p<0.001), females (OR=1.081 (95% CI: 1.048-1.114), p<0.001) and the overall population (OR=1.096 (95% CI: 1.069-1.124), p<0.001). Sarcopenia groups differed significantly (p>0.001) by gender, but no statistically significant OR was found for genders and sarcopenia (pre-, confirmed-, and severe-sarcopenia). However, this finding can be explained by the significantly higher prevalence

of pre-sarcopenia in females (OR=2.189 (95% CI: 1.48-3.239), p=0.002) and higher prevalence of confirmed and severe sarcopenia in males (OR=15.102 (95% CI: 4.461-51.131), p>0.001).

Table 1. Comparison of population, age, gender and diagnosing critters according to EWGSOP-2

	Characterise	Groups				OR (95% CI)sig	P-value
		Normal	Sarcopenia				
			Pre-Sarcopenia	Confirmed-sarcopenia	Severe-Sarcopenia		
Both Genders	**n=766 (100%)**	**n=559 (73%)**	**n=183 (23.9%)**	**n=14 (1.8%)**	**n=10 (1.3%)**		
Muscle Strength	kg	23.82±7.9	26.51±6.99	15.84±4.83	23.36±3.15	19.85±5.76	-	**<0.001**
SMMI	Kg/m²	7.17±1.16	7.36±1.17	6.75±0.97	6.1±0.72	5.99±0.87	-	**<0.001**
Muscle Performance	m/sec	0.84±0.14	0.83±0.13	0.86±0.15	0.77±0.07	1±0.08	-	**<0.001**
Gender	Male (ref)	256 (100%)	196 (76.6%)	39 (15.2%)	13 (5.1%)	8 (3.1%)	1.323 (0.935-1.871)	**<0.001**
	Female	510 (100%)	363 (71.2%)	144 (28.2%)	1 (0.2%)	2 (0.4%)		
Age	N (Year)	65.14±6.84	64.03±6.45	67.52±7.02	71.64±4.97	74.2±3.79	**1.096 (1.069-1.124)**	**<0.001**
Males	**n= 256 (100%)**	**n= 196 (76.6%)**	**n= 39 (15.2%)**	**n= 13 (5.1%)**	**n= 8 (3.1%)**		
Muscle Strength	kg	31.53±5.78	33.78±4.61	24.59±1.31	24.08±1.71	22.44±2.09	-	**<0.001**
SMMI	Kg/m²	8.11±1.03	8.35±0.86	7.97±0.79	6.15±0.73	6.22±0.82	-	**<0.001**
Muscle Performance	m/sec	0.77±0.09	0.76±0.08	0.78±0.08	0.76±0.07	0.99±0.08	-	**<0.001**
Age	N (Year)	64.91±7.12	63.56±7.09	67.64±4.61	71.23±4.92	74.5±4.11	**1.128 (1.078-1.18)**	**<0.001**
Females	**n= 510 (100%)**	**n= 363 (71.2%)**	**n= 144 (28.2%)**	**n= 1 (0.2%)**	**n= 2 (0.4%)**		
Muscle Strength	kg	19.94±5.67	22.58±4.45	13.47±1.64	14±0	9.5±0.71	-	**<0.001**
SMMI	Kg/m²	6.7±0.91	6.83±0.94	6.42±0.72	5.41±0	5.08±0.32	-	**<0.001**
Muscle Performance	m/sec	0.88±0.14	0.87±0.14	0.88±0.15	0.8±0	1.05±0.07	-	**0.369**
Age	N (Year)	65.25±6.7	64.28±6.07	67.49±7.56	77±0	73±2.83	**1.081 (1.048-1.114)**	**<0.001**

The OR of sarcopenia predictors did not assessed because of the high and confounding impact of these factors on sarcopenia as diagnosing main criterias.
OR compared between Normal and Sarcopenia Group (Pre-Sarcopenia, Sarcopenia and Severe-Sarcopenia). For gender, male was reference.
Effect estimates with a p-value < 0.05 are indicated in bold for both OR and p.value.
For OR: P.value <0.05 reported * and <0.005 reported as **.

The decision tree model of having sarcopenia (EWGSOP-2) in Figure 4 (muscle strength (kg) excluded due to its over-impact) shows that age is the main predictor of sarcopenia. People over 74 years old are at the highest risk of sarcopenia in both genders. The SMMI can predict sarcopenia regardless of gender in people aged 65 or younger, and gender is the main predictor in people aged 65-74 years old. Sarcopenia in females aged 65-74 can be predicted using SMMI≤7.093. The other findings of this study include the impact assessment of physical activity, psychological, nutritional, lifestyle, and quality of life factors.

Discussion

To the best of knowledge, this large population-based Feasibility study was conducted for the first time to evaluate the burden of sarcopenia in a healthy population. The findings indicated a considerable prevalence of pre-sarcopenia in a well-nourished, nearly healthy population that can develop into severe sarcopenia. The results may help make evidence-based policies to

develop preventive and treatment strategies. In addition, age was the main predictor of sarcopenia, and gender could have a significant impact on the severity appearance of sarcopenia.
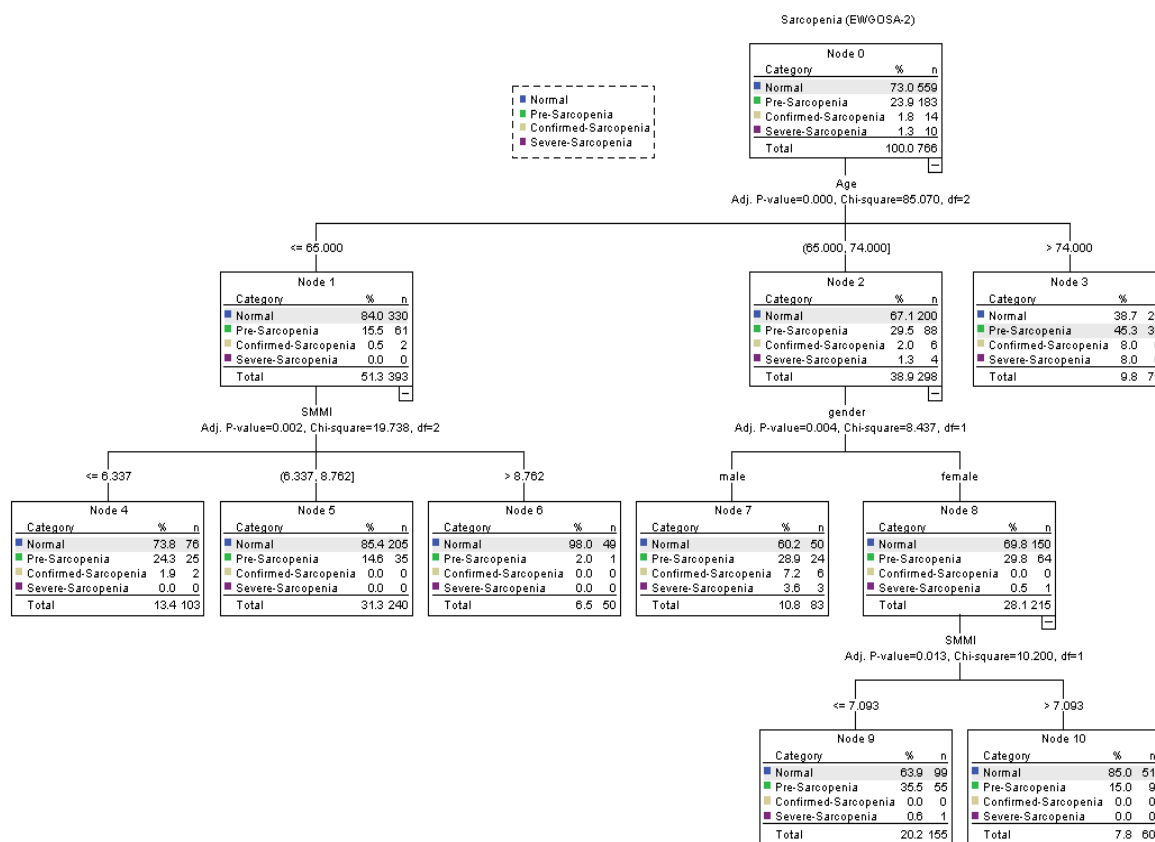

Figure 4. Gender, Age, SMMI (kg/m²), and muscle performance (m/sec) added in the model. decision three of having sarcopenia (EWGSOP-2) shows age is the main predictor of sarcopenia. Within people 65 or less years old, the SMMI can predict the sarcopenia; while in people aged 65-74 years old, gender is the main predictor.

The prevalence of sarcopenia is reported in different countries (using different definitions) between 10 to 27% in ≥60 years old and from 8 to 36% in individuals <60 years old, with a mean of 10% worldwide (7, 28). In addition, the prevalence of severe sarcopenia is expected to be ranged between 2 and 9% (7). However, no systematic review reported the worldwide prevalence of pre-sarcopenia separately (7). Based on available data, the prevalence of sarcopenia and severe sarcopenia was considerably lower than the global range in the current population. However, the prevalence of pre-sarcopenia or losing muscle strength remained unknown.

According to the current study, the prevalence of confirmed and severe sarcopenia was lowest of all studies that considered pre-sarcopenia. The prevalence of one of three stages of sarcopenia was reported in most studies between 8 to 58% that fit the current study (7, 8, 28-32). Various factors can explain this range of prevalence rates, including health, lifestyle, diagnostic cut-offs, and consideration of pre-sarcopenia (2, 3). Although pre-sarcopenia can develop into sarcopenia and severe sarcopenia, this lack of pre-sarcopenia information in other studies can be considered the most significant limitation of studies.
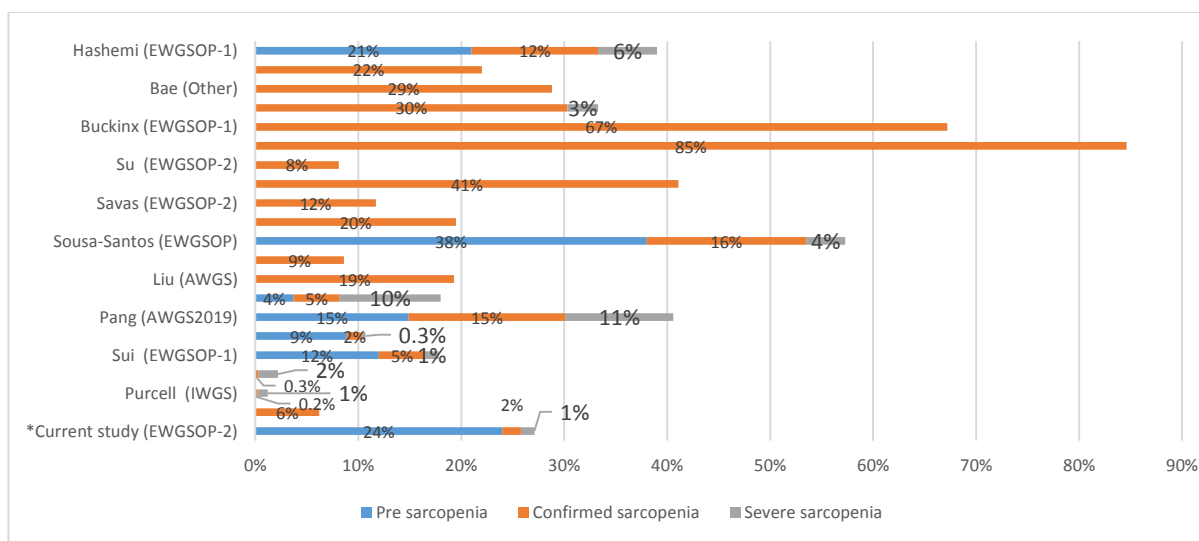
Figure 5. Illustrated comparison of the prevalence of sarcopenia in people with no considerable diseases in the world in the recent years according to finding in the table 2

One study in Japan (33) (2019) showed a prevalence of 8% and another study in Canada (34) (2020) reported 6.2% sarcopenia in big sample sizes. Both studies ignored the pre-sarcopenia assessment, which may significantly affect the reported prevalence. The prevalence of sarcopenia in all studies, which disregarded pre-sarcopenia was not limited to >10%. For example, Pelegrini et al. (35) reported a high prevalence of 30.3 and 3% for confirmed and severe sarcopenia in a Brazilian population. However, few studies considered pre-sarcopenia. Among them, one study by Sousa-Santos (32) with a sample size of 1459 individuals reported a prevalence of nearly 50% of one of three stages of sarcopenia in Portuguese people (32). Another study reported prevalence of Pre-, confirmed-, and severe-sarcopenia as much as 21%, 12.3%, and 5.7%, respectively, in

Tehran-Iran within 300 individuals (31). Table 2 and Figure 5 show the comparison of the prevalence of some studies with a close population with the current study (31-44).
Some studies already use different cut-off values. In this study, changing the sarcopenia definition to other guidelines also has been considered. The narrative comparisons of limited available data revealed that Iranians' body shape, weight, height, and composition are closer to the European population than East Asians (45-47). In addition, prioritizing strength over mass in the EWGSOP is the other advantage of this guideline. This simple comparison show that EWGSOP can be a better tool for this population than Asian cut-off points (1, 2, 45-47). Nevertheless, establishing a more comprehensive worldwide guideline based on EWGSOP-2 with a focus on cut-off points variety can be helpful.

Table 2. Literature report of the comparison of the prevalence of sarcopenia in people with no considerable diseases in the world by the time of this study.

Study first author	Country / city	Year	Total sample size	Men/ women	Population age (yr)	Diagnosis method	Prevalence			
							normal	Pre-sarcopenia	Confirmed-sarcopenia	Severe sarcopenia
Current study (Shadmand)	Iran/ Mashhad	2021	766	256/510	65.1±6.7	EWGSOP-2	559 (73%)	183 (23.9%)	14 (1.8%)	10 (1.3%)
Purcell	Canada	2020	12592	6260/6221	≥ 65	EWGSOP-2	11067 (93.8%)	NA	727 (6.2%)	0 (0%)
						IWGS	10574 (98.5%)	NA	23 (0.2%)	136 (1.3%)
						FNIH	10479 (97.6%)	NA	35 (0.3%)	213 (1.9%)
Sui	Australia	2020	665	342/323	70[1]	EWGSOP-1	546 (82.2%)	80 (12%)	30 (4.5%)	9 (1.3%)
						EWGSOP-2	594 (89.4%)	59 (8.9%)	10 (1.5%)	2 (0.3%)

Study first author	Country / city	Year	Total sample size	Men/ women	Population age (yr)	Diagnosis method	Prevalence			
							normal	Pre-sarcopenia	Confirmed-sarcopenia	Severe sarcopenia
Pang	Singapore	2020	542	228/314	58.5±18.8	AWGS2019	325 (59.3%)	82 (14.9%)	83 (15.2%)	58 (10.5%)
						EWGSOP-2	449 (81.9%)	20 (3.7%)	25 (4.5%)	54 (9.8%)
Liu	China	2020	4500	1627/2873	62.4 ± 8.3	AWGS	2071 (80.69%)	NA	869 (19.31%)	NA
Martone	Italy/ Rome	2020	11,253	4897/6356	55.6±11.5	EWGSOP-2	10280 (91.4%)	NA	973 (8.6%)	NA
Sousa-Santos	Portugal	2020	1454	613/841	65 to 100[1]	EWGSOP	620 (42.6%)	553 (38%)	225 (15.5%)	55 (3.8%)
Kim	Korea	2019	2099	1053/1046	75.9 ± 4.0	EWGSOP-2	1618 (80.5%)	NA	411 (19.5%)[2]	NA
Savas	Turkey	2019	248	55/193	70[1]	EWGSOP-2	219 (88.3%)	NA	29 (11.7%)	NA
						EWGSOP-2-T	146 (58%)	NA	102 (41.1%)	NA
Su	Japan/ Sapporo	2019	310	89/221	76±5.8	EWGSOP-2	285 (91.9%)	NA	25 (8.1%)	NA
Benjumea	Colombia / Manizales	2018	534	131/403	74.4 ± 8.2	EWGSOP-1	154 (15.4%)	NA	380 (84.6%)	NA
Buckinx	Belgium	2018	662 (247)[2]	249/413	83.2 ± 8.99	EWGSOP-1	81 (32.7)	NA	166 (67.2%)	NA
Pelegrini	Brazil/ Florianópolis	2018	439	69/369	79.9±6	Janssen et al.	292 (66.7%)	NA	133 (30.3%)	13 (3%)
Bae	Korean	2017	17968	7746/10222	Over 20[1]	Other (standard deviation of normal population)	12785 (71.2%)	NA	5183 (28.8%)	NA
Men	Australia / Sydney	2017	419	212/ 207	81.2 ± 4.5	EWGSOP-1	322 (78 %)	NA	88 (22 %)	NA
Hashemi	Iran/ Tehran	2016	300	150/150	66.8 ± 7.72	EWGSOP-1	183 (61%)	63 (21%)	37 (12.3%)	17 (5.7%)

NA: not assigned

[1] mean age or CI was not reported

[2] The prevalence is estimated according to the reported prevalence of Handgrip strength, Chair stand test, Gait speed, and SMMI.

Abbreviation: IWGS: International Working Group on Sarcopenia, FNIH: Foundation for the national institutes of health, AWGS: Asian Working Group on Sarcopenia, EWGSOP-2-T: EWGSOP-2 adjusted for the Turkish population

Many factors affect the risk of sarcopenia regarding the risks. In addition, age is the most significant factor associated with sarcopenia (2, 3, 33). The current study also had the same result for age, the findings are varied for gender. In the current study, the female group was at higher risk of pre-sarcopenia than the male, while males felt at a considerably higher risk of confirmed and severe sarcopenia, confirming the results of previous studies (35, 48). Some other studies also find a higher prevalence of sarcopenia in females (32, 34). The review of evidence revealed that the range of findings about the effect of sex on sarcopenia is more related to consideration of pre-sarcopenia in studies. Despite no robust explanation for the current finding, it seems males are at a higher risk of confirmed sarcopenia, and females are at a higher risk of pre-sarcopenia. More studies with a higher follow-up length are still required to understand this relation.

This study also aimed to understand better the risk factors for sarcopenia in an isolated from catabolic condition population. Most of the studies assessed sarcopenia within populations with a disease, malnutrition, or at least one risk factor of sarcopenia, while sarcopenia can occur in all individuals (1-4). This study can provide considerable insight regarding the importance of sarcopenia health care and risk factors as one of the pioneers by excluding all catabolism-related high-risk individuals.

Finally, sarcopenia is a complex disorder influenced by many factors, directly or indirectly as a long-term disorder, requiring more research. The world is getting older each year, and sarcopenia can become one of the world's first concerns shortly because of an inactive lifestyle and poor diet patterns enhanced during the COVID-19 pandemic (49). However, pre-sarcopenia is defined as low skeletal muscle strength, which can be controlled (1-4). The interventions can begin at the pre-sarcopenia

stage before it becomes too late. The results strongly recommend considering pre-sarcopenia assessment in further works to reach the objective.

This study aimed to determine the effect of different main lifestyle factors on sarcopenia in a well-nourished population to prevent disease and enable early diagnosis. As one of the first studies investigating the predictive factors for sarcopenia in a well-nourished population, the data are now being analyzed and will be presented in the future as one of the first studies of its kind in a well-nourished population. The strengths of this study were the large sample size, recruiting of well-nourished subjects with no catabolic diseases, and considering most of the health-related factors in one population to better understand sarcopenia and risk factors.

The first limitation of this study was related to the population. Despite reducing the risk of bias in assessing risk factors, this study cannot represent the prevalence of sarcopenia in the city because a great portion of the population, the residents with chronic diseases, are not included. Furthermore, working with these age groups is a challenging process requiring exceptional personalities. Additionally, the short form of questionnaires were used at all steps to reduce the period and provide a better assessment, which limited the data. Nonetheless, the present study's main limitation is its observational nature which cannot evaluate the directions. However, these types of studies can provide a rationale for future evaluations.

Conclusion

According to EWGSOP-2, the prevalence of pre-, confirmed- and severe-sarcopenia was 27%. Age was associated with sarcopenia, and males were at a higher risk of confirmed and severe sarcopenia, while females were at a higher risk of pre-sarcopenia. In addition, further studies are suggested to assess pre-sarcopenia as well as sarcopenia and severe-sarcopenia in further studies.

Acknowledgment

Conflict of Interest

There is no conflict of interest to declare.

Author Contribution

Study concept and design: ZH, MRSh, and PP; Drafting of the manuscript: MRSh, ZH; Study implementation: MRSh, SE, MA, RB, and KhKh; Data validation and dietary intake analysis; MRSH, SE and MA, Statistical analysis and interpretation of data: MRSh, MKhR, MGM; reviewing the manuscript, MGM, LS, FB, ZH, PP, GAF and managing the registry system: SE, MRSh. All authors approved the final version and agreed to publish the work. ZH is responsible for corresponding to the paper.

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A Pilot Randomized Comparative Study of Short-Term Response to Yoga and Diet Intervention on Myopia among College Students

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ARTICLEINFO	ABSTRACT
	Introduction: Uncorrected refractive error is the leading cause of vision impairment in India. Myopia is a common refractive error that can also be defined by a spherical equivalent (SE) ≤ -0.5 dioptre (D). To explore the combined effect of Yogic eye exercise and Diet compared with Yogic eye exercise in the management of myopia. **Method:** In a comparative study, 28 students diagnosed with myopia were randomly assigned into two groups. Group A received a naturopathic diet protocol and Yoga eye exercises, and Group B received Yogic eye exercises. Improvement in myopia was assessed as the mean change in refractive error - Spherical equivalent (SE) from baseline. **Result:** The mean SE values were reduced post-intervention in both groups. Within-group comparison of SE pre- and post-intervention showed a significant reduction in SE measures in Yoga and diet (Group A) for the right and left eyes at 95% CI with a p-value of 0.003 and 0.01 respectively. There were no significant changes in SE values in the Yoga-only group. When compared between groups, no statistically significant changes were observed in SE. **Conclusion:** The group that followed the diet combined with Yoga showed better improvement in pre- and post-assessments for both eyes. But when compared between the two groups there were no statistically significant changes in SE. Yogic eye exercise proved beneficial in both groups, but the results are better when combined with diet. The findings need to be verified further by conducting a large-scale placebo-controlled clinical trial with a wider population base on diverse age groups.

Introduction

Uncorrected refractive error is the leading cause of vision impairment in developing countries, including India (1). Myopia, also known as short-sightedness or near-sightedness is a common refractive error caused due to excessive elongation of the eye which makes light focus in front of the retina instead of focusing on the retina, making the far-away objects look blurry (2). Short-sightedness can also be defined as spherical equivalent (SE) ≤ -0.5 dioptres (D). A systematic review by Sheeladevi et al, 2019 found that the prevalence of refractive error of at least (0.50 D of spherical equivalent) ametropia was 53.1%, of which myopia and hyperopia were 27.7% and 22.9%, respectively in India (3). A meta-analysis by Agarwal et al. 2020 showed that the trend of myopia prevalence in India over the

last four decades is increasing (4). Myopia is a complex multifactorial disorder regulated by interactions between genetics and the environment or lifestyle. But the rise in myopia prevalence is possibly related to environmental factors. The modern lifestyle and high visual demands that include long periods spent on online education and other near-work activities are considered the critical causative factors (5,6). Longer smartphone use increases the likelihood of ocular and visual symptoms like blurring of vision, dry eye, myopia, and ocular surface disease, particularly in children. (7,8). Refractive correction can be performed using prescription glasses, contact lenses, and surgery(9,10). Yoga practice is considered a potential application in those occupations requiring good visual perceptual sensitivity and it reduces ocular

symptoms, visual strain, and discomfort (11,12). Studies have proven that regular yogic eye exercise has objective as well as subjective improvement in refractive errors. (13,14). Various researchers have proven that adherence to a healthy diet rich in micronutrients like vitamins, carotenoids, trace metals, and omega-3 fatty acids offers a valuable intervention against ocular diseases such as dry eye disorders, myopia progression, cataracts, glaucoma, diabetic retinopathy, or age related macular degeneration (15). Studies have shown that diet and Trataka (yogic eye exercise) individually have a beneficial effect on refractive errors but there is a lack of experimental studies on whether the combination of diet and Yogic eye exercises can be more effective than Yogic eye exercises alone in the management of refractive errors. Hence this study evaluates the combined effect of Naturopathic Diet & Yogic eye exercise compared with yogic eye exercise on myopia among College Students. We hypothesize that naturopathic diet and eye exercises are more effective than yogic eye exercise on myopia management.

Methods and Materials
Study design
A comparative study was conducted with 28 students who were already diagnosed with myopia within 5 previous years. Using a simple random sampling technique, all 28 students were randomly assigned to two groups 14 in each group. Group 'A' naturopathic diet and yoga therapy protocol were followed and Group 'B' followed only the yoga therapy protocol.

Inclusion and Exclusion
Participants taking medication for any diseases, or having undergone any surgery, including laser vision correction, within the last 5 years were excluded from the study. Since all participants included in this study were practicing general yoga as a part of their curriculum, none of the participants were exposed to the study protocol in both group and were matched for previous yoga exposure.

Assessment
After receiving informed consent, all the participants were assessed by an optometrist at the baseline for visual acuity, and diopter power was tested using Sennelln's Chart. The same procedure was repeated on the 30th day from baseline. Improvement in myopia was assessed as the mean change in Spherical Equivalent (SE) from the baseline. The SE is calculated using (spherical power plus 1/2 cylindrical power) (16).

Table 1. Diet protocol

Day	7:30 Pm	8.30pm	11.30pm	1.00pm	3.30pm	6.00pm	8.00pm	9.00 Pm
Monday	BMJ	Raw diet	LHJ	MMJ	GJ	Soup	Fruits	Decoction
Tuesday	AGJ	Raw diet	LGJ	CJ	LJJ	Soup	Fruits	Decoction
Wednesday	Decoction	Raw diet	LMJ	B	GMJ	soup	Fruits	Decoction
Thursday	BGJ	Raw diet	LHJ	BCS	LGJ	Soup	Fruits	Decoction
Friday	AJ	Raw diet	LJJ	PS	MB	Soup	Fruits	Decoction
Saturday	AGJ	Raw diet	LGJ	MMJ	GJ	Soup	Fruits	Decoction
Sunday	Decoction	Raw diet	LJJ	WMJ	LHJ	Soup	Fruits	Decoction

BMJ- Bermuda grass juice; **Raw diet-** green gram/ ground nut / Alfa alfa/ Pearl millet / Black chana sprouts + Raw vegetables (carrot, beetroot, inion, tomato, cucumber) + fruits (orange, pomegranate, guava, grapes, banana) + Dates+ grated coconut; **LHJ-** Lemon Honey Juice; **MMJ-** Muskmelon juice; **GJ-** Gooseberry Juice; **Soup-** Moringa Leaves (Clear)/ Pumpkin/ Amaranth / Cherry tomato / Carrot (Clear) / Tomato; Fruit bowl- Papaya+ Muskmelon+ Orange/ Papaya+ Grapes+ Guava/ Pineapple+ Pomegranate+ Orange/ Papaya+ Muskmelon+ Guava/ Watermelon+ Grapes+ Pomegranate; **Decoction-** Licorice Water/ Ginger Water/ Tulasi Water/ Coriander Water; **AGJ-** Ashgourd Juice; **LGJ-** Lemon Ginger Juice; **CJ-** Carrot Juice; **LJJ-** Lemon Jaggery Juice; **LMJ-** Lemon Mint Juice; **B-**Buttermilk; **GMJ-** Gooseberry Mint Juice; **BGJ-** Bottlegoard Juice; **BCS-** Beetroot Carrot Smoothie; LGJ- Lemon Gooseberry Juice; **AJ-** Aloe vera Juice; **PS-** Papaya Smoothie; **MB-** Mint Buttermilk; **WMJ-** Watermelon Juice
Juice - 250ml; Raw vegetables & fruits- 400g; Decoction – 100ml

Intervention
Naturopathic diet protocol and yoga protocol for myopia was developed by the naturopathy experts. The protocol was reviewed and redesigned after taking the expert opinion. In Group 'A' students followed the naturopathic diet, which is a sattvic diet containing raw fruits and vegetables, juices, and veg soups (Table 1.), and Yoga Protocol for 21 days (Table 2). Group 'B' students followed only the Yoga protocol for 21 days and had their routine food. (Table- 2). The naturopathic diet protocol includes raw fruits and vegetables, and juices, which are considered eliminative and soothing diets according to the naturopathy philosophy. The yoga protocol includes barefoot walking in grass,

eyewash using Triphala-soaked water, Jala neti (nasal irrigation using saline water), and Sutra Neti (passing a rubber catheter through the nose

and taking it via mouth as a means of clearing the nasal passage).

Table 2, Daily Yoga protocol

Time	Yoga	Procedure	Duration	Repetition
5.30 to 5.45 AM		Bare Foot Walking	15 mins	NA
5:45 to 6.30 AM	Eye wash	Eyewash with Triphala soaked water	5 mins	2 times in each eye using an eyewash cup
	Jala Neti*	Saline nasal irrigation	10 mins	2 times in each nostril using Jala Neti pot
6.45 to 7.00 AM	Sutra Neti**	Using rubber catheter	10 mins	2 times in each nostril
7.30 to 8.00 AM	Sungazing	Early morning sun gazing and palming	2 mins	30 seconds gazing and 30 seconds break for 2 times

The tear ducts, which connect from the eyes to the nasal passage, get the same drawing out effect as the sinuses, resulting in a brighter, clearer sense of vision.
**Sutra-Neti. passing a rubber catheter through the nose and out of the mouth as a means of clearing the airway.

Ethics
Ethical clearance was approved by the Institutional Ethics committee and written informed consent was obtained from all the students participants before the study.

Data analysis
The data were analysed using IBM SPSS 20, the mean and variance of spherical equivalent were computed and the paired t-test was computed to find any statistically significant changes before and after intervention among the groups.

Results
The average age of the students was 22.4 years in Group A and 22.3 years in Group B. In Group A, all the participants were girls and in Group B, there were 3 boys and 11 girls. All the participants in both groups completed the 21-day intervention. The average SE values were computed and were reduced post-intervention in both groups. Following the normality test, since both the groups followed the normal distribution, paired t-test for SE was computed for both groups pre-and post-intervention. A significant reduction in SE measures was found in Group A for the right eye (p=.003) and the left

eye showed a reduction but not significant at 95% CI (p= 0.12). There were no statistically significant changes in SE values in Group B which followed only Yoga Protocol. For comparing the SE measure between the Group, A, and B, an independent sample t-test was used to assess the changes between group A and Group B pre- and post-intervention. The results suggested that changes in SE measure were not statistically significant compared between the two groups Table 3. Additionally, a paired-samples t-test was conducted to compare weight of the participants in group A before and after intervention. There was a highly statistically significant difference in the weight was observed before (M=60.84 kgs, SD=11.71) and after intervention (M= 57.94 kgs, SD= 10.73); t (13) = 8.2006, p = 0.001. Similarly, A paired-samples t-test was conducted to compare weight of the participants in group B before and after intervention with a statistically significant difference in the weight was observed before (M=54.65 kgs, SD=8.06) and after intervention (M= 53.71 kgs, SD= 7.37); t (13) = 2.5291, p = 0.025, but compared to group A lesser weight reduction has been observed in group B.

Table 3. Paired sample t-test of SE pre- and post-intervention for both eyes between groups

Groups	Pre- and post-intervention*	Mean	Std. Deviation	t	Sig. (2-tailed)
Yoga and diet (Group A)	Right eye	.3839	.4506	3.188	.007
	Left eye	.2232	.4604	1.814	.093
Yoga (Group B)	Right eye	.0446	.7462	.224	.826
	Left eye	.0268	.5637	.178	.862

Spherical equivalent= SE. *Paired sample t-test (p<0.05)

Discussion
As eyes are the most important sensory organs, this study was selected to find an effective line of

treatment for myopia through modifications in the lifestyle by incorporating healthy diet and daily practice of yoga. It was projected that the prevalence of myopia will increase by 10.53% in

the next 3 decades (2020 to 2050) across all ages (17). The average age of the participants selected for this study was 22.3 years who are of developmental age, as myopia is considered a more or less developmental anomaly. This study evaluated the combined effect Naturopathic Diet & Yogic eye exercise compared with only yogic eye exercise on myopia among College Students. Two groups of students with refractive errors participated in this study for 21 days, with one group following naturopathic diet along with yoga therapy (Group A) and another group practicing only yoga therapy (Group B). Over all we observed that the effect of intervention in both groups showed a minimal change in the spherical equivalent. Previous studies have stated that different eye exercises, including yogic kriyas elicited a greater effect on a refractive error by strengthening accommodation, which is the power of changing the focus of eyes for vision, and diet can be crucial in the prevention of myopia (12–15,18). Though both the group showed improvement in SE post-intervention, Group A showed a more statistically significant difference than group B, eliciting that diet and yoga showed better improvement on myopia than practicing Yoga alone. A study by Tiwari et al. on the effect of Bates eye exercise therapy vs. Trataka Yoga Kriya found a minimal reduction in eye power, which was attributed to the stress relief phenomenon of yoga, similarly the participant in this study who practiced Yogic eye exercise also observed the reduction in SE might be attributed to the same phenomenon. The mode of action of Trataka along with diet might be attributed to the ability of eye exercise on asthenopic features and also the low calorific diet respectively (14). A statistically significant difference was obtained in the right and left eye of Group A i.e., the Yoga and diet group who had an intake of a low-calorie diet, consisting of uncooked vegetables and fruits showed a statistically significant change only in both eyes. Though many studies found there was no direct association between nutrient intake and refractive errors, high carbohydrate intake is associated with myopia (19). The reduction in SE in group A might be associated with a low-calorie diet, further studies are warranted in this area.

The limitations of the study include smaller sample size, short interventional protocol duration, and the controlled environment of the participants, the application of the intervention to patients warrants further large-scale studies applied to the wider population diagnosed with myopia. This study did not follow-up the participants to find the long-term benefits of the interventions. We used only SE as the parameter for measuring the effect, other parameters can be used along while capturing the subjective changes among the participants in further studies.

Conclusion
The results from this study can be inferred has, the group that followed the diet combined with yogic eye exercise protocol showed better improvement in pre- and post-assessment in both eyes. When compared between the two groups there were no statistically significant changes in SE, from this finding we can infer that yogic eye exercises can be beneficial for myopia but when combined with a naturopathic diet the results are even better. The diet and yoga protocol had significant impact on weight of the participants pre and post intervention. The findings need to be verified further by conducting a large-scale placebo-controlled clinical trial.

Acknowledgement

Conflicts of Interest
The authors 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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Probiotics and Their Conceivable Part within the Avoidance or Treatment of Covid-19: A Systematic Review

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A R T I C L E I N F O	A B S T R A C T
Article type: Review Article	**Introduction:** Today, the use of probiotics is very popular among the public, which has recently been tested in COVID-19. The aim of this study was to collect all studies related to the immune effects of probiotics and their possible role in the prevention or treatment of COVID-19 achieve a comprehensive result.
	Method: The article is a systematic review that uses the PRISMA checklist to extract articles. After searching for the keywords probiotics, treatment, prevention, coronavirus, and covid-19 and combining them in databases (Google Scholar, PubMed, and Science Direct) according to the inclusion and exclusion criteria, 18 articles were finally reached. Therefore, the articles of the last 10 years (2012-2012) that have been scientifically published in databases were qualitatively analyzed by reviewing both authors.
	Results: Through the qualitative analysis of most articles, we can mention that the modulation of the gut or lung microbiome is an adjuvant treatment or prevention of COVID-19, due to the immunomodulating properties of probiotics. As well as, based on the results of these articles, probiotics can be effective in reducing the secondary infection caused by Covid-19, modulating the inflammatory response, and reducing the duration of the disease. In addition, the administration of probiotics can be useful as an alternative to ameliorate COVID-19 disease and increase the effectiveness of the vaccine.
	Conclusion: Based on the evidence, probiotics boost the immune system and could be useful in the prevention or adjunctive treatment of COVID-19. More randomized trials are required to support these findings.

Introduction

The coronavirus caused by the coronavirus SARS-CoV-2 originated in Wuhan, China (1) and spread around the world in December 2019 so fast, (2) Coronaviruses are a group of viruses that are responsible for a wide range of diseases.

Diverse sorts of human coronaviruses cause different respiratory infections, for case in powerless cases side effects such as colds and in other more extreme cases indications such as pneumonia,

bronchitis, intense respiratory disorder (3). In this regard, infection can be Asymptomatic until severe symptoms vary.

May moreover influence the gastrointestinal tract, liver, and anxious framework (4). Due to the high cost of vaccines and the low availability of vaccines in many countries, therapeutic interventions that can prevent the disease or reduce the symptoms of the disease are of great importance.

Clinical perceptions of individuals with COVID-19 amid plagues around the world appear that the elderly and individuals with conditions such as diabetes mellitus, high blood pressure, cardiovascular illness, and cancer are more likely to create Corona illness and indications. They are more severe and the mortality rate is higher in these people, which shows the role of the immune system in COVID-19 (5).

The gastrointestinal tract is the body's largest immunological organ, with trillions of microbes residing in the gastrointestinal tract. Digestion can strengthen the immune system. One of the suggested mechanisms for this is that probiotics promote intestinal health and maintain the body's ecological balance, and reduce the severity of diarrhea by strengthening the intestinal flora and inhibiting harmful bacteria (6).

Current evidence suggests that the gastrointestinal microbiome may regulate the stimulation or suppression of invasive viruses, and that some gastrointestinal bacteria may

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protect the body against pathogens by interacting with human cells and enhancing positive immune responses, as well as probiotics. They reduce the number of viruses in the body and thus reduce the symptoms of viral colds, and this mechanism can also be attributed to the corona virus (7, 8).

In January 2020, the International Health Committee of China and the Guidelines of Traditional Chinese Medicine proposed the probiotics use to treat patients with COVID-19 disease to modulate the microbial flora and prevent secondary infections (9). Probiotics also increase the immunity of the host by modulating the TH1 / TH2 (T helper type 1 / T helper type 2)

ratio and have a protective effect on pathological diseases (10).

Also, the use of probiotic supplements along with vitamin D and zinc reduces the T1 / T17 ratio and pro-inflammatory factors such as IL6, IL8 and TNFα, thereby reducing inflammation of the lungs and airways in patients with COVID -19.

So far, no definitive method has been found for the prevention or cure of COVID -19 due to the lack of scientific evidence and clinical trials, and it can be said that probiotics have a possible role in the adjunctive treatment or prevention of COVID -19. The goeal of this study was to gather all papers related to probiotics and their possible role in the prevention or adjunctive treatment of COVID -19 and achieve a comprehensive result.
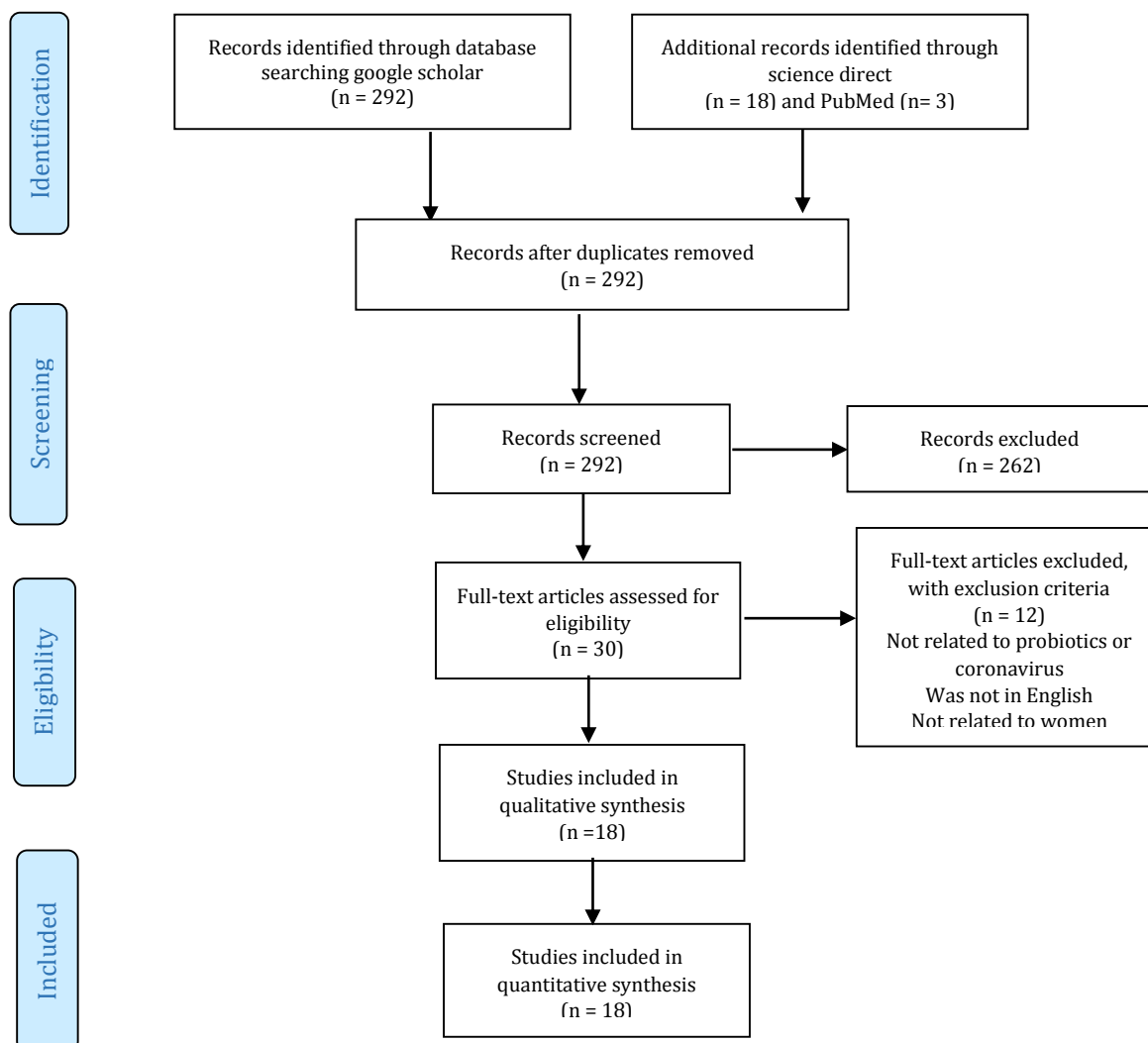

Figure 1. Article selection chart based on PRISMA checklist

Materials and Method

The strategical method followed the guidelines established through PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) (11).

Searching the keywords Probiotics, Treatment, Prevention and covid-19 and coronavirus first, and combining the keywords in the Google Scholar, PubMed and Science Direct databases in the period October 2012 to October 2022, a total of 313 articles in English were retrieved found. extract. Articles were judged on title, abstract, and body content to ensure their relevance to the prevailing research. Two independent authors conducted Data extraction, and articles indexed in two or more databases were considered only once. Then, the outcomes were checked by a third researcher to make sure that each one the eligible papers have been evaluated. Finally, 18 articles were included in the study.

The extracted data was organized by author's name, country, publication date, type of clinical trial, sample size, diagnostic criteria, patient characteristics, study duration, type and strain of probiotics used, dose of probiotics used, and positive treatment outcomes.

The inclusion criteria were: (i) articles in the period 2011-2021; (ii) access to the full text; (iii) articles in English; and (iv) clinical trial studies.

The exclusion criteria were: (i) congress papers; (ii) Irrelevant Articles not to pay attention to coronavirus; (iii) Irrelevant Articles not to pay attention to probiotics; (iv) non-English papers; and (v) studies without clear information.

Figure 1 shows the study selection chart.

Results

Probiotics can be effective in reducing the secondary infection of COVID 19, modulating the immune system and inflammatory response, as well as reducing the duration of the disease.

Based on the results of these articles, the administration of probiotics can be useful in the viral respiratory infections (VRIs) prevention, including COVID-19, and Probiotic adjuvant therapy may be considered as an alternative way to progress or prevent COVID-19 disease. Finally, the probiotic bacteria use against COVID-19 is practical, both in terms of vaccination and treatment. Many probiotics have been approved to decrease the bacterial or viral infections

duration. Table 1 shows 18 relevant articles on probiotics and their effects in COVID-19 patients.

Discussion

Qiang Li et al., showed a total of 93 out of the 123 patients (75.61) were cured by probiotics and had a median 32-day hospital stay and an average virus clearance time of 23 days, which was significantly longer than patients without probiotics, were discharged from the hospital (12).

Dynamic changes of 8 chosen laboratory variables (C-reactive protein (CRP), total T lymphocytes, IL-6, NK cells, T4 CD4 + cells, B lymphocytes, CD8 + T cells and CD4 / CD8 ratio) were monitored and as a result, the probiotics could not increase IL-6 levels but could modulate immunity and decrease the secondary infection rate in COVID-19 patients (27).

Bozkurt et al., showed that given the close association between virus replication and gastrointestinal immunity, a probiotic strategy that targets and modulates the immune response can be useful in decreasing virus replication (13). The known anti-inflammatory activity of some bifidobacteria suggests that these bacteria may play a useful role in showing the inflammatory response, which appears to be highly virus-specific. New approaches to the use of certain probiotic drugs are promising both for preventing the immune response against the virus and improving the efficacy of vaccines. (28).

Olaimat et al. reported that probiotics may decrease the incidence and severity of illnesses and can be effective in the COVID-19 adjunctive treatment or prevention. Probiotics may even prevent COVID-19 by preserving the human gastrointestinal tract or lung microbiota because dysbiosis plays an important role in susceptibility to infectious diseases. In addition, they reported that laboratory and clinical studies are needed to assess the potential prophylactic and therapeutic effects of probiotics against SARS-CoV-2 infection (14).

According to the article, the use of probiotics can reduce the tendency of viral invasion by improving the epithelial barrier ACE2. Antimicrobial peptides or bacteriocins released by beneficial bacteria, SCFAs and ACE inhibitory peptides can balance the dysregulated RAS. Therefore, blood pressure or cardiovascular complications may decrease (22, 29).

Table 1. Relative articles on probiotics based on PRISMA chart

Row	Reference	Positive results
1	(12)	• Immune response modulation • Reducing the rate of secondary infection.
2	(13)	• Immune response modulation • Increasing the effectiveness of the vaccine
3	(14)	• Reducing the incidence and severity of the disease • Prevention of COVID-19 by preserving the human gastrointestinal tract or lung microbiota
4	(15)	• Prevention of VRIs • Increasing the effectiveness of the vaccine • Use as an adjunctive therapy
5	(16)	• Reducing the tendency of the virus to enter the body by improving the epithelial barrier containing angiotensin-converting enzyme 2 (ACE2) • Balancing dysregulated renin-angiotensin system (RAS) by releasing antimicrobial peptides or bacteriocins, Short-chain fatty acids (SCFAs), and ACE inhibitory peptides • Balancing the levels of pro- and anti-inflammatory cytokines and increasing the number of T cells • Hyaluronan degradation and improvement of acute respiratory distress syndrome (ARDS)
6	(17)	• Modulating immune responses, maintaining intestinal homeostasis and interferon production • Suppression of virus-induced cytokine storm • Reduction of ventilator-associated pneumonia in patients • Overcoming intestinal dysbiosis induced by SARS-CoV2 infection • Increase the body's immune resistance
7	(18)	• Use as an adjunctive therapy that is safe, inexpensive, commercial, and readily available
8	(10)	• Modulating the immune response and reducing the cytokine storm generated during COVID-19 infection • Balancing gut microflora composition, improving gut barrier function and protective immune responses
9	(19)	• Reducing the symptoms of upper respiratory tract infections • Use as an adjunctive therapy
10	(20)	• Boosting the body's immunity • Reduction of disease symptoms
11	(21)	• Increase the body's immune resistance • Balancing gut microflora composition, improving gut barrier function and protective immune responses
12	(22)	• Use as an adjuvant treatment and reducing inflammatory complications
13	(23)	• boosting the function of natural killer cells • Stimulation of IgA antibodies • Control of mucosal barrier inflammation • Strengthening immunity
14	(8)	• Strengthening intestinal and lung immunity • Reduce intestinal inflammation • Reducing the intensity and duration of the infection
15	(24)	• Boosting the immune response by modulating gut microbiota in the lung • Inhibiting virus replication in the lung by releasing metabolites (such as butyrate, desaminotyrosine, and secondary bile acids)
16	(25)	• Enhancement of innate and acquired immune responses • Reduction of complications of VRI • Reduction of disease severity in VRIs
17	(26)	• Modulation of the immune system
18	(6)	• Adjunctive therapy to improve the immune system • Attenuation of virus replication • Helping to treat COVID-19

Reducing NO production can reduce cellular oxidative stress. This can lead to misregulation of inflammatory pathways (NLRP3 and NF-κB) and ultimately the secretion of cytokines or pro-inflammatory chemokines. Probiotics and bacteriocins can balance the levels of anti-inflammatory and anti-inflammatory cytokines and increase the number of T-cells in SARS-CoV-2 patients. Probiotics can reduce hyaluronic acid, improve ARDS (14, 30).

Conclusion

probiotic and its probable role in COVID-19 prevention or adjunctive cure were described, which affect gastrointestinal health by modulating the gut microbial flora. Since 5-10% of covid-19 patients have symptoms like vomiting, diarrhea, nausea, and abdominal pain, we believe that viruses affect the microbiome. Thus, alterations in the gut microbiome led to more severe disease and poor treatment outcomes. According to the review of various studies, we found that probiotics bolster the immune system.

In addition, they prevent the reproduction of pathogens and help create a stable and balanced microbial environment in the body, increasing the population of beneficial bacteria. Due to the different therapeutic effects of different probiotic bacteria, the strains and effective doses in the treatment of each disease should be determined so that probiotics can be used as adjunctive therapy along with the common treatments of Covid-19 diseases. Therefore, Randomized trials with a more appropriate design are needed in this field.

Acknowledgements

Conflict of Interest

There is no conflict of interest to declare.

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Cooking Process Optimization in Canned Beef Production Using the Response Surface Method

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ARTICLEINFO	ABSTRACT
Article type: Research Paper	**Introduction:** Canning food is one of the good methods of food preservation. This method will create a good shelf if the principles of preparation are observed. In this regard, the present study aimed to optimize the cooking time and pressure for canned beef production.
Article History: Received: 10 Aug 2022 Accepted: 20 Nov 2022 Published: 25 Dec 2022	**Methods:** In this study, three levels of pressure (1, 1.2 and 1.4 bars) and three cooking times (9, 15 and 18 minutes) were applied. Response surface method was used for pH, phloem weight, water-soluble solids (Brix), and protein content and sensory examinations in the produced products.
Keywords: Optimization Cooking process Canned Beef Response surface method	**Results:** The results showed that the phloem, weight and the general acceptance of the samples decreased with the increasing of processing time and pressure. Only the linear parameter of process time had a significant effect on the pH at the level of 5%, which increased slightly with the pH of the samples.
	Conclusion: The results of process optimization showed that the surface response method is an appropriate approach for optimizing the cooking process in the preparation of canned veal.

Introduction

The preservation and storage of food products have been received great attention due to the limited shelf life of various food products and growing population rate. With each day passing, the importance of food preservation and extending of its shelf life are becoming more evident mainly due to positive economic impact on marketing. The history of food preservation coincides with the evolutionary stages of the human and their nutritional requirements. Many decades, food preservation has been one of the major concerns of human especially when was trying to keep and preserve food out of the reach of intruder creatures for several season (1). Meat is one of the most important sources of animal protein. Meat is rich in valuable proteins that contain essential amino acids such as histidine, isoleucine, leucine, methionine, and tryptophan, as well as fats that are regarded as important source of energy. Moreover, meat contains fatty acids such as linoleic acid and arachidonic acid, as well as minerals (such as phosphates and sulfates), vitamins (especially B vitamins), and carbohydrates (such as glycogen). These

ingredients highlight the inevitable values of this product for human (2, 3). Considering the high nutritional values of the meat and its high perishability, there is a substantial need for optimization of long-term storage conditions for this product. Thermal treatment during canning process is one of the methods used for the long-term preservation. This method effectively destroys all the factors that may contribute to meat spoilage during storage and transportation. Canning is one of the effective ways and confers foods with a desirable shelf life if it is performed following a standard procedure (4). Considering the huge scale of the international trade and its economic value, preserving procedures with the lowest quality loss such as canning is preferred (5). In canned meat products, two-step thermal treatment is primarily used to improve the edible quality of the meat and to minimize the microbial and chemical activities (6). Canned meat is routinely sterilized through saturated steam using autoclave (7). Changing the specifications of thermal treatment (temperature and time) can affect the sterilization period (8) and thus our main qualitative considerations including

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reduced processing time (9), energy saving, nutrients preserving, and repressing the quality loss during the initial storage (10). High processing temperatures reduce the amounts of thermally-labile essential ingredients (e.g., proteins, vitamins, lipids, and minerals) and promote the production of undesirable compounds and modify the nutritional and sensory specifications (11). Studies have shown that prolonged treatments can alter the sensory and nutritional values of canned products which can be attributed to chemical interactions between food ingredients and metal. As fish products are rich of unsaturated lipids, they are more likely to undergo oxidation during the heating process and storage which deteriorates their desirable quality (12). It has been reported that thermal treatment increases the hardness and reduces the brittleness of meat mainly due to the efflux of water from meat (13). In this regard, the aim of this study was to assess the effects of different cooking periods and pressures on some quality features of canned veal using the response surface method.

Materials and Methods

Preparation of veal and canned food
Fresh veal samples with health certificates were procured from approved centers in Urmia city. Meat was manually separated from bones, fats, and skin using special knives and cut into 3x3x3 cm pieces using a slicer (Ruhler). A cooking pot (Ruhler) was used to cook the veal using steam at three pressure levels (1, 1.2, and 1.4 bars) and three time periods (9, 15, and 18 minutes). The canning process was performed using a production line (Steal Mark Mondini, Italy) and commercialized sterile horizontal autoclaves. Canned products underwent the following tests.

Measuring the pH
To measure pH, a pH meter (Meterohm, Switzerland) was initially set with two buffer solutions with pH of 4 and 7. Then 50 to 75 grams of the homogenized sample was poured into a 100 mL beaker, and its pH was read at 25 °C (14).

Determining the Strainer's Weight
First, the weight of the sieve was determined and recorded. Then the contents of the package were poured into the sieve which was held for about five minutes in a way that would facilitate the separation of the liquid phase (oil). After the complete passage of the liquid through the sieve,

the sieve and its contents were weighed and the total weight of the drained material was calculated using equation 1 (15).

Equation 1) $W = A/B$

In relation 1, W, A, and B represent the percentage ratio of the total drained weight, the weight of the contents on the sieve (grams), and the net weight of the canned product, respectively.

Soluble Solids in Water (The Brix Value)
A part of the homogenized sample was poured on a filter paper and strained. Then the Brix value was immediately measured using a refractometer (Abe, Japan) (16).

Protein quantification
The amount of protein in samples was measured using a fully automated Kjeldahl instrument following the three digestion, distillation, and titration steps. After titration, the amount of nitrogen was calculated using equation 2, where the protein factor was considered as 6.25 (17).

Equation 2) $N\ (\%) = \dfrac{(X - 14/008)}{(W)}$

In this equation, "N", "X", and "W" represent nitrogen %, the titer value, and the weight of the dried sample, respectively.

Sensory Evaluation
The sensory specifications of the samples were assessed through a taste test. Ten judges were chosen among trained individuals to evaluate the characteristics of the samples produced. For this purpose, the overall acceptance rate was determined using equation 3. The evaluation coefficients of 1, 3, 4, and 2 were applied for color, chewability, taste, and smell, respectively, on a 1-5 scale (one being the lowest and five being the highest score) (18).

Equation 3) $Q = \dfrac{\sum (P \times G)}{\sum P}$

In the equation above, "Q" indicates the overall acceptance (the quality value of the samples produced); "P" represents the rating coefficient of the specifications assessed, and "G" is the assessment coefficient of specifications.

Statistical Analysis
Response surface methodology (RSM) using a central composite design was used to evaluate the study's fixed parameters. The pressure of the cooking pot (X1), the process time (X2), strainer weight, Brix value, pH, protein content, and overall acceptance were regarded as dependent variables. Using this methodology, it was

possible to estimate all the coefficients of the quadratic regression model and the reciprocal impacts of the factors. The most important objective of the present study was to assess the reciprocal effects of factors and identify the optimal condition for producing canned veal,

therefore, the RSM statistical model was chosen for data analysis. Statistical analyses were performed in Design Expert software version 12. A total of 13 runs were considered according to our specified levels and factors. The value of $\alpha=1$ was considered for this section.
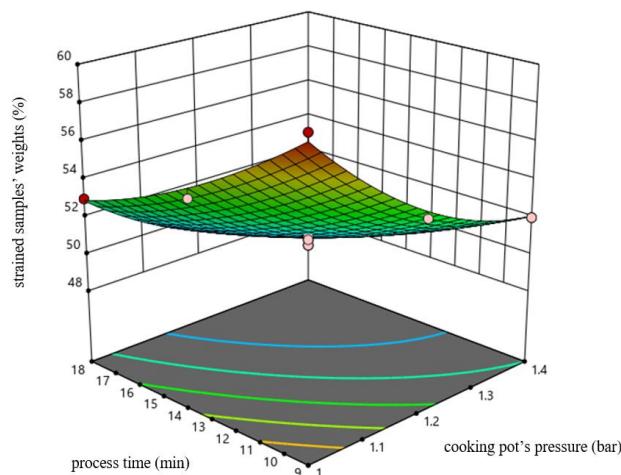

Figure 1. The effects of the cooking pot's pressure and the process time on strained samples' weights

Table 1. Model selection for the features analyzed

| Models	Strained weight		Brix		pH		Protein		Overall acceptance	
	Sum of squares	P	Sum of squares	P	Sum of squares	P	Sum of squares	P	Sum of squares	P
Intercept	35235.25		616.17		312.13		7618.16		138.94	
Linear model	94.04	<0.001	11.04	<0.001	0.003	0.0405	36.33	<0.001	4.83	<0.001
Simple quadratic model	2.25	0.25	0.001	1.00	0.0006	0.323	0.250	0.086	0.0625	0.379
Quadratic polynomial model	11.24	0.002	0.15	0.33	0.0014	0.352	0.027	0.852	0.4746	0.011
Cubic polynomial model	1.08	0.21	0.08	0.56	0.0023	0.118	0.167	0.428	0.0083	0.890
Residual	1.27		0.32		0.0017		0.412		0.1756	
Total	35345.14		627.77		312.14		7655.35		144.49	

Results

Strainer Weight

As shown in Table 1, the quadratic polynomial model was the best model for interpreting the effects of operating parameters on the weights of strained samples. The results also showed that changes in the pressure of the cooking pot, the process time, reciprocal effects, and the quadratic parameters of these variables (except for the quadratic parameter of the process time) had significant effects on the weights of strained

samples at the level of *P*<0.05 (Table 2). As shown in Figure 1, with an increase in the process time and the pressure of the cooking pot, the weights of strained samples decreased. According to the results of variance analysis (Table 3), it can be noted that the largest impact on the weight of strained samples was related to the linear parameter of the process time. Table 3 shows the model predicting the effects of the pressure and process time on the weights of strained samples.

Table 2. The analysis of variance of the parameters measured

Source	Strained weight		Brix		pH		Protein		Overall acceptance	
	Sum of squares	P	Sum of squares	P	Sum of squares	P	Sum of squares	P	Sum of squares	P
Model	107.53	<0.001	11.04	<0.001	0.0034	0.405	36.33	<0.001	5.36	<0.001
X₁	54.00	<0.001	6.00	<0.001	0.0006	0.340	8.17	<0.001	2.16	<0.001
X₂	40.04	<0.001	5.04	<0.001	0.0028	0.045	28.17	<0.001	2.67	<0.001
X₁X₂	2.25	0.036	-	-	-	-	-	-	0.062	0.167
X₁²	5.96	0.004	-	-	-	-	-	-	0.284	0.0133
X₂²	1.43	0.078	-	-	-	-	-	-	0.0402	0.256
Residual	2.36	-	-	-	-	-	-	-	0.184	-
Lack of fitness	1.09	0.480	0.23	0.793	0.0051	0.114	0.684	0.182	0.164	-
Net error	1.27	-	0.32	-	0.0009	-	0.172	-	0.020	0.0213
Sum of complete squares	109.89	-	11.60	-	0.0094	-	37.19	-	5.55	-
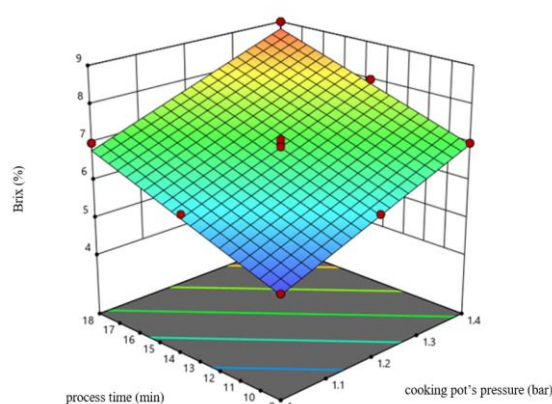
Figure 2. The effects of the cooking pot's pressure and the process time on samples' Brix values

Table 3. The fit models for the parameters analyzed

No.	The variable measured	The model obtained	R²	R²-adj	CV
1	Strained weight (%)	y= +149.65 − 114.38 X₁− 2.53 X₂− 0.833X₁X₂+ 36.72X₁² + 0.035 X₂²	0.978	0.963	1.11
2	Brix (%)	y= − 1.86 − 5.00 X₁ + 0.203 X₂	0.952	0.942	3.42
3	pH	y= + 4.77 + 0.05 X₁ + 0.04 X₂	0.763	0.636	0.499
4	Protein (%)	y= + 10.71 + 5.83 X₁ + 0.48 X₂	0.977	0.972	1.21
5	Overall acceptance	y= +19.04 − 20.37 X₁− 0.142 X₂− 0.139X₁X₂+ 8.02X₁² + 0.0059 X₂²	0.967	0.943	4.96

Brix (soluble solids)

Data analysis indicated that only the linear parameters had significant effects on the Brix values of the samples. The results showed that an increase in the process time and the pressure of the cooking pot, increases the Brix of the canned products (Figure 2). As shown in Table 2, the quadratic and reciprocal impacts of the variables on the Brix of the samples were not significant, so they were excluded from the analytic model described in Table 3.

pH

According to Table 1, the linear model fitted the best into the pH data. As shown in Table 2, only

the linear parameter of the process time had a significant effect on pH at the threshold of $P<0.05$. On the other hand, with an increase in the process time and the pressure of the cooking pot, the pH of the samples increased slightly, and the highest impact on pH was related to the linear parameter of the process time. Table 3 describes the model predicting pH trends during the canning process.

Protein Content

According to the results of model analyses (Table 1), the linear model is the best model fitted into the data related to protein content, similar to the Brix and pH values. Furthermore, the linear

parameter of the process time had the greatest impact on the protein content of the samples. Figure 4 indicates that the protein content of the

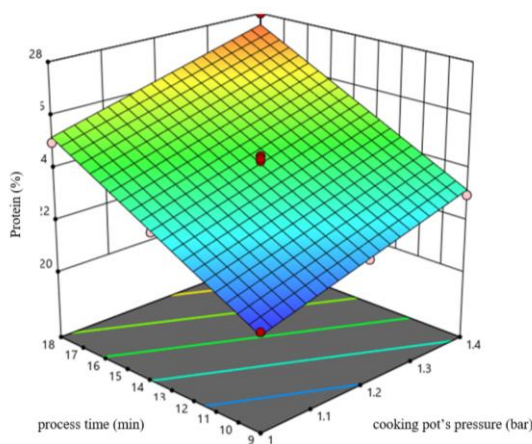samples increases with prolonged processing time and elevated pressure of cooking pot.

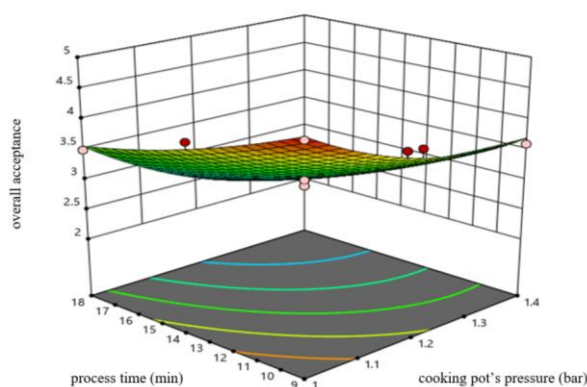**Figure 4.** The effects of the cooking pot's pressure and the process time on the protein content of the samples

Figure 5. The effects of the cooking pot's pressure and the process time on the overall acceptance of the samples

Overall Acceptance

The simple quadratic model was used for fitness analysis of the data related to the overall acceptance of the products (Table 1). Likewise, the linear parameters of the processing time and the cooking pot's pressure, as well as the quadratic parameter of the cooking pot's

pressure were the only variables showing significant impacts on overall acceptance. The results indicated that prolonged processing time and elevated pressure of cooking pot are related to decreased overall acceptance scores given by the evaluators (Figure 5).

Table 4. Comparison of the data predicted with experimental data in the optimal condition of canned veal production

	Strained weight (%)	Brix (%)	pH	Protein (%)	Overall acceptance
Predicted values	52.90	6.80	4.91	25.21	3.56
Experimental values	52.87	6.84	4.88	25.20	3.50

Optimization of Canned Veal Production

In order to identify the optimal condition for canned veal production, the cooking pot's pressures in the range of 1 to 1.4 bar and the processing time of 9 to 15 minutes were

evaluated to achieve the maximum values of strained weight, Brix, and overall acceptance. According to the results, the best outcome was achieved in a cooking pot's pressure of one bar and a processing time of 18 minutes (Table 4),

delivering the overall acceptance of 0.729. The comparison of the data retrieved by the software and those obtained from complementary tests (both at the optimal point) indicated the high accuracy of the predicted specifications.

Discussion

McAfee *et al.* (18) and Rashidi (19) stated that red meat is a good source of protein and essential elements such as iron, zinc, and vitamin B. The freshness of the primary meat can have a large impact on the quality of the final canned product. In addition, the processing steps greatly affect the quality and nutritional value of canned meat (18,19). As we observed, increased processing time and elevated cooking pot's pressure will lead to decreased weight of strained samples which can be attributed to water efflux from the veal tissue during the canning process. On the other hand, pressure elevation can dissociate water molecules from veal meat and intensify the exit of water molecules. Duranton *et al.* (20), in their study, assessed the effects of extreme pressure and temperature during processing on the quality of meat products and stated that high processing temperatures and pressures lead to the denaturation of meat proteins and resulting in a reduction in the water holding capacity (20). Ma *et al.* (21) also investigated the effects of high temperatures on canned shrimp texture and showed that high temperatures prompted the exit of water into the extracellular fluid and as a result, a reduction in the weight of the product which was consistent with the results of the present study (21). Mohammadi *et al.* (22) assessed the effects of manipulating the processing time and the cooking pot's pressure in the preparation of canned chicken and showed that increasing the processing time and the cooking pot's pressure will decrease the weights of strained samples which was in parallel with our observation (22).

Our findings indicated that with the increase in the process time and the cooking pot's pressure, the Brix values of canned products increased. This phenomenon can be explained by the enhanced veal's water-soluble material efflux following long-term processing time and cooking pot's pressure elevation. Accordingly, we noticed that the escape of soluble solids from meat tissue may be probable reason for elevated concentrations of water-soluble material (i.e., Brix) in canned food (23, 24).

As noted, the pH of the samples increased slightly following an increase in the processing time and the pressure of the cooking pot. Poulter *et al.* (25) demonstrated that the use of high processing pressures could slightly boost the pH of meat by inducing structural changes in its acidic amino acids (25). Ma *et al.* (26) also found that the use of a high-pressure technology slightly increased the pH of red thigh meat (26). Bouton *et al.* (27) reported that increasing the processing temperature boosted the pH of sheep muscle meat (27), which agreed with our findings. Fletcher *et al.* (28) stated that cooking increases the pH in chicken meat, which is consistent with the results of the present study, and in this way, the increase in pH with the increase in cooking time in our study can be justified (28).

Our results showed that longer processing time and higher pressure of the cooking pot will elevate the protein content of products. The protein content is an important quality indicator of meat and a key determinant of meat's nutritional quality. Studies have shown that storage temperature and duration, the initial status of the raw material, and thermal manipulations can affect the proteins contents of meat (29, 30). On the other hand, García-Arias *et al.* (31) believed that increased protein content after cooking is related to reduction in moisture percentage (31). Nonetheless, it is noteworthy that in most studies, protein content had been calculated based on nitrogen quantification which might not reflect the true amount of proteins (6). Consequently, an increase in nitrogen-containing non-protein compounds during the cooking process can be a contributing factor for protein content. In a study by García-Arias *et al.* (10) investigating the effects of storage temperature and duration on the chemical constituents of white fish tuna, it was reported that proteins and fats increased by 5.6% and 5.5%, respectively after canning; however, the moisture content declined by 11%. This increase in protein and fat content can be explained by their stability while moisture reduction may be related to extracellular fluid loss during thermal process in the pre-cooking stage (10).

In the present study, sensory assessments disclosed a fall in the overall acceptance of veal canned under increased processing time and the cooking pot's pressure. This assessment encompassed the organoleptic properties of the

meat, including its color, taste, and texture. In this regard, reports by Jouquand *et al.* (32) and Babatunde *et al.* (33) indicated that the cooking process could significantly affect the texture, taste, and overall acceptance of meat (32, 33). Also, according to a report by Fletcher *et al.* (34), cooking reduces the acceptance of meat in terms of color (34). Likewise, Mohammadi *et al.* (22) stated that increased processing time and elevated cooking pot pressure could diminish the overall acceptance of canned chicken which was in accordance with our results (22).

Conclusion

Considering our results, the present study showed that processing time extension and elevated cooking pot's pressure decline the weight of strained samples and the overall acceptance of meat; however, its soluble solids and proteins content increased. Overall, the surface response method is an appropriate approach for optimizing the cooking process in the preparation of canned veal.

Acknowledgment

Conflict of Interest

The authors declared no conflict of interest.

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A Correlation between Nutritional Adequacy and Clinical Outcomes among Children Critically Hospitalized with COVID-19

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ARTICLEINFO	ABSTRACT
	Introduction: Malnutrition is a prominent cause of mortality and morbidity in hospitalized children. Comorbidity of infection and malnutrition can exacerbate nutritional deficiencies and worsen healing. This study aimed to evaluate nutritional status, dietary intake adequacy, and their correlation with clinical outcomes among children diagnosed with coronavirus disease 2019 (COVID-19).
	Methods: This prospective observational study was conducted on 30 children admitted to the pediatric intensive care unit (PICU) ward of Akbar Hospital, Mashhad University of Medical Sciences, Mashhad, Iran, for eight weeks. Age, gender, and nutritional status (weight-for-lengths/heights z-scores, based on the World Health Organization child growth standards) of critically ill children with COVID-19 were recorded and evaluated upon admission. Dietary intake and its adequacy were also calculated during hospitalization. Then, the correlation between mentioned variables with clinical outcomes was examined.
	Results: Out of 30 patients, malnutrition was severe in 16.7%, moderate in 16.7%, while nutrition status was normal in 66.7% of patients. There was no significant correlation between z-scores and mortality or length of stay. However, significant differences were found between energy intake adequacy and length of hospitalization ($p<0.001$), as well as protein intake adequacy and mortality ($p=0.008$).
	Conclusion: The study showed a significant correlation between dietary intake adequacy and clinical outcomes, suggesting the role of optimizing nutrition therapy in ameliorating clinical consequences in critically ill children.

Introduction

The coronavirus disease 2019 (COVID-19) was declared as a pandemic in March 2020 by the World Health Organization (WHO) (1). This infectious disease mainly affects the respiratory system, causing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (2). Even though children are less affected by COVID-19, a report from the Centers for Disease Control and Prevention (CDC) found that young children had higher hospitalization rates than older children (3). Moreover, COVID-19 has higher mortality among certain groups, including those with poor immunity and underlying comorbidities (4). The lack of definitive treatment for COVID-19 makes it essential that the immune system be robust and efficient (1, 5). Several factors can positively affect the functioning of the immune system such as the person's nutritional status (6). A sufficient dietary intake of macro-and micronutrients is an influential factor in preserving and developing immunity. Thus, protein-energy malnutrition or subclinical deficiencies related to micronutrients can impair the immune system from responding to pathogens appropriately (7-9).

There is a bidirectional correlation between malnutrition and infection (10). On the one hand, malnutrition can increase infection susceptibility (11), which may be caused by an immune system dysfunction (12). On the other hand, infectious diseases worsen the negative energy balance and reduce fat-free mass (FFM) during hospitalization (13) such as decreased appetite,

increased catabolism, and demand for nutrients (13, 14).

Furthermore, malnutrition is associated with increased mortality, morbidity, and delayed recovery (5, 15). Therefore, assessing the nutritional status of people with COVID-19, especially vulnerable children, seems necessary. This study aimed to evaluate the nutritional quality of children diagnosed with COVID-19 disease admitted to the pediatric intensive care unit (PICU), the adequacy of energy and protein intake by the end of the first week, and the correlation of this adequacy with clinical outcomes.

Materials and Methods
Study Population
This cross-sectional study was conducted from October 2021 to December 2021 at the PICU ward, Akbar Hospital, Mashhad University of Medical Sciences, Mashhad, Iran, on children younger than five diagnosed with COVID-19 and more than 24 hours of hospitalization. The serum was screened for the coronavirus antibody by enzyme-linked immunosorbent assay (ELISA) and confirmed by Western blot or polymerase chain reaction (PCR). The exclusion criteria for the studied group were a history of viral infection, human immunodeficiency virus (HIV), hepatitis B (HBV), hepatitis C (HCV), cardiovascular, thyroid, kidney, liver, and Cushing's diseases, chronic inflammatory diseases such as multiple sclerosis and rheumatoid arthritis.

Outcome Measures and Data Collection
Nutritional status (weight (kg), height (cm), and z-scores) was recorded at the time of admission. The 24-hour dietary recall (24hDR) was calculated as mean energy and protein intake by

the end of the first week. Clinical outcomes of hospitalization (mortality and length of PICU stay) were also correlated with z-scores and adequate energy and protein intake (Figure 1).

Measurements
The weight was measured using a digital scale (Balas) to 10 g. Length or height was also assessed using a portable infantometer and stadiometer for children aged <24 and >24 months, respectively. A predictive equation was applied to estimate the stature in cases where standard measurements for length and height were impossible. Z-score was calculated as WHO weight-for-length/height (birth to 5 years) (16).

Dietary Intake
The energy and protein intake were assessed by 24hDR. The hospital dieticians performed food analysis based on the menu, hospital food analysis, and enteral and parenteral formulas. The adequate energy and protein intake based on age, weight, and gender were calculated based on nutrition guidelines (17). The energy and protein adequacy definition was the achievement of at least two-thirds of the individually determined goals regarding energy and protein requirements (18).

Statistical Analysis
The data were analyzed by the statistical package for social sciences (SPSS) software version 20. The obtained results were expressed as mean and standard deviations for quantitative data, and the qualitative data were expressed as frequency and percentage. A p-value <0.05 was considered to indicate statistical significance. The correlation between nutritional indices and clinical outcomes was measured by Spearman's test.

Table 1. Frequency distribution of malnutrition among children hospitalized with Covid-19

	Normal	Moderate malnutrition	Severe malnutrition
Nutritional status upon PICU admission [a]	20 (66.7)[b]	5 (16.7)	5 (16.7)

a Z-score (WHO weight-for-length, height) for birth to 5 years; Z > -2: natural status, -3 < Z < -2: moderate Malnutrition, Z < -3: severe malnutrition), [b] Data expressed in terms of frequency (percentage)

Results
A total of 30 hospitalized children with COVID-19 were evaluated in PICU (68.6% female, 31.4% male), whose mean age, weight, and height were 43 ± 49 (months), 13 ± 9 (kg), and 89 ± 28 (cm), respectively.

Based on the Z-score classification (Table 1), 16.7% of subjects had severe malnutrition,

16.7% had moderate malnutrition, and 66.7% were normal. In addition, the adequacy of energy and protein intake among hospitalized children was 73% and 90%, respectively, at the end of the first week.

The correlation analysis between nutritional status and intake adequacy with clinical outcomes among children hospitalized with

COVID-19 showed no significant differences between Z-scores (at the beginning of hospitalization) and length of stay and between Z-scores and mortality ($p > 0.05$). However, there were significant differences between energy intake adequacy and size of hospitalization ($p < 0.001$), as well as protein intake adequacy and mortality ($p = 0.008$) (Figure 1).
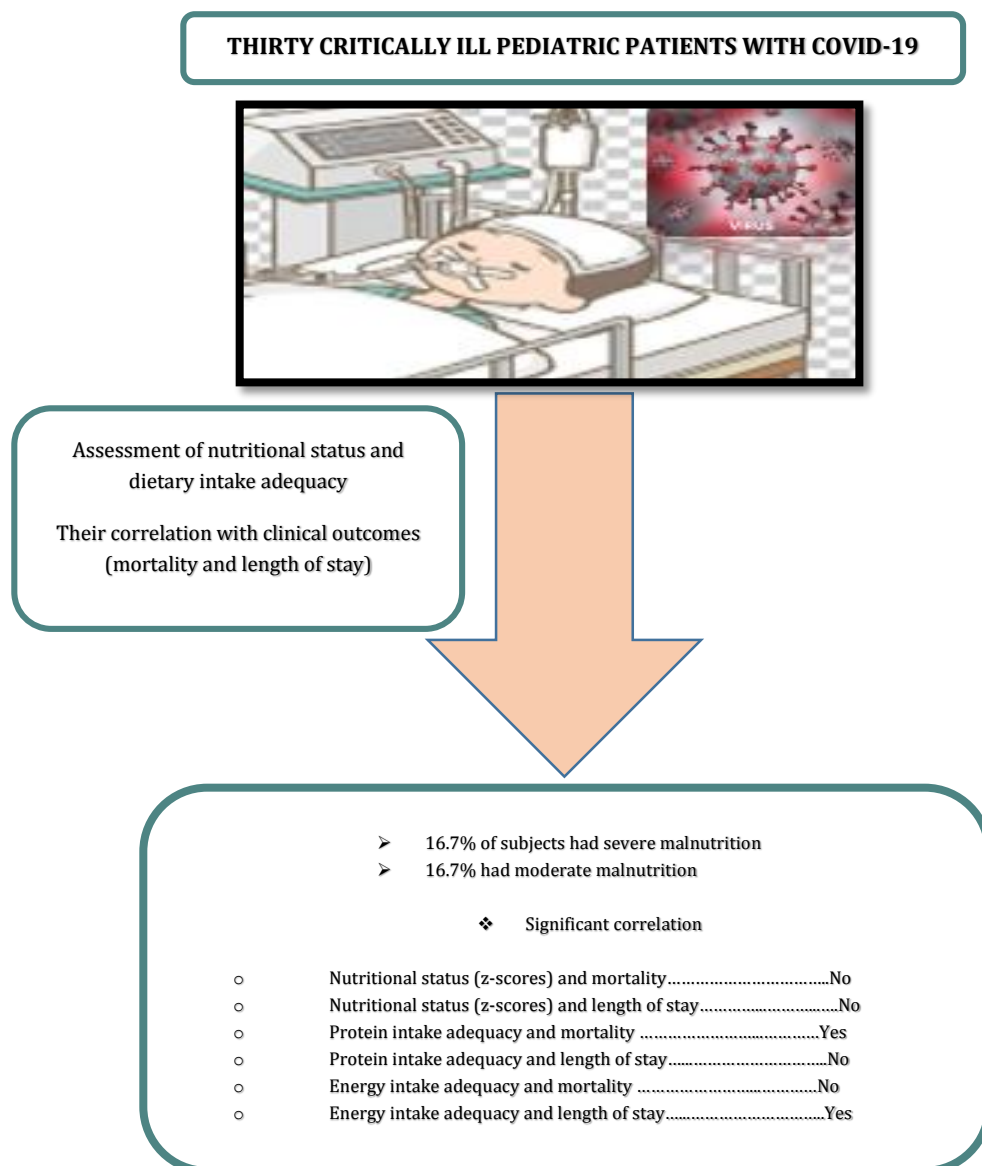

THIRTY CRITICALLY ILL PEDIATRIC PATIENTS WITH COVID-19

Assessment of nutritional status and dietary intake adequacy

Their correlation with clinical outcomes (mortality and length of stay)

➢ 16.7% of subjects had severe malnutrition
➢ 16.7% had moderate malnutrition

❖ Significant correlation

o Nutritional status (z-scores) and mortality...................................No
o Nutritional status (z-scores) and length of stay..............................No
o Protein intake adequacy and mortality ....Yes
o Protein intake adequacy and length of stay..................................No
o Energy intake adequacy and mortality ....No
o Energy intake adequacy and length of stay...................................Yes

Figure 1. Graphical flowchart of study steps and results

Discussion

The present study examined the nutritional status and dietary intake adequacy among children diagnosed with COVID-19 disease who were admitted to PICU. In addition, the correlation between nutritional quality and dietary intake adequacy was assessed with clinical outcomes.

The results indicated that malnutrition prevalence was relatively high among hospitalized children at admission, consistent with various studies that reported malnutrition among hospitalized children (19-21). This prevalence of malnutrition can disrupt the treatment and recovery process, which should be considered when effective prophylactic and

remedial treatment is unavailable in the present pandemic of COVID-19 (5). Thus, a balanced energy and protein intake can help manage viral infections, considering the bi-directional correlation between malnutrition and infection (9, 22).

The proportion of critically ill children receiving adequate energy and protein supplements in the PICU by the end of the first week was 73% and 90%, respectively. Furthermore, the findings did not demonstrate any significant correlation between Z-scores with the length of stay and mortality. Kyle et al. showed the adequacy of energy (75%) and protein (40%) intake in the first eight days of the PICU stay (23). In another study, cumulative energy and protein deficits were related to decreases in anthropometric parameters (24). Studies have shown that many critically ill children are not receiving enough protein and energy. Some of the reasons mentioned for the under-delivery of energy or protein might be the severity of the illness (23), fluid restriction (25), and the gap between energy and protein delivery and requirements (26). A practical solution can be following the nutrition support guidelines (26) or feeding protocols related to PICU (27) to improve the nutritional patterns of critically ill children.

The results did not determine significant differences between Z-scores (at the beginning of hospitalization) and length of stay, as well as Z-scores and mortality. The nutritional status at admission (using Z-scores) and clinical outcomes (as 30-d mortality, length of intensive care unit (ICU) stay, and mechanical ventilation) were examined in a prospective cohort study (28). Menezes et al. demonstrated an independent correlation between malnutrition and the size of mechanical ventilation. However, there was no correlation between malnutrition with mortality (28). In addition, Grippa et al. considered malnutrition with the Z-scores variable among 72 children hospitalized in PICU and showed that malnutrition was associated with the mechanical ventilation duration among critically ill children (29). In addition, mid-upper arm circumference (MUAC) has been shown to predict mortality in hospitalized children better than Z-scores. (30). Although the variables considered for clinical outcomes are different, assessing nutritional status on admission to the PICU is critical. Therefore, targeted nutritional rehabilitation can decrease malnutrition and later hospitalization clinical outcomes.

This study showed a significant correlation between energy intake adequacy and hospitalization length, as well as protein intake adequacy and mortality. In a prospective cohort study of mechanically ventilated children, adequate protein intake as enteral was significantly associated with mortality (19). This study did not independently examine the effect of energy on outcomes compared to energy intake (19). Moreover, Hulst et al. showed a negative association between cumulative deficits (energy and protein), ICU stay length, and mechanical ventilation days (24). Therefore, ongoing assessments of protein and energy intake are essential in PICU to avoid the under-delivery of energy or protein and the clinical outcomes. On the other hand, PICU staff training in nutritional therapy can effectively improve nutritional support among critically ill children. Moreover, nutritional adequacy during acute stress such as COVID-19 can help to reduce impaired growth and complications during children's illness.

This study was one of the few ones on nutritional adequacy regarding critically ill children with COVID-19. To our knowledge, only one study has been conducted, similar to ours (31). However, this study was limited to the PICU of one hospital and may not be generalized to other PICUs.

Conclusion

Based on the results, there was a correlation between dietary intake adequacy and clinical outcomes. This correlation was observed between energy intake adequacy and hospitalization length, as well as protein intake adequacy and mortality. However, more studies are required to confirm the role of nutritional interventions, especially the adequacy of energy and protein intake, among critically ill children.

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The Effect of High- and Low-Intensity Interval Training on Myostatin Gene Expression Levels in Muscles Fibers of Rats with Myocardial Infarction

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A R T I C L E I N F O	A B S T R A C T
Article type: Research Paper	**Introduction**: Myocardial infarction (MI) is an essential coronary artery disease, which affects mitochondrial function and causes muscle atrophy due to vessel blockage and disruption in blood transfusion and oxygen transfer. Interval exercises reduce muscle atrophy, but the appropriate exercise intensity is still unknown. This study aimed to evaluate the effect of interval training with two for six weeks on Myostatin gene expression levels in slow (ST) and fast (FT) twitch muscles in rats with MI.
Article History: Received: 09 Nov 2022 Accepted: 17 Dec 2022 Published: 26 Dec 2022	**Method**: Eighteen ten-week male Wistar rats with MI were randomly assigned into high- (HIIT) (90-85% VO_{2max}) and low-intensity interval training (LIIT) (50-60% VO_{2max}) with a control group (CG, without training). Myostatin gene expression of FT and ST was investigated as a stimulant of muscle atrophy. The training protocol was 30-minute intermittent jogging sessions on a treadmill. Each interval included 4 min of running (85-90% VO_{2max} for HIIT and 55-60% for LIIT) and 2-minute active recovery (50-60% for HIIT and 45-50% for LIIT) three days a week for six weeks.
Keywords: Myocardial infarction Muscle atrophy Interval training	**Results**: LIIT significantly decreased myostatin expression in both ST and FT while HIIT only decreased myostatin expression in ST compared to CG (P = 0.002, P = 0.016, and P=0.011, respectively). HIIT induced myostatin expression reduction was higher in FT compared to CG (P = 0.078). There was a significant difference in myostatin expression between CG (8.87) and the two training groups (HIIT [0.949] and LIIT [3.11]) in ST (P≤0.05), and between CG and LIITs and HIIT (1.22) and LIIT (0/975) in FT (P<0.05).
	Conclusion: Six weeks of HIIT and LIIT reduced myostatin gene expression and decreased ST and FT atrophy in rats with MI.

Introduction

Myocardial infarction (MI) is one of the essential coronary artery diseases, which is currently the leading cause of mortality worldwide. (1). In addition, MI affects skeletal and heart muscles (2).

Skeletal muscle atrophy because of mitochondrial dysfunction is one of the most critical complications of MI in skeletal muscle, particularly in slow-twitch muscle fibers (3). Blockage of blood vessels and disruption of blood transfusion and oxygen transfer affect tissues, including skeletal muscle, causing muscle fiber atrophy (2).

Skeletal muscle atrophy can be called muscle mass loss or damage due to injury or illness. Muscle atrophy is the loss or reduction of muscle mass affected by several stimuli and inhibitors (4). MI induces muscle atrophy by increasing the expression of the MyoD[1] gene and decreasing myogenin protein levels (4-5).

IGF-1[2] is also reduced in the case of MI, activating IRS1 and PI3K, reducing pAKT, and resulting in Gs3k beta[3]. Reducing pAkT also activates mTOR and causes atrophy in the skeletal muscle of the IGF-1/AKT/mTOR pathway (6). Therefore, any factor that can inhibit the IGF-1/AKT/mTOR[4] pathway should be effective in preventing

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[1] myogenic differentiation
[2] Insulin-like growth factor 1

[3] Glycogen synthase kinase-3 beta
[4] Mammalian target of rapamycin

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muscle atrophy. Researchers are looking for ways to reduce the amount of myostatin, one of several stimulants and inhibitory factors that promote muscle atrophy.

Myostatin is a negative regulator of muscle growth and size, which increases rapidly after an MI (7). This protein is a part of the large family of TGFβ[5] proteins, activated and inactivated by a protease, which divides the terminals of NH2 and activates the COOH terminal.

Factors such as the COOH terminal stimulate the gene responsible for regulating myostatin. Recently, myostatin has been an inhibitor of AKT as a kinase that causes muscle hypertrophy at the time of activation of protein synthesis. Therefore, myostatin can prevent muscle atrophy with stimulation of the AKT protein synthesis (8).

Regular physical activity (i.e., exercise) has a proven role in health. High-intensity interval training (HIITs) and low-intensity interval training (LIITs) are two forms of exercise, which induce positive health outcomes (i.e., in part by inducing hypoxia) (9). Hypoxia is one of the influential factors in increasing the expression of PGC-1α levels (10) and inducing muscle hypertrophy (11). HIIT and LIIT exercise training is a potent stimulator for cardiovascular and muscle adaptations, which increases maximal oxygen uptake (VO_{2max}) and metabolism, exercise performance, insulin function, fat intake, and cardiovascular fitness. In addition, HIIT and LIIT exercise training reduces carbohydrate intake and blood pressure in cardiac and hypertensive patients (12).

There has been no direct research on the effects of HIIT on the factors affecting muscle atrophy, and LIIT has been less of a concern. However, extensive studies have been conducted in which participants still need to be evolved with MI regarding the effects of strength activities on muscular atrophy.

Most previous studies have shown a positive effect of strength activity on reducing myostatin as a stimulating agent of muscle atrophy. Ruth et al. (2003) indicated that the expression of myostatin mRNA decreased after nine weeks of resistance training (13). Wilugby et al. (2004) concluded that the expression of myostatin mRNA did not significantly differ after 12 weeks of resistance training, although muscle strength and mass increased (14). Saremi et al. (2010)

observed that resistance training with and without creatine supplementation reduced the serum levels of myostatin (15). Contrary to these studies, Dale et al. (2010) examined the interactive effects between exercises and androgens on the concentration of myostatin and serum and muscle follistatin. Based on this study, none of the moderate-intensity endurance and resistance training exercises significantly changed myostatin and follistatin serum levels (16).

Further research is required considering previous studies and the lack of information about the effect of HIIT and LIIT on muscle atrophy, especially in patients with MI. Therefore, this study aimed to compare the impact of six weeks of HIIT and LIIT on the expression of myostatin gene expression levels in ST and FT (as dependent variables) in rats post-MI.

Methods

Eighteen male Wistar rats (10 weeks old) were obtained from the **R**azi **V**accine and **S**erum **R**esearch **I**nstitute. The rats underwent surgery, and their left descending artery was blocked, experiencing a MI. All rats were anesthetized and received echocardiography with doppler access (GE Healthcare brand, USA). The shortening fraction of the left ventricular (FS) during this process was relatively measured. Rats with FS ≤ 35(17) were selected as MI rats, and the MI rats were randomly divided into three groups; two experimental groups of HIIT (n=6) and LIIT (n=6) and a control group (CTRL, n=6).

After two weeks of recovery, two experimental groups were acquainted with the treadmill (*Danesh-salar-e Iranian*, Iran) with a gentle walk at a speed of 5m/s (5m a day) and four days a week for two weeks. The VO_{2max} of rats was measured by maximum physical activity based on the formula and table set out by Wislov et al. (2000) to estimate the initial speed of running rats (18). The speed of each rat was calculated on the treadmill based on the individual VO_{2max}. Then, the two experimental rat groups performed six weeks of training protocols.

In both experimental groups, the rats warmed up for 5m at 5m/s before starting the main training session. Then, 0.02 m/s per week accumulated the rats' speed, and the treadmill slope was zero

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[5] *Transforming Growth Factor-*β

degrees throughout the training period. In contrast, the rats in the control group (with MI) performed no exercise.

HIIT Protocol
The training protocol in the experimental group of HIIT comprised a 30-minute intermittent jogging session on a treadmill; each interval included 4m of running with an intensity of 85-

90% VO_{2max} and 2m of active recovery with a VO_{2max} of 50-60% intensity (Table 1). The training was performed three days a week for six weeks (Howidal et al. 2007), and the rats warmed up for 5m at 40 to 50% VO_{2max} before the start of the leading training phase. The running speed was gradually increased every second week by 0.02m/s.

Table 1. The training protocol in the experimental groups (HIIT and LIIT)

groups	weeks	Days per week	Intensity of training	Intensity of recovery	Time of exercise	each interval included	Over load
HIIT	6	3	85-90% $VO2_{max}$	40-50% $VO2_{ma}$	30 min	4 minutes of running and 2 minutes of active recovery	every second week by 0.02 m / s.
LIIT	6	3	55-60% $VO2_{max}$	40-50% VO2ma	30 min	4 minutes of running and 2 minutes of active recovery	every second week by 0.02 m / s.

LIIT Protocol
The training protocol in the experimental group of LIIT comprised a 30-minute intermittent jogging session on a treadmill; each interval of which included 4m of running with an intensity of 55-60% VO_{2max} and 2m of active recovery with a VO_{2max} of 45-50% intensity (Table 1). The training was performed three days a week for six weeks (Howidal et al. 2007), and the rats warmed up for 5m at 40 to 50% VO_{2max} before the start of the leading training phase. Running speed was gradually increased every second week by 0.02 m/s (Table 1).
Finally, the animals were sacrificed, and the samples of Soleus (ST) and Extensor digitorum longus (FT) muscle tissue were taken by a

veterinarian to measure the mRNA levels of the myostatin gene by the qRT-PCR method.
The target genes were the peroxisome proliferator-activated receptor gamma coactivator1α (myostatin forward primer: 5′-CAGGAGAAGATGGGCTGAATCC -3′ and Reverse primer: 5′- AAGCCCAAAGTCTCTCCGGG -3′) was used for normalization. (2).

Statistical Analysis
The data were analyzed using SPSS software version 18. The Kolmogorov-Smirnov test was used to determine the normality of the data. When data were of normal distribution, independent sample t-test and ANOVA were used to test for the significant level at $p \le 0.05$.

Table 2. Descriptive statistics and results of t-test and ANOVA of the myostatin gene expression of ST and FT in the control and experimental groups of the study

Type of Muscle Fiber	Group	Number	Mean±SD	(T test)	(ANOVA)
ST	CON	6	8.87±2.948	0.011	0.001
	HIIT	6	0.949±0.002		
	CON	6	8.87±2.948	0.002	
	LIIT	6	3.11±0.773		
FT	CON	6	3.91±2.035	0.078	0.0024
	HIIT	6	1.224±0.273		
	CON	6	3.91±2.035	0.016	
	LIIT	6	0.975±0.075		

Results
As shown in Table 2, the mean values of myostatin gene expression of ST in the control group (8.87) were higher than in the HIIT group (0.949) and LIIT (3.11). Further, the mean of myostatin gene expression of FT was more significant in the control group (3.91) than in the HIIT group (1.224) and LIIT (0.975).

There was a significant difference between the two groups of control and HIIT in the myostatin gene expression of ST (P = 0.11), and the myostatin gene expression of ST in control was more than the HIIT (see Table 2). In addition, the myostatin gene expression of FT in the HIIT was not significantly more than the control group (P = 0.078).

A significant difference between the control and LIIT groups was observed in the myostatin gene expression of ST (P = 0.002). The myostatin gene expression of ST contraction in the control group was greater than that of the LIIT experimental group (Table 2). There was also a significant difference between the two groups of control and LIIT in the myostatin gene expression of FT (P = 0.016). Myostatin gene expression of FT in the experimental group of LIIT was more than the control group (Table 2). A significant difference was found between the two groups of HIIT and LIIT in the myostatin gene expression of ST (P ≤0.001). The myostatin gene expression of ST was greater than that of the HIIT group in the experimental group of LIIT (Table 2).

In addition, ANOVA results showed a significant difference between the control and HIIT, the control and LIIT groups in the myostatin gene expression of ST (P≤0.05). Moreover, there was a significant difference between the control and LIIT group and the HIIT and LIIT group in the myostatin gene expression of FT (P=0.002).

Discussion and Conclusion

In this study, six weeks of HIIT and LIIT influenced the myostatin of ST and FT. Although the mitochondria in slow-twitch muscles shrank more than in fast-twitch muscles, and their performance decreased, myocardial infarction changed the phenotype of slow-twitch muscles towards glycolytic fast-twitch (2). No prior published research directly investigated the effect of the intensity of interval training on ST and FT atrophy in MI patients.

Nonetheless, the results were consistent with those of Ruth et al. (2003) and Saremi et al. (2010), which investigated the effect of resistance training on myostatin gene expressions (15-13). On the other hand, the present results differed from those of Wilugby et al. (2004) and Dale et al. (2010), expressing that neither endurance nor resistance training with moderate intensity had a significant change in serum levels of myostatin and follistatin (16-14). According to the research hypothesis, the contradiction in results resulted from different types, training protocols, and various subjects.

Based on the results, adaptability with six weeks of HIIT and LIIT improved mitochondrial function and effectively increased the size of mitochondria in muscles to promote their function (19). Positive regulation of PPAR[6] alpha and PPAR gamma, following the increase in the expression of the PGC-1 gene and its signaling, increased mitochondrial DNA replication (19-21).

Six weeks of LIIT increased the AMPK and alpha-PGC-1 by increasing ATP and AMP levels. Alpha-PGC-1 ultimately activates mTOR and suppresses myostatin by stimulating the TSC2[7] protein. On the other hand, six weeks of HIIT increased PI3K levels and activated AKT by increasing IGF-1. AKT was effective on FOXO[8] and inhibited myostatin by activating the mTOR (19).

The expression of important mitochondrial biogenetic factors such as PGC-1 alpha, NRF-1, and Tfam increased and inhibited myostatin from IGF-1/AKT/mTOR pathway due to the adaptation induced by six weeks of interval training with both intensities.

Inhibition of myostatin inactivated SMAD2 and SMAD3[9] and prevented skeletal muscle atrophy by disabling E3 ligases (19-22).

The organization should be developed for the discussion and prevent information replication that already appears in the manuscript's Introduction. Exercise of both types can be beneficial, emphasizing this point and the importance of exercising after a stroke.

Acknowledgments

Conflict of Interest

There are no conflicts of interest.

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[6] Peroxisome proliferator-activated receptor

[7] *Tuberous Sclerosis Complex 2*

[8] Forkhead box protein O

[9] SMA ("small" worm phenotype) and MAD family ("Mothers Against Decapentaplegic")

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JOURNAL OF NUTRITION FASTING AND HEALTH

Identification of *Coxiella Burnetti* and *Mycobacterium* SPP through Touch-down PCR Examination in Unpasteurized Camel Milk in North-East of Iran

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A R T I C L E I N F O	A B S T R A C T
Article type: Research Paper	**Introduction:** Food-borne illness cause major international health problems and reduce economic growth. A highly prevalent zoonotic disease is Q fever, found in many areas like New Zealand, Saudi Arabia, and Egypt. *Coxiella burnetii* is caused by an obligate intracellular bacterium that is considered in ruminants and ubiquitous and can survive in the environment for a long time. Early and reliable diagnosis of food borne pathogens through molecular methods like polymerase chain reaction is critical to find positive outcomes in eradication programs.
	Method: In this study 100 milk samples obtained from 100 camels were examined in terms of *C. burnetii* and Mycobacterium presence through a Touch-down PCR assay.
	Results: In total, there were six positive specimens of *Coxiella burnetii* in camel milk samples. No Mycobacterium was found in the samples.
	Conclusions: The findings indicated that healthy camels were major sources of *C. burnetii* in North-East of Iran. There is a need for studies on risk of *Coxiella* infection in farmers, veterinarians, milk-processing and slaughterhouse workers.

Introduction

Q fever due to *Coxiella burnetii* is a known disease caused by a ubiquitous gram-negative and resistant bacterium that can infect humans and animals including mammals (1). Q fever is a global health issue in humans with subclinical, acute and chronic forms (2). In the acute form, it appears as a self-limiting flu illness, and in chronic form, it manifests as a progressing infection (e.g., endocarditis and premature delivery in pregnant women) (3). *C. burnetti* is gram-negative resistant bacteria. It can be tolerated under pressure, heat, chemical stress, or months of hard situations. (4). many animal species are vulnerable to *C. burnetti* such as carnivores, ruminant and non- human primates (5). Sheep, goats or cattle are known as important sources of human infections (2). In these animals, *C. burnetii* infection is subclinical in most cases (6). If signs of the disease, referred to as *Coxiellosis*, manifests, reproductive complications like abortions, stillbirth, or weak newborns are observed (7). Inhalation of respiratory secretions, especially contaminated aerosols with secretions of the aborted fetus secretions are the main transmission route. During parturition in sheep and goats high load shedding of *C. burnetii* with about 10^9 bacteria per gram of placenta can be detected (6). the bacterium shed into milk, urine, and faces.

Mycobacterium tuberculosis complex is the major source of food-borne tuberculosis in humans is mostly related to the *M. Tuberculosis* complex (8). Animal tuberculosis (TB) is widely distributed with a wide range of domestic and wild reservoirs (9).

Many studies have shown that camel milk is highly identical to human milk among other animals. The milk is different from other ruminant milk with lower sugar, cholesterol,

higher minerals (potassium, sodium, copper, zinc, iron, and magnesium), and vitamin (10). Camel milk has unique specifications compared to other ruminants and it has functional effects on humans' effects. The milk can be easily digested by people who are lactose-intolerant with potential medical effects such as anti-diabetic, anti-hypertensive, and anti-carcinogenic (10).

The proteins found in camel milk create a balance of essential amino acids. The milk has immunoglobulins that fight diseases with small size so that they can enter antigens and enhance the efficiency of the immune system (11).

This study aimed to detect *C. burnetii* and *Mycobacterium spp* in raw camel milk. The samples were collected from camels of Mashhad city using a touchdown PCR assay.

Material and Methods
Experimental Procedures
DNA Extraction from Raw Milk
To remove layers and cream, the milk was centrifuged and then DNA was extracted using Berri et al (2000) method (12), with some modifications. 1ml volume of the camel milk samples was transferred to a 1 ml for three times centrifuging at 15000 rpm for 5min. every time the supernatant was removed and refilled by normal saline. The isolation of DNA was performed with the help of a genomic DNA extract kit (Dena Zist, Iran) based on the producers' instructions. The quantity of the extracted DNA was measured through spectrophotometry (Nano Drop™ 2000/2000c spectrophotometer; Thermo Scientific, USA). The concentration of DNA and purity were measured under UV absorbance at 260 nm and by the ratio of absorbance at 260 and 280 nm, respectively. (13).

DNA amplification (trans-PCR)
A pair of primers were used in the PCR assay to measure the IS*1111* gene transposase elements *C. burnetii* genome. Trans-1 (5'-TAT GTA TCC

ACC GTA GCCAGT C-3') and trans-2 (5'-CCC AAC ACC TCC TTATTC-3') primers were utilized based on the available data (14) which amplified a 687-bp fragment of the target sequence. The PCR examination was carried out as mentioned by (15). The PCR mixture (25µL) contained 2.5µL of 10×PCR buffer (100 mM Tris-HCl buffer, pH 8.3, 500 mM KCl, 15 mM MgCl2, and 0.01% gelatin, 200 µM deoxynucleoside triphosphate mix, 2µM of each primer, 0.3 U of Taq DNA polymerase, 3µL of template DNA, and deionized water. To perform the amplification, a thermal cycler (TECHNE TC- 5 UK) and the cycling program were as followed: DNA denaturation (95°C; 2min), five cycles (94°C for the 30s, 66 to 61°C; the temperature decrement by 1°C in consecutive steps) for 1 min, and 72°C for 1 min. After the initial cycling program, another 35 cycles (of 94°C for 30 s, 61°C for 30 s, and 72°C; 1min) and then a final extension step of 10 min at 72°C (14) were performed. Following electrophoresis in the agarose gel and green viewer staining (Sinaclone), they were examined using UV illumination. Following the first positive PCR product, which was detected as *C. burnetii* by sequence analysis, the product was utilized as a positive control and deionized distilled water was the USA as negative control.

To identify the genus Mycobacterium, the um, Hsp65 gen, us is used, and to identify the specific species of *Mycobacterium tuberculosis*, Primer 6110 (IS6110) Insertion sequence is used (16,17) Amplification was performed in a 25 µL reaction volume with 12.5 µL Taq DNA Polymerase Master Mix RED, 1 µM of each of the primers and 4 µL of template DNA and 6.5 µL Sterile distilled water. *Mycobacterium tuberculosis* H37Rv was considered as positive control while the negative control. The PCR outcome was examined using 1.5% agarose gel electrophoresis, after which the gel was marked using green viewer. This standard marker was supplied by Pars Toups, Iran.

IS1111 gene *(C. burnetii)*

Table1. Primer sequences for detection of C. burnetii IS1111 gene by nested PCR

Primer name	Primer Sequence	Amplicon size
Trans 1	5'-TAT GTA TCC ACC GTA GCCAGT C-3'	687bp
Trans 2	5'-CCC AAC ACC TCC TTATTC-3'	

Hsp65 (Mycobacterium)
Amplification was carried out in a thermal cycler for 35 cycles (95C for 5min as the early initial

denaturation; 95C and 30s, 62C and 30s, 72 C and 45s, and final extension at 72 C and 10 min.

Table2. Sequence and PCR primer of Mycobacterium IS6110 (Tuberculosis) Amplicon size (441 bp)

Primer name	Primer Sequence	Amplicon size
TB11F	5'-ACC AAC GAT GGT GTG TCC AT-3	441bp
TB12R	5'-CTT GTC GAA CCG CAT ACC CT-3	

Amplification was carried out in a thermal cycler with 35 cycles (95C for 5 min as starting, cycling of 95 C and 30 s, 68 C and 30 s, 72 C and 45 s, and 72 C and 10 min).

Table3. Sequence and PCR primer of Mycobacterium IS6110 (Tuberculosis) Amplicon size (123 bp)

Primer name	Primer Sequence	Amplicon size
INS1F	5'- CCTGCGAGCGTAGGCGTCGG -3'	123bp
INS2R	5'- CTCGTCCAGCGCCGCTTCGG -3	

Results

Using touch-down PCR assay to target the IS*1111* gene of the organism through Trans-1 and trans-2 primers, 6 positive *C. burnetii* out of 100 samples (6%), (in the case of positive control, the 687 base pairs of the amplified gene fragment were sequenced successfully out of the first PCR-positive sample. We compared library gene sequences of *C. burnetti* with our samples, and there was no difference between deduced amino acid and nucleotide (Figure 1).

To identify the genus Mycobacterium, all 100 DNA samples were extracted by PCR with Hsp65 primer, none of which showed a band of 441 bp. This result indicates that none of the milk samples were infected with Mycobacterium. There was no need for PCR to identify the species.
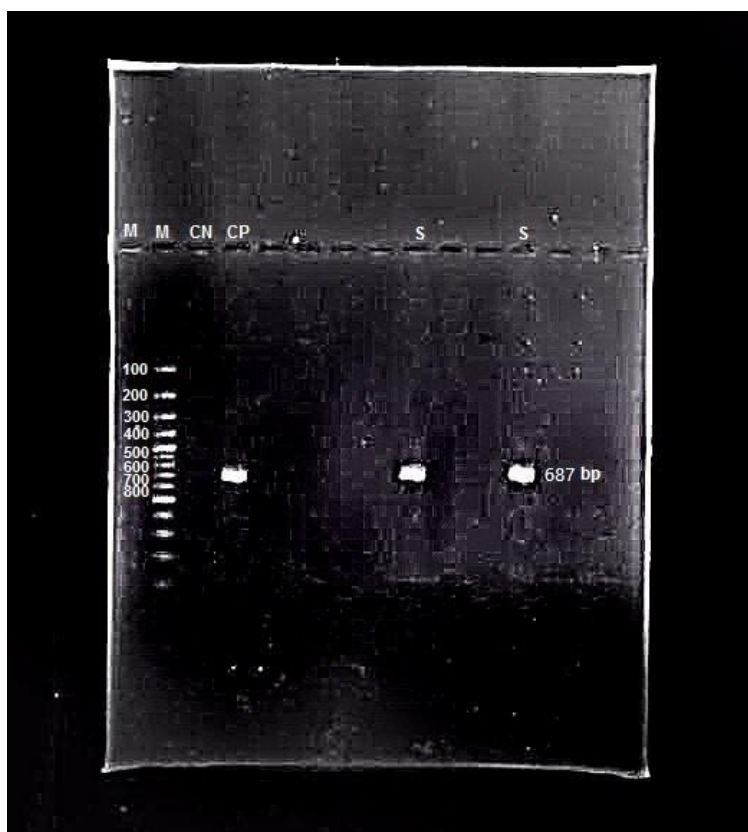

Figure 1. Electrophoresis of the amplification products of the touchdown polymerase chain reaction assay.
Detection of *Coxiella burnetii* in camel milk using touch-down PCR assay, amplifying a 687 bp
Segment of the IS1111 gene: CN= Control Negative /CP= Control positive/ Line 8 and 11 are positive samples

Discussion

Coxiella burnetii is classically a strict intracellular short (0.3 to 1.0 μm), gram-negative, and pleomorphic rod organism. It causes infection in humans through contact with sick animals*. Coxiella burnetii* resist pressure, heat, chemical stress, and even several months under stressful conditions (4). The organism is also very infectious and even one *Coxiella burnetii* can cause infection, in experimental conditions (18) *C. burnetii* can be shed from feces, urine, and milk of infected cases with a high level of concentration during parturition. It is mainly spread by birth products and the placenta in particular. When in chronic condition, the mammary glands and uterus are the main sites of infection of *C. burnetii* (19). The major part of transmission in the case of human hosts is dust containing birth fluid, urine, placenta, feces, or respiratory aerosols. Several studies have reported *C. burnetii* DNA prevalence in the blood of ruminants; so that bacterial DNA was found in two goats (5.26%) and 13 camels (15.85%) in Saudi Arabia. Still, the sheep and cattle under study showed no positive case (20). In Zambia, *C. burnetii* rates in goats and cattle were 7.55% and 7.77% (21). Another research work examined 13 blood samples in China and found that all the samples were negative (22). In Korea, 57 samples out blood samples from goats had positive amplification for *C. burnetii* DNA (23). The inconsistency of findings about the prevalence of the infection in these studies can be because of changes in study methods, types of herds, different sampling methods, and environmental conditions. The epidemiology of Q-fever in Iran is unclear. The first study was done by Rahim et al., in 2010. Using nested PCR assay, Rahimi examined 376 bulk milk samples supplied by 79 dairy ovine, bovines, and caprine herds in terms *of C. burnetii*.

In another study, (24) examined *C. burnetii* in milk samples of ruminant animals in Iran. They used the PCR technique for the identification of *C. burnetii.* In total, 9 (1.8%) milk samples were *C. burnetii* positive (6 goat milk and 3 sheep milk samples). The positive milk was collected from two villages (Daraei and Ghalebi) near the Khorramabad region in winter. Still, the collected samples of Nourabad were all negative.

In the present study, the PCR technique was used to target the repetitive transposon-like region (Trans-PCR) was used to investigate *C. burnetii* in

camel milk samples in Iran and six out of 100 samples (6%) were positive. The method's efficiency in terms of detecting Coxiella in milk has been supported by studies. The method can detect *C. burnetii*-cell in 1 ml of milk (25). In addition, trans-PCR is highly sensitive with considerable specificity (25-28). No *Mycobacterium spp.* was found in all the milk samples in this work.

Conclusion

This study found that detects *C. burnetii* and *Mycobacterium spp.* in camel milk by touch-down PCR assay. This result showed that camel milk plays an important reservoir of *C. burnetii*. *C. burnetii* prevalence in Iranian camels was about 10.76 %; this suggests that camels can have a notable role in the epidemiological study the human population in Iran.

So it is recommended that raw camel milk should be consumed with precaution and be commercially pasteurized at 74 C for public consumption.

Acknowledgment

Conflict of Interest
There is no conflict of interest to declare.

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Development and Validation of Ramadan Fasting Basic Information and Nutritional Habits Questionnaire

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A R T I C L E I N F O	**A B S T R A C T**
Article type: Research Paper	**Introduction:** Most Ramadan fasting studies are usually conducted over one-year periods and show the short-term effects of Ramadan fasting. There is no standard tool or questionnaire to evaluate people's fasting habits and Ramadan fasting long term effects. For the first time, we designed a study to develop and validate a questionnaire to evaluate people's fasting habits as a tool for the determination of short-term and long-term Ramadan fasting effects.
	Methods: After designing the questionnaire, it was sent to 10 experts in this field for judging and commenting (content validity). The validity of the questionnaire was evaluated using the Content Validity Ratio (CVR) index> 0.62, which includes the questionnaire's essentiality, relevancy, clarity, and comprehensiveness. In order to check the questionnaire reliability, the "test-retest method" was applied among 10 individuals.
	Results: After multiple drafts, the questionnaire contained 16 items, categorized into "Basic information on fasting (demographic information)", and "Fasting nutritional habits" including 13 and 3 questions, respectively. The overall CVR for relevancy, clarity, and comprehensiveness of the tool were 0.92, 0.95, 0.97, and 0.95, respectively. The Pearson correlation coefficient of the test–retest stage was 0.85.
	Conclusions: This Ramadan fasting habits questionnaire demonstrated strong content validity and test-retest reliability. It can be an appropriate instrument for the evaluation of Ramadan fasting basic information and nutritional habits in research or practice-oriented settings for the determination of short-term and long-term Ramadan fasting effects.

Introduction

Ramadan is the 9th month in Islamic calendar which is known as a month of fasting by all Muslims around the world, during which every healthy and mature Muslims fast from dawn to sunset (about 12 hours based on geographic location) to fulfill this religious obligation(1). During the month of Ramadan, which lasts between 29 to 30 days, Muslims have two large meals after sunset and before first light and they abstain from eating and drinking for the rest of the day (2). Therefore, not only eating habits changes during this month but also other factors like sleep duration and pattern get altered (3). Abstinence from eating, drinking, smoking and intercourse for such a long period of time, as well as life style alternation could affect body in many different ways (4-13).

It has been shown that caloric and dietary restrictions besides lifestyle changes during Ramadan can be considered a two-edged sword, as there is no consensus on its effects (4-9). Also, most of the related-studies are usually conducted in one-year period and show the short-term effects of Ramadan fasting (4-13). Ramadan fasting long term effects investigation require a questionnaire to evaluate people's fasting habits. New surveys must be extremely tested to ensure the validity of the instrument (14, 15). Validity is the extent to which any tool measures what it is

asserted to (16). Content validity is the extent to which items fit or show a particular domain and are assessed by quantitative methods (14, 16).

The aim of current study is to develop and validate a questionnaire to evaluate people's fasting habits and as a tool for determination of short-term and long-term Ramadan fasting effects.

Method

The present research developed and validated a Ramadan fasting questionnaire in 3 stages: (1) questionnaire development, (2) content validity, (3) reliability.

Item Generation and Development

The content questions relevant to the current study were based on expert viewpoint, related literature, and previous surveys between 2000 and 2016 (4-13).

Overall, 16 items were recognized for the primary questionnaire version. The questionnaire and the data form for rating the content were sent via e-mail to 10 experts with experience in the field of nutrition, Ramadan researches, psychometrics, and community medicine.

Content Validity

There are several techniques to test content validity. The current research applied one technique which included empirical methods for

calculating the content validity ratio (CVR) and the content validity index (CVI) (17, 18). These empirical methods have been explained below:

CVR

For calculating CVR, each item of the questionnaire was scored by the experts using a 3-point Likert scale as follows: 1. Essential, 2. Useful but not essential, and 3. Unessential. CVR values range varies between 1 and -1. The higher score shows further agreement of panelists on the necessity of an item in an instrument. The CVR formula is: CVR = (Ne - N/2)/(N/2), in which N is the total number of panel members and the Ne is the panelists numbers showing "essential". According to Lawshe's table, when there are 10 members in expert advisory panel, selected items are those with CVR= 0.62 or above (19). In other words, the content validity of the items will be verified if the CVR for each item equals to or higher than 0.62.

CVI

CVI is the most commonly offered measure of content validity. Panelists was asked to score the items according to clarity and its relevancy with structure of the underlying research in accordance with the theoretic descriptions of the structure itself and its dimensions on a 4-point grade (1: irrelevant, 2: somewhat relevant, 3: quite relevant, 4: highly relevant). Table 1 was attached to the cover letter to lead the experts for grading methodology.

Table 1. The table added to the cover letter to guide experts for scoring method

Relevancy	Clarity
1: not relevant	1: not clear
2: item need some revision	2: item need some revision
3: relevant but need minor revision	3: clear but need minor revision
4: very relevant	4: very clear

There are 2 kinds of CVI: item level CVI (I-CVIs) and the scale level CVI (S-CVI) (18). I-CVI is calculated as the number of experts who rate the relevancy of each item as 3 or 4, divided by the experts' number. Values between 0 and 1 while I-CVI is higher than 0.79, the item is relevant, between 0.70 and 0.79, the item requires editing, and if the value is less than 0.70 the item is removed (18). Likewise, S-CVI is computed by the number of items in an instrument that have got a rating of "very relevant" (18). Two techniques are existed to calculate S-CVI including, the Average CVI (S-CVI/Ave) and the Universal Agreement (UA) among experts (S-CVI/UA), the first is a less conservative technique

(18). S-CVI/UA is computed via summing all items with I-CVI =1 divided by the total number of items, whereas S-CVI/Ave is computed via dividing the sum of I-CVIs by the total number of items (18). A S-CVI/Ave ≥ 0.9 and S-CVI/UA ≥ 0.8 show great content validity (20).

Asking panel members to review the tool for comprehensiveness would be the final phase for estimating the content validity. The panelists are asked to determine whether the instrument's items and each of its dimensions are a perfect and comprehensive sample of content as far as the theoretic meanings of the concepts and its dimensions are concerned. Is it necessary to add or remove any item? Regarding members'

judgment, agreement proportion is assessed for the comprehensiveness of each dimension and the whole tool. Therefore, the number of experts who found the tool comprehensiveness as desirable is divided by the total number of experts.

Reliability of the Questionnaire

The test–retest method was used to determine the reliability of the questionnaire under the same conditions at different times. The questionnaire was provided to 10 employees of Mashhad University of Medical Sciences and was required to fill out the questionnaire. After 10 days, the questionnaire was provided to the same employees, and they again filled in the questionnaire. Then, statistical analysis was carried out by SPSS software (IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp). To assess the reliability of all questions via the test–retest technique, Pearson correlation coefficient was used to evaluate the agreement between primary and secondary responses. Pearson correlation coefficient is commonly used to describe the direction and strength of the linear relationship

between variables and it assesses the linear relationship between quantitative variables (21). This coefficient ranges between −1 and 1 which explains the linear dependence degree between two quantitative variables. Pearson's correlation coefficient is calculated as below (22):

$$r = \frac{n(\sum xy) - (\sum x)(\sum y)}{\sqrt{[n\sum x^2 - (\sum x)^2][n\sum y^2 - (\sum y)^2]}}$$

In this formula, r is the Pearson correlation coefficient, n is the valid responses number, x shows the score of an item, and y shows the total score of each respondent with valid responses; Assuming that the distribution of both variables (x and y) are normal. The values of r are distributed as follows: r = 0 to 0.25, very low correlation; r = 0.26 to 0.49, low correlation; r = 0.5 to 0.69, moderate correlation; r = 0.7 to 0.89, high or strong correlation; r = 0.9–1.0, very high or very strong correlation (23).

Ethics

The study was approved by the Ethics Committee of Mashhad University of Medical Sciences (decision number IR.MUMS.fm.REC.1396.293).

Table 2. Content validity and test-retest reliability for Ramadan fasting basic information and nutritional habits questionnaire.

Number	Question	Content validity			Test-Retest Reliability*	
		CVR	Relevancy	Clarity	Pearson Correlation	P value
	Basic information on fasting (demographic information)					
1	Gender and age	1	1	1	1	<0.001
2	Have you ever fasted during your lifetime? a) YES, I have fasted for at least one day so far b) NO, I have never fasted	1	0.9	1	1	0.003
3	**In general**, what times of the year have you fasted? a) I have fasted only in the month of Ramadan b) I have fasted only outside of Ramadan c) In addition to Ramadan, I have fasted at least one day at other times of the year	1	1	1	1	<0.001
4	How has your fasting status been in the past five years? (table: Year / How many days have you fasted in Ramadan? / How many days have you fasted outside of Ramadan? / About how many days of your fasting days were without sahari meal?) * Please answer the questions in this table as long as you remember. * If you do not remember, please enter the number 100 in the table.	0.8	0.9	0.9	1	<0.001
5	If you have not fasted in the last 5 years, what was the reason? a) Chronic underlying disease	1	1	0.9	0.723	0.006

Number	Question	Content validity			Test-Retest Reliability*	
		CVR	Relevancy	Clarity	Pearson Correlation	P value
	b) Acute illness or surgery in Ramadan c) Pregnancy d) Breastfeeding e) Weakness and fatigue f) Travel g) Individual unwillingness for any reason					
6	Have you ever fasted without a doctor's permission when you are sick? a) Yes b) No	0.8	1	1	1	<0.001
7	Have you had a history of acute problems (those mentioned in the next question) that led to going to the emergency room during Ramadan fasting? a) YES b) NO	1	1	1	1	<0.001
8	In case of an acute problem, which case happened during Ramadan fasting? a) Myocardial Infarction b) Stroke c) Loss of consciousness **In diabetic patients:** d) Symptomatic severe hypoglycemia e) Diabetic ketoacidosis (DKA) f) Hyperosmolar Hyperglycemic State (HHS) g) Please name other problems…	1	1	0.8	0.848	0.004
9	At what age did you fast for the first time?	1	1	1	0.962	<0.001
10	Since what age have you fasted at least 20 days of Ramadan almost regularly?	1	0.9	0.9	0.835	0.003
11	**In general**, have your parents were fasting? a) YES b) NO (for any reason) c) I don't know	0.8	0.8	1	0.791	0.011
12	**If you know**, did your mother fast during your fetal period? a) YES b) NO c) I don't know	0.8	0.9	1	0.377	0.317
13	**If you know**, did your mother fast during the first year after your birth (while breastfeeding)? a) YES b) NO c) I don't know	0.8	1	1	0.243	0.217
	Fasting nutritional habits					
1	If you fast, when do you **generally** break your fast? a) After the Azan or less than an hour after the Maghrib Azan b) Between one and two hours after Maghrib Azan c) More than two hours after Maghrib Azan	1	0.9	1	0.896	0.001
2	If you fast, what do you generally break the fast with? (What is the first thing you eat to break the fast?) a) Tea b) Milk c) Fruit juice d) Water e) Beverage f) Date g) Soup h) Dessert i) Solid food j) Other (please name) …	0.8	0.9	1	0.899	0.001
3	**In general**, which of the following meals do your meals include in Ramadan? a) Eftar + Sahari b) Eftar+Sahari+Dinner c) Eftar + Dinner d) Eftar	1	1	1	1	<0.001

*Pearson Correlation

Results

After multiple drafts, the questionnaire consisting of 16 items was developed, categorized into "***Basic information on fasting (demographic information)***", and "***Fasting nutritional habits***" including 13 and 3 questions respectively.

Content Validity

All experts' feedbacks on essentiality, relevancy, clarity, and comprehensiveness of the 16 questions were collected in 4 weeks (response rate= 100%). The CVR, relevancy, clarity, and comprehensiveness of the final instrument were 0.92, 0.95, 0.97 and 0.95 respectively. Table 2 shows the CVR, clarity and relevancy of each item.

Reliability

The test–retest reliability was applied to assess the reliability. The Pearson correlation coefficient (r = 0.85) represented the good reliability of the questionnaire.

Discussion

In this research, a questionnaire was developed to address the short-term and long-term Ramadan fasting effects. This questionnaire has excellent content validity and substantial test-retest reliability as an instrument that can be used to examine Ramadan fasting basic information and nutritional habits. To the best of our knowledge, there is no other questionnaire available for evaluating people's fasting habits. There are around 1.9 billion Muslims around the world (24). Fasting is mandatory for all Muslim adults. Regarding the season and geographic location, fasting period may last up to 20 hours (25). However, no questionnaire is exited to evaluate people's fasting habits and determine the of short-term and long-term Ramadan fasting effects.

The Lawshe methodology is one of the most acceptable instruments for determining content validity (26). Considering that 5-10 experts were sufficient (27), the content validity was determined by a group of 10 expert specialists. Lawshe CVRs of 1.0 showed the consensus of the experts. All questions were essential.

The test-retest Pearson Correlation for the entire questionnaire was acceptable. This tool will produce repeated and consistent measurements for evaluation of Ramadan fasting basic information and nutritional habits and will be consistent for a given individual. Of note, In 2 questions (**If you know**, did your mother fast during your fetal period?; **If you know**, did your mother fast during the first year after your birth (while breastfeeding)?), the P value was above 0.05, which indicates that the answers were not reliable. It is possible that after the initial filling of the questionnaire, they asked their mother and their answer is different when filling the questionnaire again. The purpose of raising these questions is to evaluate the long-term effect of a mother's fasting during pregnancy and breastfeeding on the child's health. Another limitation of current study is that according to the questionnaire items, this questionnaire may only be used in Iran or countries with similar dietary habits not for examining dietary habits of Muslims residing in other regions with different dietary habits.

Implications

This Ramadan fasting questionnaire shows strong content validity and test-retest reliability. It is an appropriate instrument for evaluation of Ramadan fasting basic information and nutritional habits in research or practice-oriented settings for determination of short-term and long-term Ramadan fasting effects.

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Conflict of Interest

There is no conflict of interest to declare.

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