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Prevalence of Feeding Intolerance in PICU: A Cross-sectional Study

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ABSTRACT

Introduction: Malnutrition, as defined by the World Health Organization (WHO), occurs when the body does not receive sufficient nutrients or energy to meet growth, maintenance, and functional needs. Severe malnutrition in children increases the risk of death, disease episodes, complications, and prolonged illnesses. Therefore, early nutritional support is crucial in pediatric critical care settings. In cases where oral feeding is not feasible, enteral feeding (EN) becomes necessary to provide adequate energy. However, despite its advantages, feeding intolerance remains a significant challenge. This study aims to determine the prevalence of feeding intolerance among critically ill children in the Pediatric Intensive Care Unit (PICU).

Method: This cross-sectional study was conducted at the Akbar Pediatric Subspecialty Center's PICU in Mashhad, Iran between March and April 2022. The evaluation focused on nutritional adequacy and feeding intolerance.

Results: A total of 72 patients were included in this study with a majority being girls. Approximately 30 percent of patients exhibited severe malnutrition based on their BMI Z-score (<-3). Boys were more affected than girls in this regard. Most patients received a combination of EN and parenteral nutrition (PN) to fulfill their energy and protein requirements successfully. In most cases, children consumed over 66% of their energy needs through these methods. Feeding intolerance primarily manifested as vomiting and regurgitation (47%), followed by high gastric residual volume (GRV) (36.1%) and abdominal distention (34.7%).

Conclusion: The findings from our study highlight the prevalence of malnutrition within PICU settings along with common complications associated with feeding intolerance such as vomiting and regurgitation. Standardizing a definition for feeding intolerance could prove beneficial for improving research protocols aimed at effectively managing this condition.

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Introduction

Malnutrition is a condition characterized by insufficient nutrient and energy intake to meet the body's growth, maintenance, and functional requirements, as defined by the World Health Organization (WHO) (1). Severe malnutrition in children significantly increases the risk of mortality compared to well-nourished children. Additionally, severe disease episodes are more likely to occur among malnourished children, leading to increased complications and prolonged illness durations for each episode (2). Furthermore, malnutrition can also develop during hospitalization or after discharge, with pediatric intensive care units (PICUs) posing a higher risk setting for such cases (3).

Early nutritional support plays a vital role in pediatric critical care management and should be tailored to individual patient needs (4). Nutritional interventions should be initiated promptly following admission and emphasize early assessment of nutritional status. Preferential oral intake over parenteral feeding is recommended whenever possible, and a

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preference for enteral feeding over parenteral feeding (3). However, when patients are unconscious or unable to swallow or drink due to sedation or other factors, enteral feeding becomes necessary for adequate nutrition delivery (5).

Enteral nutrition offers several physiological advantages including maintenance of gut integrity through gut-associated lymphoid tissue stimulation that supports motility and prevents intestinal mucosal atrophy. It also helps reduce hyperglycemia levels and decreases hospital stay duration. Nonetheless, enteral nutrition may present complications as well (6). The most common complication associated with enteral feeding is enteral tube feeding intolerance (ETFI), which occurs due to impaired gastroduodenal motility and absorption resulting in inadequate enteral nutrient intake (7). Features indicative of ETFI include large gastric residual volumes (GRVs), abdominal distension or increased girth; vomiting; diarrhea; or subjective discomfort (2, 8). These conditions lead not only to insufficient nutrient intake but also result in extended stays in intensive care units and increased mortality rates (9).

Numerous studies conducted worldwide have explored EN intolerance specifically in PICUs. Some studies have found higher rates of ETFI among critically ill patients receiving EN, as indicated by GRV measurements, particularly within the PICUs of hospitals compared to general wards (6).

Although there are no agreed-upon definitions of EFI, it occurs frequently and can have adverse consequences (10). Nutrition by enteral route (EN), the preferred method of nutrient delivery, may be an integral part of the care provided in the PICU, with the potential to modify the response to critical illness or injury, thus enhancing survival (11). The international PICU community needs to agree on a consistent definition of feeding intolerance so that practice and research are consistent. Further education may help healthcare professionals to better understand the limitations of the markers used to define feeding intolerance (12).

Given the importance of addressing enteral feeding intolerance to prevent malnutrition within the PICU setting, this study aims to determine the prevalence of feeding intolerance among critically ill children in Iran.

Methods

Study design and participants

This cross-sectional study was conducted at the PICU of Akbar Pediatric Subspecialty Center in Mashhad, Iran. Data collection took place from March to April 2022. Patients admitted to the two general PICUs of Akbar Hospital were included using simple sampling based on our inclusion criteria. The study enrolled patients under 18 years old who received ETF and were hospitalized in the PICU for a minimum of 48 hours. Patients who had already been receiving enteral tube feeding prior to hospital admission, those on an oral diet with supplemental ETF, individuals transitioning from PN to an oral diet, or cases where medical records were unavailable were excluded from further analysis.

Patient characteristics, daily nutrition intake (EN or PN), and outcome data were collected during their stay in the ICU. Nutrition data encompassed information such as the type and quantity of prescribed and received nutrition (both calories and protein). These details were recorded daily for a maximum duration of 42 days or until death/discharge from the PICU. The determination of optimal nutrition prescription was not standardized but left to individual provider judgment, which was documented accordingly. Nutrition adequacy was calculated by comparing the percentage of prescribed proteins or calories with the actual amount received.

Data extracted for each patient included demographic characteristics, diagnosis, route of EN delivery, specific enteral formula used, time taken in days following initiation of feeding to achieve nutritional goals, occurrence of feeding intolerance episodes along with associated complications if any occurred during subsequent management.

For children aged 2 to 5 years old standing upright height measurements were recorded while length measurements lying down were taken for those under 2 years old unable to stand independently. Existing digital scales available on-site measured weight in kilograms (kg). Height and weight measurements followed centimeters (cm) units. CDC charts served as references for calculating z-scores.

The criteria defining enteral tube feeding intolerance (ETFI) encompassed the development of symptoms necessitating changes in the feeding protocol and/or specific

treatments, such as antiemetic or prokinetic medication administration. Symptoms could include one or a combination of the following: nausea, vomiting, diarrhea, abdominal pain/distension, and GRV. Nausea, vomiting, or abdominal pain were based on patient-reported symptoms while diarrhea was determined by assessing bowel charts for passing three or more loose bowel movements per day.

Statistical Analysis

Data analysis was performed in Statistical Package for the Social Sciences (SPSS) (Chicago, USA) (version 16) using descriptive statistics to define the baseline characteristics. The obtained results were expressed as mean and standard deviations or median and 25th to 75th interquartile in the case of quantitative data, and the qualitative data were expressed as frequency and percentage.

Result

We studied 72 critically ill patients admitted to PICU (39 males and 33 females) with a mean age of 31 months. According to Table 1, the profile of the patients included in the plan is displayed, which shows that among the seventy-two patients studied, the number of hospitalized male patients is more than female patients. However, their mean age, height, and weight at admission are lower than girls.

Abbreviations

BMI: Body Mass Index
SD: Standard Deviation
WAZ: Weight for Age Z-score
WHZ: Weight-for-length/Height Z-score

Table 1. Characteristics of the Patients admitted to PICU.

Baseline Characteristics			
	Sex		
	Male	**Female**	**Total**
N (percent)	39 (% 54.2)	33 (% 45.8)	72 (100%)
Age (months)	22.6	40.8	31
Mean Weight (range)	8.75kg (2.7-35)	13.5kg (2-88)	10.9kg
Mean Height (range)	74.2cm (5-147)	84.6cm (45-155)	79cm
Mean WAZ	-1.9	-2.18	-2
Mean WHZ	-2.19	-3.16	-2.6
Mean BMI (for Age More than 2) (range)	14.1 (10-17)	16.8 (9-36)	15.6
Mean BMI for age z score	-2.3	-2.1	-2.2

According to Table 2, patients were divided into three groups based on WHZ, WAZ, and BMIZ in terms of low, moderate, and severe malnutrition, and it was observed that most patients have severe malnutrition (Z score <-3).

Table 2. Malnutrition Characteristics

	Total		
	Mild **-1 to -2**	**Moderate** **-2 to -3**	**Severe** **< -3**
WAZ	19%	20%	27%
WHZ	12%	12%	29%
BMI z score (For Age greater than 2)	4%	13%	30%

In Table 3, the state of malnutrition was analyzed separately for girls and boys, and severe malnutrition was observed in boys more than girls.

Table 3. Malnutrition Characteristics

Sex	**Male**			**Female**		
	Mild **-1 to -2**	**Moderate** **-2 to -3**	**Severe** **< -3**	**Mild** **-1 to -2**	**Moderate** **-2 to -3**	**Severe** **< -3**
WAZ	9%	9%	15%	9%	11%	12%
WHZ	6%	4%	18%	5%	8%	11%
BMI z score (For Age greater than 2)	0%	4%	13%	4%	8%	17%

As shown in Table 4, most patients receive EN and PN together to achieve their energy and protein goals. A total of 15 patients receives parenteral nutrition while six patients receive only enteral nutrition.

Table 4. Route of nutritional intervention

Route	N	Percent
Enteral Nutrition (EN)	6	8.3 %
Parenteral nutrition (PN)	15	20.8 %
EN + PN	51	70.8 %
Total	72	100%

Table 5 provides information on receiving nutrition goals or nutritional adequacy. Approximately 86% of children receive more than two third of their energy goals. The ratio for protein adequacy was approximately 70%.

Table 5. Nutritional Characteristics of Energy and Protein

	N (%total)	Mean Percent	Interquartile Range (IQR)
Energy Tolerance (>66.6%)	62 (86.1%)	83.3%	22.75
Energy Intolerance	10 (13.9%)	16.6%	22.75
Protein Adequacy (>66.6%)	51 (70.8%)	73.7%	40
Protein Inadequacy	21 (29.2%)		

According to Table 6, there were several reasons for enteral feeding interruptions. The frequency of vomiting and regurgitation was higher among these patients (47.2%). Following these symptoms, high GRV (36.1%) and abdominal distention (34.7%) occur more often. Children receiving enteral nutrition were less likely to suffer from diarrhea (30.6%) or constipation (13.9%) compared to other symptoms.

Table 6. Prevalence for Each Identified Reason for EN feeding Interruptions.

Reasons for EN feeding interruptions	Case (total=75)	Percent	Episode
Vomiting or Regurgitation	34	47.2 %	63
Excessive GRV	26	36.1 %	53
Abdominal distention	25	34.7 %	46
Diarrhea	22	30.6 %	36
Constipation	10	13.9 %	12

Discussion

In this study, we observed a higher rate of severe malnutrition, particularly among boys compared to girls. Most patients in our study received a combination of enteral nutrition (EN) and parenteral nutrition (PN) to meet their energy and protein goals. Approximately 86% of children obtained more than two-thirds of their energy requirements, while the protein adequacy ratio was around 70%. Feeding intolerance, resulting in vomiting and regurgitation, was found at a frequency of 47.2% among these patients.

A previous study by Kunrong Yu et al. investigated food intolerance during the first seven days of hospitalization and reported the highest level on the second day (1). They defined ETFI as the presence of two or more combinations of symptoms such as GRV with abdominal distension/pain, nausea/vomiting, diarrhea, and subjective discomfort. In contrast, we defined ETFI based on the presence of any single symptom.

Yahyapoor et al., in their research on causes of EFI, found that approximately two-thirds (66%) of critically ill patients experienced EFI. This condition was associated with higher APACHE II scores, SOFA scores, mechanical ventilation duration, large GRV (77.9%), vomiting (33.8%), and abdominal distension in ICU-admitted patients. The most common symptom identified was vomiting (6).

Wang et al.'s study showed that the prevalence of nutritional intolerance among ICU-admitted patients (35.6%) was significantly higher compared to those admitted to general wards within the hospital population (27.4%)(9). Similarly, our study revealed that 16.6% suffered from energy intolerance.

Our study examined the effects of feeding intolerances, such as vomiting, gastrointestinal reflux disease, diarrhea, constipation, and abdominal distention. We found that vomiting accounted for the highest percentage of cases among other symptoms. A review article indicates that vomiting is not an appropriate

marker for evaluating gastrointestinal dysfunction because it is affected by several factors, such as nasogastric aspiration, enteral feeding, and the patient's status. According to some studies evaluating vomiting in critically ill patients, its prevalence ranged from 6-12% (2), while our study found that the prevalence reached 47.2%.

A systematic review of seventy-two studies highlighted that most of them defined feeding intolerance based on a large GRV(1). However, there are challenges in considering GRV as a sole factor for intolerance since each article had its own definition of high GRV. Regular measurement of GRV during enteral feeding is commonly used as an indicator for gastric emptying, feeding success, and aspiration risk according to feeding protocols (2).

While a gastric residual volume below 150 mL is typically considered safe for continued intragastric feeding (2), recent studies suggest that enteral nutrition can still be continued even at residual volumes up to 5 ml/kg or 250ml (13). However, there is conflicting evidence regarding the accuracy and significance of this measurement in assessing gastric emptying. Our study considered multiple factors related to intolerance which provides an advantage over other studies.

In recent research conducted by Liauchonak et al., they aimed to establish an evidence-based definition for enteral nutrition intolerance (14). Their prospective cross-sectional cohort study included patients who received EN during their ICU stay lasting more than 24 hours. The authors modified their nutrition algorithm by incorporating two symptoms of ENI (in contrast to our single symptom criterion) and compared the time required to achieve 60% EN adequacy and the number of interruptions before and after this intervention. In critically ill children, implementing the modified nutrition algorithm did not result in changes in either the time taken or total interruptions required for achieving adequate EN intake.

Solana et al.'s study examined the prevalence of enteral nutrition interruption among critically ill children admitted to PICU along with its associated risk factors. They found that procedures performed outside PICU were the most common cause contributing to ENIs. Caloric and protein intake decreased in the PICU, especially among children with higher Pediatric Risk of Mortality Scores (PRISM), longer PICU stays, and cardiopathy. Their study considered factors such as procedures performed outside the PICU which strengthens their findings. Similarly, our study was influenced by the lack of consistent definition for feeding intolerance and varying interpretations and thresholds for gastric residual volume (15).

Abdominal distension was observed in 13% of patients according to a review article. Studies examining outcomes related to feeding intolerance have shown its association with increased morbidity, mortality, and longer ICU stays. Factors such as large gavage volume received, high rate of gavage infusion, inappropriate gavage temperature or head angle, and medications causing intolerance were taken into account in this study.

Lee et al.'s investigation explored various potential causes of feeding intolerance including respiratory methods such as high positive end-expiratory pressure (PEEP). They found that these methods accounted for most cases of feeding intolerances along with their duration (16).

To reduce food intolerance among patients hospitalized in special care units following certain tips including optimizing gavage administration technique involving considerations like appropriate head angle/temperature/volume infusion rate can be beneficial when combined with training provided to nurses and doctors. However, research in this area has been limited due to the lack of consensus definitions regarding gastrointestinal dysfunction monitoring relying on indirect indicators rather than objective uniform definitions.

It is important to note that none of the GI symptoms alone can predict gastrointestinal function accurately; therefore, further studies are required to develop a simple reproducible scoring system combining clinical symptoms and measurable parameters for evaluating GI tract function.

Conclusion

In conclusion, our study revealed a significant prevalence of EFI among critically ill children admitted to intensive care units. Vomiting and regurgitation were identified as the most common symptoms associated with EFI. Following specific guidelines regarding gavage

administration techniques including considerations like appropriate volumes, infusion rates, temperature, head angle, and medications can help reduce the occurrence of food intolerance in patients hospitalized in special care units. However, further research is needed to establish consensus definitions for gastrointestinal dysfunction and develop effective strategies for managing feeding intolerance.

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A Comparison of the Effects Continuous and Interval Exercises on Fibrillin-1 and Asprosin in Obese Male Rats

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ABSTRACT

Introduction: Obesity is still a health problem for humanity. Although the favorable role of exercise on weight loss has been reported. But the effect of the type of exercise is still unclear. The present study compared the effects of continuous exercise (CE) and interval exercise (IE) on fibrillin-1 and asprosin in obese male rats.

Methods: Forty- eight male rats were divided into six groups including 1) obese IE, 2) obese CE, 3) healthy IE, 4) healthy CE, 5) obese control and 6) healthy control. Groups 1- 4 performed exercises for 8 weeks and 72 hours. Insulin resistance index, fasting glucose, insulin, fibrillin-1 and asprosin were measured after the last training session. Data analysis was performed by Two-way analysis of variance and Kruskal-Wallis tests with SPSS software (P≤0.05).

Results: There were significant differences in insulin resistance (P=0.001), fibrillin-1 gene expression (P=0.001), fasting glucose (P=0.001), asprosin serum levels (P=0.001), and insulin (P=0.002) levels between obese IE, obese CE, healthy IE, healthy CE, obese control and healthy control groups.

Conclusions: Although obesity increased fibrillin-1 and asprosin, but IE and CE decreased fibrillin-1 and asprosin. Thus, IE and CE can be used for controlling fibrillin-1 and asprosin levels. IE and CE can be considered as effective methods to reduce weight in obesity.

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Introduction

Many factors are involved in human obesity.In the same vein, more than 40 diseases are attributed to obesity and the most important of which are cardiovascular disease, type 2 diabetes, stroke, hypertension, hyperlipidemia, respiratory diseases, gastrointestinal complications, and liver disease (1,2).Many adipokines have been identified so far, which directly and indirectly affect the control of obesity and type 2 diabetes(3)Therefore, the investigation of the fat cells and the hormones secreted from them with a direct effect on increasing blood glucose is very important for obese people(4). Most physical education and medical sciences specialists agree on the method of controlling diets along with physical activity as the most basic and scientific method of weight loss, however, there is still no general agreement on the rate of the role of exercise and diet in weight loss (5). The present study used sports intervention to consider the positive changes in gene expression level and the secretion of hormones affecting obesity(5).Obesity causes changes in the levels of some hormones secreted by adipose tissue, which can severely activate the production of glucose in the liver in the long-term. The increased levels of glucose in the long term reduce the sensitivity of muscle cells to insulin and lead to insulin resistance (6). Insulin resistance and its complications are very effective in developing obesity(7).Insulin is an axial regulatory hormone, which is responsible for the energy balance and glucose homeostasis.Since insulin is no longer able to get glucose into the cells in obese people,blood glucose levels are constantly rising and both glucose and insulin in the blood are rising at the same time(8). The recently identified adipokine, called 'asprosin' hormone is synthesized through

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FBN1 gene expression and enters the blood circulation. Asprosin can increase glucose release in liver via move towards liver. Controlling the asprosin secretion among people with diabetes is very important, since the effect of asprosin on glucose release in liver is reduced by decreasing its synthesis and secretion (9,10). Exercise along with diet are considered as very important factors in controlling obesity and weight loss. Different exercises can improve glucose control via increasing glucose uptake in muscles (11). Some studies indicated that blood pressure is lowered after exercise, especially strenuous exercise and the metabolic stress caused by sport activities can improve the oxygen uptake following trainings and carbohydrates utilization during training. The type of training protocol is still under consideration (12). Considering a few studies conducted on the asprosin secretion and FBN-1 gene responsible for its secretion among healthy and obese people, the need for conducting study is direly felt in this regard.Further, given the positive effect of exercise on the improvement of the obesity, review the impact of sport activities on the asprosin is very critical. According to investigations, in the less researches reviewed the effects of continuous exercise (CE) and interval exercise (IE) on fibrillin-1 and asprosin. Thus present study performed to compare the effects of continuous exercise (CE) and interval exercise (IE) on fibrillin-1 and asprosin in obese male rats.

Materials and Methods

Animals Caring

This experimental study, 48 two-month Wistar male rats (mean weight of 180.23±7.59 g) were prepared from Mashhad University of Medical Sciences (MUMS) and transferred to Ferdowsi University of Mashhad. The situation of lab was under standard conditions (temperature: 20-24˚C, humidity: 40-50%, and 12 hours cycle of light/darkness). All ethical principles were according to Research Ethics Committee of Medical Kerman University with code IR.KMU.REC.1399.688.

Induction of Obesity

After transferring to the laboratory, the high-fat diet used for animals and kept until they reached the desired weight.In the present study, some rats became obese for two objectives(2).

Exercise Training Protocol

During two weeks, the rats were familiarized with IE and CE protocols for 10 sessions (in separated training protocols). At the beginning of training sessions all animals were placed on the treadmill in calmness situation with uniform and very low speed. In later sessions, when the rats were well on schedule, the treadmill speed was gradually increased equal to running protocol. Further, the training time was increased during two weeks in order that the rats could reach the actual training time in the main part of the training at the end of two weeks.After two weeks, without any problems with the protocols and familiarity of the rats, the main training started and ended for eight weeks(13). IE performed with intensity near to VO_{2max} with zero incline to improve oxidative capacity, oxygen absorption and aerobic power in skeletal muscles. For performing IE, the animals ran 2- 4 minutes (with 1- 2 minutes active recovery) for 5 sessions per week. In the first week, the training was started with 35% Vo2max and a speed of 14 m/min at a low intensity and 85% VO_{2max}. Similarly, the training period was increased by one meter per minute every two weeks and it reached 18 m/min and 38 m/min at the end of the eighth week(13).

Sampling

3 days after last IE and CE, all animals anesthetized by xylazine (90 mg/kg) and ketamine (90 mg / kg). Blood sampling gathered directly from heart and immersed in sodium citrate as well as centrifuged for 10 minutes. After tissue sampling, all tissues kept in liquid nitrogen and transferred to lab.

Measuring Variables

1. Asperosin test was performed using HANGZHOU EASTBIOPHAR.withCat.No : CK-E91570,ELISA kit. (Sensitivity : 0.23ng/ml)

2. First, RNA was extracted using the instructions of Trizol Kit (Bioneer made in South Korea) in white Adipose tissue and then cDNA was synthesized using Takara Jaben Kit. Also, the primers of studied genes for reverse transcription are presented in Table 1. In order to quantify the ratio of the desired gene to the reference gene, the formula 2-ΔΔCT was used.

3. Enzymatic colorimetric method was used for measurement of glucose concentration by Pars Azmoun company kit (Tehran, Iran) with measurement sensitivity of 5 mg/dL.

Table 1. Sequence of primers used in the research

Genes	Primer Sequences	Sizes (bp)
β Actine	*Forward: 5'TGGCCCACATATTCCTTGGT 3'* *Reverse: 5'-GTAGCTGCGGACATTTCAGG -3'*	147
FBN1	*Forward: 5'- AGCCTTCCTTCCTGGGCATGG -3'* *Reverse: 5'- AGCACTGTGTTGGCGTACAGGTC-3'*	183

Table 2. The test results of two-way analysis of variance

Source of changes	Sum of squares	DF	Mean square	F value	Significance level
Obesity	2854.400	1	1096.341	94.700	P=0.001
Exercise	1711.876	2	855.938	73.935	P=0.001
The interaction of obesity and age	46.183	2	23.091	1.995	P=0.149
Error factor	486.233	42	11.577		
Total	58299.500	48			

*Significance level: P <0.05

4. ELISA method was used for measurement of serum insulin by Demeditec Diagnostic insulin ELIZA kit (Germany).

5. HOMA- IR formula was used for measurement of insulin resistance according to below formula:

$$\text{HOMA} - \text{R} = \frac{\text{Fasting Insulin } \left(\mu\frac{\text{U}}{\text{ml}}\right) x \; Fasting\; Glucose \left(\frac{mmol}{l}\right)}{22.5} (14).$$

Data Analysis Procedure

Two-way analysis of variance (for asprosin) and Kruskal- Wallis (insulin resistance, FBN1 gene expression, fasting glucose and insulin, levels) tests were used for statistical analysis of data by SPSS software (P≤0.05).

Table 3. The mean asprosin among obese and non-obese rats

Group	Mean	Standard error	95% confidence interval	
			Lower bound	Upper bound
Obese	38.61	0.695	37.21	40.01
Non-obese	29.05	0.695	27.65	30.46

Table 4. The paired comparison of asprosin hormone level in untrained, high intensity interval training and continuous aerobic training groups by post hoc test

Exercise i	Exercise j	Meandifference (i-j)	Standard error	Significance level	Lower bound	Upper bound
Untrained	**-**	9.28	1.20	P<0.001	6.28	12.28
CE	**Untrained**	-9.28	1.20	P<0.001	-12.28	-6.28
	IE	5.14	1.20	P<0.001	2.14	8.14
IE	**Untrained**	-14.43	1.20	P<0.001	-17.43	-11.43
	CE	-5.14	1.20	P<0.001	-8.14	-2.14

Results

As shown in Table 3, the mean asprosin was lower in non-obese rats compare to obese rats. Further, obesity increases asprosin in male rats.

Based on the Table 4, asprosin hormone levels were not the same in CE and untrained rats (P=0.001). Further Considering the difference between the mean asprosin of CE and untrained rats; CE significantly reduced the level of asprosin hormone in rats as well as IE significantly reduced the level of asprosin hormone in rats. Also the results showed that IE reduced asprosin levels more than CE. Mean weights of rats in pre and posttest are presented in Figure 1.

The results of the Kruskal- Wallis test showed that there are significant differences in FBN1 levels between groups. The results of Mann–Whitney U-test showed that obesity control increased FBN1 (P=0.001) nevertheless CE control, IE control, CE Obese and IE Obese significantly reduced FBN1 (P=0.001) in obese rats. Indeed exercise did not significantly changed FBN1 in healthy rats. IE reduced asprosin secretion in obese rats compared to CE. This has been shown in Table 5.

The results of Kruskal- Wallis test showed that in obese control rats, the insulin levels were significantly higher than non-obese rats (P=0.002) as well as CE and IE (CE control, IE control, CE Obese and IE Obese) significantly increased insulin in healthy rats and decreased in obese rats. IE reduced insulin in obese rats compared to CE.

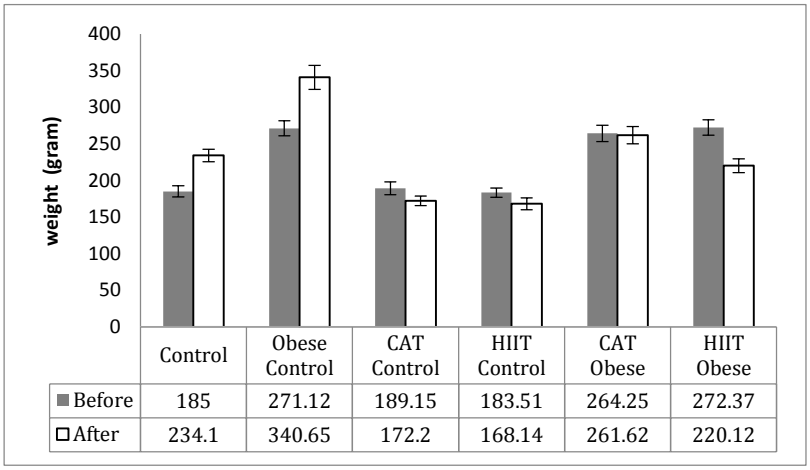

Figure1. The mean weight of the studied rats

Table 5. A comparison of the mean and standard deviation of FBN1 gene expression, insulin, fasting glucose levels, and insulin resistance index in the study groups by Kruskal- Wallis test

Variable	Study Groups						Kruskal-Wallis test results
	Control	Obeseity control	CE	Obesity and CE	IE	Obesity and IE	
FBN1	Mean±SD 0.00±1.00	Mean±SD 0.50±3.19	Mean±SD 0.26±1.16	Mean±SD 0.41±2.24	Mean±SD 0.97±0.97	Mean±SD 0.41±1.73	X2=38.01، df=5، P=0.001
Insulin	0.27±1.96	0.77±3.14	0.19±2.03	0.92±2.27	0.20±2.10	1.92±2.29	X2=19.55، df=5، P=0.002
Fasting Glucose Level	10.05±198.62	28.22±313.5	10.14±179.37	38.12±282.75	9.77±179.62	43.34±267.12	X2=42.27، df=5، P=0.001
Insulin Resistance Index	2.55±17.32	9.63±43.87	1.80±16.24	6.28±28.53	2.05±15.95	6.10±27.05	X2=36، df=5، P=0.001

In healthy rats, the fasting glucose levels were lower than obese rats (P =0.001) as well as IE and CE (CE control, IE control, CE Obese and IE Obese) significantly decreased fasting glucose in obese and healthy rats. IE reduced glucose in obese and healthy rats compared to CE.

The results of Kruskal-Wallis test showed that insulin resistance levels in healthy rats were significantly lower than obese rats (P =0.001) as well as IE and CE (CE control, IE control, CE Obese and IE Obese) significantly decreased insulin resistance in obese and healthy rats. IE insulin resistance in obese and healthy rats compared to CE.

Discussion

In present study insulin resistance, fasting glucose, insulin, FBN1 and asprosin levels were different in all research groups. Progress in metabolic diseases can be due to imbalance in factors secreted from adipose tissues. According to results of present study in obese training, healthy training, obese control and healthy control groups, the insulin resistance, fasting glucose, insulin, FBN1 and asprosin levels were significantly different. In recent years, the central elements regulating energy homeostasis, such as food intake behavior and energy expenditure, have been considered to better understand the pathophysiological mechanisms of obesity, as the main cause of metabolic disorders (15).The studies conducted on the mechanisms associated with the obesity and the occurrence of cardiovascular disease identified increased fat mass as the most important factor(16). In recent studies, adipose tissue is known as an active

endocrine and paracrine organ by synthesizing and secreting a set of adipocytokines and bioactive mediators, which controls body weight balance and explains the association of overweight and obesity with insulin resistance, diabetes, and cardiovascular disease by influencing the lipid, metabolic, and inflammatory profile (14). The recently discovered hormone, called asprosin, is secreted from fat cells during fasting. Via cAMP signaling pathway asprosin can affect liver to release glucose into the bloodstream (17). As a result, the hormone insulin is secreted.It is worth noting that asprosin is secreted during starvation and in response to an excessive decrease in blood glucose, aiming to regulate glucose homeostasis(17).Therefore, the this research initially reviewed whether obesity in rats could cause changes in asprosin levels. Based on the results, obesity in rats significantly increased asprosin secretion levels. Asprosin levels in healthy rats were significantly lower than obese rats. Thus reducing the asprosin for obese people, is very important to prevent the effect of this hormone on the release of liver glucose into the bloodstream. Given that increased plasma glucose levels, followed by increased insulin secretion, has a negative effect on the secretion of hormones influencing lipolysis, a strategy for weight loss in obese people is to lower their glucose levels during fasting. Also fasting glucose and insulin levels in healthy rats were lower obese rats, so that it may be related to lower levels of asprosin in healthy rats compared to obese rats (14). It has been reported that increase in insulin secretion and blood glucose levels in obese people can be due to the secretion of more than normal level of asprosin. Indeed high levels of asprosin hormone in obese people can affect liver to increase the release of glucose into the bloodstream. Due to dysfunction in GLUT4 which is a special glucose transporter, glucose cannot enter into the muscle cells. Therefore for decrease the secretion and synthesis of asprosin, adjustments in nutrition and exercise interventions can be important.

Results showed that CE significantly decreased blood glucose and asprosin levels in obese and healthy rats. Accordingly, obese people can prevent the increased asprosin via performing CE and managing their glucose levels in bloodstream. FBN1 gene (in humans) can encode the Fibrillin-1 glycoprotein. Researchers recently pointed that Fibrillin-1 can plays a important role in adipogenesis. The researchers reported that in inbred mouse species the FBN1 levels are associated with the changes in adipocyte levels. Further, a decrease or increase in fibrillin-1 levels in fat cells, caused by gene mutations or lifestyle, is associated with the changes in fat cell levels and FBN1 gene expression in obese mice more than normal-weight or healthy mice with adipocyte size. The relationship between asprosin and obesity largely depends on its upstream gene, namely, FBN1(18).

In present study obesity in rats raised FBN1 levels. Thus, the synthesis and secretion of asprosin increased in response to the enhancement of gene expression. Finally the main aim of controlling glucose and asprosin levels is to reduction of FBN1. In present research IE and CE significantly decreased FBN1 levels as well as FBN1, increase in obese rats. In order to explain the impact of obesity and trainings on fasting glucose homeostasis, it should be noted that obesity is a disease-causing change in fat cells.Therefore, there may be changes in the level of liver cells, leading to the release of more glucose into the bloodstream (19). The obese people may experience increased insulin secretion level and insulin resistance during fasting if their blood glucose levels rise. Based on the results, the insulin resistance, insulin, glucose, asprosin and FBN1 levels in healthy rats were significantly lower than obese rats. In addition, exercise reduced asprosin levels and FBN1 gene expression in adipocytes. Therefore, long-term exercise helps the glucose homeostasis in obese people by reducing the activity of glycogenolysis and hepatic gluconeogenesis pathways. Decreased plasma glucose means the decreased insulin secretion and reduced insulin resistance.Additionally, exercise increase glucose uptake by muscle cells in obese individuals by increasing AMPK and GLUT4 activity(20).Therefore, exercise through changes in the levels of fat and muscle cells leads to a reduction in insulin resistance and glucose in the long term. Results showed that IE lowered insulin resistance, glucose, asprosin secretion, and FBN1 gene expression compared to CE,which can be attributed to the impact of exercise on GLUT4 activity. The long-term aerobic exercise contributes to the activation of GLUT4s on the surface of muscle cells by increasing intracellular calcium levels and calmodulin

calcium(21).However, HIIT increases AMPK levels sharply, due to the high conversion rate of ATP to AMP.AMPK has a greater effect on the activation of GLUT4s on the surface of muscle cells(22).Therefore, the greater effect of HIIT protocol compared to CAT on lowering blood glucose levels and insulin resistance for more activation of AMPK is due to the high intensity of exercise in HIIT. As FBN1 and asprosin recently discovered, less studies found regarding to effect of IE and CE. Although in present study IE had high positive effects on FBN1 and asprosin in obese rats compared to CE.

Conclusion

Although obesity increased fibrillin-1 and asprosin, but IE and CE decreased fibrillin-1 and asprosin. Thus IE and CE can be used for controlling fibrillin-1 and asprosin levels as well as an intervention method contributing to the reduction of weight and obesity.

Statements

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Conflict of Interest

There is no conflict of interest.

Animal Rights

All ethical principles were according to Research Ethics Committee of Medical Kerman University.

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Risk Stratification for Fasting in Diabetic Patients Based on the IDF-DAR Guideline

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ABSTRACT

Introduction: Fasting during Ramadan is a religious obligation and may pose a risk to diabetic patients. This study aimed to stratify diabetic patients based on the International Diabetes Federation- Diabetes and Ramadan International Alliance (IDF- DAR) guideline for the risk of fasting before Ramadan.

Methods: A total of 317 diabetic patients attending the endocrinology clinic at Ghaem Hospital, Mashhad, Iran, participated in this cross-sectional study. The American Diabetic Association (ADA) criteria were applied to diagnose diabetes. Various parameters, including diabetes type and duration, complications, and risk factors were recorded to determine IDF-DAR-based risk. Then patients were stratified into high (IDF-DAR>6), medium (IDF-DAR: 3.5-6) and low (IDF-DAR: 0-3) risk groups accordingly.

Results: Out of the 317 patients included in the study, 115 (36.3%) patients were categorized as low risk, 127 (40%) as moderate-risk, and 75 (23.7%) as high-risk groups.

Conclusions: According to the IDF-DAR guideline, majority of the patients fell into the low and moderate risk categories, suggesting that they should not be entirely exempted from fasting during the holy month of Ramadan. However, the validity of this patient stratification in various fasting populations needs to be evaluated through prospective longitudinal studies.

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Introduction

Diabetes mellitus (DM) is a prevalent metabolic disorder with a heavy socioeconomic burden on public health. The prevalence of DM has increased throughout the recent decades in most developed and developing countries [1-5]. According to the International Diabetes Federation (IDF), the number of people affected by diabetes, without implementing effective prevention measures, is estimated to rise to 783 million by 2045 [4]. Diabetes ranks among the ten leading causes of death and its prevalence has increased by over 70% since the year 2000 [6].

The fasting practice during the month of Ramadan is considered as one of the five pillars of Islam. The obligatory period of fasting typically lasts between 29-30 days and is applicable to all healthy Muslim individuals who have attained puberty. Adherents to this practice are expected to abstain from all food and drink from the time of dawn to sunset, while also refraining from additional activities such as taking oral medications, sexual activity, or smoking. Pregnant women, geriatrics, children, and disabled individuals may be exempted from fasting, otherwise, fasting in certain vulnerable people such as those with uncontrolled DM and those experiencing co-morbidities may increase the likelihood of complications [7]. Fasting complications in diabetic patients may include hyper- and hypoglycemia, diabetic ketoacidosis, hypovolemia, and thrombosis [8].

On the other hand, it has also been proposed that fasting in type 2 DM patients may reverse insulin resistance and improve the glycemic control status [9]. The eligibility of diabetic patients for Ramadan fasting practice used to be defined based on expert opinion rather than medical evidence. However, the introduction of the first edition of the International Diabetes Federation-Diabetes and Ramadan International Alliance (IDF-DAR) guidelines 2016, a practical tool to help healthcare professionals (HCPs) to safely and scientifically guide people with diabetes who tend to fast during the holy month of Ramadan [7]. Considering the increasing population of

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people with diabetes in the Middle East and North Africa (MENA) as well as Southeast Asia, necessitates employing the evidence-based guidelines (EBM) in supporting patients with DM who wish to fast during Ramadan [4].Hence, researches pertaining to diabetes and Ramadan have increased greatly in the last five years [10]. According to the IDF-DAR guideline [11], patients with DM are classified into three groups based on their risk factors: low, medium, and high-risk for possibility of fasting. Given that fasting may lead to unfavorable complications in diabetic patients, the present study has aimed to stratify diabetic patients based on IDF-DAR guideline for the risk of fasting before the Ramadan.

Methods

This cross-sectional study was conducted on all eligible diabetic patients (n=317) who were referred to the endocrinology clinic at Ghaem Hospital, Mashhad, Iran, from 2022 to 2023. The study protocol was approved by the Ethics Committee of Mashhad University of Medical Sciences (# IR.MUMS.MEDICAL.REC.1401.619). The American Diabetic Association (ADA) criteria were applied to confirm the diagnosis of diabetes. A comprehensive collection of demographic and clinical information including detailed medical history, comorbidities, and diabetic risk factors were recorded in a relevant checklist.

Table 1. Risk score for diabetic patients that tend to fast during Ramadan. (Adopted from Diabetes and Ramadan: Practical guidelines 2021. Diabetes Res Clin Pract. 2022 Mar; 185:109185. doi: 10.1016/j.diabres.2021.109185.)

Risk Element	Risk Score	Risk Element	Risk Score
1. Diabetes type and duration		**8. MVD Complications/Comorbidities**	
Type 1 diabetes 1	1	Unstable MVD	6.5
Type 2 diabetes	0	Stable MVD	2
		No MVD	0
2. Duration of Diabetes		**9. Renal Complications/Comorbidities**	
A duration of ≥ 10	1	eGFR < 30 mL/min	6.5
A duration of < 10	0	eGFR 30–45 mL/min	4
		eGFR 45–60 mL/min	2
		eGFR >60 mL/min	0
3. Presence of hypoglycaemia		**10. Pregnancy**	
Hypoglycaemia unawareness	6.5	Pregnant not within targets	6.5
Recent Severe hypoglycaemia	5.5	Pregnant within targets	3.5
Multiple weekly Hypoglycaemia	3.5	Not pregnant	0
Hypoglycaemia<1 time per week	1		
No hypoglycaemia	0		
4. Level of glycaemic control		**11. Frailty and Cognitive function**	
HbA1c levels > 9%	2	Impaired cognitive function or Frail	6.5
HbA1c levels 7.5–9%	1	> 70 years old with no home support	3.5
HbA1c levels < 7.5%	0	No frailty or loss in cognitive function	0
5. Type of treatment			
Multiple daily mixed insulin Injections	3		
Basal Bolus/Insulin pump	2.5	**12. Physical Labour**	
Once daily Mixed insulin	2	Highly Intense physical labour	4
Basal Insulin	1.5	Moderate Intense Physical Labour	2
Glibenclamide	1	No physical labour	0
Gliclazide/MR or Glimepride or Repeglanide	0.5		
Other therapy not including SU or Insulin	0		
6. Self-Monitoring of Blood Glucose (SMBG)		**13. Previous Ramadan Experience**	
Indicated but not conducted	2	Overall negative experience	1
Indicated but conducted sub-optimally	1	No negative or positive experience	0
Conducted as indicate	0		
7. Acute complications		**14. Fasting hours (location)**	
DKA/ HHS in the last 3 months	3	≥ 16 h	1
DKA/ HHS in the last 6 months	2	< 16 h	0
DKA/ HHS in the last 12 months	1		
No DKA or HHS	0		

DKA—Diabetic Ketoacidosis. HHS— Hyperosmolar Hyperglycaemic Syndrome/. Hyperglycaemic Hyperosmolar Nonketotic Coma (HONC). MVD—Macrovascular disease (Cardiac-cerebral or peripheral). EGFR—estimated glomerular filtration rate.

The IDF- DAR guideline-based factors such as the type and duration of diabetes, the presence of hypoglycemia, the characteristics of hyperglycemia, self-monitoring of blood glucose (SMBG), acute and chronic complications, pregnancy, frailty and cognition function, physical labor, previous Ramadan experience, and type of diabetic treatment were recorded (Table1) [11].

Then the patients were stratified based on the calculated risk score. Risk scores of 0-3, 3.5-6, and >6 were defined as low, moderate, and high risk, respectively. Patients with missing information for risk stratification based on IDF-DAR guideline were excluded.

Normal distribution of data was checked using the Kolmogorov–Smirnov test. Descriptive statistical analysis including mean±Standard Deviation (SD) and frequency (Percent) as well as inferential tests including independent t-test/Mann-Whitney, Chi-square, and/or Fisher's exact test were used. All statistical analyses were conducted using SPSS software version 22.0 (Chicago, IL, USA) and a $P< 0.05$ was considered statistically significant.

Table 2. The baseline characteristic of participant in total and stratified based on the IDF-DAR criteria are presented in Table 1. Based on the IDF-DAR risk stratification criteria

Variable		Total n=317	IDF-DAR risk stratification			P-value
			Low n=115 (36.3)	Moderate n=127 (40.0)	High n=75 (23.7)	
Age		58.58±11.76	55.77±10.34	59.59±11.89	60.86±12.79	0.006
Gender	Male n (%)	108 (34.8)	30 (26.3)	55 (43.3)	23 (30.7)	0.017
	Female n (%)	209 (65.2)	85 (73.9)	72 (56.7)	52 (69.3)	
Diabetic type	I	22 (7.14)	1 (0.9)	4 (3.1)	17 (22.7)	<0.001
	II	295 (92.6)	114 (99.0)	123 (96.9)	58 (77.3)	
Diabetic duration (years)		10.54±8.14	6.93±5.59	10.62±7.25	15.56±9.46	<0.001
Diabetic duration	<10 y	152 (47.8)	80 (69.6)	50(39.4)	22(29.3)	<0.001
	>10 y	165 (52.2)	35 (30.4)	77(60.6)	53 (70.7)	
Hypoglycemia		84 (27.1)	9 (7.9)	41 (32.3)	34 (45.3)	<0.001
SMBG		230 (72.6)	106 (92.2)	83 (65.4)	41 (54.7)	<0.001
History of DKA/HHS		12 (3.7)	4 (3.5)	4 (3.1)	4 (5.3)	0.664
Cardiovascular disease		60 (19.3)	14 (12.3)	25 (20.0)	21 (28.0)	0.025
Chronic complication (CVD and GFR)	No	288 (90.7)	114 (99.1)	118(92.9)	56(74.7)	<0.001
	Stable CVD/eGFR 45-60	24 (7.8)	1(0.9)	9(7.1)	14(18.7)	
	eGFR 30-45	1 (0.3)	0 (0)	0 (0)	1(1.3)	
	UA/HF/eGFR≤30	4 (1.2)	0 (0)	0 (0)	4 (5.3)	
Frailty and Cognitive Function		53 (16.9)	4 (3.5)	24 (18.9)	25 (33.3)	<0.001
Pregnancy		2 (0.9)	0 (0)	2 (1.6)	0 (0)	0.346
Physical labor (intensive)		4 (1.2)	1 (0.9)	2 (1.6)	1 (1.3)	0.999
Previous Ramadan Experience (negative)		67 (20.9)	19 (16.5)	30 (23.6)	18 (24)	0.315
Hypertension		130 (41)	46 (40.4)	50 (39.7)	34 (45.3)	0.711
Diabetic drugs	insulin secretory	127(39.7)	44 (38.2)	71 (55.9)	12 (16)	<0.001
	Oral agents	68 (25.2)	0 (0)	22 (17.3)	56 (74.7)	
	Insulin	112(35.1)	71(61.7)	34(26.8)	7(9.3)	
	others					
FBS		160.57±56.44	142.07±44.75	164.54±52.74	185.38±69.84	<0.001
Hba1c		8.07±1.65	7.05±1.22	8.17±1.61	9.03±1.47	<0.001
CR		0.98 ±0.36	0.93±0.24	1.02±0.44	0.96±0.27	0.298
LDL		84.23±32.87	86.59±30.97	81.00±31.31	83.65±35.78	0.452
HDL		45.19±12.56	44.70±10.98	45.00±13.09	46.38±13.97	0.687
TG		149.35±68.26	148.78±53.67	147.17±67.64	151.77±87.54	0.916
AST		21.94±10.23	22.53±11.32	22.06±10.44	20.63±8.07	0.654
ALT		25.23±16.39	27.55±19.09	26.19±17.39	20.71±8.19	0.107
Microalbuminuria		33.47±48.89	27.18±37.95	34.97±52.51	34.00±50.44	0.807

Results

The baseline characteristics of participants are presented in Table 2. The patients (n=317) were assigned into three categories according to their risk level: low-risk (n=115), moderate-risk (n=127), and high-risk (n=75) groups.

The mean age was found to differ significantly amongst the risk groups with the mean age being highest in the high-risk group (60.86±12.79

years) and lowest in the low-risk group (55.77±10.34 years) (P=0.006). There were significant differences in gender distribution amongst the risk groups, with the highest proportion of males being in the moderate-risk group (43.3%) (P=0.017).

Our results revealed that patients with type I diabetes were significantly belonged to the high-risk group (22.7%) when compared to both the low-risk (0.9%) and moderate-risk (3.1%) groups, with a significant statistical difference (P<0.001).

The highest proportion of participants who were on insulin alone were found in the high-risk group (74.7%), whereas the moderate-risk group had the highest proportion of participants taking insulin secretory oral agents (55.9%). On the other hand, the low-risk group had the highest representation of individuals taking other diabetic medications (61.7%) (P<0.001). Additionally, participants in the high-risk group exhibited the highest mean levels for HbA1c, FBS, and cholesterol levels, while those in the low-risk group had the lowest levels (P<0.05).

Discussion

The important challenge with diabetic patients and fasting practice in Ramadan is to define which patient is eligible for fasting and who can be exempted. The present study has aimed to stratify diabetic patients based on IDF-DAR guideline for the risk of fasting before the Ramadan.

Among a total of 317 diabetic patients, 115 were found to be in low-risk, 127 in moderate-risk, and 75 in high-risk groups.

According to a large multicenter study conducted in 13 Islamic countries, it was found that 42.8% of patients with type-1 and 78.7% of patients with type-2 diabetes practiced fasting during Ramadan. However, it was noted that some of these patients did not consult their physicians before starting their fasting practice, nor did they adjust their drug dosages and timing or monitor their blood glucose levels even when they developed acute complications. Also, a higher rate of acute complications was reported in fasting patients with diabetes and several deficiencies in the care and awareness of such patients were identified [12].

From this point of view, our study was consistent with the baseline point of the prospective study conducted by Nagi Mohammed et al. on validating the accuracy of the IDF-DAR risk stratification tool in predicting adverse outcomes in patients with diabetes [13].In line with our results, the majority of patients were stratified in low (339 (51.4%)) and moderate (173 (26.3%)) risk groups. As expected, hypoglycemia was reported in the low, moderate, and high risk groups by 6.3%, 21.9% and 35.0%, respectively. Also, severe hypoglycemia was observed in 3(2.1%), 3(1.8%), and none (0%) patients in the high, moderate, and low risk groups, respectively. Based on the results, they concluded that new IDF-DAR risk assessment can be a reliable tool to predict both the ability to fast during Ramadan and the likelihood of hypoglycemia or hyperglycemia.

In light of these information, a risk assessment conducted in this study revealed that 127 (40%) patients were at moderate risk and require extra education and percussion for fasting. The remaining 115 (36.3%) low risk patients can proceed with caution during fasting. Recent studies have shown that a large proportion of patients with diabetes (50-80%) lack proper knowledge and skills to effectively manage their condition [14-15]. Some studies have found that individualized Ramadan education can significantly reduce diabetes complications [16-17]. A study by A.B.M. Kamrul-Hasan showed that the risks of hypoglycemia and hyperglycemia were significantly higher in the high-risk group compared to the low-risk group [18].

Current consensus suggest that diabetic patients who plan to fast should discuss their risk score for fasting based on their comorbidities, glucose status, and risk of adverse events [9, 19, 20].In our study, 27.1% of diabetic patients had a history of hypoglycemia, which is a significant barrier to fasting. Fortunately, 72.6% of cases recorded self-monitoring blood glucose levels before Ramadan, which can help ensure safe fasting. Additionally, 19.3% of our patients had established cardiovascular diseases and were advised against fasting to prevent adverse events.

Ideally, individuals should be educated to plan for fasting during Ramadan at least 4–6 weeks in advance to allow for nutritional and medication adjustments, especially for medications that may induce hypoglycemia (such as insulin or sulfonylurea) [21].Patients taking medications that increase the risk of hypoglycemia should be encouraged to regularly monitor their blood

glucose levels during fasting and advised to break their fast once hyperglycemia (blood sugar >300 mg/dl) or hypoglycemia (blood sugar <70 mg/dl) happens [11].

Diabetic patients are recommended to consult healthcare professionals to evaluate their blood glucose control and other metabolic parameters through laboratory tests before fasting.

Lack of patients' follow-up after fasting is the main limitation in the current study that should have been taken into account when interpreting the results.

Conclusions

Majority of the patients in the present study fell into the low and moderate risk categories, suggesting that they should not be necessarily exempted from fasting during the holy month of Ramadan. However, it is crucial to identify the patients who are at a higher risk of fasting associated complications. However, the validity of this patient stratification in various fasting populations needs to be evaluated through prospective longitudinal studies.

Statements

[illegible]

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The authors declare that they have no competing interests.

Author's Contributions

ZMK, designed the study. ZMK, MAY contributed in data gathering and ZMK, HMM, MAY interpreted the results. ZMK, HMM, MAY, wrote and edited the manuscript. All authors read, commented and approved the final manuscript.

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Association of Covid-19 Infection with Physical Activity and Food Intake; Mashhad PERSIAN Cohort Results

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ABSTRACT

Introduction: The emergence of the COVID-19 pandemic in December 2019 had a profound impact on global public health. This study investigates the effects of the coronavirus on dietary intake and physical activity among the Mashhad PERSIAN cohort population in Mashhad, Iran.

Method: This nested cohort study was done among the PERSIAN Cohort Study in Mashhad University of Medical Sciences population who confirmed COVID-19 infection through PCR testing. Participants were assessed for physical activity using the International Physical Activity Questionnaire (IPAQ) short form, as well as changes in appetite and food intake during the COVID-19 infection. All measurements were compared during the disease period to pre-infection.

Results: This study comprised 381 confirmed COVID-19 patients (average age of 42.51 ± 7.31 years) of which 154 (40.4%) were male. There was a significant reduction in the levels of vigorous and moderate physical activity, walking, and total sitting time (P<0.001 for all). Also, food intake was reduced during the COVID-19 infection compared to before the infection.

Conclusion: In summary, this study demonstrates a significant decrease in physical activity and food intake during COVID-19 infection.

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Introduction

In December 2019, an unknown-cause pneumonia outbreak occurred in Wuhan, Hubei Province, China, which alarmed the medical and scientific community. The causal culprit was ultimately identified as SARS-CoV-2, a new beta coronavirus that targets the lower respiratory system and causes bilateral pneumonia in humans (1). The World Health Organization (WHO) has given the name this pathology COVID-19, and it has infected and injured thousands of human beings worldwide. Many countries, including Spain, Italy, Brazil, Chile, and Colombia, have taken extraordinary measures. Quarantine, or the entire confinement of the populace in their houses, has been one of these containment strategies. This, however, has caused interruptions in most daily activities, including lifestyle aspects like nutrition and physical activity (2).

A healthy diet, according to the World Health Organization and the Spanish Academy of Nutrition and Dietetics (2020), can aid in disease prevention and treatment (3). The population's diet quality and their health have a strong relationship (4). It's worth mentioning that obtaining fresh food during quarantine periods may be challenging, and there may be shortages of certain food products. The Food and Agriculture Organization (FAO) acknowledges that the COVID-19 epidemic has disrupted global food supply systems, impacting both supply and

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demand. Furthermore, COVID-19 has increased social inequalities, with the poorest households bearing the brunt of the burden (2).

While quarantine and physical isolation have positive effects on preventing and reducing the transmission of the virus and protecting individuals' physical health, these measures can have long-lasting and widespread negative psychological impacts (7-5). Several studies have demonstrated various negative psychological effects of social isolation, including higher levels of anxiety, stress, fear, and even symptoms of depression that can persist beyond the isolation period (8-5). Positive social connections are important human requirements, and their deprivation may result in social cravings akin to food desires (9). As a result, physical isolation and quarantine may interfere with vital psychological requirements. They can make it challenging for people to try out and build interpersonal connections and relationships, as well as to self-regulate their activities (10).

Adequate physical activity leads to a reduction in the incidence of hospitalization related to COVID-19. Interestingly, engaging in at least 150 minutes per week of moderate-intensity aerobic activity, 75 minutes per week of vigorous-intensity aerobic activity, or an equivalent combination of moderate and vigorous activity, is associated with a 34.3% lower prevalence of COVID-19 and hospitalization due to COVID-19 (11).

This study aimed to investigate the relationship between COVID-19 infection with physical activity and dietary intake.

Materials and Methods

This study had a nested cohort design that was done among the Prospective Epidemiological Research Studies in Iran (PERSIAN) Cohort Study in Mashhad University of Medical Sciences) study population. The PERSIAN cohort study of Mashhad City is currently being implemented among Mashhad University of Medical Sciences employees. This population-based prospective organizational cohort study was conducted among participants aged 30 to 76 in Mashhad, Iran, as part of the "PERSIAN Cohort" research project (12). All Mashhad PERSIAN cohort population who confirmed COVID-19 infection through PCR testing was enrolled before the vaccination. Between 2 and 8 weeks after recovery, people were referred to the Mashhad PERSIAN cohort center and were evaluated for physical activity, anorexia, and reduced food intake during disease duration and compared with the collected data from before the disease.

The level of physical activity was assessed using the International Physical Activity Questionnaire (IPAQ) short form for one week (13). This questionnaire's reliability and validity in PERSIAN have been confirmed (14, 15). The frequency and duration of their exercise, their level of activity (vigorous, moderate, or strolling), and the amount of time they had spent sitting during the preceding seven days were also evaluated. Appetite levels and changes in food intake (evaluated through a 4-item dietary recall assessment (16)) were assessed during COVID-19 infection and compared to before the onset of COVID-19.

Statistical Analysis

All statistical analysis was done, using SPSS statistical software version 23. The normal distribution of the variables was evaluated by the Kolmogorov-Smirnov test. Qualitative and quantitative variables were described as frequency by percentage and mean ±standard deviation respectively. Quantitative variables were compared before and during corona infection by student paired t-test. The comparison of the qualitative variables in the two mentioned periods was made by the McNemar test. The significance level was considered less than 0.05.

The Mashhad University of Medical Sciences ethics committee approved the protocol of the study (IR.MUMS.MEDICAL.REC. 1400.631). Informed consent was completed by all participants before the study.

Results

The baseline characteristics of the study population are summarized in Table 1.

A total of 381 confirmed COVID-19 infected patients (mean age of 42.51±7.31 years) enrolled in to study. Among the participants, 154 individuals (40.4%) were male.

As indicated in Table 2, there was a significant reduction in the levels of vigorous and moderate physical activity, walking, and total sitting time compared to before the onset of COVID-19 ($P<0.001$ for all).

Table 3 shows the amount of oral food intake compared to before the onset of COVID-19. Most of our study participants reported a decreased

food intake during the period of disease. Specifically, 80% of them received only half or less than 50% of their usual food intake during COVID-19 infection. Only 2.9% of participants received 100% of their usual food intake.

Table 1. Characteristics and clinical outcomes of patients with COVID-19

Characteristic	Age, years	Gender, n (%)		Last Education, n (%)								Height (cm)
		Female	Male	Elementary	Middle school diploma	Diploma	Associate Degree	Bachelor	Master Degree	PHD	Illiterate	
N=381	42.51±7.31(30-76)	227(59.6%)	154(40.4%)	8(2.1%)	1(0.3%)	42(11%)	36(9.4%)	184(48.3%)	72(18.9%)	38(10%)	0(0%)	164.06±9.81(140-189.5)

Table 2. Association between COVID-19 infection and physical activity in the pre-and post-COVID-19 infection periods

	Before COVID-19 infection	After COVID-19 infection	P Value
Intense physical activity (min/day)	92.57±84.32	5.90±40.02	<0.001
Moderate physical activity(min/day)	135.89±107.05	18.24±53.35	<0.001
Walking physical activity (min/day)	36.23±18.50	4.08±13.56	<0.001
sitting (min/day)	406.40±141.64	311.21±168.18	<0.001

Paired t-test (The mean of physical activity in each level were compared before and after COVID-19 infection by paired t-test.)

Table 3. Association between COVID-19 infection and food intake during COVID-19 infection compared to before the onset of COVID-19.

	Number	Percent
No amount of food (%) 	92	24.1
25% of food (%) 	55	14.4
50% of food (%) 	157	41.2
75% of the food (%) 	66	17.3
100% or all food (%) 	11	2.9
Total	381	100

Discussion

Physical Activity

The results of this study showed a significant reduction in vigorous and moderate physical activity, as well as walking, and total sitting time during the period of COVID-19 infection compared to before the onset of COVID-19. The reported decrease in sitting time among individuals after contracting COVID-19 was attributed to spending more time lying down and resting during their illness.

Numerous studies have highlighted exercise as a protective factor against the disease (17). This protective factor has been associated with reduced mortality, improved lung function, and alleviation of disease symptoms (17). Our findings align with those of a conducted study by Antunes et al. (2020) which demonstrated that COVID-19 quarantine measures may lead to reduced physical activity levels in individuals (10). Conversely, the results of a cross-sectional study by Lesser et al. (2020) showed significant

changes in physical activity behavior between active and inactive participants since the implementation of COVID-19 restrictions. Among inactive individuals, 40.5% reported a reduction in physical activity levels, whereas only 22.4% of active individuals experienced decreased activity levels. Interestingly, 33% of those who were inactive got more active, while 40.3% of active people increased their physical activity even further (18).

In 2020, Grazia Maugeri and colleagues conducted a cross-sectional study on physical activity during the COVID-19 pandemic. They found that individuals with low physical activity levels before the pandemic increased their activity during the quarantine, while highly active and moderately active participants reduced their exercise. Overall, physical activity significantly decreased across different age groups. The study's results align with our findings, emphasizing the decrease in physical activity levels among highly active and moderately active individuals during the pandemic. The imposed quarantine restrictions compelled many people, especially those who were typically more active; to reduce their regular physical activity levels (19). Furthermore, in a systematic review, a majority of studies reported a decrease in time spent in all physical activity subgroups, including light, moderate, vigorous, and walking (if specified), during the relevant quarantine period. Additionally, an increase in sedentary behaviors was observed across various populations, including children and patients with different medical conditions (20).

While publications on the influence of the COVID-19 pandemic on physical activity have been published, no research concentrating on the COVID-19 infection impacts on physical activity has been reported.

The present study showed a significant decrease in food intake during COVID-19 infection compared to before infection. There was no similar study to compare food intake during COVID-19 infection with before infection. Dietary patterns and the amount of food intake during COVID-19 widespread among healthy individuals have been investigated in previous research.

Regarding the association of COVID-19 infection on food intake and nutrition, there were a total of 1,383 studies, out of which 6 articles were similar to our study (2, 21-25).

Dietary pattern research revealed that the COVID-19 dietary pattern consumed more energy and had lower dietary quality than pre-COVID-19 dietary patterns. In terms of food composition, the COVID-19 food basket contained fewer beverages (particularly alcohol and coffee), a slight increase in the availability of eggs and red meat, and a significant increase in plant-based foods (particularly processed vegetables, fruits, nuts, and pasta/rice) when compared to food baskets in 2019. However, intake of plant-based food products remained below the dietary standards' suggested levels, while consumption of red meat remained high. The COVID-19 diet's daily energy intake was 2,509 kilocalories, a 6% increase over 2019 and 27% more than the recommended level (21).

Another study found that 43.5% of those polled reported eating more during the quarantine, and 51.8% acknowledged eating extra snacks between meals. The highest frequency of daily meals during the quarantine was reported as three meals (30.3%) and four meals (39.3%), while for snacks, it was one snack (28.1%) and two snacks (36.1%). Comparatively, individuals with higher BMI reported increased food and snack consumption. Finally, individuals who were more obese had the highest frequency of consuming more snacks during the quarantine (25).

In general, based on the results of the conducted studies, a significant reduction in the consumption of rice, meat, chicken, fresh vegetables, fresh fruits, soy products, and dairy products were observed. The frequency of food intake also differed between genders, with women consuming more rice, fresh vegetables, and fresh fruits but less meat, chicken, soy products, and dairy compared to men. There was a notable increase in the consumption of wheat products, other main dishes, and preserved vegetables (23).

In another study, the number of meals consumed during the day increased significantly during the quarantine, with 11.2% of respondents consuming five or more meals. The percentage of individuals eating snacks between meals increased by 5.1% during the quarantine. Overall, two-thirds of the respondents reported changes in body weight, with 45.86% of participants gaining weight during the quarantine, primarily due to increased body fat mass. Finally, individuals who were overweight,

obese, and older (aged 36 to 45 years and over 45 years) tended to gain weight, while those underweight were more inclined to lose weight (22).

Conclusion

In this study, a significant decrease in physical activity and daily food intake during COVID-19 infection was observed.

Declarations

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Conflict of Interest

The authors declare no conflict of interest.

Strengths

This study is the first longitudinal study to investigate the relationship between COVID-19 infection and food intake and physical activity. The study was conducted within a university cohort, and the sample size was acceptable. We were able to exclude individuals who had received vaccination before follow-up. There are no reports on the impact of COVID-19 infection on physical activity, and all published studies have only examined the effects of the COVID-19 pandemic on physical activity. Our study is the first to report the impact of COVID-19 on physical activity.

Limitations

The design of this study was observational, so causality cannot be determined. Assessing individuals' physical activity using a pedometer instead of a physical activity questionnaire could have provided a better assessment of changes in physical activity. Assessing food intake using a 24-hour recall during COVID-19 infection could have provided a better assessment of changes in food intake.

Suggestions

In future studies, it is recommended to measure the evaluated indices multiple times and conduct regular follow-ups of study participants. This longitudinal approach will provide a more comprehensive understanding of the changes and trends in the studied indices over time. Furthermore, it is important to include a control group in addition to the outcome group to allow for comparison and better interpretation of the results. Investigating these indices in the long term will provide valuable insights into the long-term effects and implications.

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The Effectiveness of Endurance Training and Nano Curcumin Supplementation on the Expression of Mir-21and P53 Genes in Brain Tumor Tissue in an Animal Model of Glioblastoma Multiform

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ABSTRACT

Introduction: Glioblastoma multiforme is the foremost common harmful tumor of the central nervous system that specifically influences the brain and is resistent to common therapies such as surgery, radiotherapy and chemotherapy. The aim of this study was to examine the viability of perseverance preparing and Nano-curcumin supplementation on the expression of miR-21 and P53 qualities in brain tumor tissue in a creature demonstrate of glioblastoma multiforme.

Methods: In this experiment, 35 8-week-old male Wistar rats were divided into seven groups with 5 rats each: healthy control group, 4-week-old healthy, control group cancer, 4-week-old cancer group and training group, Nano-curcumin group and training-Nano-curcumin group. Cancer cells were injected into the right frontal cortex of mice using a pump at a depth of 2.5 mm. One week later, mice entered the treadmill training program (4 weeks) and Nano-curcumin was administered orally at a dose of 80 mg/kg (28 days). Gene expression was measured using real-time fluorescent quantitative PCR and used for analysis.spss software.

Results: The expression of miR-21 gene in the training group, Nano-curcumin, and training group Nano-curcumin was lower than that of the control cancer at 4 weeks (P = 0.001). Moreover, the expression of P53 gene in the Nano-curcumin training group and Nano-curcumin training group was higher in cancer cells and 4-week blood-eating cancer than in the control group (P = 0.001).

Conclusion: Endurance training and curcumin administration appear to reduce tumor growth in mice with brain tumors by modulating the expression of miR-21 and p53 genes.

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Introduction

Brain tumors, particularly dangerous tumors, are among the foremost terrifying maladies that straightforwardly influence the brain. Brain tumors are isolated into two sorts: essential and auxiliary (systemic) brain tumors (1). Glioblastoma multiforme (GBM), the most dangerous tumor of the central nervous system, originates from glial tissue and is resistant to commonly used therapies such as surgery, radiotherapy, and chemotherapy (2). The normal survival time of glioblastoma multiforme patients who get standard care treatment is roughly 15 months (1, 2).

Later studies have resulted in the belief that microRNAs play a crucial part within the start and movement of cancer (3). A few miRNAs act as tumor silencers or oncogenes and play key parts in tumor cell expansion and tumor cell apoptosis (4) MicroRNAs (miRs) suppress their target genes through binding the 3′ untranslated regions of messenger RNAs. (5). One of the

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biomarkers in cancer determination and treatment is miR-21; which is found on chromosome 17 and is known as an oncogene protein activator. It has been detailed in numerous threatening tumors, counting glioblastoma, and is included in tumor movement (6). Laboratory studies considers on a few sorts of cancer have appeared that the devastation of miR-21 stifles cell expansion and tumor development, and decreases the intrusion of metastasis (4). The results of a study showed that the diminishment of miR-21 expression moreover decreases tumorigenic potential in glioblastoma cell lines (7). A few miRs have been reported to be included within the p53 pathway, which are either controlled by p53 or act specifically to quell the expression of p53 or its downstream effectors, showing the significance of miRs within the p53 pathway (5). P53 could be a translation factor and tumor silencer protein that plays a part amid the cell cycle, counting transmission of cell messages, cell reaction or harm and recovery, DNA stability and genomic Stability (9, 8). The P53 quality is found on the brief arm of chromosome number 17 and its disturbance and inactivation leads to cancer (6). Later investigate has revealed that P53, in expansion to having an administrative part in apoptosis, cell cycle capture, and cell maturing, conjointly through supporting the activity of cancer prevention agents, too plays a skillful role (10). MiR-21 applies its oncomiR work by hindering apoptosis. Erasure of miR-21 sensitizes cells to apoptosis actuated by DNA-damaging specialists, within the nearness or nonattendance of p53 (5).

On the other hand, curcumin, the organically dynamic substance of turmeric, has different possibilities in terms of usefulness, which influences the work of numerous translation components, development variables, and receptors due to its polyphenolic dynamic substance. In addition, curcumin has low poisonous quality and it has been appeared that it performs a wide run of therapeutic capacities, counting antioxidant, anti-inflammatory, antimicrobial impacts, treatment of diabetes, Alzheimer's, and anti-cancer through numerous cellular pathways (10, 11). It moreover can change the cell cycle and pathways included in multiplication, apoptosis, relocation, intrusion, angiogenesis, and metastasis. Mechanically, curcumin balances a few atomic targets and applies its anticancer properties either by changing quality expression, actuation, or signaling pathways or by coordinating interaction and diminishes a few side impacts related with chemotherapy (12). A few prove has affirmed the antitumor impact of curcumin and its work on tumor cells (13, 14). Ponders have appeared that the utilization of curcumin and nano-curcumin, in expansion to a critical increment within the expression of the P53 quality, leads to cell cycle capture and apoptosis (15,10). In a study, Wang et al. (2017) appeared that curcumin can initiate apoptosis and diminish the movement of cancer cells by reregulating miR-21/Akt (16). Too, sports and cancer considers appear that regular exercise can have useful impacts in preventing and controlling cancer (17). Aerobic exercises is a vital non-pharmacological instrument that has an anti-tumor impact and can diminish tumor development, act as a tumor silencer, and control quality expression through distinctive components such as quieting quality expression (18). in a research, it was appeared that sports exercises increase P53 (19). In a review article that investigated the aerobic metabolism of P53 and cancer, it was expressed that the components directed by P53 control mitochondrial breath additionally offer assistance keep up genomic soundness (20). The results of a study showed that endurance training together with nano curcumin supplementation caused cell cycle capture and apoptosis by influencing the p21-p53 pivot (10). The study of Soltani et al (2019) appeared that Aerobic exercise can can diminish MIR-21 (4). Moreover, 8 weeks of high-impact work out diminished P53 and MIR-21 in individuals with prostate cancer (6). Nowadays, one of the critical issues that analysts have focused on is examining the impact of diverse intercessions such as Aerobic exercise and home grown supplements and their impacts on the development, improvement and conceivably restraint of the cancerous mass (21). Considering the useful impacts of preparing action on the avoidance of Cell senescence and apoptosis handle as well as positive and antioxidant and anticancer impacts of nanocurcumin supplement (10). This question is raised whether the combination of sports movement and the utilization of nanocurcumin supplements can have a more noteworthy impact on the diminishment of glioblastoma brain tumor tissue

than either of them alone. According to the searches, no comprehensive ponder was found that explored this issue. In spite of the fact that no study was found in this field, a few ponders have examined the synchronous impact of Aerobic exercise and curcumin on brain work. Therefore, the purpose of this study is the effectiveness of endurance training and nanocurcumin supplementation on miR-21 and P53 quality expression in brain tumor tissue of a creature show of glioblastoma multiforme.

Methods

The present study is experimental; which was done at the Creature Center of Research facility Sciences, Baqiyat Elah University of Medical Sciences. This study has been looked into and endorsed by the ethical committee of the Borujerd Islamic Azad College with code IR.IAU.B.REC.1400.29. Since it was not conceivable to get to human subjects due to space, ethics, and time limitations, subsequently, animal subjects (male Wistar rats) were utilized. Concurring to the enlightening of the Iranian Society for the Protection of Laboratory Animals, the examined creatures were kept independently in cages. The statistical population of male Wistar rats and its sample size included 35 rats. The test estimate was decided with G*Power program based on the factual strategy of investigation of change and alpha blunder level of 0.05 and control of 0.85 with 35 tests (10). Rats with an approximate age age of eight weeks and a weight extend of 200±20 grams were gotten from the Pasteur Research facility Creature Breeding Center (Tehran, Iran) and after the animals were transferred to the sports physiology laboratory. All creatures were kept within the same and ideal conditions of research facility creatures (temperature 22±2 °C, relative mugginess 45-50 and light-dark cycle 12:12) and they had free access to standard research facility nourishment and water. After a week of getting utilized to the environment, the rats were arbitrarily partitioned into 7 bunches of 5 included: Basic healthy control group, healthy control for 4 weeks, Basic cancer control, 4-week cancer control, cancer + nanocurcumin, cancer + endurance exercise and cancer + aerobic exercise + nanocurcumin group were divided. One week after the Induction of cancer cells within the frontal cortex of rats, nanocurcumin supplement was arranged and gavage was performed concurring to the informational (22). For endurance training, rats were to begin with arranged for running for one week on a treadmill for rodents (DSI 5-lane show, made in Iran). At that point, a 4-week exercise protocol was executed with an escalated of 18 meters per minute for 25-40 minutes per day with reiteration of 5 sessions per week (balanced high-impact convention) (Table 1) (23).The control groups (basic healthy, healthy 4 weeks, basic cancer and 4 weeks cancer) did not get any movement or supplements. At the end of the interventions of the exercise and supplementation groups, the mice were transferred to the laboratory. The basic healthy control and basic cancer control groups were anesthetized and sacrificed with peritoneal injection of xylosin and vaccinia 48 hours after the last training session (at the end of 4 weeks) to determine the basic values of the studied genes in the first week. At that point, the brain tissue of all rats was expelled beneath sterile conditions and instantly solidified in fluid nitrogen at -70 degrees Celsius. At that point for tissue staining and processing, tumor estimate estimation and RNA (RiboNucleic Corrosive) extraction and cDNA (Complementary deoxyribonucleic corrosive) as well as primers designing and the examination of gene expression utilizing Real-time PCR method, the tests were data analysis and Information examination was kept up.

Table 1. Endurance training protocol

Endurance Training	Weeks	Intensity	Duration	frequency
Aerobic Treadmill	1	18 m/min	25 min/day	3 day/ week
	2	18 m/min	30 min/day	3 day/ week
	3	18 m/min	35 min/day	3 day/ week
	4	18 m/min	40 min/day	3 day/ week

Cancer Cell Culture

C6 mouse glioblastoma cells were gotten from the National Center for Hereditary Assets. Cells were to begin with refined. To culture C6 cells, an RPMI cell culture medium was utilized. To begin with, C6 cells were refined in a carafe in RPMI medium (Roswell Stop Dedication Founded), 300 mg/ml penicillin (Sigma, America), 720 mg/ml streptomycin (Jabarban Hayan Pharmaceutical), and 2 g/L sodium bicarbonate 10% (Merck,

Germany). The ultimate volume of the cell culture medium was 1000 ml, and its pH was balanced to1.7. At that point it was neutralized with PBS (buffered saline Pho) (Gibco, America) and 0.025% trypsin-EDTA arrangement and with 10% FBS medium. The arrangement was centrifuged at 1200 rpm for 5 minutes and the cells were isolated. The Initial density for cell culture was considered to be 100,000 cells/cm2. At long last, 10 microliters of trypan blue color (0.4% weight-volume) and 90 µl of cell suspension and neobar slides were used for cell counting and viability. The rate of recolored cells (blue) was decided as the rate of dead cells.

Cancer Tumor Induction

To induce cancer, the refined cells of C6 creature glioma blastoma were utilized (24). In such a way that for tumor induce, the animals were anesthetized by intraperitoneal infusion of ketamine (100 mg/kg) and xylazine (10 mg/kg) (4). To begin with, animal hair was shaved. At that point, the creature was settled by putting the bars interior the ears and upper teeth to the stereotaxic gadget (Stelting 1, show 200195504). After making a skin cut within the back of the cranium and evacuating the periosteum concurring to Swansen's enlightening, utilizing an mixture pump and a stereotaxic gadget, the proper frontal cortex of the rats was decided on the bone with the taking after facilitates and gradually infused into the rats brain for 10 minutes in a volume of 10 microliters with a concentration of 5x105 cells/30 µL. At that point the bone was closed utilizing wax and the skin was sutured utilizing cotton string. After relinquishing the creatures, the brain tissue was sent to the research facility for recoloring and tissue preparing (25).

Preparation and Gavage of Nano Curcumin Supplement

One week after the affirmation of cancer within the rats, concurring to the instructions, nano curcumin supplement was given 5 days a week for 28 days with a dose of 80 mg/kg for cancer + nano curcumin, cancer + Exercise + nano curcumin groups utilizing an affront syringe based on the weight of each mouse. To get ready chitosan nanoparticles supplement, chitosan (500 mg) was Solved in 2% v/v acidic corrosive arrangement (50 ml) and blended with curcumin in ethanol (1 mg/ml).15 ml of 1% weight-volume TPP arrangement was included to it. At that point the arrangement was blended for 1 hour and centrifuged at 10,000 rpm for 30 minutes. To get chitosan nanoparticles encased in curcumin. Commercial nano curcumin fabricated commercially by Exir Nano Sina Company (Tehran, Iran) was utilized as a comparative test of item quality. For each creature, after planning the item, 80 mg per kilogram of body weight was utilized (22).

RNA Extraction and cDNA Production

To extricate add up to RNA, it was homogenized at a proportion of 1 to 10 in Isol RNA-reagent Lysis concurring to the enlightening of the pack (Qiagen, Germany). In arrange to remove protein components, the resulting product was centrifuged at 4C for 10 minutes at 12000 rpm. The supernatant was expelled and blended with chloroform with essential Isol at a proportion of 0.5 to 1. The item was centrifuged at 4C for 15 minutes at 12,000 rpm and the mineral and watery parts were isolated, the RNA containing portion was evacuated and blended with isopropanol at a proportion of 0.5 to 1 and cleared out for 10 minutes at room temperature and after that centrifuged at 4C for 10 minutes at 12,000 rpm. The plate containing RNA was broken down in 20 µL of Free-RNAs water. RNA concentration was measured employing a nono drop gadget and the proportion of 260 to 280 between 1.8 and 2 was characterized as ideal immaculateness. After extricating RNA with tall virtue and concentration from the hippocampus of all examined tests, cDNA amalgamation steps were performed according to the manufacturer's convention (Fermentas, USA) and after that the synthesized cDNA was utilized to perform the invert translation response.

Designing Primers and Checking the Expression of miR-21 and P53 Genes

Refined water containing lyophilized preliminary 10 microliters, Primer Forward and Primer Revers 0.5 microliters each, cDNA, 1 microliter and DEPC Water 8 microliters were utilized to plan groundworks. For Biagen, the entire RNA of the cells was extricated concurring to the Cinagen convention utilizing the q RT-PCR strategy utilizing Kiazol arrangement. The quality of extricated RNAs was assessed by spectrophotometry. To plan single-stranded cDNA, Oligo dt groundwork and invert translation chemical were performed agreeing to the pertinent convention. Each PCR response was

performed in an ABI Step One machine agreeing to the manufacturer's protocol. Real-time PCR response cycles for qualities were performed at three temperatures of 94, 60, and 72 degrees Celsius. The Melting chart was done to check the precision of PCR responses. Dissolving chart was done to check the exactness of PCR responses. GAPDH (Glyceraldehyde-3-phosphate dehydrogenase) and U6 were utilized as reference qualities for P53 and miR-21. The expression levels of control and exploratory qualities were measured together (Table 2).

Table 2. Sequence of primers used

Gene	Forward primer	Reverse primer	Product lenght	Accession number
U6	5′-GCGCGTCGTGAAGCGTTC-3′	5′- G TGCAGGGTCCGAGGT-3′		
mir-21	5′-TGGATACGACTCAACATCA-3′	5′-TGGATACGACAAAAATATGGA-3′		
GAPDH	5′-CAAGTTCAAGGGCACAGTCA-3′	5′-CCCCATTTGATGTTAGCGGG-3′		
P53	5′-TCCCCTCCTTTCTTGCCATT-3′	5′- CAGAGACCCAGCAACTACCA-3′	170 nt	NM_030989.3

Statistical Analysis

To begin with, the Shapiro-Wilk test was utilized to check the normality of the data dispersion. Considering the normality distribution of the data, a one-way investigation of change was utilized to check the difference between the groups, Levene's test was utilized for the homogeneity of changes, and Tukey's post hoc test was utilized on the difference that there was a contrast. data analysis was done utilizing the SPSS software version 26 and charts from Excel with significant level (p≤0.05).

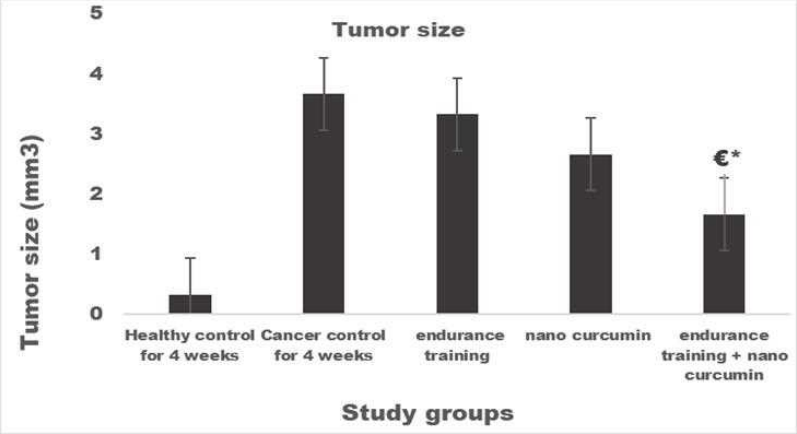

Figure 1. Tumor size in different groups.(* p ≤ 0.05)*A significant decrease in the cancer + endurance training + nano curcumin , compared to the 4-week cancer control group , (€ p ≤ 0.05)€ Significant reduction in the training of the cancer + endurance training + nano curcumin compared to the cancer + endurance training.

Results

According to the results of one-way analysis of variance of the one-way examination of fluctuation for tumor development rate, it appeared that there's a noteworthy distinction between the bunches (sig=0.001, F=16.50). Agreeing to the results of Tukey's post hoc test, the tumor growth rate within the preparing + nano curcumin gather was altogether to the results diminished compared to the 4-week cancer control group and preparing cancer (P=0.001) (Figure 1). In any case this diminishment within the nano curcumin group and the preparing gather was measurably noteworthy (P ≤ 0.05).

In addition, there was a significant difference between groups in the expression of miR-21 (sig = 0.001, F = 55.79), thus exercise and nanocurcumin each had a different expression of miR-21 (P<0.001) and exercise had. + nanocurcumin interaction showed a decrease in the expression of miR-21 gene in Tukey post hoc test (P=0.001) (Table 3) (Figure 2)

Regarding the expression of the p53 gene, there was a difference between groups in a single analysis of different experiments (sig = 0.001, F = 22.123). Tukey's post hoc test showed that the training effect, nanocurcumin effect, and training effect caused an increase in P53 gene expression in the nanocurcumin group (P = 0.001) (Table 3) (Figure 3).

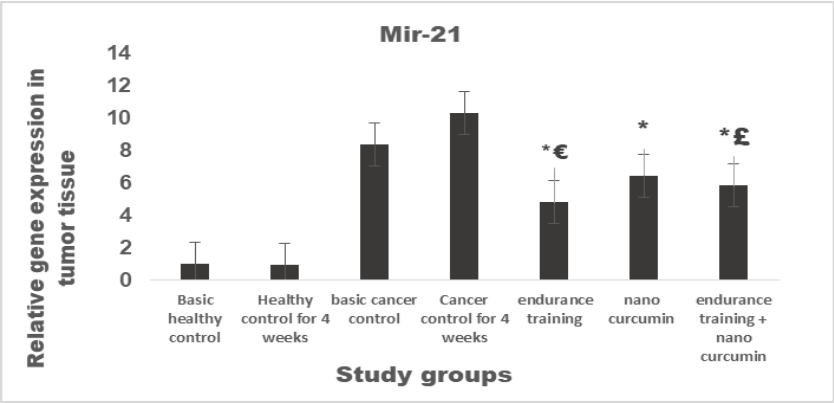

Figure 2. miR-21 gene expression in different groups.(* p ≤ 0.05)*significant reduction of cancer + endurance training, cancer + nano curcumin and cancer + endurance training+ nano curcumin compared to the Cancer control for 4 weeks.(€ p ≤ 0.05) € significance of cancer + endurance training and cancer + endurance training + nano curcumin compared to the basic cancer control.

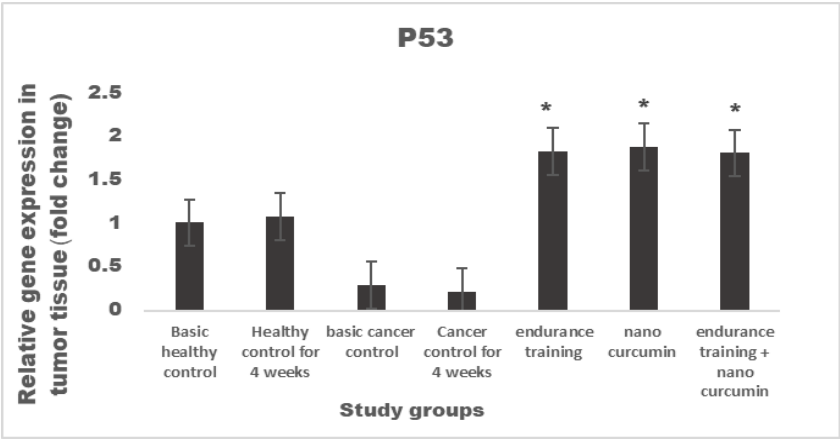

Figure 3. p53 gene expression in different groups. (*p≤0.05) *significant Increase in cancer + endurance training, cancer + nano curcumin and cancer + endurance training + nano curcumin compared to basic cancer control and Cancer control for 4 weeks

Table 3. Tukey's post hoc test for gene Mir-21 and P53

Groups	Groups	Mir-21 P Value	P53 P Value
Basic Cancer Control	Cancer control for 4 weeks	0.070	0.950
	endurance training	0.001*	0.001*
	nano curcumin	0.091	0.001*
	endurance training + nano curcumin	0.011*	0.001*
Cancer Control for 4 Weeks	endurance training	0.001*	0.001*
	nano curcumin	0.001*	0.001*
	endurance training + nano curcumin	0.001*	0.001*
Endurance Training	nano curcumin	0.195	1.000
	endurance training + nano curcumin	0.691	1.000
Nano Curcumin	endurance training + nano curcumin	0.966	1.000

Discussion

The results appeared that 4 weeks of perseverance preparing and nano-curcumin supplementation altogether diminished tumor development and did not alter (non-significant diminish), mir-21 expression quality and expression P53 expression within the preparing, nano-curcumin, and preparing + nano-curcumin groups compared to the 4-week cancer control gather.

The discoveries of the think about expressed that the induction of cancer in rats caused a diminish in tumor development, which was more significant within the preparing + nanocurcumin gather than within the training group and the

nanocurcumin group; which appears the impact endurance exercise together with the utilize of nanocurcumin. In line with the results of the present study, Betof et al. (2013) showed that high-impact endurance exercise can decrease tumor development in cancer groups up to two times compared to other groups (26). Additionally, regarding the effect of two interventions (physical activity and nanocurcumin), Delfan et al. (2020) showed that 5 weeks of endurance training and taking 100 mg/kg curcumin inhibited the growth and development of cancer cells by affecting intra-tissue mechanisms (27). Parvareh et al. (2023) expressed in their study that the synchronous utilize of sports exercises (resistance-endurance) and the utilization of nano curcumin supplement by hindering mRNA and STAT5 quality through the JAK-STAT pathway driven to the decrease of GBM tumors in rats (28). exercises and curcumin, as components that cause apoptosis, by ceasing mitosis, anticipate the movement of the cell cycle in dangerous tumors through distinctive signaling pathways, counting the pathway of cell multiplication, tumor silencer qualities and passing receptors (DR4, DR5) and advance restrains tumor cells (29,30,31).

Agreeing to the research results, the variable sum of miR-21 within the endurance exercise group, nano curcumin gather, and nano curcumin + exercise group was essentially lower than the 4-week cancer group. which is reliable with the results of Dufresne et al. (2018), Amani-Shalamzari et al. (2020), Soltani et al. (2019) and Esmatabadi et al. (32,33,34,4). But it was conflicting with the results of Jio et al. (2019) and Baggish et al. (2014) (35, 36). Within the studies, it has been found that the expression of miR-21 diminishes altogether after exercise (32, 33, 6, 4). The instrument of the impact of sports movement and particularly high-impact exercise on miRs has not been well characterized. But likely sports action controls miRNAs included in cell multiplication, intrusion, and metastasis (17). In expansion, Mir-21 may be a negative tumor suppressor of modified passing (PDCD4) and downstream direction of signaling targets in cell lines. By decreasing Mir-21 expression inside cancer cells, work out reestablishes PDCD4 action and limits cancer cell multiplication (4). Curcumin, having a dynamic component by interatomic with proteins and adjusts their expression and action, such as translation components, cell survival variables, angiogenesis, and different signaling pathways, can possibly direct the expression of a few miRNAs included in cancer (34). Prove has appeared that nano curcumin represses the development of cancer cells by down-regulating the expression of miR-21 and diminishing the number of cells within the G2/M stage of the cell cycle (34, 37, 38). The number of available studieson the combined impact of both intercessions, physical action and curcumin on mir-21 is exceptionally few, but a few studies have detailed the impact of these two mediations on diminishing the expression of miR-1, miR-133, miR-30, and mirR-199 (39, 40).

The results of the current research about the expression of the P53 quality within the cancer + exercise group, nano curcumin group and nano curcumin + preparing gather were significantly higher than the fundamental cancer control group and 4 weeks cancer. The results of this study are in line with The results of Akbarpour et al. (2022), Ju et al. (2019), Asghari Rekabard et al. (2018(, Nakayama et al. (2011), Pourjafarian et al. in conjunction with endurance training on p53 quality (6,10,35,41,42). But it was conflicting with our results of Ma et al. (2013) (5). The particular biological role of P53 is exceptionally complex, but P53 primarily secures the genome from transformations or hereditary changes. When defects are found in DNA, the P53 protein is phosphorylated and stabilized and actuates downstream pathways to halt the cell cycle, repair DNA, or actuate apoptosis (9). In an investigation, it was found that hereditary pathways are controlled by P53 amid sports exercises (43). In a review article on P53, aerobic metabolism and cancer. They expressed that the mechanisms controlled by p53 can interfere in the genes that coordinate the two main energy production pathways of aerobic metabolism and also help maintain genomic stability (9). Curcumin influences the function of numerous transcription factors, development factors and their receptors, cytokines, proteins and qualities directing cell expansion and apoptosis; and represses the expansion and survival of tumor cells (10). In line with the current study, in a research, they explored the utilization of curcumin at a dosage of 360 mg three times a day for 10 to 30 days in patients with colorectal cancer, which come about in a diminish within the serum concentration of TNF-α, an increment

within the apoptosis of tumor cells, and an increment within the event of p53 within the tumor got to be (42). A study has also shown that tolerance and promotion of nanocurcumin, in addition to increasing p53, may also cause cancer death by inhibiting cell growth and development, arresting the cell cycle and apoptosis, possibly affecting cell death (10).

Conclusion

According to the current findings, regulation of miR-21 and p53 gene expression and consumption of nanocurcumin after endurance training can reduce tumor volume through cell arrest cycle and apoptosis in mice with tumor cells. It prevents and slows down the development of cancer.

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Conflict of Interest

The creators thusly announce that there's no conflict of intrigued within the show consider.

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JOURNAL OF NUTRITION FASTING AND HEALTH

The Effect of Saffron Extract Consumption Along with Aerobic Training on Glycemic Indices in Streptozotocin (STZ)-Induced Diabetic Male Wistar Rats

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ABSTRACT

Introduction: Diabetes is one of the most common chronic diseases and one of the main causes of death all over the world. This study aimed to investigate the effect of consumption of saffron extract combined with aerobic exercise on glycemic indices in streptozotocin induced diabetic male rats with.

Method: This experimental study was conducted on 40 adult male rats aged 10-12 weeks weighing 220-250g. Rats were randomly allocated to diabetic control, diabetic exercise, diabetic saffron, and diabetic exercise + saffron groups after the induction of diabetes. Moderate intensity exercise was administered five days a week for eight weeks. Before and after eight weeks glycemic indices were measured, and the data were analyzed with Kolmogorov-Smirnov, one-way analysis of variance, and Tukey's post hoc tests.

Results: The results showed that fasting glucose in the saffron extract + aerobic exercise was significantly lower than the control and the aerobic training groups (p=0.014). Glycosylated hemoglobin in the saffron extract + aerobic exercise group was lower than the control group (p=0.001), and insulin resistance in the saffron group was lower than the control group with aerobic exercise (p=0.001).

Conclusion: Both an aerobic exercise and a consumption of saffron extract can probably prevent diabetes by reducing hyperglycemic indicators. Aerobic exercise and consumption of saffron extract co-administration can exert more beneficial effects.

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Introduction

Diabetes mellitus (DM) is a very severe metabolic endocrine disorder worldwide (1) and includes a heterogeneous group of disorders usually characterized by varying degrees of insulin resistance, impaired insulin secretion (3,2), and increased glucose production. The increase in blood sugar is called hyperglycemia (4).

Diabetes is known as one of the most common chronic diseases and one of the main causes of death worldwide. The prevalence of this disease is increasing, and it is estimated that the number of individuals involved will reach from 171 million people in 2000 to more than 336 million people in 2030 (5). In addition, more than 220 million people worldwide have diabetes, which is estimated to double by 2030 (6). Currently, 11% of Iranians have diabetes. Every 10 seconds, a person in the world dies due to a lack of awareness of diabetes and its control methods. Every 39 seconds, a person in the world loses their leg due to the lack of knowledge about diabetes and its control method. The inappropriate lifestyle of people has caused the spread of this disease (6).

The criteria for diagnosing diabetes mellitus are established based on the agreement between the National Diabetes Information Group and the World Health Organization (3). Normal methods for diagnosing diabetes are based on different urine and blood chemical tests (7).

The main diabetes treatment methods are insulin therapy, diet, and exercise. There is a need to adjust the diet, drug treatment, and exercise to properly treat this complex disease, along with the patient's cooperation.

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Exercise is one of the critical interventions in managing diabetes treatment. Interestingly, abnormalities that cause insulin resistance can be reversed by weight loss, diet, and physical activity (8). However, when exercise is performed simultaneously with nutritional interventions, it can create more effects. Special attention has recently been paid to medicinal plants, including saffron (9).

In addition to being a widely used food seasoning, saffron has many pharmacological effects. It has been reported that a small oral consumption (daily 100mg of saffron or 30mg of saffron hydroalcoholic extract powder) can cause many pharmacological effects in humans (10).

In folk medicine in different parts of the world, saffron is used as a sedative, antispasmodic, fatigue-resistant, glucose and blood-decreasing agent (11). On the other hand, various methods of treatment are recommended for the treatment or control of diabetes, including the use of natural plants, exercise activities, or lifestyle modification (12).

Some plants, such as saffron extract (13), almond (14), and dill (15), have been studied as interventions to reduce blood sugar and fat in people with diabetes; in this regard, one of the effective medicinal plants is saffron (13).

Skourtis et al. (2020) investigated the effect of using saffron supplements on oxidative stress, which plays a critical role in the pathogenesis of diabetes, and showed that saffron extract has an antioxidant effect in diabetic rats (16). In a review study, Sunny (2022) examined the effects of saffron supplementation in diabetic patients and concluded that the use of saffron supplementation in diabetic patients improves metabolic factors, blood sugar control, lipid profile, oxidative stress, and inflammation (17).

Another review study evaluated the effect of aerobic exercise and the consumption of saffron supplementation and showed that consumption of saffron and short-term aerobic physical activity increases antioxidant capacity and cardio-respiratory function and reduces muscle pain. Consumption of saffron and long-term aerobic exercise activity causes increased antioxidant capacity, improved blood sugar, strengthened respiratory diseases, and improved glycemic indices (18).

This study aimed to determine the effect of saffron extract consumption on glycemic indices in male Wistar rats treated with streptozotocin (STZ).

Some limitations the researcher could not control include the impossibility of precise control of the food consumed by rats and the Impossibility of precise control of rats' sleep.

Material and Methods

This study was conducted at the student's expense and with the support of the university's vice chancellor for research and approved by Islamic Azad University, South Tehran Branch.

This experimental study was conducted on male Wistar rats, and the animals were selected randomly and controlled accurately, this research is an experimental type with a post-test design and a control group.

The statistical population of this research included male Wistar rats purchased from the Laboratory Animal Center of Shiraz University of Medical Sciences. The sampling method was random, and 50 rats weighing 200-250g were randomly selected from among the animals.

Then, the selected rats were divided into five groups (n = 10 in each group), including healthy control, diabetic control, diabetic training, diabetic saffron, and training + diabetic saffron. The independent variables of the present study included aerobic training and consumption of saffron extract, and the dependent variable included blood sugar.

All standard conditions were considered, including temperature, relative humidity, free access to water and standard food, and dark/light cycle (12 hours). In addition, the ethical principles of maintaining and working with laboratory animals were observed based on the instructions of the National Institute of Health for the care and use of laboratory animals during the whole period of the study.

All animals were allowed to adapt to the laboratory environment for two weeks. At the beginning of the experiment, there was no statistical difference in the animals' weight and blood glucose levels. The intervention was five days a week and eight weeks with moderate intensity.

The aerobic training protocol consisted of 10 minutes of warm-up (soft running, combined movements of arms and legs, and stretching), 40 minutes of the main training (running with an intensity of 60 to 75% of the maximum heart

rate), and finally, 5 minutes of cooling down and returning to the initial state (19).

The target heart rate of the training was obtained from the Karvonen formula:

Target Heart Rate = [(max HR – resting HR) × Intensity%] + resting HR

The maximum heart rate was obtained from the formula (220-age) (20). A hand-held heart rate monitor (Polar watch) was used, and the subjects' heart rate was controlled. Besides, the Rockport walking test was used to obtain the subjects' VO2 max. Before starting the training protocol, two preparatory training sessions were considered to familiarize the subjects with the training protocol and heart rate counting.

Aerobic training consisted of fast walking in the first two weeks (the intensity in these two weeks was about 60% of the heart rate, and the duration was between 15 and 20 minutes) due to the lack of regular exercise and low physical fitness. The intensity and duration of training increased gradually and continuously every week. The placebo group and saffron supplement group were asked not to do any exercise during this period.

Oral administration of saffron extract was performed by gavage. The rats were taken by the researcher every day to consume aqueous saffron extract and received 25 mg/kg body weight of aqueous saffron extract at 11:00 a.m. using needle gavage (21).

Serum glucose levels were measured using a biochemical kit and enzymatic (glucose oxidase) method (22). In addition, fasting insulin was measured by the competitive enzyme immunoassay method.

The index based on HOMA-IR was used to investigate insulin resistance. High-performance liquid chromatography (HPLC) was also used to measure glycosylated hemoglobin (HbAIC) using the Nycorard (Norway) system.

The rats were sacrificed to measure the studied parameters 24 hours after the last training session, at the end of the 8th week, and the biochemical changes caused by the effect of aerobic training and saffron extract could be investigated.

The rats were sacrificed, and their tissue samples were paraffin-embedded and frozen at -60°C. Laboratory analysis was conducted to obtain the data, and gene expression was determined using the RT-Real Time PCR method.

The measurement tool for this study comprised various equipment and instruments, which included a laboratory digital scale sensitive enough to weigh subjects with a precision of 0.001 grams. In addition, a treadmill specifically designed for the physical activity of laboratory rats was made in Iran as a part of the measurement tool. Various surgical instruments such as carpal blades, scissors, forceps, and others were also included. A timer also adjusted the laboratory environment's darkness and brightness, and a thermometer regulated the temperature. Other necessary equipment included laboratory tubes, Erlen, Sten, alcohol, surgical gloves made of latex, two and a half and five cc syringes, a dissection tray, and cotton. Descriptive and inferential statistics were used to analyze the data. The Kolmogorov-Smirnov statistical test, one-way analysis of variance, and Tukey's *post hoc* test were used for inferential statistics. The significance level was considered $P \leq 0.05$, and SPSS statistical software (version 26) was used for statistical analysis.

Results

The results showed that fasting glucose in the saffron extract group with exercise Aerobics was significantly lower than in the control group and lower than in the aerobic training group. Glycosylated hemoglobin in the saffron extract group with aerobic exercise was lower than the control group. Insulin resistance was lower in the saffron consumption group, which was lower than the control group with aerobic exercise.

The results indicated a significant difference in fasting glucose levels in different groups (P=0.001). Fasting blood sugar in the aerobic training group, the saffron extract consumption group, and the saffron extract consumption along with the aerobic training group were lower than the control group (P=0.001). Moreover, blood sugar was lower in the saffron extract and aerobic training group than in the aerobic training group (P=0.01).

According to the results, there was no significant difference in fasting insulin levels in different groups ($p>0.05$), while there was a significant difference in the ranks of glycosylated hemoglobin in the studied groups (p=0.001). Levels of glycosylated hemoglobin in the aerobic

training group, the saffron extract consumption group, and the saffron extract in the aerobic training group were lower than those of the control group (p=0.001). In addition, there was a significant difference in the levels of insulin resistance in different groups (p=0.001). Levels of insulin resistance in the aerobic training group, the saffron extract consumption group, and the saffron extract consumption along with the aerobic training group were lower than in the control group (P=0.001) (Table 1).

Table 1. Comparison of the mean and standard deviation of fasting glucose, insulin, glycosylated hemoglobin and insulin resistance levels in the studied groups.

Group	Glucose (milligrams per deciliter)	Insulin (microunits per milliliter)	Glycosylated hemoglobin (percentage)	Insulin resistance (HOMA-IR)
Control	395.2±19.06	6.61±0.93	9.58±0.78	6.45±0.92
Aerobic training	315.33±20.91*	6.15±0.9	7.39±0.44*	4.78±0.78*
Consumption of saffron extract	305.44±4.21*	6.20±0.36	6.97±0.22*	4.67±0.31*
Consumption of saffron extract + aerobic training	291.88±10.69*	6.84±0.70	6.93±0.26*	4.92±0.49*

* Significant difference compared to the control group (p<0.05).

Discussion

Most studies have shown that the consumption of saffron extract and aerobic physical activity significantly affect the glycemic indices of people with diabetes (23-26).

This study aimed to investigate the effect of aerobic training, consumption of saffron extract, and simultaneous consumption of saffron extract and aerobic training on fasting glucose, insulin, insulin resistance, and HbAIC.

Aerobic training led to a significant decrease in blood sugar, insulin resistance, and glycosylated hemoglobin. These results are consistent with those of (20-27).

Exercise activity has been less addressed as a primary factor in the treatment of type 1 diabetes to improve glycemic control. Several studies have failed to show the independent effect of exercise activity in improving glycemic control by measuring HbAIC in type 1 diabetic patients. In contrast, most studies have reported the improvement of glycemic indices in this regard (19, 20, and 27).

Many studies have shown that aerobic training effectively improves glycemic indices, which is in line with the results of the present study. For example, Haji-Hasani et al. (2012) showed that eccentric and concentric exercises cause a significant decrease in blood sugar, HbAIC, and blood lipids in diabetic patients (28). David et al. (2006) reported that following aerobic training, HbAIC significantly decreases in diabetic patients (29).

Researchers in Fenicchia et al. examined diabetic women's glucose and insulin responses to glucose loading using short-term aerobic training. According to (2004), short-term aerobic training improved total glucose concentration but did not significantly change insulin concentration (30).

The present findings showed that resistance to insulin in the aerobic training group rats was significantly lower than in the control group, meaning that eight weeks of aerobic training improved insulin resistance.

Exercise training reduces the amount of insulin resistance in cells. Moreover, exercise training increases insulin sensitivity, resulting in less insulin needed to regulate blood glucose after exercise.

The results of the present study showed that the consumption of saffron leads to a significant decrease in fasting glucose levels and resistance to insulin. This finding is consistent with previous research of Hazman and Ovalı (2015) and Tajaddini et al. (2015). Hazman and Ovalı reported that saffron injection significantly improved FBS and insulin in diabetic rats (31,32).

The antidiabetic effects of saffron on diabetic patients are probably exerted through different mechanisms, such as stimulating glucose uptake and increasing insulin sensitivity in skeletal muscle cells by activating AMP-activated protein kinase (AMPK) and mitogen-activated protein kinases (MAPKs) pathways (33). However, further research is still needed to establish this statement for clinical recommendations.

Further, consuming saffron extract and aerobic training has a more significant effect on reducing fasting glucose than consuming saffron extract alone.

The results of this study were consistent with those of Rajabi et al. (2022) and Shavandi et al. (2010). Rajabi et al. (2022) showed that saffron at a concentration of 400mg has a significant effect on homeostatic model assessment for insulin resistance (HOMA-IR) and serum levels of insulin and adiponectin (34), which were consistent with the results of other studies in this regard (23).

Conclusion

Based on the results, both a period of aerobic training and a period of saffron extract consumption could probably control diabetes by reducing hyperglycemic indices. Aerobic exercise and consumption of saffron extract simultaneously can lead to more beneficial effects. However, this was the first time that the impact of aerobic training on the consumption of saffron extract was examined in diabetic rats, and further research is needed to draw more accurate conclusions in this regard.

Declarations

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Conflict of Interest

The authors declare the existence of any conflict of interest in this study.

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The Effects of Quarantine and Corona Virus on Dietary Habits, Physical Activity, and Anthropometric Indices

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Anthropometric

ABSTRACT

Introduction: Numerous changes occurred in economic, social, and medical fields following the onset of Coronavirus (COVID-19), including lifestyle and dietary habits. This study examined dietary habits, physical activity, and individuals' anthropometric indices during quarantine due to the lack of research on quarantine's effects on lifestyle changes.

Methods: This cross-sectional study was conducted on adults based on inclusion and exclusion criteria during the COVID-19 quarantine period. Participants were asked to complete a 10- to 15-minute online survey regarding their experiences during the COVID-19 pandemic. Demographic variables and surveys related to eating habits, lifestyle behaviors, and the impact of COVID-19 on physical activity and health were investigated. SPSS 22.0 was used to analyze all data, and the significance level was less than 0.05.

Results: A total of 630 participants were enrolled in this study, of whom 537 (85.2%) were women, and their mean age was 38.12 + 3.25 years (range: 19-58). The average of all anthropometric profiles, in general and gender-specific, was significantly higher during quarantine than before (P<0.001 for all). Moderate and high levels of activity were significantly higher before quarantine than during quarantine, while low levels of activity increased significantly during quarantine (P<0.001 for all). The most consumed food groups included fruits (72.9%) and meat (63.8%). Carbonated drinks (16.7%) and fats and oils (21%) were less consumed than other food groups. The most critical factors affecting BMI during quarantine included fat-free body weight before quarantine (importance coefficient (IC) = 0.4097), weight before quarantine (IC = 0.2398), and gender (IC = 0.4097).

Conclusions: Based on the results, quarantine probably increased obesity prevalence, altered diet habits, and decreased physical activity levels among individuals. Expanding health development programs is essential to promote healthy lifestyles in communities during quarantine.

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Introduction

The new Coronavirus disease (COVID-19), which originated from SARS-CoV-2 and was first reported in December 2019 in Wuhan, China, resulted in a newfound and quickly evolving situation and inevitably extended outside of China and Asia and a pandemic was announced in March 2020 (1, 2). Meanwhile, the countries that reported their first cases began to enforce strict hygiene rules and eventually national quarantine (3).

Quarantine and isolation are two actions to minimize the spread of infectious diseases (4). Quarantine in public health is defined as separating individuals or populations susceptible to an infectious disease from infected individuals (4). According to SARS experiences, mandatory quarantine could cause high levels of psychological distress, which usually appears as low mood and agitation, emotional disturbances and exhaustion, anger, insomnia, post-traumatic stress disorder, and depression symptoms (5). Therefore, quarantine during pandemic diseases

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could be categorized as a stressful event, and generally, such events affect diet patterns (6). Concerning acute or chronic stress, hypophagia or hyperphagia could occur, leading to significant weight changes. Meanwhile, total quarantine due to a pandemic could alter dietary habits because of forcing most people to stay in their houses longer. The result is usually fewer food options and less physical activity, which is very worrying in people with prior dietary problems.

There is a lack of information regarding the effects of quarantine on obesity. However, there is agreement that it is similar to a period of severe stress, and stress could increase obesity risk. An outcome of quarantine stress is a change in lifestyle and dietary habits. Stress-related eating (defined as an effort to feel better by drinking or eating in stressful situations) is related to obesity, especially in women (7). There may be a sex-specific response to stress, where women usually use food to overcome stress. In contrast, men mostly use other oral behaviors, such as alcohol consumption and smoking, as strategies against stress (8, 9). These factors, along with obesity, were considered public health issues before the pandemic, and COVID-19 could make them worse (10). In addition, studies have shown the negative effects of low physical activity on physical and mental health (11). Therefore, fear related to COVID-19 is having an indirect impact and increases obesity by affecting sleep quality and negative emotions (12).

Social distance and lifestyle changes have also affected the Iranian population. All activities in sports centers, gyms, and swimming pools were suspended (13). A worrying matter is the long-term effects of the pandemic on body weight management in adults. Dietary habits could protect health and weight gain (14). In the last decade, obesity prevalence among adults has increased and affected 650 million people (39% of the population) around the world. In general, the percentage of obese women (40%) is higher than that of obese men (7.33%) (15). However, a study recently showed that weight gain and obesity are related to COVID-19 risk and its consequences (16). As a result, obese individuals are identified as a high-risk group in need of actions and social isolation (17). There may be a great deal of importance to population health, and physical activity, stress, hygiene, and diet quality could differ in countries (18). However, the effects of restricted activities on body weight remain unknown, with studies showing results from around the globe (19).

There is a need for further investigation regarding obesity-related risks, especially since obesity management treatments have been reduced in many parts of the world due to quarantines. In addition, conflicting findings regarding quarantine's effects on COVID-19 have been reported. According to Cicero et al., quarantine did not significantly impact body mass index in 359 patients (20). Meanwhile, Al-Domi et al. showed that quarantine led to weight gain among participants (21). Therefore, this study aimed to evaluate how quarantine and COVID-19 affected people's eating habits, physical activity, and anthropometric indicators despite the dangers associated with obesity, the contradictions in the studies, and the limited number of studies investigating this issue in Iranians.

Materials & Methods

The present study is a cross-sectional study that was performed using an online questionnaire to gain information on the effects of the COVID-19 pandemic on people over 18. This study has an ethical code with the number IR.IAU.TMU.REC.1400.123 approved in Islamic Azad University, Science and Research branch.

The participants were asked to complete a 10-to-15-minute questionnaire about their experiences during the COVID-19 pandemic related to their hygiene behaviors and lifestyle. The people who agreed to participate signed a consent form that enabled the researchers to call them to obtain their information. Participants answered questions about dietary habits, lifestyle behaviors, and COVID-19's effects on physical activity and mental health. This study was performed using the survey method with an online questionnaire. The participants were chosen using convenience sampling. The inclusion criteria were consent to participate, age over 18, Iranian nationality, not having special diets during the pandemic and quarantine, and not suffering from endocrine disorders or disorders leading to loss or gain of weight. Participants who did not fully cooperate (filed less than 70% of the questions) were excluded from the study.

This study was performed using convenience sampling, and the sample size was calculated using the Cochrane formula with a 95%

confidence interval. Participants were asked about their gender, ethnicity, age, marital status, and primary anthropometric information (height and weight before quarantine and current). Information about their families, including the number of children, educational level, and whether the participants or other family members or relatives were affected. Nutritional intake, food consumption, and diversity in their diet were evaluated through a questionnaire.

Besides demographic variables, the COVID-19 pandemic significantly changed people's everyday lives. The participants were asked about these changes (Do you feel like your size has grown compared with before the quarantine?) (Did you have a special diet during the quarantine?) (Which food group have you consumed most during the quarantine?) (Has diversity in your foods been increased during the quarantine?) (Have the consumption of herbal essences and teas been increased during the quarantine?) (Do you take weight loss drugs?) (Have your mealtimes changed during the quarantine?) (Did you experience microphagier during the quarantine?) And how they acted in response to the orders to stay at home and the amount of their food waste during the weeks. The James formula calculates the participants' lean body mass (LBM). The daily physical activity of the individuals was assessed using the online questionnaire. Questions consisted of pre-quarantine physical activity (low, moderate, and high), current physical activity, types of physical activity (including hula hoop, rope skipping, pull-ups, morning exercises, and others), and the site of physical activity (gym, park, home or neither) and were reported as self-declaration.

All data were analyzed using SPSS software version 22. The Kolmogorov-Smirnov test was used to assess the normality of the variables. The histogram diagram was drawn, and the Chai-squared test was used to determine the relation between the dependent qualitative variables. Additionally, the Independent T-test and the Mann-Whitney test were used to compare quantitative variables with qualitative variables based on the normality of the data. The effects of the confounding factors of age, gender, history of illnesses, consumption of supplements, and energy intake were modulated in modeling and the regression test. In this study, a P-value less than 0.05 was considered significant.

Table1. General characteristics of the participants in the study

variable		Frequency N (%) =630
gender	female	537 (85.2)
	male	93 (14.8)
marital status	single	321 (51)
	married	309 (49)
infected with Corona virus	no	618 (98.1)
	yes	12 (1.9)
Follow a special diet	no	555 (88.1)
	yes	75 (11.9)
number of children	0	362 (57.6)
	1	96 (15.2)
	2	147 (23.3)
	>2	24 (3.8)
level of education	diploma and lower	246 (39)
	diploma	30 (4.8)
	bachelor or master's degree	312 (49.6)
	doctorates degree or higher	42 (6.7)

Results

In this study, an overall of 630 individuals with a mean age of 38.12 + 3.25 years (range of 19-58) were assessed, of whom 537 were women (85.2%), and 93 were men (14.8%). About 51% of the participants were single, and 49% were married. Table 1 demonstrates the frequency and percentage of the participants' general features. In addition, 39% of the total population's educational level was diploma and lower, 4.8% of the participants had a diploma, 49.6% had a bachelor's or master's degree, and 6.7% had a doctorate or higher. Further, 11.9% of the

participants followed a specific diet, and only 1.9% were infected with Coronavirus.

Table 2 shows the anthropometric indices of the studied population before and after the quarantine. Mean±standard deviation of the participants' weight was 65.77±15.75kg before the quarantine and increased to 67.43±15.75kg. During the quarantine, weight, body mass index (BMI), lean body mass, and percent of body fat significantly increased (P=0.001).

Table 2. Comparison of anthropometric indices before and after quarantine

Variable	Quarantine		P-value
	after (Mean±SD)	before (Mean±SD)	
weight	65.77 ± 15.75	67.43 ± 15.75	<0.001
body mass index	24.60 ± 5.96	25.22 ± 5.98	<0.001
lean body mass	49.71 ± 7.23	50.45 ± 7.13	<0.001
percent of body fat	22.66 ± 10.37	23.41 ± 10.31	<0.001

Table 3. Comparison of the anthropometric indices before and after the quarantine separated by gender

variable	female		P-value	male		P-value
	after quarantine (Mean±SD)	before quarantine (Mean±SD)		after quarantine (Mean±SD)	before quarantine (Mean±SD)	
weight	63.31 ± 12.64	65.04 ± 12.81	<0.001	80.15 ± 22.97	81.44 ± 22.65	<0.001
body mass index	24.36 ± 5.89	25.01 ± 5.93	<0.001	25.98 ± 6.18	26.42 ± 6.16	<0.001
lean body mass	49.15 ± 6.92	49.96 ± 6.89	<0.001	52.95 ± 8.13	53.34 ± 7.85	<0.001
percent of body fat	20.63 ± 9.22	21.42 ± 9.21	<0.001	34.49 ± 8.74	35.02 ± 8.63	<0.001

Table 3 shows the anthropometric indices before and after quarantine, separated by gender. Results showed that weight, body mass index, lean body weight, and percent of body fat significantly increased in both genders (O=0.001), and weight gain after quarantine was more in women than men.

High and moderate physical activity levels before quarantine were significantly higher than during quarantine (high activity: 18.6% before quarantine compared to 9.7% during quarantine and moderate activity: 55.2% before quarantine compared to 34.8% during quarantine). In comparison, low levels of activity showed a more significant increase during quarantine (55.6% during quarantine and 26.2% before quarantine) (P<0.001 for all).

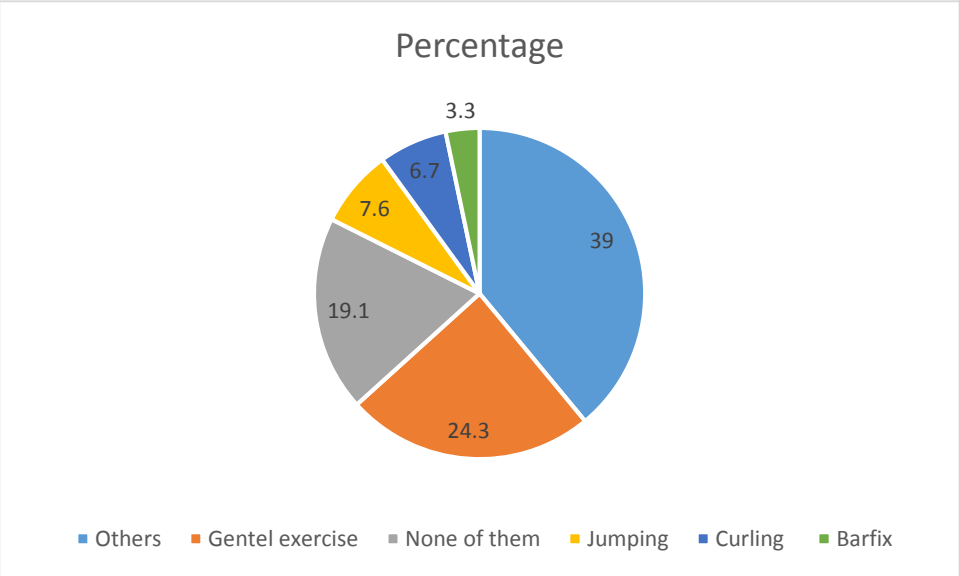

Figure 1. Percentage of frequency of pre-covid-19 physical activity in participants in the study

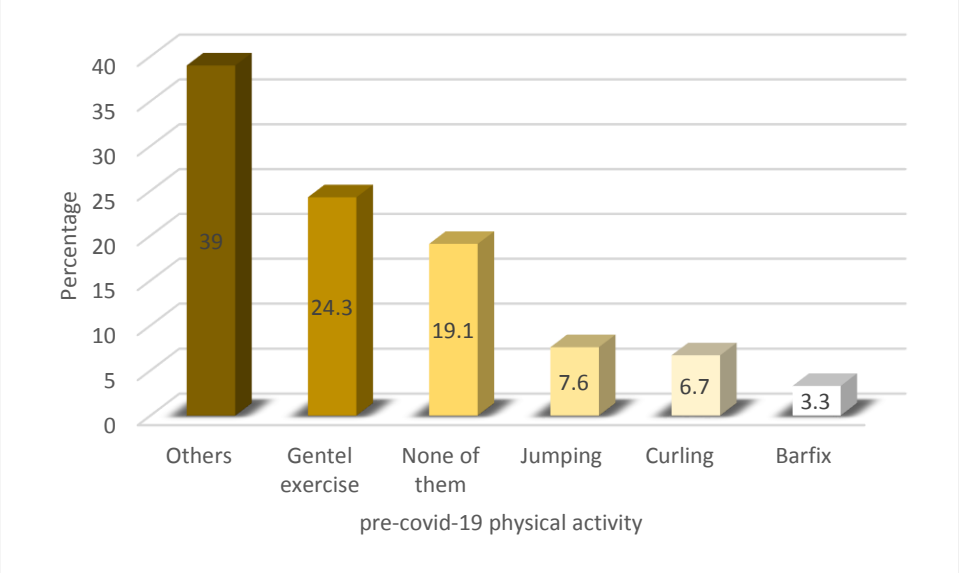

Figure 2. Prevalence of pre-covid-19 physical activity type in participants in the study (Unit: Percentage)

Figures 1 and 2 show the percent frequency of physical activity and the type of physical activity before Coronavirus in participants, respectively. According to these diagrams, 44.4% exercised at home, 22.9% at the gym, and 17.6% in parks. Among different types of physical activity, 24.3% of the participants did morning exercises.

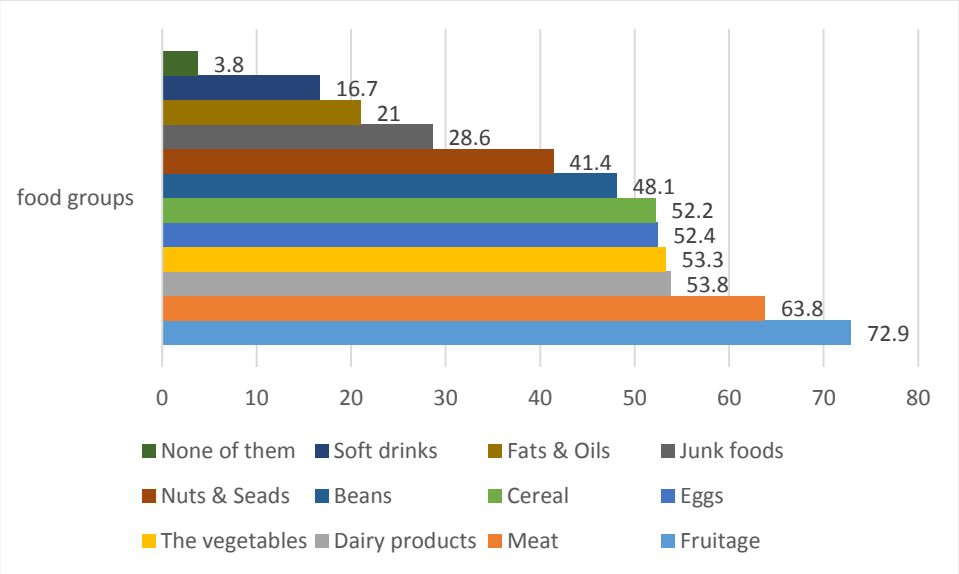

Figure 3. The frequency of the type of food groups consumed during quarantine in the participants in the study (Unit: Percentage)

Figure 3 shows the frequency percent of food groups consumed during quarantine in this study population. According to this diagram, the most popular food group among individuals was fruits (72.9%), meat (63.8%), dairy products (53.8%), vegetables (53.3%), eggs (52.4%), and grains (52.2%) were eating more than others. Carbonated drinks (16.7%) and fats and oils (21%) were consumed less than other food groups during the quarantine.

The effects of different factors on body mass index (BMI) after quarantine are indicated in Figure 4. Results show that lean body mass before the quarantine had the most significant

impact on BMI during the quarantine (Importance coefficient= 0.4097). Then, weight before the quarantine (Importance coefficient= 0.2398) and gender (Importance coefficient= 0.1751) were the most critical factors, respectively. Among the factors, grains and red meat had the least effect on BMI after quarantine.

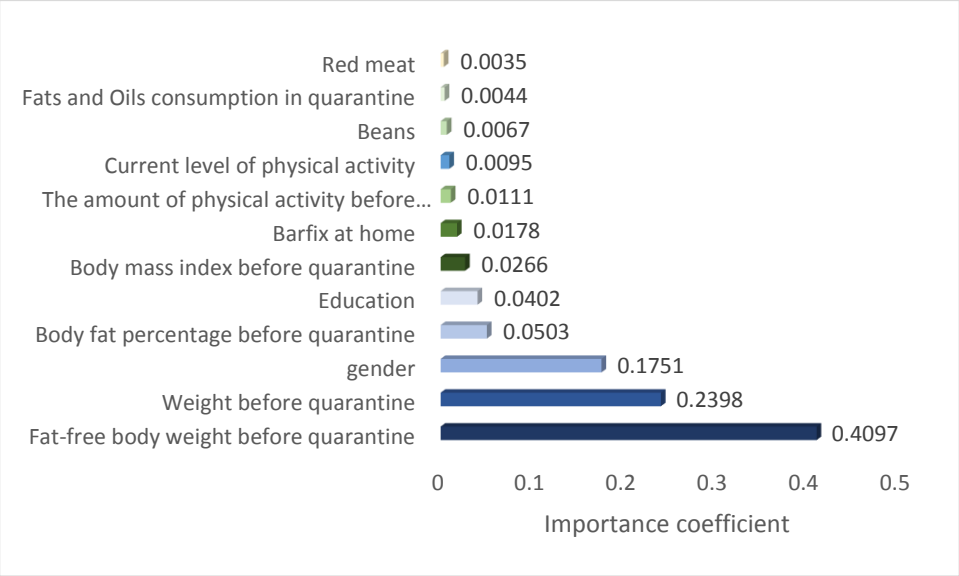

Figure 4. Comparison of the effect of different factors on body mass index after quarantine (Units: BMI= Kg/m^2; physical activity variables= low, moderate, and high; weight variables= Kg)

Discussion

A cross-sectional study evaluated quarantine and Coronavirus's effects on dietary habits, physical activity, and anthropometric indices. According to the present study, weight, body mass index, lean body mass, and percent of body fat significantly increased in both genders during the quarantine compared with before the quarantine. Precision and fast physical distances were key to fighting the COVID-19 pandemic worldwide. Various social interventions, including quarantines in public and private areas, were implemented to facilitate these actions. Companies faced bankruptcy, and the unemployment rate rapidly increased due to the economic crisis, even though the wanted outcomes were isolation and inhibiting virus transmission. Socioeconomic challenges exacerbate social and economic inequality and polarization of the population in countries with liberal markets and no cohesion-based welfare programs (22).

Obesity is a chronic, non-infectious disease and might seem unlikely to be related to COVID-19. Instructions to stay at home, as well as efforts to isolate high-risk populations and those infected or suspected of being infected with COVID-19, had a severe effect on obesity patients' hygiene behaviors and welfare (23). Combining forced isolation with economic hardships and worsening mental and social status could lead to long-term metabolic effects (24). Increasing social inequality as a result of political interventions against COVID-19 could worsen socioeconomic status, contributing to obesity and metabolic disorders in different populations (25). A possible explanation for this increase is the abundance of processed foods, which are high in energy, tasty, cheap, and easily available.

Global pandemics may cause mental stress, but socioeconomic conditions may exacerbate anxiety. Feeding patterns are generally affected by such events. Stress, sleep disturbances, and increased snack consumption could lead to weight gain (25). Food-related behaviors may be negatively affected by social-mental disorders. Food hoarding is a cultural example that is temporary. Social-mental stress increases energy consumption (26). In addition, obesity is more likely to occur in people with a low level of social interaction. Quarantine changed food exposure, which may have challenged cognitive limitations and caused binge eating behaviors to increase (27). Staying at home for long periods could also

increase the consumption of diverse food, meals, and snacks (27, 28). In addition, emotional eating, which usually helps to alleviate negative feelings, could increase in these situations (23). Patients usually choose cheaper foods with poor energy and nutritional content due to recent economic challenges. Although more patients cook at home, processed foods are mostly stored because of their longer shelf life, leading to more fat, carbohydrates, calorie consumption, and weight gain than moderate diets (29). Obesity affects pulmonary functionality and inflammation, and COVID-19's effects on obese patients are not much. Obesity leads to decreased exhalatory reserve volume, functional capacity, and adaptability of the respiratory system. Lying down may compromise pulmonary function and complicate ventilation in patients with high abdominal obesity. In addition, increased inflammatory cytokines related to obesity could increase COVID-19 infection risks (30, 31).

The results showed intense and moderate physical activity was significantly more before than during quarantine. Individuals mostly did mild activities during quarantine. Other studies have also confirmed the present results (32, 33). A lack of fitness centers, limited organized sports, and the need for physical distance may make it difficult to maintain an active lifestyle. Keeping weight in check requires more than 300 minutes of physical activity each week. Thus, disturbances in physical activity could have negative outcomes for individuals trying to lose or gain weight (32, 33).

Obesity is the abnormal or excessive fat accumulation that endangers human health. While obesity threats are increasingly recognized, worldwide obesity prevalence continues to increase, possibly because of low activity in our living environments after modern industrialization and the increase in alternatives (34, 35), which may have been intensified due to the quarantine because of COVID-19. Additionally, schools play an important role because they provide physical education classes and extracurricular activities that reduce adolescent obesity. School closures may have inappropriate effects on adolescents' activity patterns and weight during the quarantine. For example, an Italian study found that obesity affected children's exercise time and how much time they spent sleeping and using monitors after the COVID-19 quarantine (36, 37), which could probably remain after quarantine. A study by Jia et al. (35) demonstrated that all three educational levels saw increases in young people's weight during the COVID-19 quarantine and spent more time sedentary and less active. Young Chinese people were wary of being outside after the quarantine despite most cities being designated low-risk areas in May. Additionally, most universities and high schools began teaching online between March and April, resulting in more idle time.

According to many studies, children, teenagers, and adolescents changed their eating habits during COVID-19 and gained weight (38, 39). More specifically, 41.7% of teenagers in Palestine stated that their weight gain during the quarantine was due to the consumption of more fried foods, sweets, sugary beverages, and dairy products (40). A meta-analysis showed that the COVID-19 pandemic has altered daily life and eating habits. The number of meals increased during the quarantine, with men eating more potatoes, meat, and sugary beverages than women (41). Women and teenagers in Spain, Brazil, and Chile ate more vegetables and fruits compared with the week before the quarantine. Teenagers whose mothers had higher educational levels consumed even more vegetables and fruits (42). During the quarantine, 20.7% of teens consumed fried foods and sweets, which is related to a higher BMI and a younger age. A moderate increase in high-calorie or salty food consumption was observed (24%) in younger women who were married, had a history of psychiatric disorders, and were related to their living location. Parents were more worried about their children's food intake and tried to control it by limiting some foods and pressuring their children to take healthier and more nutritious foods (42, 43).

According to our knowledge, the present study is the first in Iran to investigate the effects of quarantine and Coronavirus on eating habits, physical activity, and anthropometric indices. This study collected important information to increase awareness regarding future comprehensive healthcare for obese patients. Access to healthcare services should continue during these times, especially if the pandemic leads to more quarantine regulation, as staying at home has consequences for long-term health. This cross-sectional study limited our ability to determine cause and effect. A self-administered

questionnaire was another study limitation, which could lead to measurement errors. Another limitation was that behavioral changes were based on the patient's perception when completing the questionnaire, which was not according to the quantification of these behaviors.

Conclusion

Based on the results, quarantine probably increases the prevalence of obesity, changes dietary habits, and lowers individuals' physical activity. The development of health programs to promote healthy lifestyles in societies during quarantine is being supported. However, more studies are required to confirm these findings.

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Conflicts of Interest

The authors declare no competing interests.

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JOURNAL OF NUTRITION FASTING AND HEALTH

The Trend of Nutritional Adequacy and Nutritional Routs among Imam Reza Teaching Hospital: A NutritionDay Review in 2019-2021

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ABSTRACT

Introduction: Adequate nutrition is essential for the well-being of hospitalized patients. Assessing nutritional adequacy significantly affects patient-centered care. The nutritionDay (nDay) project, supported by ESPEN, conducts a global audit to evaluate nutritional risks. Imam Reza Teaching Hospital in Mashhad is one of the participating hospitals. This study examined the trend of nutritional adequacy in Imam Reza Teaching Hospital from nDay 2019 to 2021.

Method: This cross-sectional study analyzed data collected from the nDay database of Imam Reza Teaching Hospital in Mashhad, Iran, between 2019 and 2021. Written consent was obtained, and the study followed international standards and the nDay questionnaire. Factors such as nutritional routs, energy goals, and energy intake were considered. All statistical analyses were performed using the Statistical Package for Social Sciences version 19.0. The three-year trend of nutritional adequacy was compared using the Chi-Square test.

Result: A total of 414 patients from 14 departments of Imam Reza Hospital were included in the study during 2019-2021. There was no significant trend of energy goal among the wards during 2019-2021, except in women's burn, orthopedic surgery, and general surgery wards ($P<0.001$,<0.001,<0.001, respectively). There was a significant variation in energy intake during 2019-2021 in men's burn, oncology, cardiac surgery, gynecology surgery, and general surgery wards ($P<0.001$, <0.001, <0.001, <0.001, <0.001, respectively).

Conclusion: This study showed that burn and oncology patients did not achieve their energy goals, indicating the importance of nutritional care.

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Introduction

Nutrition is crucial to individuals' overall health and well-being, particularly those receiving medical care in hospitals (1). In the most critically ill patients, there is a difference between the amount of nutrients needed and the amount consumed. According to previous studies, low-calorie intake is associated with worse outcomes (2). Proper and sufficient nutrition is necessary for the patient's health and recovery in managing a hospitalized patient and medical procedures. Adequate nutrition supports recovery, improves patient outcomes, and contributes to healthcare quality (3). Monitoring and improving nutritional adequacy have become essential aspects of healthcare delivery with an increasing emphasis on patient-centered care (4). Insufficient food intake in patients leads to the risk of chronic conditions, including malnutrition. Previous studies have shown that malnutrition affects 20-50% of hospitalized patients (5). Malnutrition has costs for the patient and the hospital, which leads to more use of resources and increased costs associated with longer hospital stays and may lead to increased mortality, pressure ulcers, and

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infections (6). Moreover, studies have indicated that the need for energy and protein increases during most acute illnesses (7).

Different nutritional routes based on the patient's clinical conditions include oral intake, Oral Nutrition Supplements (ONS), fortified/enriched food, enteral nutrition, and parenteral nutrition. The initial approach for the patient should prioritize oral nutrition. Implementing oral nutritional support, such as ONS and fortified/enriched food, can enhance clinical outcomes. Enteral nutrition should be considered when the calorie intake is inadequate or oral nutrition is not feasible. Enteral nutrition is preferred over parenteral nutrition due to its lower risk of infectious and non-infectious complications. Ultimately, parenteral nutrition is considered for patients with contraindications for oral and enteral nutrition (8).

Nutritional adequacy is the intake of essential nutrients to meet nutritional needs and prevent malnutrition (9), obtained from comparing the need for specific nutrients and their intake in a person or population (10). There are several criteria to define nutritional adequacy, which are related to preventing deficiency diseases, preventing chronic diseases related to nutrition, reducing the risk of diet-related diseases, clinical health related to nutrition identified by biochemical tests, and maintaining physiological balance (9).

nDay is a yearly one-day cross-sectional audit of hospital wards and nursing homes (11). NutritionDay started in Iran in 2010 and has been continuously implemented every year in Mashhad since 2019 (11). This study aimed to evaluate the trend of nutritional adequacy and nutritional routs in Imam Reza Teaching Hospital from 2019 to 2021.

Materials & Methods

This cross-sectional study used the data from the nDay database of Imam Reza Teaching Hospital. The energy intake and energy goals were used to analyze the trend of nutritional adequacy.

All statistical analyses were performed using the Statistical Package for Social Sciences version 19.0 (SPSS Inc., Chicago, IL, USA). The Kolmogorov-Smirnov test was used to assess the variables' normal distribution, and the variables' frequency was expressed with descriptive analysis. The three-year trend of nutritional adequacy was compared using the Chi-Square test.

The Research Ethics Committee of Mashhad University of Medical Sciences approved the study protocol (IR.MUMS.MEDICAL.REC.1402.371).

Results

The study population included adult patients (n=414, 49.3% male, with a mean age of 44.20 years, range 12 – 81 years old) from 14 units of Imam Reza Teaching Hospital in Mashhad, Iran.

Table 1 demonstrates basic information on the study population. The lowest BMI in 2019 and 2021 was related to the oncology ward, associated with the orthopedic surgery ward in 2020.

Table 1. Basic information on study population among 2019-2021

	2019				2020				2021			
Ward Name	**Number**	**Age (Min-Max)**	**Weight**	**BMI**	**Number**	**Age (Min-Max)**	**Weight**	**BMI**	**Number**	**Age (Min-Max)**	**Weight**	**BMI**
Burn Female	10	36 (24-40)	65.4±18.1	25.6±6.2	8	38(31-52)	60.4±11.6	23.1±4.8	10	40 (12-43)	61±24.1	25.3±5.2
Burn Male	9	29 (23-36)	57.8±8.6	19.9±2.5	8	29 (19-37)	68.3±18.8	22.8±6.1				
Internal Medicine/ Gastroentero logy	17	60 (54-65)	60.3±16.2	23.4±5.9					15	55 (50-62)	64.5±11	22.9±2.7
General Internal	19	64 (56-75)	65.6±15.2	23±4.9					20	58 (47-71)	67.9±14.8	25±5.1
Internal medicine/ oncology	9	25 (19-31)	59.7±11.1	18.9±2.4					24	45(25-52)	63.2±11.4	25.6±11.1

Ward Name	2019				2020				2021			
	Number	Age (Min-Max)	Weight	BMI	Number	Age (Min-Max)	Weight	BMI	Number	Age (Min-Max)	Weight	BMI
Internal Med	23	73 (59-81)	65.4±13.7	24.4±5.5					22	62 (49-70)	67.8±14.4	24±4.8
Surgery-General	30	47 (36-64)	65.1±15.5	23.6±5.0	11	33 (30-39)	70.5±15.3	25.6±6.4	24	42 (33-53)	70.5±11.4	25.7±3.6
Gynecology Surgery	10	43 (37-45)	78±13.5	30±5.3	10	29 (22-35)	72.6±14.2	28.9±5.3	20	31 (27-34)	71.9±13.4	30.7±12.2
Orthopedic Surgery	40	46 (27-59)	66.2±16	23.7±4.4	10	33 (24-53)	63.8±15.6	22.4±5.2	33	44 (30-53)	67.4±14.7	24.7±4.8
Urology	14	58 (40-61)	69.5±14.2	23.7±4.5					18	55(43-63)	68.6±11.7	24.7±4.3

Figure 1 shows the frequency variation of nutritional routes between 2019-2021. Patients from male burn and urology wards received regular hospital food in 2019. In addition, all hospitalized patients in men's burn and general Internal wards had regular hospital food in 2020 and 2021, respectively.

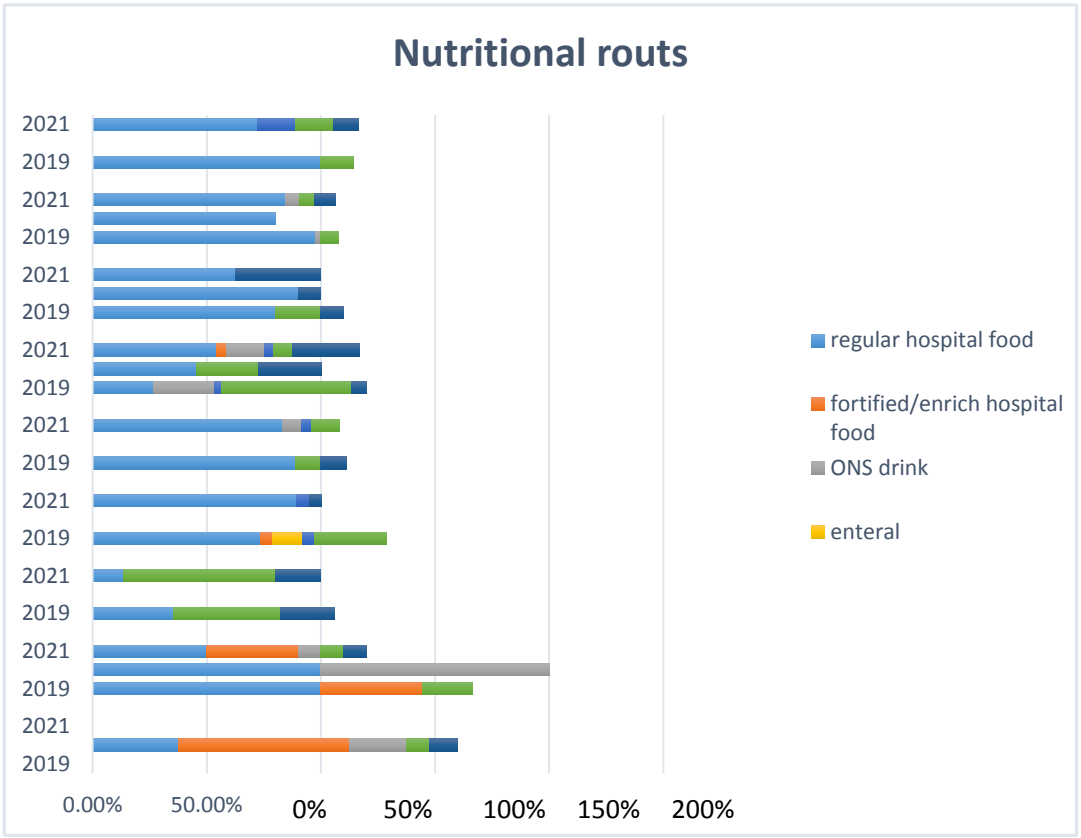

Figure 1. Nutritional routs during 2019-2021

The men's burn ward had the highest prevalence of fortified/enriched hospital food (44.4%) in 2019, followed by women's burn (75%) in 2020 and men's burn (40%) in 2021.

Regarding oral nutrition supplementation, the general surgery ward had the highest frequency (26.7%) in 2019, followed by the men's burn ward (100%) in 2020 and the general surgery ward (16.7%) in 2021.

Enteral nutrition was applied in 13% of the general internal medicine ward patients in 2019. There was no report of enteral nutrition in nDay 2020 and 2021.
In addition, parenteral nutrition was reported in 5.3% of general internal medicine ward patients. There was no report of parenteral nutrition among patients evaluated in nDay 2020. However, urology was the most prevalent ward regarding parenteral nutrition in nDay 2021 (16.7%).

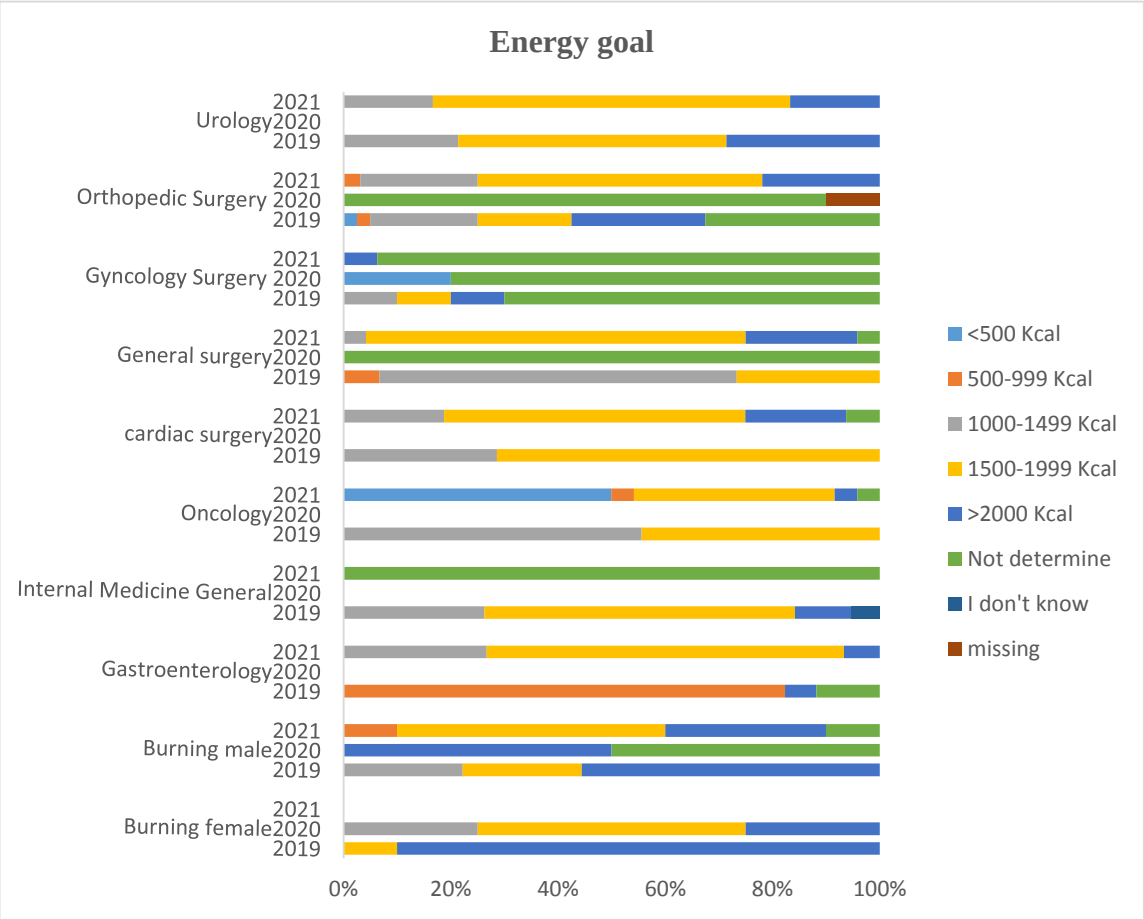

Figure 2. Trend of energy goal during 2019-2021

The trend of energy goals during 2019-2021 is demonstrated in Figure 2. There was no significant variation in energy goals among the wards during 2019-2021, except in women's burn, orthopedic surgery, and general surgery wards (P<0.001, <0.001, and <0.001, respectively). The highest frequency of <500kcal energy goal was reported for the gastroenterology ward (82.4%) in 2019, the gynecology surgery ward (20%) in 2020, and the oncology ward in 2021 (50%). The highest frequency of >2000 kcal energy goal was related to the women's burn ward in 2019 (90%), while it was reported in the men's burn ward in 2020 and 2021 (50% and 30%, respectively).

Figure 3 presents the trend of energy intake during 2019-2021. There was a significant variation in energy intake during 2019-2021 in men's burn, oncology, cardiac surgery, gynecology surgery, and general surgery wards (P<0.001, <0.001, <0.001, <0.001, and <0.001, respectively).
The highest frequency of <500 kcal energy intake was reported in the cardiac surgery ward (28.6%) in 2019, while it was related to gynecology surgery (10%) and oncology (50%) in 2020 and 2021, respectively. The oncology ward had a high prevalence (66.7%) of energy intake in the range of 500- 999 kcal in 2019, and it was observed in women's burn (62.9%) and

orthopedics surgery (34.4%) in 2020 and 2021. For the range of 1000-1499 kcal energy intake, the high frequency was reported in the general surgery ward (63.3%%) in 2019, but it was related to women's burn (37.5%) and orthopedics surgery (25%) in 2020 and 2021 respectively. The highest frequency for energy intake ranging from 1500-2000kcal was reported in the gynecology surgery ward, with a frequency of 70% and 10% in 2019 and 2020, respectively. However, the highest frequency of energy intake in this range was reported in the cardiac surgery ward (54.2%) in 2021. Furthermore, the gastroenterology ward was the most prevalent ward for energy intake >2000 kcal (41.2%%) in 2019, and it was related to the men's burn ward (50% and 20%, respectively) in 2020 and 2021.

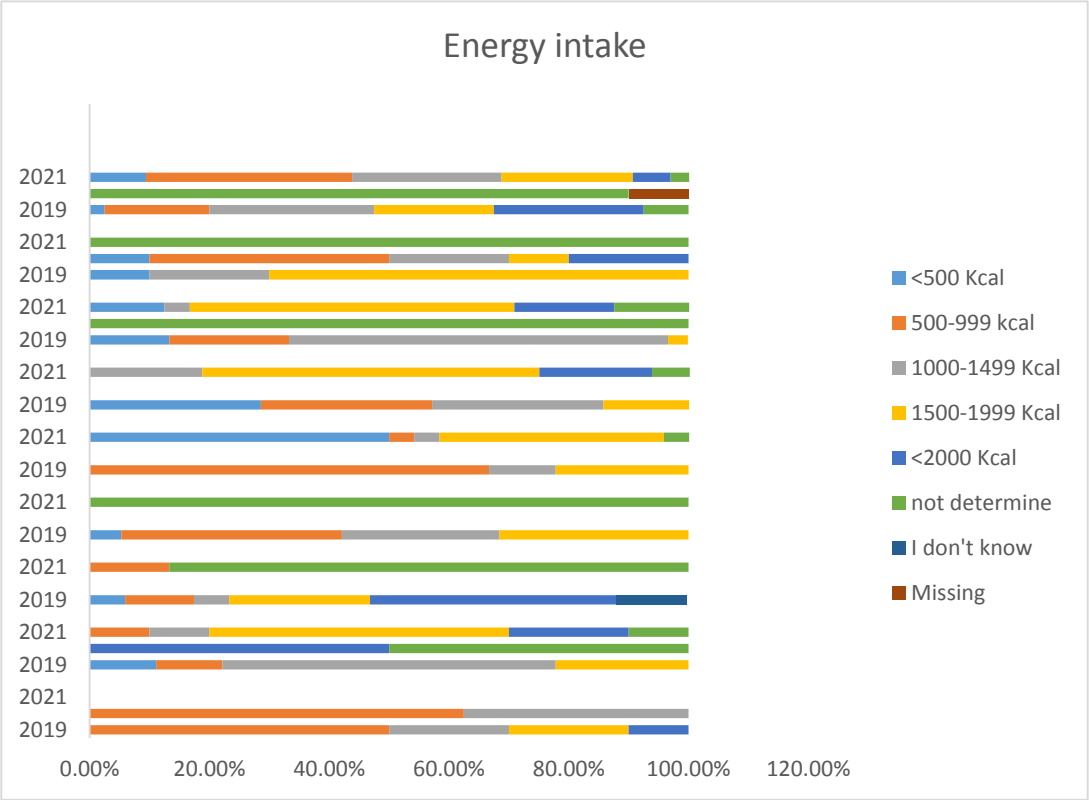

Figure 3. Trend of energy intake during 2019-2021

Discussion

There was no specific trend for energy intake and energy goal, but there was a significant variation in energy intake and energy goal between 2019 and 2021. The lowest energy goal was reported in patients of the gastroenterology ward. In addition, the most insufficient energy intake was reported in patients in oncology and women's burn wards. Among the hospital wards, a high percentage of patients in the women's burn and men's burn wards had regular hospital food, fortified/enriched hospital food, and oral nutrition supplements. The general internal medicine ward reported the highest prevalence of enteral nutrition. The highest frequency of <500 and >2000kcal energy goals were reported for gastroenterology and women's burn wards, respectively. A high frequency of <500 and >2000kcal energy intake was reported in gynecology surgery and men's burn wards, respectively. The lowest BMI was related to the patients in the oncology ward.

Saure et al. (2019) showed that a high percentage of patients (22.7%-38.7%) received a special diet 5.3% of patients received oral nutrition supplement (ONS) in combination with special food, and 0.3% of patients received alone, and 7.3% of patients received enteral or parenteral or combination enteral-parenteral nutrition (12). Another study conducted in 2016 showed that 44% of patients receive nutritional support, and among the types of nutritional routes,

parenteral nutrition had a higher prevalence than other routes (13). Hiesmeyer et al. reported that 59% of patients received a regular hospital diet, 15% received an enriched diet, 2% received protein-energy supplements, and 9% received enteral or parenteral nutrition (14). There were contradictory studies related to the nutritional routs, which can be due to reasons including the strategy of diet therapy at the hospital level, the strategy of diet therapy at the national level, the socio-economic development conditions of the country, and the hospital level.

Several studies have shown inadequate nutritional intake among oncology patients. The prevalence of malnutrition among them was 30.9%. Oral food intake of oncology patients was often insufficient due to appetite and factors affecting it, such as nausea, vomiting, and change in sense of taste. A previous study (on behavior and dietary knowledge among inpatients in oncology wards) indicated that the daily energy intake is significantly lower than the energy goal. Further, hospitalized patients consumed only 65.3% of their daily energy requirement. Nausea, anorexia, vomiting, and change in taste were among the most critical factors affecting appetite, which accounted for 68% (15). Bye et al. (2016) conducted another study on changes in inflammatory biomarkers and energy intake in cancer patients. This study showed that the median energy intake of the patients was 26.2 kcal/Kg, which decreased to 23.7 kcal/Kg during the 12-month follow-up (16). Another study on food intake, readmission, and length of stay of oncology patients showed that only 9.1% of the patients received their energy needs by consuming the main meal. The patients who were at risk of malnutrition (MST≥2) consumed less energy compared with normal-nourished patients (17).

In the present study, oncology patients had the lowest body mass index. Low energy intake was observed in oncology patients, and low energy intake and low BMI can be caused by anorexia following illness and treatment.

Some studies have demonstrated macronutrient and micronutrient deficiency in burn patients. Khorasanchi et al. (2018) studied burn patients' nutritional needs and actual intake and demonstrated a significant difference between energy intake and required energy (18). Another study was conducted in 2022 on the relationship between biochemical indicators and food intake with wound healing in burn patients and showed that energy intake is much less than the recommended energy goal. This same applies to protein intake, as 87% of patients didn't receive their required energy and protein (19).

Like other studies, the present study showed that energy intake was much less than the energy goal in burn patients. In burn patients, the energy goal increases due to increased catabolism and the nature of diseases.

Despite numerous screening tools, patients have had insufficient food intake during the last few decades. Inadequate food intake leads to malnutrition and leads to adverse outcomes, including increased length of hospital stay and mortality. Screening at the beginning of admission to identify patients needing medical nutritional therapy may improve patient outcomes (20, 21).

Conclusion

Based on the results, burn and oncology patients did not consume their energy goal, indicating the importance of nutritional care in these patients. Moreover, oral and enriched nutrition strategies and ONS were more common in burn patients.

Declarations

Conflict of Interest

The authors declare no conflict of interest.

Funding

This work was founded by Mashhad University of Medical Science (MUMS), Iran (Project Number: 4020935)

Ethical Approval

This study was conducted following the guidelines laid down in the Helsinki Declaration. The Research Ethics Committee of Mashhad University of Medical Sciences approved all procedures on human volunteers (IR.MUMS.MEDICAL.REC.1402.371).

Limitation and Strength

This study used a standard questionnaire to evaluate the patient's nutritional status and food intake. Moreover, the questionnaires were completed by experts or nutrition students. The trend report during consecutive years helps to provide better nutritional care.

The limitation of the present study is the use of a cross-sectional study design. In addition, the total number of non-COVID-19 patients who

could be evaluated was around 50 due to the COVID-19 pandemic 2020.

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JOURNAL OF NUTRITION FASTING AND HEALTH

Evaluation of Sausage Products Properties by Chemical, Microbial, and Histological Techniques in Qom Province, Iran

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ABSTRACT

Introduction: Sausages consist of principal processed meat products, including meat and other additives, making special texture and flavor. Consumption of these products has increased nowadays. This study aimed to evaluate the chemical, microbial, and histological properties of sausage products manufactured and distributed in Qom province, Iran.

Methods: In this study, 100 samples of varied types of sausage products were randomly collected from Oct-2021 to Jun-2022. Chemical, microbial, and histological techniques were applied to analyze samples. All measurements were implemented in triplicate, and the data were analyzed by SPSS software version 25.

Results: The highest amounts of total fat, sodium nitrite, and starch were observed in 40-50% of meat sausages and total protein and phosphate in 60-70%. *Salmonella spp. and Escherichia coli*, as dangerous pathogens, were not detected in collected samples. Total Bacterial Count, Coliform, *Clostridium perfringens*, Yeast and Mold were lower than the acceptable limit of the Iranian National Standard Organization (INSO 2303: 2021) ($p<0.05$). The histological techniques showed that lymphatic, skin, peritoneal fat and plant tissue, trachea, bone, and hyaline cartilage were used to produce sausage samples with no significant statistical differences ($p<0.05$).

Conclusion: The microbial properties of samples indicated suitable hygienic conditions of production and distribution. There was a strong consequence of dramatic variation in the processing and manufacturing conditions of collected sausage samples, due to the high standard deviation value in the chemical, microbial, and histological analysis results. However, the overall quality of production and distribution of analyzed sausage products were suitable.

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Abbreviations

AOAC=Association of Official Analytical Chemists
BPA=Baird-Parker Agar
MUG=MacConkey sorbitol agar-MU
PCA=Plate Count Agar
RVB=Rappaport-Vassiliadis Broth
SPS=Sulfite Polymyxin Sulfadiazine
TBARS=Thiobarbituric Acid Reactive Substances
TSB=Trypticase Soy Broth
TVBN=Total Volatile Basic Nitrogen
XLD=Xylose Lysine Deoxycholate

Introduction

Meat and meat products contain many minerals and nutrients recommended as daily intakes, but consuming these products should be associated with some nutritional problems. The nutritional value of sausages has changed due to the variation in meat composition, ingredients, and processing characteristics (1). Generally, meat products are manufactured from different raw materials, producing varied chemical, microbial, and sensory properties. The main composition of meat products associated with used raw materials includes protein, pigment, fat, and water contents (2). Sausage products are a varied group of meat products made from various meat species, including beef, buffalo, chicken, pork, and fish, with different spice formulations.

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Protein functionality is a critical characteristic in meat processing, directly related to the meat composition of raw material (3). In addition to safety and nutritional benefits, other characteristics of meat products consumers consider are sensory, price, and convenience properties (4). There should be more about appropriate safe and standard manufacturing strategies to produce healthier meat products such as sausages. Suitable quality properties of final sausage products, including chemical, microbial, texture, and sensory properties, are evidence of good manufacturing process, shelf life, and safety characteristics (5).

Many studies have demonstrated the hazards of sausage products for the consumer. The most important pathogens that have been detected in sausage products were *Clostridium botulinum, Clostridium perfringens, Listeria monocytogenes, Escherichia coli,* and *Salmonella typhimurium.* However, the products' salt, nitrite, thermal processing, and dryness significantly lead to antibacterial effects. In addition to foodborne pathogens, spoilage micro-organisms cause problems in consuming these products. Raw material properties, processing characteristics, and hygienic conditions of manufacturing are the key factors for sausage production with high-level safety. Furthermore, sensory attributes are essential for consumer acceptability (6).

Various factors affect the sensory properties of sausage products. Fat, water contents, and raw meat quality directly affect these products' texture and rheological properties (7). Since sausage products primarily comprise raw meat, high-quality meat without unauthorized tissue is essential. Because of the high price of meat in sausage production, manufacturers tend to use unauthorized tissue instead of meat in the formulation of these products.Animals' skin, fat, bone, and hyaline cartilage usually are used as unauthorized tissue in sausage manufacturing. Histological methods have been employed by researchers to detect unauthorized tissues in sausage products (8). Previous studies have investigated the histological methods to detect unauthorized tissues in the texture of sausage manufactured by Iranian producers (9), but their findings are controversial. This study aimed to evaluate the chemical, microbial, and histological findings of unauthorized tissue of sausage products manufactured and distributed in Qom province, Iran.

Material and Methods

Sampling Procedure

In this study, 100 samples of varied types of manufactured sausage were collected from markets located in different parts of Qom province, Iran. Samples were immediately transported by icebag at 4˚C to the laboratory for chemical, microbial, and histological analysis after coding (9).

Chemical Analysis

Chemical characteristics, including moisture, fat, protein, starch, ash, phosphate, and nitrite contents, were determined through the Gravimetric, Soxhlet, Kejldahl, Fehling, Dry, and Spectrophotometric methods, respectively, by AOAC (2005). All measurements were implemented in triplicate. Chemical characteristics including moisture, fat, protein, starch, ash, phosphate, and nitrite contents were determined by the methods suggested by AOAC (2005), (10). All measurements were implemented in triplicate.

Microbial analysis

About 10g of each sample was subjected to a homogenizer (IUL Instruments, Barcelona, Spain) and diluted with 90ml of a sterile solution of 0.1% (w/v) peptone water, 0.85% NaCl and 1% tween 80 as an emulsifier (Merck, Germany). Thus, ten-fold serial dilution was obtained by mixing 1m1 of a homogenized sample with a 9ml sterile solution of 0.1% peptone water. *Total Bacterial Count*, *Molds and yeasts*, and *Staphylococcus aureus* were determined using plate count agar: 37˚C/24-48h (PCA, United Kingdom), Baird-parker agar; 37˚C/24h (BPA, Merck, Germany) and Malt Extract Agar (Merck, Germany); 25˚C/5 days, respectively. Salmonella was detected using the method described below. Pre-enrichment media was done by Lactose Broth (25g of each sample added to LB)incubated at 37˚C/20h. Then, selective enrichment was performed by transferring 0.1ml of pre-enriched incubated media into the Rappaport-Vassiliadis broth (RVB, Oxoid) incubated at 42˚C/24h. Finally, enriched samples were streaked on Xylose Lysine Deoxycholate agar (XLD agar) (Merck, Germany) and incubated at 37˚C/48h. Lysine agar, Triple Sugar Iron agar as differential media, and Urea broth were used as complement media (37˚C/24h). Clostridia colonies were detected by inoculating 10ml of the sample dilution into 20ml SPS Agar (Sulfite Polymyxin Sulfadiazine) (Merck, Germany) tubes and

overlaying with 2ml of paraffin oil to provide anaerobic conditions. Colonies were grown in violet-red bile Agar (Merck, Germany), which was used to enumerate Coliforms via incubation at 37˚C/24h. Each sample was first enriched in Trypticase Soy Broth (TSB, Merck, Germany) in shaker incubator at 37˚C/18h to detect Escherichia coli. Then, the samples were linearly cultured on MacConkey sorbitol agar-MUG (MUG, Sigma-Chemical, USA) at 37˚C/24h. All experiments were implemented in triplicate (11).

Histological properties

Three similar parts were separated from each sample and then divided into four pieces for tissue analysis. The pieces were fixed in a 10% buffered formalin solution for light microscopy processing and then embedded in paraffin blocks. The blocks embedded with paraffin and processed samples were cut into pieces with a diameter of 6µm and stained using Hematoxylin and eosin (Vector, USA). An optic microscope (N-180 M, Novel, China) (MD-130, OME-TOP, Taiwan) was used to examine slides and detect unauthorized tissue in sausage samples. Then, the images were processed using Photoshop software CS (Adobe system, CA, USA). All measurements were carried out in triplicate (9).

Statistical Analysis

All analyses were performed in triplicate. One-way Analysis of Variance (ANOVA) was used to determine the difference ($P<0.05$) between treatments, and the contrast between means (Duncan`s multiple range test for chemical and microbial analysis) was used to assess the differences between the variables. Statistical analyses were conducted using SPSS software version 22 for Windows (Chicago, USA).

Table 1. Chemical properties of sausage samples manufactured and collected from Qom province, Iran

Meat content (%)	Chemical Properties						
	Total Moisture (%)	Total Fat (%)	Total Protein (%)	Starch (%)	Total Ash (%)^{NS}	Phosphate (%)	Sodium Nitrite (mg/kg)
40 – 50	54.31 ± 1.33^{a}	21.97 ± 1.54^{c}	11.24 ± 1.00^{a}	8.60 ± 0.65^{b}	1.97 ± 0.45^{b}	0.28 ± 0.12^{b}	44.75 ± 22.48^{b}
50 – 60	59.08 ± 4.21^{a}	16.96 ± 4.16^{b}	14.35 ± 2.47^{a}	5.30 ± 0.56^{a}	2.01 ± 0.46^{b}	0.29 ± 0.15^{b}	37.84 ± 26.58^{a}
60 – 70	61.68 ± 4.07^{a}	14.60 ± 2.71^{b}	18.30 ± 1.27^{b}	3.75 ± 1.76^{a}	1.83 ± 0.47^{a}	0.41 ± 0.13^{a}	32.87 ± 09.23^{a}
80 – 90	68.34 ± 4.24^{b}	9.17 ± 3.37^{a}	17.36 ± 1.04^{b}	3.17 ± 1.51^{a}	1.42 ± 0.28^{a}	0.37 ± 0.18^{a}	34.18 ± 15.68^{a}

Different letters between groups in each column denote significant differences ($p<0.05$).
^{NS} Not Significant

Results

Chemical Analysis of Sausage Samples

The chemical properties of sausage products with different meat content collected from different areas of Qom province are demonstrated in Table 1. These results showed that the total moisture content in group 89–90% meat content was higher than other groups ($p<0.05$). In addition, the nitrite and starch content of sausage samples included in the 40–50% meat content group was higher ($p<0.05$). The maximum fat content included 21.97% for the 40-50% meat content group ($p<0.05$). Further, the percent of total ash, phosphate, and protein were higher in the (50–60 and 60–70%) meat content group, respectively.

Table 2. Microbiological quality of sausage products produced and collected from Qom province, Iran

Microorganism	Mean	SD[a]
Total bacterial count (*Log CFU/g*)	+3.701	+2.621
***Coliform* (*Log CFU/g*)**	+2.155	+1.025
***E. coli* (*Log CFU/g*)**	0	0
***Salmonella* (*Log CFU/25 g*)**	0	0
***S. aureus* (*Log CFU/g*)**	+0.696	+0.060
***Clostridium perfringenes* (*Log CFU/g*)**	+0.954	+0.146
Yeast and Molds (*Log CFU/g*)	+1.568	+0.318

[a] SD = Standard Deviation

Microbial Analysis of Sausage Samples

The microbial properties of sausage product samples manufactured and collected from different areas in Qom province, Iran, are summarized in Table 2. *Salmonella* and *Escherichia coli* (E. coli) were not detected, but (*Log CFU/g*) (Mean±SD) of *Total Bacterial Count*, *Coliform*, S. aureus, *Clostridium perfringenes*, *Yeast, and Molds* were 3.701±2.621, 2.155±1.025, 0.696±0.060, 0.954±0.146 and 1.568±0.318, respectively.

Table 3. Histological properties of sausage samples collected from Qom province, Iran

Histological parameter	Mean (%)	SD[a]
Abdominal Cavity Tissue	0	0
Genitourinary Tissue	0	0
Nervous Tissue	0	0
Lymphatic Tissue	0.01	0.103
Skin Tissue	0.19	0.396
Peritoneal Fat Tissue	0	0
Bone and Hyaline Cartilage	0.16	0.368
Plant Tissue	0.02	0.145
Trachea	0.03	0.177
Breast tissue	0	0
Chicken Gizzard Tissue	0	0
Face Meat	0	0
Spleen Tissue	0	0
Lung Tissue	0	0
Liver Tissue	0	0

[a] SD = Standard Deviation

Histological Study for Detecting Unauthorized Tissue in Sausage Samples

Histological parameters of sausage samples are demonstrated in Table 3, revealing that collected samples detected lymphatic, skin, peritoneal fat, plant tissue, trachea, bone, and hyaline cartilage. The non-standard raw materials as unpermitted tissue, such as skin tissue (A), plant tissue (B), adipose tissue (C), connective tissue (D), and soy protein, are shown in Figure 1.

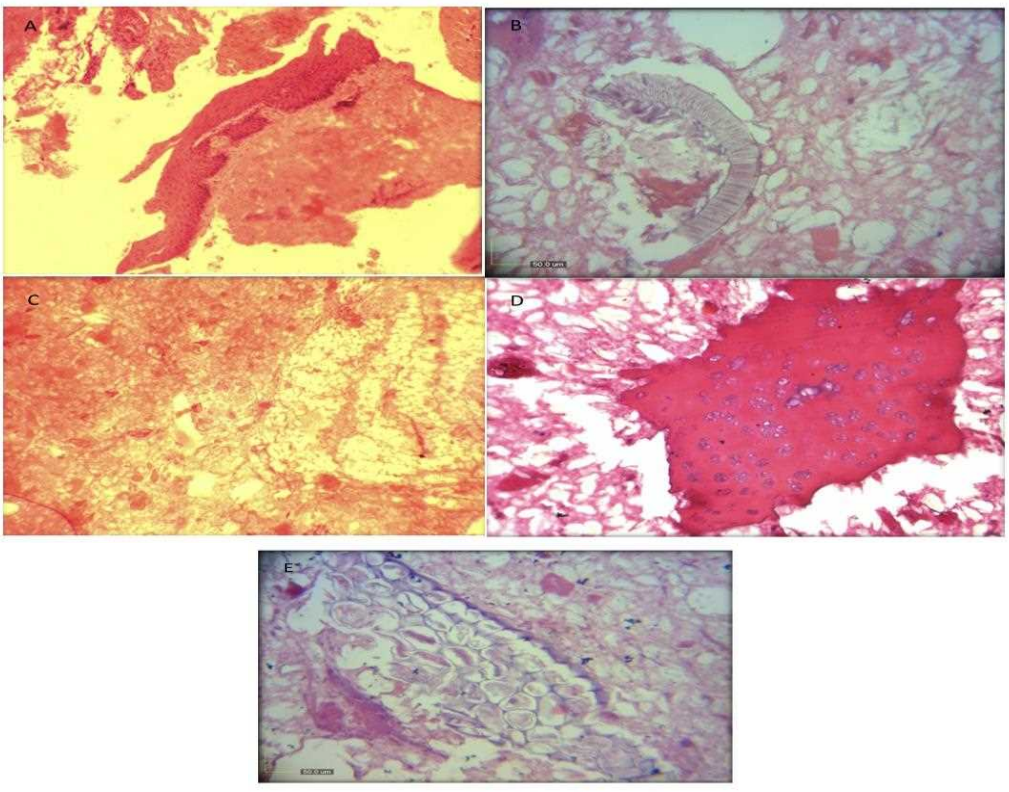

Figure 1. Microscopic image of skin tissue (A), plant tissue (B), adipose tissue (C), connective tissue (D) and soy protein (E) in sausage sample detected by histological method.

Discussion

Chemical Properties

Table 1 shows the chemical properties of sausage products collected from different regions of Qom province, Iran, with varying meat contents. The results showed that the total moisture in the 80-90% meat content group increased, and the amount of nitrite and starch decreased. In the 40-50% meat group, the amount of nitrite and starch was higher than in other groups, and the moisture content was also reduced ($p<0.05$). The results obtained for total protein indicate that the total moisture of sausage products increased as meat content increased and starch and nitrite contents decreased. Alamin et al. (2015) reported more than 70% moisture content in sausage samples manufactured by goat, camel, and beef meat (12). Moreover, there was a significant positive correlation between the meat and moisture contents of the samples. A maximum fat content of 21.97% was detected for the 40-50% meat content group, but Alamin et al. (2015) reported 2.31–3.45% fat content for sausage samples, which was lower than those of the present study (12). A higher fat content was observed in the present study, which decreases the nutritional value of meat products. Mallika and Prabhakar (2011) investigated the chemical characteristics of low-fat pork sausage. A lower fat content was found in produced samples in comparison with controls. In addition, the moisture content reported for low-fat pork sausage was significantly higher than our findings. Lower fat content promotes consumers' health and decreases cardiovascular diseases. The type of meat used to manufacture sausage products gives rise to variations in the fat content of final products that must be balanced before the formulation and processing. This high variation was observed due to different processing conditions and properties of employed meat in the formulation (13).

Zohdi et al. (2021) compared the safety and quality of high-priced (premium) and low-priced (economic) meat products. A total of 200 samples collected from the various grades of Egyptian beef luncheon sausage and burger patties commonly distributed in Egyptian markets were tested. All examined beef samples included the fatty acids profiles of chicken and buffalo meat. Moreover, the results revealed that the samples that exceeded the permissible limits of TBARS and TVBN values were more premium than those of economic products for luncheon and burger patties. Conversely, the economic luncheon indicated a higher percentage of samples that exceeded the permissible limit of residual nitrite compared to the premium one (14).

Microbial Properties

The microbial properties of sausage product samples manufactured and collected from different areas in Qom province, Iran, are summarized in Table 2. As shown in Table 2, yeast, moulds, and coliform counts were higher than in other studies. In addition, *Salmonella* and *E. coli* as dangerous pathogens were not detected in collected samples, indicating the suitable safe condition for consumption of these products. In the present study, *Staphylococcus aureus* (*S.aureus*) 0.696 *Log CFU/g* was lower than Drosinos et al. (2005). This study focuses on 2 *Log CFU/g* of *S.aureus* in traditional Greek fermented sausage products. In addition, 3.79 *Log CFU/g E. coli* were observed in sausage samples, indicating the low hygienic condition of the manufacturing process. The growth of other bacteria and lactic acid bacteria prevents increasing coliform and *E. coli* in meat products (15). Sohrabi et al. (2020) checked 101 samples of bovine minced meat (Group 1) and ready-to-cook meat products (Group 2) were collected from supermarkets in Turin, Italy. A higher presence of bacteria and inflammatory cells was detected in Group 1. Bacterial strains associated with inflammatory cells were detected with a higher score in Group 2. *Sarcocystis spp.* was present in 83.3% of Group 1 samples and 49.1% of Group 2 (16). In another study, more than 1.5 *Log CFU/g S.aureus* was found in processed buffalo sausage samples by Sachindra et al. (2005), indicating lower hygienic conditions of processing. In addition, 4–5 Log *CFU/g* coliforms were found in samples, while 2-3 *Log CFU/g Coliforms* should be attributed to employing higher microbial quality raw meat for the product's formulation. There is no considerable difference between yeast and mold counting of samples in this and the present study. The lower quality of raw materials should be attributed to increased *yeast, moulds*, and *Coliform* counting in meat-processed products such as sausages (17). Findings demonstrated that raw materials and the processing conditions of sausage products manufactured and

contributed to in Qom province, Iran, were at a suitable and standard level, considering the lack of salmonella detection in any collected sample.

Histological Findings

Histological techniques can be a simple, fast, economical, decisive, and conclusive tool for the quality control of some foodstuffs (18). Histologically, the meat comprises skeletal striated muscle fibers, adipose, water, and connective tissue. The histological examination allows the identification of tissue structures in meat products and, to a certain extent, the presence of unauthorized plant and animal ingredients. For this reason, the histological examination was adopted in some developed countries as a complementary method of assessing the integrity of the products. The histological examination provides concrete images regarding the tissues' morphological integrity in the meat's composition (19). The histological parameters of sausage samples are presented in Table 3. The collected samples detected Lymphatic, skin, peritoneal fat, plant tissue, trachea, bone, and hyaline cartilage. Izadi et al. (2016) investigated fraud in minced and processed meat by histological analysis, and they successfully implemented this method to detect unauthorized tissues (20). Latorre et al. (2015) analyzed unauthorized tissue in Kabab loghmeh, Kabab koobideh, handmade hamburgers, and sausage samples. In addition, plant tissue (soya), hyaline cartilage, and lymph nodes were also found in sausage product samples collected from different areas in Yazd province, Iran (9). Unauthorized tissue in formulating sausage and meat products indicated a lack of standard and hygienic conditions in food manufacturing regulation, leading to lower quality for consumers (21). The non-standard raw materials as unpermitted tissue are shown in Figure 1. Latorre et al. reported that histological methods and microscopic examination are valuable procedures for detecting unpermitted tissue in meat products (9). Malakauskiene et al. (2016) reported blood vessels, adipose, and connective tissues in sausage samples collected from Kaunas, Lithuania, by histological analysis. In addition, histological methods were introduced to detect unauthorized tissue in processed meat products (22).

Moghtaderi et al. (2019) applied histological methods to detect unauthorized tissues in the Iranian sausage samples, such as skeletal muscle fiber (100%), fat tissue (100%), and plant material (97.70%). A wide range of unauthorized tissues was detected, including dense connective tissue (6.66%), cartilage (28.30%), bone (8.30%), skin (51.60%), smooth muscle (1.66%), and blood vessels (11.66%). The results confirmed that the histological methods with Masson's trichrome staining are practical techniques for routine assessment of the authenticity and quality of sausage to protect consumers from adulteration (23).

In another study by Maghami et al. (2022), 34 samples of minced meat, hamburger, and sausage were randomly collected from the markets in northeast Iran. The results showed a distinctive difference in meat percentage compared to the labeled rate. Skeletal and smooth muscles, blood vessels, nerves, gizzard, adipose tissue, glandular tissue, cartilage, bone, tendon, skin, lymphatic tissues, and plant materials were observed (24).

This study showed that histological methods are helpful for the detection of unauthorized tissue in sausage products. However, the hygienic and safe sausage products consumed in Qom province, Iran, at an appropriate level, but the variation in production and distribution of these products were observed in higher values by other researchers.

Conclusion

The dramatic variation observed in results obtained for chemical, microbial, and histological analysis indicated various manufacturing and distribution conditions for these products in Qom province, Iran. The microbial characteristics of the samples can be due to the lower quality of raw materials and improper time-temperature conditions during distribution and storage in supermarkets. Histological parameters of sausage samples showed that applying unauthorized tissue in the formulation of sausage products can cause food loss of nutritional value. The chemical properties did not show a specific change in the amount of protein, fat, moisture, and carbohydrates, and other chemical tests, such as TVN, TBARS, and peroxide, are recommended to show spoilage. Therefore, health surveillance organizations should perform more accurate monitoring based on histological methods and chemical and microbial properties to prevent fraud, and new methods such as PCR should be recommended.

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JOURNAL OF NUTRITION FASTING AND HEALTH

Air Pollution and Nutrition

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Dear Editor

Air pollution is the fourth leading cause of premature death after hypertension, smoking and malnutrition and is the second leading cause of death from non-communicable diseases after smoking (1-3).

Increased air pollution can increase the vulnerability of the respiratory system, and poor air quality is a serious risk for acute and chronic respiratory diseases and cardiovascular disease. Also Exposure to air pollution causes underlying diseases such as high blood pressure, diabetes, cardiovascular and respiratory diseases, and as a result these underlying diseases increase the vulnerability to Coronavirus disease 2019 (4).

People with the underlying disease and living in contaminated areas are at higher risk for coronary heart disease, and studies show that air pollution is a contributing factor to diseases such as the flu and covid-19. Airborne particles increase Coronavirus disease 2019 mortality by up to 6%, ozone up to 7% and nitrogen dioxide up to 15%. When different dust particles increase in the air and the virus spreads in the air; For example, The virus has more chance to spread to other people when talking, coughing and sneezing(5).

Polluted air usually consists of carbon monoxide, sulfur dioxide, nitrogenous oxides, unburned hydrocarbons, fine suspended particles, lead bromide and other lead compounds. Although polluted air can be dangerous for everyone, children, the elderly, pregnant mothers, and heart and lung patients are more at risk. It is better to follow a number of nutritional tips that play an important role in reducing the effects of air pollution (6).

Eating fresh fruits and vegetables and consuming less fast food and prepared foods can reduce the harmful health effects of air pollution to some extent. One of the fruits that is recommended to be eaten during times of increased air pollution is apple. Apples are rich in pectin. Pectin is an anti-toxin and absorbs lead and prevents damage to body cells. Of course, oranges and strawberries, lemons, grapefruits, carrots and whole grains also have this property (7).

Consumption of food sources of vitamin E and vitamin D can neutralize the toxic effects of polluting gases. Vitamin E is found in vegetable oils, walnuts, almonds, olives, and wheat germ, liver and green leafy vegetables. Suitable sources of vitamin D are milk, butter and egg yolks, which should be consumed more on days of air pollution. In fact, milk and dairy products, especially cheese and yogurt, can reduce the absorption of heavy elements such as lead due to the calcium they contain. Milk enriched with bacteria called probiotics balances the level of beneficial bacteria in the human intestine. Vegetables such as cantaloupe, mango, pumpkin, pepper, spinach, cabbage and apricot have beta-carotene. Beta-carotene is also one of the important carotenoids and the precursor of vitamin A, which is converted into vitamin A in the body, and includes yellow and orange fruits and vegetables. These fruits and vegetables have high beta-carotene, which helps the body to reduce the harmful effects of fine dust. Selenium is necessary for the functioning of enzymes in the body that can deal with bad and damaging

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environmental conditions. This nutrient can be obtained through food sources such as fish, red meat, grains, legumes, eggs, chicken, and garlic.
In the conditions of air pollution, it is recommended to eat foods that contain selenium and beta-carotene antioxidants. (5).
The consumption of more antioxidants provides a basis for the body to easily eliminate harmful free radicals. Foods that contain vitamin E and C are a rich source of antioxidants. On the other hand, vitamin C can help eliminate lead that enters our body with polluted air. Citrus fruits, green peppers, broccoli, cauliflower, kale, tomatoes, green leafy vegetables, spinach, kiwi, cantaloupe and strawberries have a lot of vitamin C. Of course, it is better to eat these foods raw and fresh (4).
Green tea or pomegranate juice are suitable for days of increased air pollution, green tea is rich in vitamin C and antioxidants. Pomegranate juice can also remove toxic substances from the body. Oily fish, which are rich in omega-3 fatty acids, reduce shortness of breath and the harmful effects of inflammatory compounds (6).
When faced with air pollution, it is necessary to take measures to deal with the adverse effects of this condition. One of these actions that can have positive and useful effects in dealing with this condition is the importance of proper nutrition in polluted air.

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