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The Malnutrition Frequency Trend in Imam Reza Teaching Hospital: Results from Nutrition Day 2019-2021

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ABSTRACT

Introduction: Malnutrition is a global health issue affecting nearly 40% of the population. Hospital malnutrition is a severe medical problem, which is often overlooked and has negative effects on both patients' health and the economy. In addition, malnutrition increases the risk of clinical complications and mortality. On Nutrition Day (nDay), patients in hospital wards and nursing homes worldwide participate in a cross-sectional survey using a standard questionnaire to assess their nutritional status. This study aimed to investigate the prevalence of malnutrition among patients at Imam Reza Teaching Hospital from 2019 to 2021.

Method: This study analyzed nDay data from Mashhad's Imam Reza Teaching Hospital from 2019 to 2021. The study included demographic and nutritional data from patients in fourteen different wards/units of the hospital. Over the past three years, an overview of malnutrition trends was presented by examining patient-reported responses to questions about malnutrition and its risk factors.

Results: The frequency of malnutrition in Burn units was the highest at 37.5% in the male unit in 2020 (P-value: <0.0001). The lowest BMI was observed in Oncology patients, averaging 18.9±2.4 in 2019. The most significant decrease in nutritional intake occurred in the Gastroenterology department, at 37.5% in 2019. The most significant change in hospital nutrition intake one week before admission was in the General Surgery ward, with a 47.4% decrease in 2019.

Conclusion: The significance of malnutrition, particularly in hospitals, could assist the health system in addressing this issue by using nDay as a standard questionnaire and screening tool. This study indicated that Burn units, Gastroenterology, Oncology, and General Surgery departments are the most susceptible to malnutrition and thus require increased attention.

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Introduction

Malnutrition, a critical global health challenge, has been approached from two distinct angles. Initially, attention was directed toward undernutrition, food security, and micronutrient deficiencies, while the focus later shifted to obesity, overweight issues, and excessive dietary intake (1). This multifaceted problem transcends national boundaries, affecting countries across the development spectrum (2). Prevalence rates of malnutrition vary significantly among communities, ranging from as low as 0.8% to as high as 40% (3). Given the growing exposure of individuals to both forms of malnutrition at different life stages, it is essential to identify risk factors (4). Various determinants contribute to malnutrition, including diminished appetite, eating difficulties, respiratory conditions, and gastrointestinal disorders. Furthermore, socioeconomic status, social isolation, advancing age, and physical limitations can also play a role (5, 6). Investigating hospital malnutrition is crucial, given that hospitalized patients are particularly vulnerable to these risk factors (7).

The pervasive issue of malnutrition within hospital settings is a significant medical concern that is often overlooked, yet it profoundly affects both patient health and the broader economic

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landscape. Malnutrition has been correlated with a surge in clinical complications, mortality rates, hospital admissions, and readmissions, alongside escalated healthcare expenditures (8). A comprehensive approach integrating robust medical treatment with intensive nutritional interventions is imperative to address hospital malnutrition (9) effectively. In certain instances, hospital malnutrition may stem from underlying medical conditions. Additionally, inadequate monitoring, identification, and assessment procedures within hospital environments may contribute to malnutrition occurrences. Furthermore, some healthcare professionals lack sufficient training to identify individuals at risk of malnutrition (9, 10). Over recent decades, several tools have been devised to detect malnutrition. Examples include the Mini Nutritional Assessment (MNA, 2009), the Malnutrition Universal Screening Tool (MUST, 2003), the Subjective Global Assessment (SGA, 1987), the Nutritional Risk Screening (NRS, 2002), and the Malnutrition Screening Tool (MST, 1999), for malnutrition assessment (11). Nonetheless, most screening tools are tailored to specific demographics, such as patients in healthcare settings or particular communities (12). A straightforward screening test for nutritional risk is utilized by participants of the nDay initiative, enabling the evaluation of various hospitals worldwide and their departments over time (10).

Nutrition Day (nDay) is an annual event during which hospital wards and nursing homes undergo cross-sectional audits for a single day. The nDay initiative has collected data from various wards and units supported by ESPEN (European Society for Clinical Nutrition and Metabolism) since its inception in 2006. Local unit staff use a standardized questionnaire to gather and input data into the nDay database. Globally, Nutrition Day aims to enhance awareness and understanding of malnutrition within the healthcare system and improve overall nutrition care quality (10, 13).

Mashhad University of Medical Science joined for the first time in 2010 and continued participating until 2023 as part of the Nutrition Day project. (14). This study aims to evaluate trends in malnutrition prevalence and associated factors observed from 2019 to 2021 at Imam Reza Teaching Hospital.

Materials & Methods

The data for this cross-sectional study was obtained from the nDay database at Imam Reza Teaching Hospital in Mashhad, Iran, from 2019 to 2021. All patients provided consent for their participation in the study. Throughout the study, the standardized protocol was followed, and a specific questionnaire was tailored to nDay (10). Various factors were considered during the data analysis, including 1) frequency of malnutrition, 2) frequency of being at risk for malnutrition, 3) body Mass Index (BMI), 4) weight loss within the past three months, 5) reduced food intake in the week before admission, 6) changes in food intake since admission, and 7) the amount of food consumed by patients on nDay. The data related to nutritional status were utilized to analyze malnutrition frequency trends.

The data were analyzed using SPSS (Version 19, Chicago, IL, USA). The Kolmogorov-Smirnov test showed that all variables had a normal distribution. Descriptive analyses were used to report variable frequencies. PRISM software was used to create charts illustrating trends in malnutrition frequency from 2019 to 2021, providing a comprehensive understanding of the issue.

The project was approved by the Mashhad University of Medical Sciences Ethical Committee (Code: 4020808).

Results

Demographic Findings

A total of 414 adult patients from 14 units within Imam Reza Teaching Hospital in Mashhad participated in the study.

In 2019, 14 wards contributed to the survey, covering Infection, Oncology, General Medicine, Rheumatology, Pulmonary, Gastroenterology, Internal Medicine, Dermatology, Burns male, Burns female, General Surgery, Orthopedic Surgery, Gynecological Surgery, and Cardiac Surgery. However, participation was limited due to the COVID-19 pandemic in 2020, with only five wards involved: Burns male, Burns female, General Surgery, Orthopedic Surgery, and Gynecological Surgery. In 2021, ten wards joined the study, including Infection, Oncology, Rheumatology, Gastroenterology, Internal Medicine, Burns male, General Surgery, Orthopedic Surgery, Gynecological Surgery, and Cardiac Surgery. Table 1 presents the frequency of malnutrition and at-risk cases in different units between 2019 and 2021.

The Burns male hospital unit exhibited the highest proportion of malnourished patients, nearly 40% in 2020. In 2019, the Pulmonary ward closely trailed the Burns male unit 2021, rated at 30.8% and 30%, respectively. However, in 2019, the Burns female unit recorded the highest percentage of patients at risk of malnutrition (100%), followed by Gynecological Surgery (53%) and the Burns male unit (50%) in 2020.

Table 1. Frequency of malnutrition and at-risk cases for malnutrition

Unit Name	2019 N (%)		2020		2021		P-value malnutrition	P-value At Risk
	Malnourished	At Risk	Malnourished	At Risk	Malnourished	At Risk		
Infection	-		-		-	-		
Oncology	2 (22.2%)	4(44.4%)			2(8.3%)	7(29.2%)	0.00*	0.02*
General Medicine	-	3(15.8%)			1 (4.5%)	8(36.4%)		0.00*
Rheumatology	1 (10.0%)	3(30.0%)			-	-		
Pulmonary	4 (30.8%)	1 (7.7%)						
Gastroenterology	2 (11.8%)	4(23.5%)			2(13.3%)	3(20.0%)	0.41	0.60
Internal Medicine	2 (16.7%)	1 (8.3%)				9(47.4%)	< 0.0001*	
Dermatology		1(12.5%)						
Surgery								
Burns (Female)		10(100%)	1 (12.5%)	2(25.0%)				< 0.0001*
Burns (Male)	1 (11.1%)	4(44.4%)	3 (37.5%)	4(50.0%)	3 (30.0%)	1 (10.0%)	0.00*	< 0.0001*
General Surgery		1 (5.3%)	-	-	1 (4.2%)	10(41.7%)		< 0.0001*
Orthopedic surgery	2 (5.0%)	1 (2.5%)	1 (10.0%)	1(10.0%)	1 (3.1%)	-	0.09	0.01*
Gynecological Surgery			-	-	1 (6.3%)	-		
Cardiac Surgery		3 (42.9%)			-	-		

Table 2. BMI in different units between 2019-2021

Unit	BMI (kg/m²)		
	2019	2020	2021
Infection	24.7±6.2		24.9±4.8
Oncology	18.9±2.4		25.6±11.1
General Medicine	23.0±4.9		
Rheumatology	25.3±5.2		23.0±2.4
Pulmonary	25.7±11.3		
Gastroenterology	23.4±5.9		22.9±2.7
Internal Medicine	24.4±5.5		24.0±4.8
Dermatology	29.3±10.4		
Burns (Male)	19.9±2.5	22.8±6.1	25.3±5.2
Burns (Female)	25.6±6.2	23.1±4.8	
General Surgery	24.9±3.9	25.6±6.4	25.7±3.6
Orthopedic surgery	23.7±4.4	22.4±5.2	24.7±4.8
Gynecological Surgery	29.5±7.7	28.9±5.3	30.7±12.2
Cardiac Surgery	26.9±4.1		25.3±4.7

Conversely, the lowest prevalence of malnourished patients was observed in the surgery orthopedic unit in 2021, accounting for only 3%, followed by surgery general in 2021 at 4.2%. Figures 1 and 2, respectively, demonstrate the frequency of malnutrition and the risk for malnutrition in different wards between 2019 and 2021.

As shown in Table 2, the Oncology unit had the lowest BMI in 2019, with a median of 18.9±2. Following closely behind was Burns's male unit in 2019, with a BMI of 19.9±2.5. In contrast, Gynecological Surgery had the highest BMI among all units in 2021, with a BMI of 30.7±12.2.

Weight Loss in the Last Three Months

Table 3 indicates that Gastroenterology patients had the highest rate of unintentional weight loss among all units, at 76.5%. Internal Medicine followed this in 2021 at 68%, and Orthopedic Surgery in 2020 at 60%. Figure 3 illustrates the weight loss trend in the three months following admission.

Food intake a week before hospitalization

According to Table 4, 37.5% of patients admitted to the Gastroenterology unit in 2019 had consumed a quarter or almost none of their food, followed by 36.7% in General Surgery and 33.3% in Oncology. Figure 4 illustrates the trends in food intake before hospitalization from 2019 to 2021.

Food Intake in nDay

According to Table 5, General surgery had the lowest food intake in 2019 at 47.4%, almost none. Conversely, Rheumatology had nearly all their food intake in 2019 at 50%, Burns males in 2020 at 50%, and Rheumatology again in 2021 at 50% (the supplementary tables are at the end of this file). Figure 5 illustrates the trends in food intake during this period.

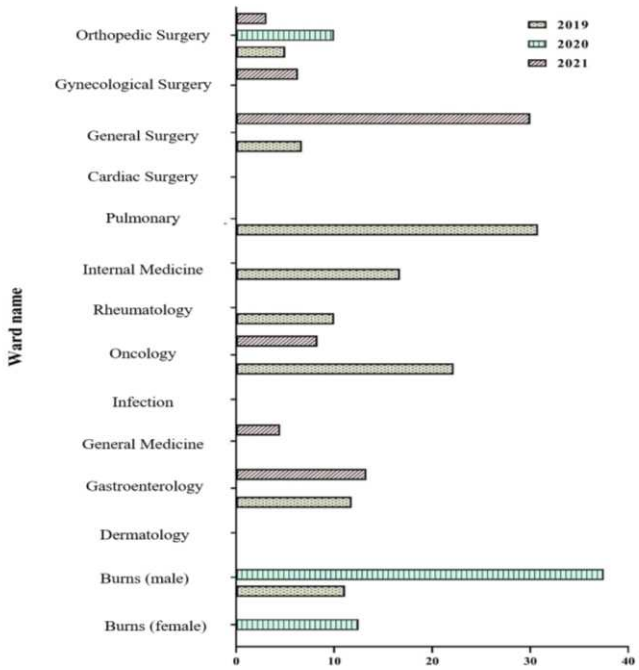

Figure 1. Trend in the frequency of malnutrition

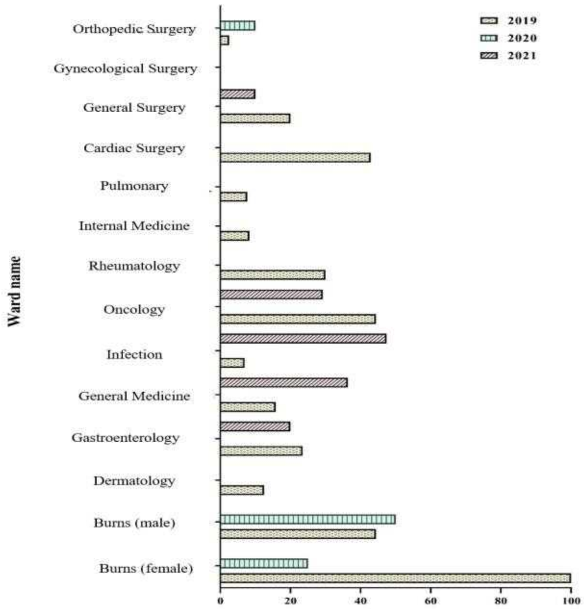

Figure 2. Trend of individuals at risk for malnutrition

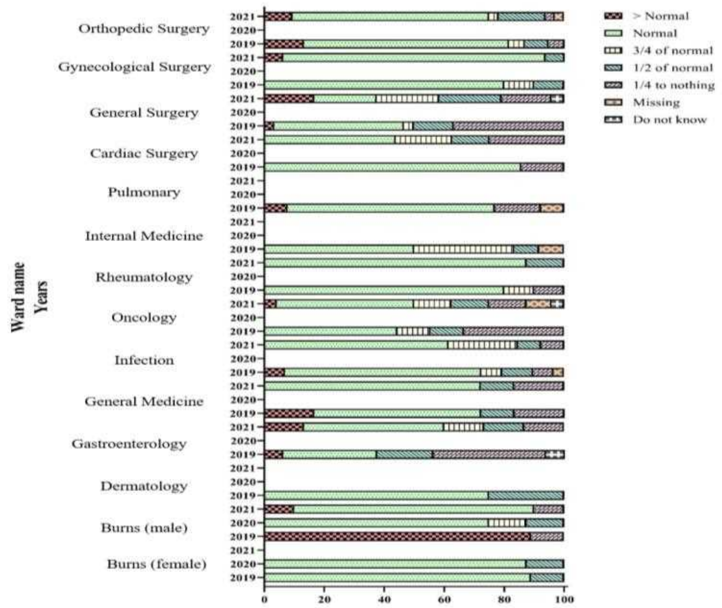

Figure 4. Trend of eating well before admission

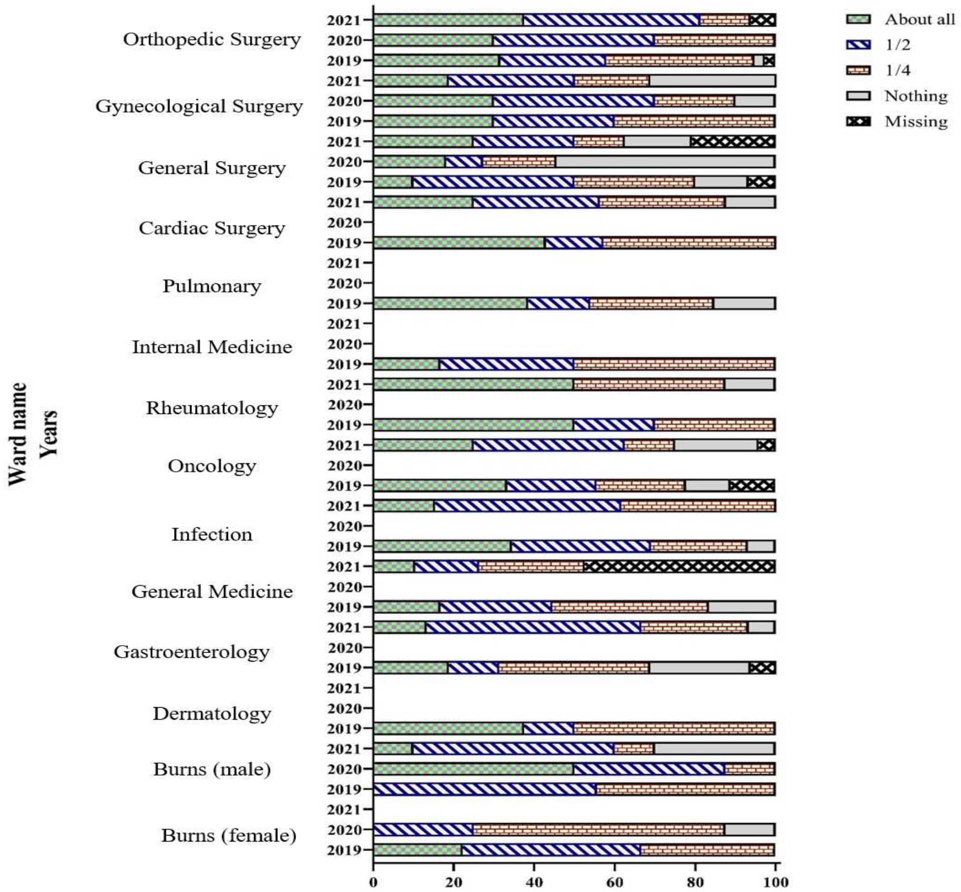

Figure 5. Trend of food intake on nDay

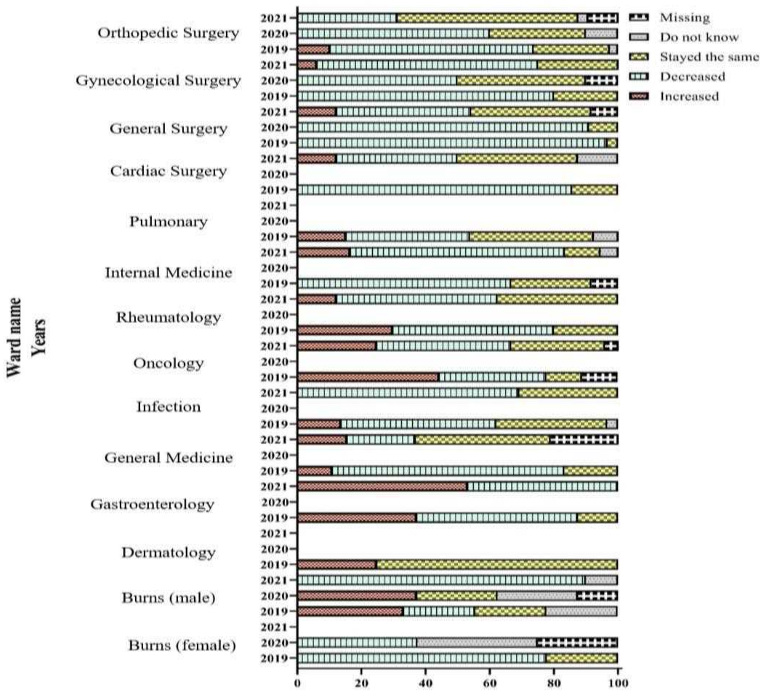

Figure 6. Trend of food intake in the hospital

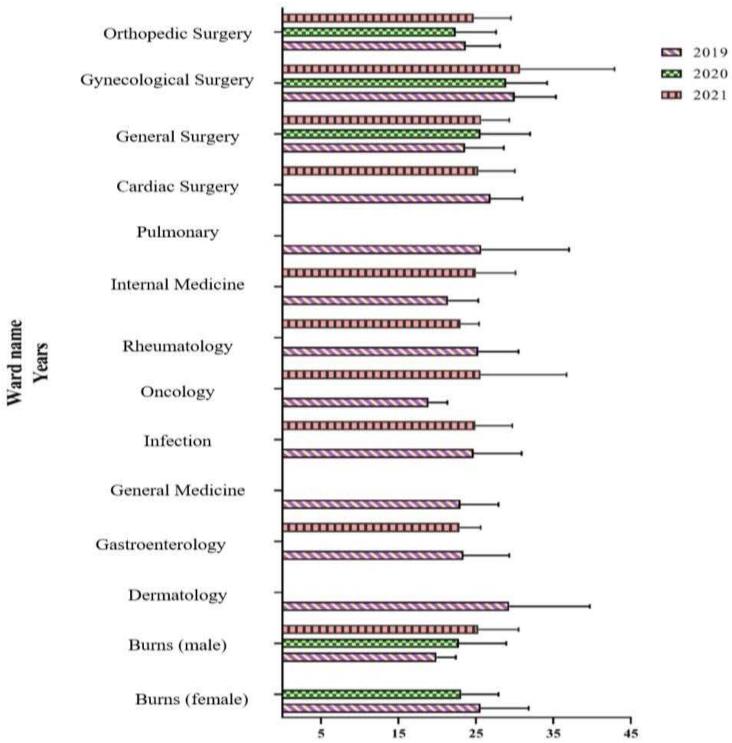

Figure 7. Trend of BMI (Body Mass Index) in various units

Food Intake Changes in the Hospital

As shown in Table 6, patients in the Burns male unit in 2021 experienced a 90% decrease in food intake, while patients in the Cardiac Surgery unit in 2019 had a 55% decrease. Oncology patients in 2019 saw a 44.4% decrease, while those in the Burns male unit in 2020 and Gastroenterology in 2019 had an increase of 37.5% in food intake compared to before hospitalization.

Discussion

A consistent trend was not identified for malnutrition within the Imam Reza Teaching Hospital after analyzing the data from various departments. Frequencies of malnutrition varied across different years within the same department. However, when considering all departments collectively, the Burns department, Pulmonary department, and General Surgery exhibited the highest rates of malnourished patients. Additionally, the Burns and Gynecological Surgery units showed the highest risk of malnutrition.

The lowest Body Mass Index (BMI) was observed among oncology patients. Gastroenterology recorded the highest instances of weight loss in the three months preceding admission and a reduction in nutrition intake before hospitalization. Comparatively, the general surgery unit reported the lowest food intake during nDay and admission.

Malnutrition in the Burn Unit

According to our findings, the Burns unit showed the highest incidence of malnutrition. A survey conducted in 2021 involving 109 patients across various hospitals in Iran also found that a majority of patients in the Burns ward were malnourished (15). The prevalence of malnutrition in Burns units is not surprising, given the critical condition of individuals with burn injuries. These patients were prone to hypermetabolic responses, which could intensify the use of bodily resources and potentially lengthen hospital stays when combined with preexisting malnutrition (16). This study also noted an increase in overall malnutrition and malnutrition risk among Burns ward patients over the years studied.

Burn victims experience a significant increase in metabolism, requiring a higher intake of energy, carbohydrates, protein, fats, vitamins, minerals, and antioxidants. Early nutritional intervention through parenteral or enteral feeding is crucial to reduce infection risks, speed up recovery, and minimize long-term complications (17).

The persistent hypermetabolism and catabolism from burn injuries led to muscle wasting, cachexia, impaired wound healing, organ dysfunction, and increased susceptibility to infection (18).

Despite ongoing research, the causes of hypermetabolism after burns remain complex and are not fully understood. Active investigations can uncover the intricate dysregulation of metabolism, hormonal pathways, and inflammatory processes. Oxygen consumption is linked to increased ATP turnover and cellular-level thermogenesis by the body (19).

Several studies have implicated catecholamines as the primary mediators of hyper metabolism (19, 20). Catabolic hormones such as epinephrine, cortisol, and glucagon inhibit protein synthesis and fat production (21). As a result, an imbalance between protein synthesis and breakdown leads to skeletal muscle cachexia. Dysregulation of muscle kinetics may persist for up to three years following severe burns, resulting in reduced lean body mass (18).

The timing of treatment, including nutrition, significantly impacts patient outcomes post-severe burns. Burn injuries cause severe damage to the intestinal mucosa, heightening bacterial translocation and impairing nutrient absorption. Hence, initiating nutritional support within 24 hours of injury is crucial (22). Given burn patients' hypermetabolic state, meeting their caloric requirements while avoiding overfeeding is essential, with nutritional support being the primary objective (23).

As nitrogen constitutes the fundamental building block of amino acids, ensuring adequate protein provision is another crucial aspect of post-burn nutritional support. Thus, monitoring nitrogen inputs and losses facilitates the study of protein metabolism (18).

Malnutrition and BMI

The study's Oncology ward had the lowest Body Mass Index (BMI). Cachexia, characterized by unintentional weight loss and muscle wasting, affects approximately 30% of all cancer patients. Individuals experiencing cachexia endure severe fatigue and weakness, significantly impacting their quality and length of life. Cachectic patients face a negative energy balance due to increased energy expenditure and decreased energy intake.

Tumor-secreted factors and systemic inflammatory responses may elevate the basal metabolic rate, leading to inefficient energy production pathways and heightened energy demand by both the tumor and the host (24).
Cachexia is defined as a body weight loss exceeding 5%, or >2%, in patients with a BMI <20 kg/m^2 or the presence of sarcopenia. Patients with refractory cachexia often exhibit substantial catabolism. Various factors, including tumor type, cancer stage, food intake, inflammation levels, and response to chemotherapy, can influence the progression of cachexia (25). Identifying and effectively treating cachexia may improve symptoms and treatment outcomes for affected patients (26). Common interventions typically revolve around nutrition and physical therapy, alongside addressing underlying causes of reduced food intake, such as anorexia and nausea (26, 27).
Moreover, inflammatory factors and mediators implicated in cachexia contribute to systemic inflammation. Immune checkpoints modulate adipocyte differentiation and systemic metabolic balance during cachectic development. A comprehensive understanding of cachexia encompasses the immunometabolism axis, immune-gut axis, and immune-nerve axis (28).

Weight Loss before Admission

The findings revealed that patients admitted to the Gastroenterology ward experienced the most significant weight loss three months before their hospitalization.
Various gastrointestinal diseases are characterized by weight loss, often accompanied by clinical manifestations of malassimilation, maldigestion, and malabsorption. Patients with conditions such as celiac disease, lambliasis, small bowel Crohn's disease, common variable immunodeficiency syndrome (CVIDS), and Whipple's disease may exhibit a loss of absorptive surface. Persistent intestinal pseudo-obstruction can lead to weight loss due to postprandial pain and dysmotility. In addition, there may be a primary or secondary association between exocrine pancreatic insufficiency and various protein-losing enteropathies. Surgery-related symptoms, including dumping syndrome, bile acid malabsorption, and short bowel syndrome, contribute to chronic intestinal issues. Intestinal injuries such as radiation-induced enteritis, persistent intestinal ischemia, and intestinal diabetic polyneuropathy also lead to chronic intestinal problems (29).
Similarly, recent weight loss can be indicative of clinical manifestations and a cornerstone in the diagnosis of some gastrointestinal diseases, such as Crohn's disease (30). The underlying physiological disturbances caused by disease can also be considered as the cause of weight loss as a marker of disease activity (31)
Weight loss can be a crucial diagnostic marker for certain gastrointestinal diseases, such as Crohn's disease, and may indicate disease activity. Many gastrointestinal diseases have an inflammatory basis, resulting in a generalized catabolic state. During the acute phase of the disease, resting energy expenditure increases, while proinflammatory cytokines exert anorexic effects. This inflammatory state impacts leptin, adiponectin, and ghrelin levels, potentially affecting satiety. Moreover, deficiencies in both macronutrients and micronutrients occur due to malabsorption in these disease processes (32, 33). Symptoms such as a solid gastrocolic reflex and food-related pain can lead patients to avoid eating, putting them at risk of malnutrition (31). Patients suffering from any of these conditions are at risk of malnutrition.

Conclusion

Malnutrition among hospitalized patients remains under-recognized despite efforts to screen for it systematically over the years. Inadequate nutritional treatment during hospitalization contributes to its persistence (34).
Early screening for malnutrition is crucial for the timely initiation of nutritional therapy. Regular monitoring of nutritional status using a nutrition evaluation score facilitates prompt nutritional intervention as recommended by nutrition guidelines (35, 36).
Targeted nutritional care for patients admitted to wards where malnutrition risk is highest can help reduce the incidence of malnutrition and associated complications.

Strength and Limitation

The significant strength of this study lies in its comprehensive sampling approach, encompassing a wide array of hospitals and specialties within those hospitals. Consistency and comparability were ensured across different settings utilizing a standardized questionnaire as a global tool for assessing malnutrition frequency. A detailed breakdown of the questions and factors related to malnutrition was provided, enhancing understanding and facilitating targeted interventions. The reporting of trends further enhances our understanding of the prevailing situation, enabling us to deliver improved nutrition care to our patients. Moreover, collaborating with dieticians and nutrition students in compiling the questionnaire adds credibility to the findings.

The study has limitations, however, which need to be acknowledged. The cross-sectional design and reliance on self-reported survey responses introduce inherent limitations, including the potential for recall bias. Moreover, the inability of some wards to participate in the study due to the COVID-19 pandemic in 2020 restricts the generalizability of the findings to some extent. Furthermore, the study's restriction to a single teaching hospital, albeit the largest in northeast Iran, may limit the generalizability of the findings. Despite these limitations, this study provides valuable insights into the prevalence and factors associated with malnutrition, paving the way for future research and interventions in this critical area of healthcare.

Declarations

Conflict of Interest

The authors declare that they have no conflict of interest.

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Ethical Approval

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

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Supplementary Tables

Table 3. Weight loss in the last 3 months

Unit	2019						2020						2021						p-value					
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Infection	3 (10.3%)	10 (34.5%)	5 (17.2%)	6 (20.7%)	5 (17.2%)																			
Oncology	1 (11.1%)	5 (55.6%)	1 (11.1%)	1 (11.1%)	1 (11.1%)																			
General medicine		8 (42.1%)	10 (52.6%)	1 (5.3%)																				
Rheumatology		5 (50.0%)	4 (40.0%)	1 (10.0%)									1 (6.7%)						< 0.0001*					
Pulmonary	1 (7.7%)	8 (61.5%)	3 (23.1%)	7 (31.8%)	1 (7.7%)																			
Gastroenterology	1 (5.9%)	13 (76.5%)		2 (11.8%)	1 (5.9%)								1 (6.7%)						0.77					
Internal Medicine	2 (12.5%)	2 (12.5%)	3 (18.8%)		5 (31.3%)								1 (6.7%)						0.22					

Unit	2019						2020						2021						p-value					
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Dermatology	2 (25.0%)	1 (12.5%)	3 (37.5%)	2 (25.0%)																				
Surgery																								
Burns(male)		1 (11.1%)	4 (44.4%)		1 (11.1%)	3 (33.3%)		3 (37.5%)	1 (12.5%)				1 (10.0%)	2 (20.0%)	2 (20.0%)			5 (50.0%)		< 0.0001*	< 0.0001*			
Burns(female)	1 (11.1%)	4 (44.4%)	3 (33.3%)	1 (11.1%)		3 (37.5%)			2 (25.0%)												0.21			
Surgery General	1 (3.3%)	10 (33.3%)	8 (26.7%)	3 (10.0%)	1 (3.3%)	7 (23.3%)		4 (36.4%)	4 (36.4%)				1 (4.2%)	13 (54.2%)	10 (41.7%)				0.70	0.00*	0.02*			
Orthopedic Surgery	9 (23.1%)	6 (15.4%)	14 (35.9%)	2 (5.1%)	2 (5.1%)	6 (15.4%)		6 (60.0%)	3 (30.0%)				2 (6.3%)	5 (15.6%)	13 (40.6%)	4 (12.5%)	1 (3.1%)	7 (21.9%)	0.0007*	< 0.0001*	0.26	0.07	0.47	0.20
Genecology Surgery	1 (3.2%)	1 (3.2%)	3 (9.7%)	24 (77.4%)																				

Unit	2019						2020						2021						p-value					
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Cardiac Surgery	1 (14.3%)	2 (28.6%)	4 (57.1%)											12 (75.0%)	2 (12.5%)			2 (12.5%)		< 0.0001*	< 0.0001			

1- intentionally/ 2- unintentionally/ 3- steady weight/ 4- gained weight/ 5- does not know

Table 4. Food intake one week before hospital admission

Unit	2019							2020							2021							p-value						
	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Infection	2 (6.9%)	19 (65.5%)	2 (6.9%)	3 (10.3%)	2 (6.9%)	1 (3.4%)										8 (61.5%)	3 (23.1%)	1 (7.7%)	1 (7.7%)				0.55	0.00*	0.62	0.78		
Oncology		4 (44.4%)	1 (11.1%)	1 (11.1%)	3 (33.3%)										1 (4.2%)	11 (45.8%)	3 (12.5%)	3 (12.5%)	3 (12.5%)	2 (8.3%)	1 (4.2%)		1	0.82	0.82	0.0004		
General Medicine	3 (16.7%)	10 (55.6%)		2 (11.1%)	3 (16.7%)											13 (72.2%)		2 (11.1%)	3 (16.7%)				0.01*		1	1		
Rheumatology		8 (80.0%)	1 (10.0%)		1 (10.0%)											7 (87.5%)		1 (12.5%)					0.18					
Pulmonary	1 (7.7%	9 (69.2%)			2 (15.4%)	1 (7.7%)																						
Gastroenterology	1(6.3%)	5(31.3%)		3(18.8%)	6(37.5%)		1(6.3%)								2 (13.3%)	7 (46.7%)	2 (13.3%)	2 (13.3%)	2 (13.3%)			0.09	0.02*		0.24			

Unit	2019							2020							2021							p-value						
	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Internal medicine		6 (40.0%)			5 (33.3%)		4 (26.7%)									9 (47.4%)	2 (10.5%)	2 (10.5%)	5 (26.3%)		1 (5.3%)		0.31		0.27			< 0.0001*
Dermatology		6 (75.0%)		2 (25.0%)																								
Surgery																												
Burns(male)		8 (88.9%)				1 (11.1%)			6 (75.0%)	1 (12.5%)	1 (12.5%)				1 (10.0%)	8 (80.0%)			1 (10.0%)				0.06					
Burns(female)		8(88.9%)		1 (11.1%)					7 (87.5%)		1 (12.5%)												0.66		0.82			
Surgery GeneraG	5 (26.3%)	7 (36.8%)	3 (15.8%)	2 (10.5%)	2 (10.5%)										1 (9.1%)	4 (36.4%)	3 (27.3%)	2 (18.2%)	1 (9.1%)			0.00*	1	0.0589	0.10	0.80		
Orthopedic Surgery	5 (13.2%)	26 (68.4%)	2 (5.3%)	3 (7.9%)	2 (5.3%)										3 (9.4%)	21 (65.6%)	1 (3.1%)	5 (15.6%)	1 (3.1%)	1 (3.1%)		0.36	0.65	0.47	0.08	0.47		
Gynecological Surgeryy		8 (80.0%)	1 (10.0%)	1 (10.0%)											1 (6.3%)	14 (87.5%)		1 (6.3%)					0.18		0.29			
Cardiac Surgeryu		6 (85.7%)			1 (14.3%)											7 (43.8%)	3 (18.8%)	2 (12.5%)	4 (25.0%)				< 0.0001*			0.0502		

1-more than normal/ 2-normal/ 3- about ¾ normal/ 4-about half of normal/ 5- about a quarter nearly nothing/6- I don't know/7-missing

Table 5. Food Intake on Nutrition Day

Unit	2019					2020					2021					pvalue				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Infection	4 (13.8%)	14 (48.3%)	10 (34.5%)	1 (3.4%)								9 (69.2%)	4 (30.8%)				0.00*	0.65		
Oncology	4 (44.4%)	3 (33.3%)	1 (11.1%)		1 (11.1%)						6 (25.0%)	10 (41.7%)	7 (29.2%)		1 (4.2%)	0.00*	0.18	0.54		0.06
General Medicine	2 (11.1%)	13 (72.2%)	3 (16.7%)								3 (15.8%)	4 (21.1%)	8 (42.1%)		4 (21.1%)	0.30	< 0.0001*	0.00*		
Rheumatology	3 (30.0%)	5 (50.0%)	2 (20.0%)								1 (12.5%)	4 (50.0%)	3 (37.5%)			0.00*	1	0.00*		
Pulmonary	2 (15.4%)	5 (38.5%)	5 (38.5%)	1 (7.7%)																
Gastroenterology	6 (37.5%)	8 (50.0%)	2 (12.5%)									8 (53.3%)	7 (46.7%)				0.67	< 0.0001*		
Internal Medicine		8 (66.7%)	3 (25.0%)		1 (8.3%)						3 (16.7%)	12 (66.7%)	2 (11.1%)	1 (5.6%)			1	0.01*		
Dermatology	2 (25.0%)		6 (75.0%)																	
Surgery																				
Burns (male)	3 (33.3%)	2 (22.2%)	2 (22.2%)	2 (22.2%)		3 (37.5%)		2 (25.0%)	2 (25.0%)	1 (12.5%)		9 (90.0%)		1 (10.0%)		0.55	< 0.0001*	0.61	0.01*	

Unit	2019					2020					2021					pvalue				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Burns(female)		7 (77.8%)	2 (22.2%)				3 (37.5%)		3 (37.5%)	2 (25.0%)							< 0.0001*			
Surgery general	1 (5.3%)	7 (36.8%)	8 (42.1%)	2 (10.5%)	1 (5.3%)															
Orthopedic Surgery	4 (10.5%)	24 (63.2%)	9 (23.7%)	1 (2.6%)			6 (60.0%)	3 (30.0%)	1 (10.0%)			10 (31.3%)	18 (56.3%)	1 (3.1%)	3 (9.4%)		< 0.0001*	< 0.0001*	0.03*	
Gynecological Surgery		8 (80.0%)	2 (20.0%)				5 (50.0%)	4 (40.0%)		1 (10.0%)	1 (6.3%)	11 (68.8%)	4 (25.0%)				< 0.0001*	0.00*		
Cardiac Surgery		6 (85.7%)	1 (14.3%)								2 (12.5%)	6 (37.5%)	6 (37.5%)	2 (12.5%)			< 0.0001*	0.00*		

1-about all / 2-1/2/ 3-1/4 / 4-nothing / 5-missing

Table 6. Change in food intake upon admission

unit	2019					2020					2021					P-value				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Infection	10 (34.5%)	10 (34.5%)	7 (24.1%)	2 (6.9%)							2 (15.4%)	6 (46.2%)	5 (38.5%)			0.00*	0.08	0.03*		
Oncology	3 (33.3%)	2 (22.2%)	2 (22.2%)	1 (11.1%)	1 (11.1%)						6 (25.0%)	9 (37.5%)		5 (20.8%)	1 (4.2%)	0.21	0.02*		0.0544	0.0609

Orthopedic Surgery	General Surgery	Burns(female)	Burns (male)	Dermatology	Internal Medicine	Gastroenterology	Pulmonary	Rheumatology	General Medicine
12 (31.6%)	7 (36.8%)	2 (22.2%)		3 (37.5%)	2 (16.7%)	3 (18.8%)	5 (38.5%)	5 (50.0%)	3 (16.7%)
10 (26.3%)	2 (10.5%)	4 (44.4%)	5 (55.6%)	1 (12.5%)	4 (33.3%)	2 (12.5%)	2 (15.4%)	2 (20.0%)	5 (27.8%)
14 (36.8%)	1 (5.3%)	3 (33.3%)	4 (44.4%)	4 (50.0%)	6 (50.0%)	6 (37.5%)	4 (30.8%)	3 (30.0%)	7 (38.9%)
1 (2.6%)	9 (47.4%)					4 (25.0%)	2 (15.4%)		3 (16.7%)
1 (2.6%)						1 (6.3%)			
3 (30.0%)			4 (50.0%)						
4 (40.0%)		2 (25.0%)	3 (37.5%)						
3 (30.0%)		5 (62.5%)	1 (12.5%)						
		1 (12.5%)							
12 (37.5%)			1 (10.0%)			2 (13.3%)		4 (50.0%)	2 (10.5%)
14 (43.8%)			5 (50.0%)			8 (53.3%)			3 (15.8%)
4 (12.5%)			1 (10.0%)			4 (26.7%)		3 (37.5%)	5 (26.3%)
			3 (30.0%)			1 (6.7%)		1 (12.5%)	
2 (6.3%)									9 (47.4%)
0.55			< 0.0001*			0.1491		1	0.14
0.02*		0.00*	0.02*			< 0.0001*			0.03*
0.00*		< 0.0001*	< 0.0001*			0.13		0.29	0.0503
						0.00*			
0.30									

Cardiac Surgery	Gynecological Surgery
3 (42.9%)	3 (30.0%)
1 (14.3%)	3 (30.0%)
3 (42.9%)	4 (40.0%)
	3 (30.0%)
	4 (40.0%)
	2 (20.0%)
	1 (10.0%)
4 (25.0%)	3 (18.8%)
5 (31.3%)	5 (31.3%)
5 (31.3%)	3 (18.8%)
2 (12.5%)	5 (31.3%)
0.00*	0.12
0.00*	0.25
0.07	0.00*
	0.00*

1-increased 2-decreased 3-stayed the same 4-does not know 5- missing

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Synergistic Effect of High Intensity Interval Training and Atorvastatin in Treatment of NAFLD in Rats Fed High Fat/Fructose Diet

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ABSTRACT

Introduction: Non-alcoholic fatty liver disease (NAFLD) is a prevalent chronic liver disease that ranges from steatosis to non-alcoholic steatohepatitis (NASH), fibrosis, cirrhosis, and liver carcinoma. With the increasing prevalence of NAFLD, there is a growing need for effective prevention and treatment strategies. Stable pharmaceutical compositions containing atorvastatin have been developed to treat hypercholesterolemia and related conditions. In addition, high-intensity interval training (HIIT) can have positive effects on NAFLD.

Methods: Twenty-one male Wistar rats were divided into 2 groups: 1) high fat-fructose diet (HFFD) + HIIT, 2) HFFD + HIIT + atorvastatin. The groups received HFFD for 15 weeks to induce NAFLD. Atorvastatin was administrated at the dose of 2 mg/kg/day. The interventions (atorvastatin and HIIT) were done for 8 weeks.

Results: Triglyceride (TG), Alanine transaminase (ALT), and aspartate transaminase (AST) were significantly reduced in the HFFD + HIIT + atorvastatin. The groups had no significant difference in weight, low-density lipoprotein (LDL), alkaline phosphatase (ALP), and HDL/LDL ratio.

Conclusion: Although atorvastatin along with HIIT reduced aminotransferase, HIIT has benefits in improving lipid profile. Combining atorvastatin and HIIT may offer synergistic benefits in managing NAFLD by targeting both liver enzymes and inflammation.

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Introduction

Non-alcoholic fatty liver disease (NAFLD) arises as a consequence of an excessive buildup of fat in the hepatocytes, the main functional cells of the liver, resulting in impaired liver function and subsequent adverse health outcomes (1). The global prevalence of NAFLD has witnessed a steady rise owing to the prevalent lifestyle changes characterized by sedentary behavior, unhealthy dietary patterns, and the increasing burden of obesity, thereby necessitating urgent attention and the implementation of preventive measures to curtail its escalating prevalence (2). Furthermore, epidemiological investigations have revealed that the prevalence of NAFLD in Iran is estimated to be approximately 31.15% and 21.5%, highlighting the alarming magnitude of the disease burden in this specific geographical region (3) (4). Alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), and alkaline phosphatase (ALP) are of paramount importance in assessing the well-being of the liver. Patients with NAFLD often exhibit elevated levels of these liver enzymes, namely ALP, AST, GGT, and ALT, in their serum. Additionally, these enzymes experience an increment in situations such as diabetes and alcoholism (5).

In light of the intricate pathophysiological mechanisms underlying NAFLD, characterized by the upregulation of triglyceride (TG), total cholesterol (TC), and low-density lipoprotein (LDL) levels, it is crucial to devise effective strategies aimed at mitigating the aberrant lipid metabolism implicated in the pathogenesis of NAFLD (6). Consequently, the identification and deployment of therapeutic agents that possess

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potent antioxidant properties emerge as a promising avenue to combat the multifaceted challenges posed by obesity and its associated complications. Specifically, the administration of atorvastatin, a pharmacological agent renowned for its well-documented minimal side effects profile, holds tremendous potential in fortifying the body's antioxidant defenses, thereby exerting a pivotal role in the prevention and management of obesity-induced inflammation and its subsequent sequelae. By harnessing the inherent antioxidant capacity of atorvastatin, healthcare providers and researchers can potentially unlock novel therapeutic approaches to tackle the burgeoning epidemic of NAFLD with utmost efficacy and safety, ultimately striving towards the attainment of improved public health outcomes and a better quality of life for affected individuals (7, 8).

Physical activity plays a crucial role in managing inflammation-related diseases like NAFLD by reducing the mass of visceral fat, enhancing lipolysis, and subsequently decreasing the release of pro-inflammatory cytokines, thus establishing an anti-inflammatory environment (9). High-Intensity Interval Training (HIIT) has been shown to effectively reduce fat tissue and promote increased oxygen consumption post-exercise through enhanced oxidation. This highlights the significance of physical activity in mitigating the detrimental effects of inflammation and underscores the potential of HIIT as a therapeutic approach in combating NAFLD and other related conditions (10). HIIT has been shown to effectively reduce fat tissue (11) and promote increased oxygen consumption post-exercise through enhanced oxidation. This highlights the significance of physical activity in mitigating the detrimental effects of inflammation and underscores the potential of HIIT as a therapeutic approach in combating NAFLD and other related conditions (8). Atorvastatin is a key enzyme involved in cholesterol synthesis. By reducing cholesterol production, atorvastatin effectively lowers LDL levels and improves lipid profiles (12). Atorvastatin treatment also led to a decrease in intracellular ATP levels and an increase in cytotoxicity in HepG2 cells (13). This drug has been found to inhibit 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductase, leading to up-regulation of LDL receptors and increased clearance of LDL-cholesterol from the plasma (14). Additionally, atorvastatin has been shown to modulate microRNA expression, with up-regulation of miR-124a and down-regulation of GAMT expression in hepatocytes (15). Repression of hsa-mir-20a-5p, a microRNA, has been associated with increased LDL receptor expression in HepG2 cells (16). High doses of statins, including atorvastatin, were found to induce key enzymes involved in VLDL production in rat liver, potentially through overexpression of SREBP-2 (17). However, it is important to note that higher doses of atorvastatin have been associated with hepatotoxicity, as evidenced by increased liver enzyme activity and degeneration of liver cells (18). Overall, the mechanism of action of atorvastatin on liver enzymes involves modulation of gene expression and potential disruption of VLDL production.

These findings suggest that atorvastatin can effectively improve lipid profiles, reduce fatty liver, and regulate liver enzymes in NAFLD. However, it is important to note that the effects on different lipid parameters may vary, and careful monitoring of liver enzymes is necessary during treatment. The potential synergistic effects of combining HIIT and atorvastatin in the treatment of NAFLD have also been explored. However, research in this area is limited, and further studies are needed to fully understand the benefits and mechanisms of combined therapy. It is possible that the combination of HIIT and atorvastatin may provide additive or synergistic effects in improving liver health and lipid metabolism in individuals with NAFLD. So, we aimed to assess the simultaneous effect of HIIT and improving NAFLD in rats fed HFFD.

Material and Methods

Animal and Design

Twenty-one male Wistar rats weighing between 180-200 grams were obtained from Shahid Mirghani Research Institute (Golestan, Iran). These rats were selected as the subjects for this experiment due to their suitability for research purposes. Throughout the duration of the experiment, the rats were provided with unlimited access to both feed and water, ensuring that their nutritional needs were met adequately. In order to maintain a consistent environment, the rats were housed in a controlled setting with a 12-hour dark/light cycle and a temperature ranging from 20 to 24 C.

To ensure accurate results, a period of one week was allotted for the rats to acclimate to their new surroundings and become familiar with their living conditions. Following this adaptation period, the rats were subjected to the induction of NAFLD in alignment with the protocol developed by Eslami et al. (19). Normal diet will contain 4.30 kcal per gram including 3.87% fat (soy oil), 17.46% casein protein, 68.7% carbohydrates, 8.97% minerals, and 1% vitamins (20). For inducing NAFLD, the rats consumed HFFD (45% fructose and 35% olive oil (gavage)) (18) for 15 weeks. In addition to consuming HFFD, the groups had free access to normal diet. At the end of the 15th week, a selection of blood samples and liver tissue were taken at random from 5 rats in order to assess the levels of serum ALT and observe any changes in the liver tissue. The biochemical and histopathological findings indicated that NAFLD had been induced in the rats. Following this, the animals were divided into two distinct groups; 1) HFFD + HIIT (n=8), HFFD + HIIT + atorvastatin (n=8). The interventions, consisting of administering atorvastatin at a dosage of 2 mg/kg (dissolved in 6% DMSO, gavage) (Raha Pharmaceutical co, Iran) (21) and implementing HIIT, were maintained for a duration of eight weeks.

Measurement of Biochemical Indices

The rats were rendered unconscious through the administration of ketamine (50 mg/kg) and xylazine (5 mg/kg, Merck, Germany) via intraperitoneal injection (22), thereby inducing anesthesia. In order to assess the presence and quantity of aminotransferases and ALP, standard enzymatic techniques were employed, while levels of TG, LDL, and HDL were measured using an auto-analyzer (BT-3500, Biotecnica Instruments, Italy).

Table 1. Protocol of HIIT

Week	1st	2nd	3rd	4th	5th	6th	7th	8th
Repetition	4	5	6	8	8	10	12	12
Min	2	2	2	2	2	2	2	2
%	90%	90%	90%	90%	90%	90%	90%	90%

HIIT Protocol

The protocol of HIIT is mentioned in Tables 1 (23).

Statistical Analysis

The distribution of the data was determined using the Shapiro-Wilk test, while the homogeneity of variances was assessed using Levene's test. Furthermore, ANOVA was employed to compare the means of the desired variables. The analysis was conducted using SPSS software version 16, with a significance level set at $P \leq 0.05$.

Ethical Statement

The investigation was conducted in compliance with the guidelines outlined in the publication "Guide for the Care and Use of Laboratory Animals" issued by the National Institutes of Health (NIH publication No. 85–23, revised 1996). The research protocol was granted approval by the ethics committee of the local institution (IR.SSRC.REC.1402.121). Diligent measures were taken to mitigate animal distress and minimize the quantity of animals employed.

Table 2. Mean and standard deviation of weight changes in groups during 8 weeks

Group	W1	W2	W3	W4	W5	W6	W7	W8
HFFD + HIIT	377.60 ± 28.90	375.35 ± 26.08	372.25 ± 31.42	378.81 ± 34.09	362.68 ± 27.54	352.79 ± 26.88	362.68 ± 29.61	368.49 ± 30.49
HFFD + HIIT + Atorvastatin	379.05 ± 30.88	373.18 ± 36.78	379.58 ± 40.82	381.48 ± 39.94	368.78 ± 36.33	363.87 ± 29.98	372.06 ± 28.81	378.70 ± 31.26
F	0.006	0.01	0.10	0.01	0.09	0.37	0.25	0.27
P	0.94	0.97	0.75	0.91	0.77	0.55	0.62	0.61

Table 3. Mean and standard deviation of biochemical parameters in groups during 8 weeks

Group	TG	LDL	HDL	LDL/HDL	ALT	AST	ALP
HFFD + HIIT	47.32 ± 4.49	1.10 ± 0.43	31.12 ± 4.53	0.03 ± 0.01	87.82 ± 11.12	146.12 ± 14.86	232.33 ± 43.60
HFFD + HIIT + Atorvastatin	57.00 ± 5.78	3.02 ± 2.25	36.60 ± 4.18	0.08 ± 0.07	54.24 ± 1.99	98.22 ± 2.58	299.60 ± 42.71
F	7.48	2.74	3.54	1.63	45.30	51.75	5.41
P	0.02*	0.14	0.10	0.24	0.00*	0.00*	0.05

* P<0.05

Results

The results of ANOVA and the average of weight and biochemical parameters are mentioned in Table 2 and 3, respectively. The findings of the analysis of the two groups reveal that the levels of TG (P= 0.029), ALT (P= 0.000), and AST (P= 0.000) in the HFFD + HIIT + Atorvastatin are notably diminished in comparison to the HFFD + HIIT. While there was no significant difference in LDL, HDL and ALP indices between the two groups. On the contrary, the evaluation of weight comparison over the course of eight successive weeks during the implementation of the intervention similarly indicated that there were no statistically significant disparities between the two groups. The results of the study showed significant improvements in the lipid profile and liver enzymes in the HFFD + HIIT + atorvastatin compared to the HFFD + HIIT. These findings suggest that HIIT along with atorvastatin may be a promising strategy for managing NAFLD.

Discussion

The aim of this investigation was to determine the effect of HIIT on NAFLD with the intention of ascertaining its efficacy. Furthermore, considering the HIIT + atorvastatin group, the objective was to determine whether there was a change in the effectiveness of the interventions on NAFLD with the simultaneous administration of atorvastatin and HIIT. By comprising two groups, we wanted to assessment these changes are due to the effect of HIIT alone or the HIIT + atorvastatin can cause an increasing effect on these changes.

HIIT has gained significant attention as an exercise intervention for various health conditions, including NAFLD. HIIT involves short bursts of intense exercise alternated with periods of rest or low-intensity exercise. This type of training has been shown to improve metabolic health, cardiovascular fitness, and body composition (24). Our results showed that HIIT for 8 weeks could improve TG, LDL, and LDL/HDL ratio which it considered as a potential approach for preventing fat accumulation in hepatocytes. This results along with the study by Mirghani et al (25). In addition, administration of atorvastatin along with HIIT, reduced aminotransferase. HIIT and Moderate Intensity Training (MIT) possess the capability to counteract the obesity epidemic. Nevertheless, investigations that compare the impacts of these training methods on obesity present contradictory discoveries pertaining to the reduction of body weight (26). HIIT has been found to exhibit superior efficacy compared to MIT in decreasing certain indicators of obesity in obese rats fed a high-fat diet (HFD). Nevertheless, this particular mode of exercise does not yield any notable influence on insulin resistance (27).

In a study by Kalaki-Jouybari et al., the researchers investigated that HIIT significantly decreased the expression of fatty acid synthase (FAS), Acetyl-CoA carboxylase (ACC), and sterol regulatory element-binding protein-1c (SREBP-1c) compared to the diabetic control group. Additionally, HIIT partially increased the expression of miR-122, suggesting its potential role in improving NAFLD. These findings indicate that HIIT can alleviate NAFLD features through the induction of miR-122 in the liver (24). Eslami et al., investigated that receiving atorvastatin 10 mg/kg for eight weeks, alongside the HFFD exhibited significant reductions in TG, cholesterol, aminotransferases, gamma-glutamyl transferase levels compared to HFFD control (22). In a separate investigation, it was demonstrated that the ingestion of atorvastatin 10 mg/kg/day in rats afflicted with NAFLD is correlated with a reduction in TG and cholesterol concentrations. Conversely, in healthy rats, the consumption of atorvastatin results in heightened cholesterol and HDL levels (5).

HIIT can increase fatty acid oxidation-related gene expression and decrease adipogenesis-related gene expression, leading to improved liver metabolism and lipid metabolism disorders (28). HIIT also increases liver mitochondrial biosynthesis-related gene expression and restores mitochondrial dynamics-related gene expression, which may contribute to the improvement of lipid metabolism (29). Additionally, it can reduce liver inflammation by decreasing the expression of inflammatory factors and macrophage markers associated with M1 macrophages, while increasing the expression of markers associated with M2 macrophages (30).

Motta et al. investigated the effect of HIIT on improving metabolism in obese rats with NAFLD. HIIT significantly reduced body mass, blood glucose, glucose tolerance and lipid profile,

improved insulin sensitivity, reduced visceral fat and hepatic steatosis (31). Another study found that four weeks of HIIT did not result in significant changes in liver enzymes in overweight women (30). Additionally, a study on men with metabolic syndrome (MetS) found that eight weeks of HIIT led to significant improvements in liver enzymes, with greater improvements observed when combined with sodium alginate supplementation (32). Endurance training and adenosine may serve as probable stimulants for the expression of the UCP-1 gene and exhibit efficacy as lipolytic agents in the context of obesity. By adhering to a nutritious diet and engaging in aerobic exercises, the MAPK p38 pathway can augment insulin-mediated glucose uptake and subsequently instigate oxidative phosphorylation in mitochondria (33, 34). In a study by Mirghani et al, indicated that Endurance training and the administration of vitamin D have the potential to yield substantial reductions in certain anthropometric indices (35).
Guiyuan Ji et al. showed that both dietary control and atorvastatin effectively improved serum and liver lipid metabolism and liver function compared to the HFD control. Interestingly, the combination of atorvastatin and dietary control led to further reductions in liver weight, hyperlipidemia, and liver steatosis compared to atorvastatin or dietary control alone. However, the combination therapy did not significantly improve TG and FFA metabolism compared to dietary control alone. These findings suggest that combining atorvastatin with dietary control may have synergistic effects in improving lipid profiles, liver function, and liver steatosis. However, the specific mechanisms underlying this synergy require further investigation (36).

Conclusion

The management of NAFLD requires a comprehensive approach that addresses abnormal lipid profiles, liver function, and associated health complications. Atorvastatin, a medication known for its cholesterol-lowering effects, has shown promise in improving lipid profiles and liver function in individuals with NAFLD. Additionally, the efficacy of atorvastatin therapy may vary depending on the dosing regimen. HIIT has also emerged as an effective exercise intervention in reducing visceral fat and promoting overall metabolic health. This suggests that HIIT could be a potential approach for preventing fat accumulation in hepatocytes. The combination of atorvastatin and HIIT may offer synergistic benefits in managing NAFLD by targeting both lipid metabolism and hepatic enzymes. By combining the antioxidant properties of atorvastatin with the anti-inflammatory effects of exercise, healthcare professionals can develop comprehensive treatment strategies to improve the lipid profiles, liver function, and overall health outcomes of individuals with NAFLD. Further research is needed to elucidate the optimal dosage, duration, and timing of combined training interventions. Additionally, long-term studies are required to assess the sustainability and long-term effects of this approach. Nevertheless, the integration of atorvastatin and HIIT in the management of NAFLD holds promise and warrants further investigation for its potential impact on public health and the prevention of NAFLD-related complications.

Declarations

Ethical considerations

The research protocol was granted approval by the ethics committee of the local institution (IR.SSRC.REC.1402.121).

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Author's contribution

Raouf Moradian and Amir Haji Ghasem conceived and planned the experiments, Raouf Moradian carried out the experiments, Saleh Rahmati and Lida Moradi contributed to the interpretation of the results. Raouf Moradian wrote the manuscript, Amir Haji Ghasem helped supervise the project.

Conflict of Interest

The authors declare that there is no conflict of interest regarding publication of this article.

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JOURNAL OF NUTRITION FASTING AND HEALTH

Sustainable Diets: Bridging between Climate Change and Food Security: A Systematic Review

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ABSTRACT

There is a complex relationship between climate change and the security and sustainability of the food system, which significantly affects human health. Different factors of climate change can have a positive or negative effect on the food system and pattern. This article aimed to discuss various strategies to control or avoid these effects.

Studies published from the beginning to 2024 analyzing "the effect of climate change on food security and role of sustainable diets" were searched in Google Scholar, Pubmed, and Web of Science. Related articles were reviewed among the screened articles.

The effects of climate change on food production and availability, as well as extreme weather events, affect both the physical and economic accessibility of food. Climate change also affects the stability and resilience of food systems, which has long-term implications for food security. Additionally, efforts to achieve food security through agricultural intensification and land expansion contribute to greenhouse gas emissions from deforestation and land use changes.

The solution lies in establishing a sustainable nutrition and food system that ensures food security for all without compromising future generations' economic, social, and environmental well-being. Numerous scientific sources have investigated and approved some of these nutritional and dietary patterns, such as Eat-lancet, vegetarian, and Mediterranean diets. Certainly, more interventions focusing on sustainable food patterns and other adaptation methods and necessary strategies for their widespread implementation are needed.

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Introduction

Climate change has emerged as one of the most pressing global challenges in recent decades, which encompasses long-term shifts in temperature, weather patterns, global warming, and greenhouse gas emissions [1]. The impacts of climate change are wide-ranging, including droughts, water scarcity, rising sea levels, wildfires, melting polar ice caps, and loss of biodiversity [2]. Various frameworks have been developed to address these risks, such as the Sustainable Development Goals (SDGs). Sustainable development aims to ensure a prosperous future for society while safeguarding the environment and the rights of future generations [3]. The SDGs, established in 2012 to succeed the Millennium Development Goals, encompass critical objectives such as poverty eradication, hunger alleviation, disease prevention, and literacy promotion [4].

Two specific SDGs focus on the nutrition sector: SDG2 aims to achieve zero hunger, improve food security, eliminate malnutrition, and promote sustainable agriculture, while SDG12 emphasizes responsible production and consumption [5]. Despite efforts made in recent decades, food insecurity remains a significant issue in many countries, particularly developing nations. The Food and Agriculture Organization (FAO) defines food security as ensuring access to safe and nutritious food, meeting dietary needs and preferences, and enabling individuals to lead active and healthy lives. The State of Food Security and Nutrition in the World 2019 report revealed that over 820 million people suffered from hunger in 2018, primarily in Africa, Latin America, and Asia. Additionally, more than two billion people experienced some level of food insecurity, with 8% of the population affected in developed regions like North America and

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Europe, which underscore the dysfunctionality of the global food system, exacerbated by climate change [6, 7].

Climate change directly impacts food security through various channels, including loss of rural livelihoods, degradation of marine and coastal ecosystems, terrestrial and inland water ecosystems, and food systems. Projections have indicated that by 2080, 15% of the world's population could face a water crisis [8]. As the global population continues to grow, expected to reach 9.7 billion by 2050, the availability of resources for food production diminishes, further aggravating food insecurity [9]. However, progress towards achieving SDGs aimed at eliminating malnutrition and poverty by 2030 is inadequate, with only a quarter of countries on track to meet targets related to stunting, wasting, and overweight [6, 7].

This study provides an overview of the effect of climate change on food security and nutrition, encompassing physical effects on agroecosystems and livelihoods, underscoring the urgency of addressing climate change to eradicate hunger and enable the agricultural sector to adapt. Furthermore, the imperative of mitigating climate change to levels is stressed to ensure food security and nutrition for all, advocating for "sustainable diets." This paper explores the intricate relationship between diet, climate change, and food security by providing examples and describing their components. The structure of the paper is organized as follows: firstly, the challenges posedby climate change and potential adaptation solutions are discussed; secondly, sustainable food systems and proposed policies are presented; thirdly, sustainable diets are introduced and comprehensively reviewed; and finally, the issue of food wastage is addressed alongside potential solutions due to its significant impact on food security.

Method and Materials

A comprehensive systematic search was conducted on PubMed, Web of Science, Google Scholar, and Scopus to identify relevant literature published between 2000 and 2024. The search utilized Medical Subject Headings (MeSH) vocabulary and appropriate terms, including ("food security" OR food insecurity OR food safety OR food supply OR "food availability" OR "food system" OR "agriculture") AND ("climate change*" OR "weather" OR "temperature" OR "precipitation") AND ("sustainable diet" OR "sustainable nutrition" OR sustainable diets OR diet* pattern). Additionally, reference lists of included studies and previous review articles were scrutinized to ensure comprehensive coverage and inclusion of eligible records. Articles were assessed based on titles, abstracts, and full texts to determine eligibility.

Results

Agricultural Productivity

Changes in temperature and precipitation patterns in the United States accounted for 70% of the variability in agricultural productivity growth from 1981 to 2010. Liang et al. found that continued trends could lead to an annual decrease in agricultural productivity ranging from 2.84% to 4.34% [10]. In a study across 35 African countries, rainfall variability significantly impacted agricultural productivity, whereas temperature showed no discernible effect [11]. Conversely, in an Asian context, increased CO_2 emissions and rising temperatures had a detrimental long-term effect on agricultural productivity, despite a short-term positive correlation between CO_2 and productivity [12].

Food Distribution

Global food manufacturers and retailers are actively addressing climate-related risks, including heightened variability in raw material supplies, increased costs, water scarcity, disruptions in distribution networks, workforce challenges, and evolving consumer preferences, which collectively influence global food system stability and security [13].

Access to Food Resources

Climate change-induced reductions in land productivity are projected to decrease food crop production in South Asia, potentially escalating food prices and exacerbating regional food insecurity [14]. In Sub-Saharan Africa, climate shifts are compelling reliance on markets due to diminished crop yields and extended periods of food scarcity, placing added pressure on market efficiency and operational costs [15, 16]. Studies in various European regions have indicated that climate change contributes to increased food prices; for instance, the 2003 heatwave in France resulted in a 25% decline in fruit harvests, while rising temperatures in Ukraine caused a 13% reduction in grain production [17, 18].

Food Preferences and Dietary Needs

Dietary preferences vary globally and are deeply rooted in cultural practices. Research by Long et al. highlighted that traditional pork-based meals in Japan incorporate diverse ingredients such as abundant vegetables and modest meat portions, effectively reducing the overall carbon footprint of the dish [19]. In contrast, dietary patterns in the USA reflect lower diet quality and higher greenhouse gas emissions, necessitating shifts towards diets that substitute plant-based proteins for beef, pork, and poultry [20]. The Danish adaptation of the EAT-Lancet reference diet, known as the Danish plant-rich diet, serves as the basis for climate-friendly food-based dietary guidelines (FBDGs). Transitioning Danish adults from their current average diets to the Danish plant-rich diet could reduce carbon footprints by 31% [21].

Discussion

Climate change profoundly affects human health, mainly through associations with infectious diseases, mortality rates, and respiratory and cardiovascular diseases. Elevated temperatures and extreme heat correlate with increased incidences of cardiovascular and respiratory ailments, stroke, neurological disorders, myocardial infarction, childhood asthma, and pediatric respiratory diseases [22]. Additionally, changes in temperature, extreme heat, aridity, and cold temperatures are linked to heightened healthcare utilization, including emergency department visits, hospital admissions, and ambulance services.

In addition, climate change contributes to undernutrition, malnutrition, child stunting, and deficiencies in both children and adults due to extreme weather events. Environmental factors also play a role in childhood stunting [23]. Climate-induced disruptions to ecosystems diminish both the quantity and quality of food, exacerbating food insecurity, which, in turn, heightens disease susceptibility and mortality rates [5]. Projections have suggested that climate change may reduce global food availability, potentially leading to decreased consumption of fruits, vegetables, and red meat and predicting 529,000 deaths globally over the next three decades [13, 24]. Climate change and variability threaten food safety by affecting the occurrence and persistence of disease-causing microorganisms, thereby increasing the risk of foodborne illnesses [1].

Climate Change Adaptation Strategies (CCAS)

Farmers employ diverse strategies to adapt to climate change, such as utilizing modified crops, adjusting planting or harvesting times, planting trees, increasing fertilizer or pesticide use, conserving soil and water resources, diversifying livelihoods, and adjusting livestock management practices. The adaptation strategies are influenced by age, gender, income, livestock ownership, and access to weather-related information. Women are more interested in applying adaptation strategies, whereas age shows an inverse relationship [26-28]. The adverse impacts of climate change on productivity often result in higher commodity prices and necessitate more intensive management practices, expanded cultivation areas, reallocation through international trade, and reduced consumption [29]. For instance, adopting altered cultivation methods, implementing modern irrigation techniques, and promoting crops with lower water requirements effectively mitigate climate change effects in Iran. Addressing greenhouse gas emissions and combating desertification are critical to reducing climate change impacts [30]. Local agricultural knowledge and practices contribute to achieving sustainable agriculture. Workshops that facilitate knowledge sharing among farmers play a crucial role in climate change adaptation, enhancing productivity and sustainability. Low awareness of the health benefits of locally produced organic food often leads to the widespread consumption of cheaper imports, undermining local markets. Integrated land use planning transportation projects and affordable housing initiatives can improve low-income individuals' financial stability and mental health, ensuring access to nutritious food and mitigating adverse impacts on natural conditions, economic welfare, and food security [31].

Sustainable Diets: A Solution

Over the past 50 years, changes in food production have alleviated hunger, increased life expectancy, reduced infant and child mortality, and alleviated global poverty. However, increased food accessibility, urbanization, and rising incomes have led to a nutritional transition crisis, manifesting in:

- More than 820 million undernourished individuals
- 151 million stunted children
- 51 million wasted children
- Over 2 billion people suffering from micronutrient deficiencies
- 1-2 billion adults overweight or obese
- Nearly doubling global diabetes prevalence in the past 30 years [5, 32].

A sustainable food system integrates all elements and activities related to food production, processing, distribution, preparation, and consumption to provide food security and ensure health while minimizing ecological impact and safeguarding the rights of future generations [5]. The sustainable food system comprises four interconnected dimensions, each crucially linked to agriculture: health, environmental sustainability, economic viability, and social equity. Thus, dimensions should shift in the food basket to prioritize nutritious, culturally appropriate, accessible, affordable, and low-waste diets [1, 5, 22, 32-42]. Aligning with the transition in the food basket, sustainable diets offer a pivotal approach to achieving a sustainable food system. Dietary guidelines are critical in addressing sustainability challenges within diets and food systems by promoting improved nutrition and health outcomes while reinforcing ecological, economic, and cultural resilience. Identifying gaps in sustainability within dietary guidelines helps integrate multiple sustainability dimensions into evolving food systems. Tailoring national dietary recommendations to encompass distinct sustainability sub-dimensions through targeted programs tailored to each country's unique attributes enhances policy coherence and stakeholder engagement across food system development [31, 43].

Sustainable diets optimize individual health and well-being across all dimensions, minimize environmental impact, ensure accessibility, affordability, safety, and equity, and uphold cultural acceptability. They are designed to support optimal growth and development at all life stages, minimize all forms of malnutrition (undernutrition, micronutrient deficiencies, overweight, and obesity), reduce dietary-related noncommunicable diseases, and preserve biodiversity and planetary health. Energy consumption and carbon footprint are evaluated using a hybrid Input-Output Analysis-Life Cycle Assessment (IOA-LCA) method to assess the environmental footprint of different diets [38]. Dietary greenhouse gas emissions, contributing to climate change, can be reduced by eliminating meat from diets [44].

GRAPHICAL ABSTRACT [45, 46]....

Eat-lancet

A group of scientists (2019) presented a series of reference values for all food groups to show that adhering to these values can make food production sustainable and stick to 1.5°C changes in temperature, which is responsible for 10 billion people.

EAT-Lancet References: Protein Intake

The provided values are based on prospective studies conducted on the consumption of red meat **[**37**,** 39**]**:

Various dietary patterns, including vegan, vegetarian, pescatarian, or semi-vegetarian, have been associated with a 12% lower overall mortality risk compared to omnivores. A plant-based dietary score has shown a linear inverse relationship with the risk of type 2 diabetes and coronary heart disease [39]. In middle and low-income countries, the recommended intake ranges from 0 g/day to approximately 28 g/day of red meat, with a midpoint of 14 g/day for the reference diet.

Diary

The recommended calcium intake of 1200 mg/day in the USA is derived from studies lasting three weeks or less. Regions with low dairy consumption and calcium intake have demonstrated lower fracture rates than areas with high dairy consumption (WHO, 2003). There is no significant reduction in fracture risk among adults who consume more than 500 mg of calcium daily. The reference diet provided in the EAT-Lancet report contains 718 mg/day of calcium. A report such as that published by EAT-Lancet offers valuable information, but it has some limitations as well, which primarily focuses on the production and consumption stages of the food system, neglecting the social and economic aspects [34, 42, 47, 48].

Vegetarian Diet

Vegetarian diets have been associated with a lower incidence of type 2 diabetes, obesity, coronary heart disease, and other noncommunicable diseases, as well as greater life expectancy. Shifting to ovolactovegetarian and vegan diets could result in median reductions of -35% and -49% in greenhouse gas emissions, respectively. Shifting to ovolactovegetarian and vegan diets could also reduce land use by -42% and -49.5%, respectively. Adopting an ovolactovegetarian diet can achieve a median decrease of -28% in water consumption. The vegetarian diet outperforms the national average diet regarding overall environmental footprint, with 3.14% lower energy consumption and 12.7% lower carbon footprint. All vegetarian diets are not equally sustainable, and there is substantial variability among the vegetarian diets. The flexitarian diet is often recommended due to its lower environmental impact and proven health benefits [35, 37, 39, 41].

Considerations during Complementary Feeding Period

A vegetarian diet, against a healthy omnivorous diet like the Mediterranean diet, carries several risks, such as the risk of critical micronutrient deficiency or insufficiencies or the risk of growth retardation resulting in different outcomes in neurophysiological development and growth.

There is no data documenting the protective effect of vegetarian or vegan diets against communicable diseases in children aged six months to 2-3 years [40].

Mediterranean Diet

Claims have been made in several international reports that the Mediterranean diet offers the best consumption pattern regarding both the environment and health. However, a specific assessment of the Mediterranean diet in comparison with other dietary patterns is lacking [49]. The Mediterranean diet is more than just a nutritional model and involves all stages before food consumption, including crop selection, growing, harvesting, fishing, processing, and food preparation. These activities are carried out to respect lands and landscapes, conserve traditional practices, and preserve fishing and farming activities in Mediterranean communities. The Mediterranean diet was added to the Representative List of the Intangible Cultural Heritage of Humanity in 2010 due to its cultural and sustainable characteristics [38].

Food Loss and Food Waste

Food loss occurs before reaching the consumer, while food waste occurs after consumption. Food loss and waste negatively affect food security and nutrition and contribute significantly to greenhouse gas emissions, environmental pollution, degradation of natural ecosystems, and biodiversity loss. For instance, in 2010, food loss and waste during the retail and consumer stages in the United States resulted in approximately 160 million metric tons of CO_2-equivalent greenhouse gas emissions [5, 50]. Individuals are responsible for improving consumption habits, increasing awareness and information, and encouraging interdepartmental cooperation and systemic thinking among professionals to address these issues.

Conclusion

The current global context is characterized by various crises, such as obesity, undernutrition, and climate change, all of which present significant health risks. Nutrition, health, and the environment are intricately linked across the lifespan. Climate change and pandemics have a detrimental effect on food systems, worsening global food insecurity. Agricultural and food systems significantly affect the environment, climate, dietary habits, and health. Yet, proactive management, agricultural investments, and dietary adjustments can effectively alleviate the effects of climate change. Educating farmers on local production and sustainability practices within the food system, alongside addressing financial challenges, could serve as a viable strategy and policy to combat climate change and food insecurity.

Declarations

Limitations and Strengths

This study is the first comprehensive review of the effect of climate change on food security, sustainable diets, and their role. However, this investigation focused primarily on the impact of factors such as temperature, precipitation, and greenhouse gases on specific subgroups of the food system due to limited access to sufficient information. The publication bias could not be entirely excluded. There was considerable heterogeneity between the included studies. In addition, all included studies were not methodologically appropriate. The potential methodological problems of studies were bias regarding selection, performance, detection, and reporting. Further studies are needed to examine the interaction between sustainable diets and climate change, compare sustainable diets in terms of health and overall utility, and delve into the details of these topics.

Authors' contributions

A. Ashabi designed and supervised the study, and M. Soltanian conducted research and wrote the paper; M. Soltanian was primarily responsible for the final content. All authors read and approved the final manuscript.

Conflict of Interests

There is no conflict of interest to declare.

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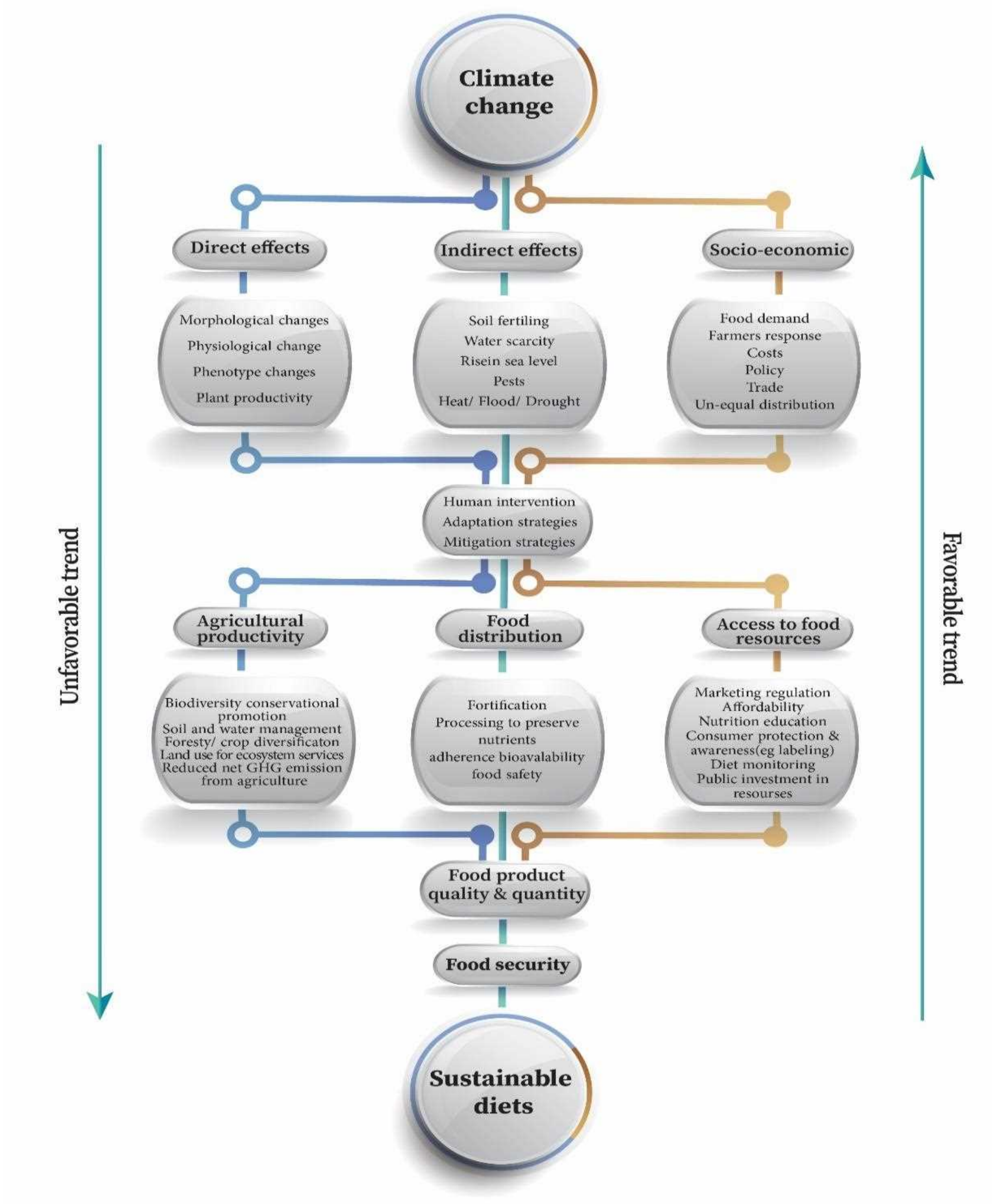
GRAPHICAL ABSTRACT



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The Effect of Interval Training on PPARy-GLUT4 Expression in Subcutaneous Adipose Tissue of Male Wistar Obese Rats

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ABSTRACT

Introduction: Genetic and metabolic dysfunctions are primary contributors to the onset and intensity of type 2 diabetes (T2D) through the promotion of insulin resistance. The objective of this research was to examine the impact of high-intensity interval training (HIIT) on the expression levels of PPARγ and GLUT4 in the subcutaneous adipose tissue, as well as its influence on insulin sensitivity among obese rats.

Methods: Obesity was induced by high fat diet (HDF) in 14 male wistar rats. Then rats were divided randomly into exercise (HIIT, n = 7) or control (n = 7) groups. The exercise group completed an 8 weeks HIIT (5 days / weekly) and the control group received no training. Fasting blood glucose, insulin sensitivity and PPARγ and GLUT4 gene expression in subcutaneous adipose tissue were measured 48 hours after last exercise session. Independent t-test was used to compare variables between groups.

Results: HIIT resulted in a significant decrease in fasting blood glucose (P = 0.001) and increase in insulin sensitivity (P = 0.001), PPARγ expression (P = 0.038) and GLUT4 expression (P = 0.019) in subcutaneous adipose tissue compared with control rats.

Conclusion: HIIT can be improve insulin sensitivity in obese rats. This improvement may be attributed to increased PPARγ and GLUT4 expression in subcutaneous adipose tissue.

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Introduction

Over many years, researchers have come to believe that obesity is the result of complex interactions between hormonal and environmental factors acting on fat and glucose metabolism, such as liver and muscle insulin function defects, adipose tissue metabolism, and lipolysis of the whole body (1). Recent research over the past ten years has highlighted the significance of genetic contributors alongside other factors in the development of obesity and its associated metabolic conditions. The alteration in the expression of certain genes or proteins, which act as transcription factors, can impact carbohydrate and lipid metabolism through their effects on lipolysis or insulin function. Notably, genetic components like FOXO1, PPARγ, and FTO are involved in regulating energy balance as well as glucose and lipid metabolism within specific tissues, including skeletal muscle and adipose tissue (2, 3). Moreover, numerous studies have documented a correlation between the levels and expression of these proteins with obesity, lipid profiles, and insulin resistance (3, 4).

Among the genetic components, the effective role of PPARy in controlling insulin action and glucose homeostasis has been mentioned (3). PPARγ is expressed in white and brown adipose tissue, colon and spleen. Nonetheless, its levels are markedly elevated in adipocytes, where it serves a crucial function in controlling the formation of adipose tissue, maintaining energy equilibrium, and synthesizing lipids (5, 6). The mechanisms responsible for the effect of PPARy on insulin sensitivity are complex and adipose tissue, skeletal muscles and liver are its target points, but it seems that adipose tissue is the most important target tissue of PPARy-TZDs, which is manifested by increasing insulin sensitivity (7). Although PPARy is often expressed in adipose tissue, it is also expressed in some other tissues such as skeletal muscle and liver, which are involved in glucose homeostasis

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(3). It has been found that PPARγ activity directly regulates the expression of GLUT4 as the main glucose transporter in adipose tissue and skeletal muscle (8). These evidences somehow support the interaction of PPARγ and GLUT4 in glucose homeostasis in the target tissue. Laboratory studies have revealed that the expression of PPARy is reduced in obese animal species (9) and the use of PPARy agonists by increasing its expression in type 2 diabetic rats is associated with the improvement of glucose metabolism and insulin function (10).

During the last two decades, a multitude of research endeavors have explored the impacts of various therapeutic treatments with the aim of improving carbohydrate and lipid metabolism or inflammatory profile in healthy or sick obese populations, but among them, there are few studies aimed at the effect of non-pharmacological interventions such as exercise. It has been done on genetic or transcription factors or their polymorphisms in healthy or sick obese people. In this context, the study of Lee et al (2014) showed that 8 weeks of low-intensity strength training leads to an increase in PPARγ expression in adipocytes of obese Sprague Dawley rats, but glucose response and insulin resistance were not mentioned in this study (11). Rufino et al, (2016) also reported that exercise increases the expression of some genetic markers in macrophages by increasing the activity of the PPARy transcription factor, which is associated with anti-inflammatory properties and improving insulin sensitivity to prevent insulin resistance and type 2 diabetes. (12). On the other hand, in Garley's study (2016), 4 weeks of aerobic running in obese mice fed a high-fat diet was associated with an improvement in fasting insulin, but did not affect the expression of GLUT4 in skeletal muscle (13). In another study, GLUT4 expression in adipose and muscle tissue of type 2 diabetic rats was not affected by regular aerobic exercises (14). In addition, In Benafar's study (2018), 6 weeks of resistance exercise led to an increase in GLUT4 expression in the biceps muscle of type 2 diabetic rats (15). In Bagheri et al.'s study (2020), 8 weeks of intense interval training led to an increase in hepatic PPARy expression and hepatic triglyceride content in rats with fatty liver (16).

The review of research evidence on the one hand points to contradictory findings regarding the response of the expression of these transcription factors to exercise training and on the other hand to the lack of sufficient studies regarding their response to high intensity interval training (HIIT) in obese rats. Consequently, the current research was undertaken to ascertain the impact of HIIT over a period of 8 weeks, with a frequency of 5 times per week, utilizing treadmill running, on the expression of PPARγ and GLUT4 in the subcutaneous adipose tissue of rats consuming a high-fat diet (HFD). Additionally, this study evaluated alterations in glucose concentrations and insulin sensitivity.

Materials and Methods

Experimental Animals

The research sample for this controlled experiment comprised exclusively male Wistar rats housed at the Pasteur Institute of Tehran's animal facility. From this population, 14 rats (aged 10 weeks, weighing 220 ± 10 grams) were subjected to a high-fat diet (HFD) for 8 weeks, leading to obesity. Subsequently, these rats were allocated into two groups: a control group (n=7) and a high-intensity interval training (HIIT) group (n=7). The conditions for the rats included a regulated lighting environment with 12-hour cycles of light and darkness, and a stable climate maintained at 22 ± 3°C with relative humidity between 30% and 60%.The high-fat diet for the group continued until the end of the study.

Induction of Obesity

To induce obesity, a HFD was used for 8 weeks. In order to prepare high-fat food, first, standard food was prepared from Pars Animal Feed Company, then it was kneaded and added to 1% cholesterol powder and 1% pure corn oil (100%) and made into pellets again (17).

Training Protocol

Following the obesity induction, the cohort of 14 obese rats was split into two groups: control and HIIT groups. The HIIT group underwent an 8-week regimen of HIIT, consisting of five weekly sessions that involved treadmill running, as detailed in Table 1. The control group rats were not included in this exercise regimen. Forty-eight hours subsequent to the final exercise session, all rats from both groups were subjected to dissection post an overnight fast.

Sample Collection and Biochemical Assay

48 hours subsequent to their final exercise session, having fasted for a period of 10 to 12 hours, the experimental rats from each group

were anesthetized via intraperitoneal injection with a solution comprising 10% ketamine at a concentration of 50 mg/kg and 2% xylazine at 10 mg/kg. Following this, samples of subcutaneous adipose tissue were collected from the rats, rinsed with saline solution, and then preserved in microtubes of 1.8 ml capacity filled with a 20% solution of RNAlater for subsequent genetic analysis. The extraction of RNA was carried out utilizing the RNeasy Mini Kit provided by QIAGEN. For the quantification of gene mRNA levels, RT-Real Time PCR was conducted using the Rotorgen 6000 system and the One Step SYBR Green Kit by Takara, in accordance with the manufacturer's protocol (17). RNA Polymerase II served as the control gene. The specific sequences of the primers utilized are detailed in Table 1.

Table 1. High intensity interval training protocol according to speed and time of running in interval obese group

Exercise session (weeks)	**Exercise phase**		**Resting phase**	
	Time (S)	**Speed** (m/min)	**Time** (S)	**Speed** (m/min)
1 -2	40	20	120	14
3 – 4	40	25	120	14
5 – 6	40	30	120	14
7 - 8	40	35	120	14

* Running time in the exercise phase is 40 seconds and in the active rest phase is 2 minutes and the speed is in meters per minute

Table 2. Primer sequence

Genes	**Primer sequence**
PPARγ	**For**: ACAACAGGCCACATGAAGAGC **Rev**: AAGCTTCAATCGGATGGTTCTTCG
GLUT4	**For**: CTTGGCTCCCTTCAGTTTGG **Rev**: CCTTTCTCTTCCCACCACCTG
RNA Polymerase II	**For**: ACTTTGATGACGTGGAGGAGGAC **Rev**: GTTGGCCTGCGGTCGTTC

Statistical Analysis

The Shapiro-Wilk test was employed to verify the data's normality. Descriptive statistical methods were utilized to characterize the data and graphical representations, while independent t-tests were applied to assess differences between groups concerning the variables under investigation. A significance threshold was set at an alpha level of less than 0.05. All statistical analyses were conducted using the SPSS for Windows, version 22 software.

Results

Alterations in body weight for both groups pre- and post-exercise program are detailed in Table 3. The independent t-test revealed no significant difference in baseline weight between the cohorts (P = 0.632). Conversely, while the paired t-test indicated a significant increase in weight from start to finish of the intervention, the independent t-test demonstrated that this increase did not result in a significant difference in final body weight when comparing the two groups (P = 0.126).

Statistical analysis revealed significant differences between the two groups concerning their glucose levels, insulin sensitivity, and the expression of PPARγ and GLUT4, as detailed in Table 4. In other words, HIIT resulted in significant decrease in fasting blood glucose (P = 0.001) and increase in insulin sensitivity (P = 0.001), PPARγ expression (P = 0.038, Fig 1) and GLUT4 expression (P = 0.019, Fig 2) in subcutaneous adipose tissue compared with control rats.

Table 3. Pre and post-training of body weight of 2 groups (Mean ± SD).

Group	**Pre-training**	**Post-training**	**sig***
Control	295 ± 8.5	370 ± 4.9	*0.001*
Exercise	297 ± 6.6	364 ± 7.5	*0.001*
sig¥	*0.632*	*0.126*	-----

** Significant changed based on paired t test*
¥ Significant change based on independent t test

Table 4. Clinical characteristic and genes expression after HIIT intervention of exercise and control groups (Mean ± SD).

Variable	Control group	HIIT group	*sig*
Fasting glucose (mg/dl)	120 ± 4	96 ± 5	*0.001*
Insulin sensitivity (HOMA-IS)	0.56 ± 0.01	0.66 ± 0.05	*0.001*
PPARγ expression	1	1.57 ± 0.65	*0.038*
GLUT4 expression	1	1.26 ± 0.25	*0.019*

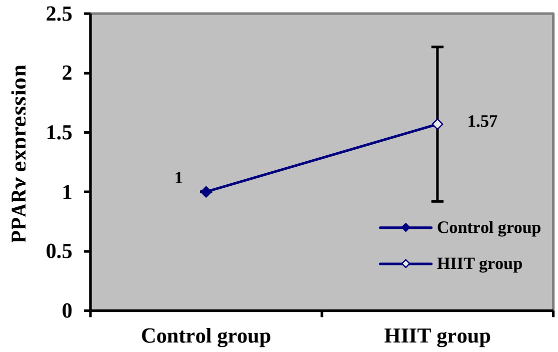

Figure 1. PPARγ expressions in subcutaneous adipose tissue in exercise rats compare to control group.

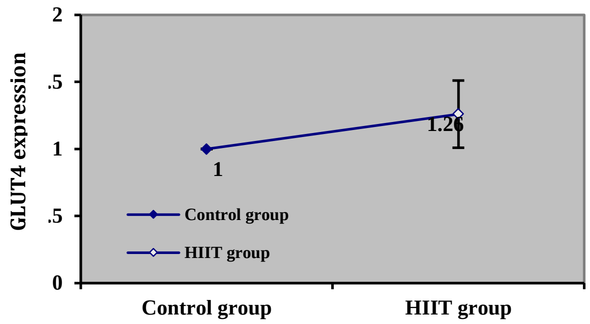

Figure 2. GLUT4 expressions in subcutaneous adipose tissue in exercise rats compare to control group.

Discussion

The research identified a notable elevation in PPARγ and GLUT4 expression in the subcutaneous adipose tissue as a key outcome. Specifically, subjecting obese rats, whose condition was prompted by a HFD, to HIIT five times per week over an eight-week period resulted in enhanced PPARγ and GLUT4 expression in their subcutaneous adipose tissue relative to a control group that did not engage in the exercise intervention. Additionally, the HIIT was linked with a marked reduction in fasting blood glucose levels and an improvement in insulin sensitivity when compared to the control group. This finding of reduced fasting glucose following diverse exercise protocols aligns with the results documented in prior research. In line with the present study, in Bai et al.'s study (2013), 2 months of aerobic exercise led to a significant reduction in fasting blood glucose in overweight male and female students (18). Also, in Di Raimondo's study (2013), 24 weeks of exercise training in the form of 1 hour of brisk walking on a treadmill for 5 sessions per week led to a significant reduction in glucose and glycosylated hemoglobin in patients with metabolic syndrome (19). However, contrary to the findings of this study, in another study, 6-week exercise training with an intensity of 60 to 80% of VO2max did not lead to a significant change in glucose (20). Also, in another study, 20 weeks of sports activity in the form of 3 to 5 sessions with an intensity of 70% of VO2max per week did not lead to a change in glycosylated hemoglobin (21). In the study of Maltais et al. (2016), 4 months of resistance training, although it was associated with a decrease in body fat mass, did not lead to a change in insulin and glucose in overweight elderly men (22).

Despite the contradictions in the mentioned findings, which are often rooted in differences in the type, duration and intensity of training or differences in the type of population studied, most studies support the improvement of blood glucose or glycemic profiles following exercise, especially those that have continued for a long time. In the meantime, most studies have attributed this improvement to the reduction of insulin resistance following exercise. Thus, in the present study, in addition to improving fasting glucose, insulin sensitivity was also increased in response to HIIT. In confirmation the outcomes of this research, which identifies the enhancement of insulin sensitivity as a key outcome in response to HIIT, a parallel investigation by Ho (2015) explored the impact of a year-long weight loss initiative involving dietary limitations. This study focused on obese and overweight individuals, assessing insulin resistance, insulin sensitivity, and inflammatory indicators. The results indicated that the 12-month regimen led to a significant reduction in insulin resistance and an elevation in insulin sensitivity (23). Drawing from their results, these scholars have highlighted the advantageous therapeutic influence of exercise in diminishing risk factors associated with the development of insulin resistance within obese individuals.

Despite the mentioned evidence, some other studies have reported the non-alignment of their findings with our findings. For example, in Legat's study (2012), 6 sessions of HIIT in a two-week period was not associated with a remarkable change in insulin sensitivity in obese men (24). In Dongz's study (2013), 12 weeks of endurance and resistance training was not associated with significant changes in insulin function and cellular glucose transport in middle-aged obese men (25). Longitudinal studies have also indicated that changes in protein levels or expression of some genes in the target tissue strongly affect insulin action in adipose and muscle tissue. Some of them, such as GLUT4, also affect glucose transport directly or by affecting insulin signaling mechanisms in the target tissue (26). On the other hand, the findings of this study revealed that the expression of GLUT4 and PPARγ is affected by HIIT. In other words, 8 weeks of HIIT increased PPARγ and GLUT4 expression in subcutaneous adipose tissue of obese rats. In this context, in the study of Li et al, (2014) the protein and expression of PPARγ in subcutaneous adipose tissue of male Sprague Dawley rats were increased in response to 8 weeks of low, moderate and intense resistance training compared to the control group (11). Some other studies have also reported the improvement of blood glucose with an increase in PPARγ expression in response to relatively intense aerobic exercise (11).

In the study of Pala et al, (2018) although acute exercise for 30 minutes led to a decrease PPARγ expression in liver and muscle tissue of albino Nejard rats, continued exercise for 6 weeks significantly increased PPARγ and GLUT4 and GLUT2 in liver and muscle tissue (27). Laboratory evidence has supported the effective role of GLUT4 protein levels in fat and muscle tissue in glucose regulation (28). In patients with insulin resistance, the metabolic process of glucose in adipose and muscle tissue is damaged, and the response of GLUT4 to insulin is impaired (29). In confirmation of our findings, Lennon et al (2010) have mentioned that relatively intense exercise leads to an increase of 34 and 22% of GLUT4 in the heart and adipose tissue of laboratory rats (30). Hashi et al (2011) also lead to a 36 and 20% increase in GLUT4 expression in skeletal muscles and adipose tissue of type 2 diabetic patients (31).

The mechanisms responsible for the effect of PPARγ on insulin sensitivity and resistance are complex and adipose tissue, skeletal muscles and liver are its target points, but it seems that adipose tissue is the most important target tissue of PPARγ-TZDs, which is manifested by increasing insulin sensitivity (7). In this context, it has been determined that in type 2 diabetic patients, PPARγ activity through binding to thiazolidinediones (TZD) leads to a significant improvement in insulin sensitivity of the whole body, which is associated with a decrease in insulin and glucose levels (7). It is also possible that the change in the activity or expression of PPARγ in response to exercise due to the effect on other transcription factors effective in insulin signaling pathways, such as GLUT4, leads to a decrease in insulin resistance or an improvement in glycemic profile(8). It should be noted that although measuring the expression of the mentioned genes is the strengths of the present study, this evaluation alone does not represent the response of the glycemic profile to exercise because many hormonal and genetic components such as inflammatory and anti-inflammatory mediators and stress agents Oxidative agents are effective in this process and their lack of measurement is one of the limitations of the present study.

Conclusion

HIIT improves glucose in obese Wistar rats. This improvement may be attributed to increased GLUT4 and PPARγ expression in subcutaneous adipose tissue along with an increase in insulin sensitivity in response to this training method. However, understanding the mechanisms responsible for changes in insulin action in response to exercise requires more studies.

Declarations

Acknowledgments

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Authors' Contributions

Each author contributed equally to the composition of this article.

Conflict of Interest

The authors have reported no potential conflicts of interest.

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The Effect of Aerobic Training Combined With Martighal Consumption on Vascular Endothelial Growth Factor and Homocysteine in Sedentary Women with Metabolic Syndrome

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ABSTRACT

Introduction: Recently, the role of using medicinal plants during exercise training has been expanded with the aim of improving antioxidant and cardiovascular function in healthy and sick obese populations. The aim of this study was to investigate the effect of aerobic training combined with Martighal extract on the serum levels of vascular endothelial growth factor (VEGF) and homocysteine in females with metabolic syndrome.

Methods: In this clinical trial, 48 obese females (30 ≤ BMI ≤ 36, mean weight: 84.45 ± 5.41 kg) diagnosed with metabolic syndrome aged 30-45 years were randomly allocated into four distinct groups: control (no intervention); Martighal extract (280 mg /daily); aerobic training (alternate days) and combined (aerobic training + Martighal) groups. Baseline concentrations of fasting VEGF and homocysteine, along with anthropometric measurements, were recorded prior to and 48 hours after the final training session for each group. To analyze the data, Analysis of Covariance (ANCOVA) was employed for inter-group comparisons, while the paired-sample t-test was utilized to assess within group changes (P ≤ 0.05).

Results: Compared to the control group, serum VEGF significantly increased in the aerobic, Martighal and combined groups (P=0.001). There was no significant difference in homocysteine levels in response to the interventions (P=0.919).

Conclusion: Despite the mentioned evidence, the effectiveness of Martighal consumption during aerobic exercise cannot be emphasized only by improving VEGF, and it is suggested to measure other markers of vascular endothelial function in order to obtain the mechanisms responsible for Martighal supplementation during exercise in these patients.

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Introduction

Metabolic syndrome is an obesity-related disease that often includes symptoms of chronic diseases such as type 2 diabetes, high blood pressure, and cardiovascular diseases. In other words, people with metabolic syndrome also suffer from diabetes and cardiovascular disorders (1). In another definition, abdominal obesity, insulin resistance, hyperglycemia, high systolic and diastolic blood pressure, low HDL levels, and increased triglycerides, cholesterol, and LDL are indicators of this syndrome (2). People with metabolic syndrome also suffer from cardiovascular disorders and oxidative stress or vascular endothelial disorder, like other abnormalities related to obesity (3). Thus, obesity and metabolic syndrome, due to the direct or indirect damage to the vascular dilatation characteristics of the endothelium, lead to endothelial dysfunction, which has been introduced as the first step in the spread or progression of cardiovascular diseases (4). Obesity is also related to endothelial dysfunction and blood pressure due to other factors such as

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narrowing of blood vessels, increased sympathetic activity and hyperactivity of the renin-angiotensin system and increased insulin resistance (5).

It is well known that the endothelium is one of the important areas in the control and regulation of vascular function and some hormonal and enzymatic components play a key role in this process. Among the hormonal components, clinical studies have reported homocysteine as one of the cardiovascular risk factors and supported it as an indicator of heart attack. higher serum homocysteine, especially in the presence of obesity, is associated with increased risk of heart diseases such as atherosclerosis and coronary artery disease (6, 7). Homocysteine is produced from the demethylation of methionine, which is introduced as a homologue of cysteine. Its high level is associated with the risk of heart attack, arteriosclerosis. Homocysteine has been introduced as a risk factor for coronary and peripheral vascular disease, and can lead to atherosclerosis due to damage to the inner wall of the arteries, interference in blood coagulation pathways, and the oxidation of lipoproteins (8). As a vascular repair factor, the protective effect of VEGF on blood vessels in cardiovascular disease has been reported in a large number of studies (9, 10). Homocysteine can downregulate the expression of VEGF in endothelial cells. Furthermore, the inhibitory effect of homocysteine on the migration of endothelial cells was achieved by downregulating the expression of VEGF using small interfering RNA transfection (11).

In summary, disruption in the systemic levels of components such as homocysteine and VEGF is associated with damage to vascular endothelial function in obese people or those with diseases related to obesity, which include metabolic syndrome. These components may provide suitable solutions with the aim of prevention, control, and improvement of metabolic syndrome in health and wellness science studies. Based on the mentioned evidence, the improvement of vascular endothelial function by pharmaceutical or non-pharmacological treatments is in the focus of health science researchers. In this context, the effective role of exercise training as one of the non-pharmacological treatments on the metabolic, hormonal and genetic components of obesity-related chronic diseases has been frequently proposed, although the findings are more or less contradictory. Farahti et al (2012) reported that 8 weeks of aerobic exercise increased nitric oxide and decreased weight and fat percentage in postmenopausal women (12). In addition, although Vale et al, (2023) reported that 8 weeks of aerobic training leads to a significant decrease in nitric oxide and a decrease in myeloperoxidase in women with metabolic syndrome (13), but Shekarchizadeh et al (2012) reported no change in nitric oxide and VEGF in response to 4 weeks of resistance training (14). In a study by Soori et al, (2016), 10 weeks of aerobic training was associated with a decrease in homocysteine, but no change in cardiovascular risk factors such as LDL and TG in obese women (15).

Apart from the contradictory effects of exercise training on the aforementioned components, which often depend on the difference in the type, duration, intensity, and population studied (12, 13, 14, 15), some recent studies have investigated the effect of exercise training with nutritional supplements, especially antioxidant supplements on cardiovascular function markers. Among these supplements, Martighal plant products in the form of plant extracts or medicines made from it such as silymarin, which is made of elements called flavonolingan with antioxidant properties play an important role in inhibiting free radicals and lipid peroxidation along with increasing antioxidants (16). Reducing the levels of malondialdehyde is one of the most important indicators of oxidative stress (16, 17) and increasing antioxidant capacity (18) and other enzyme antioxidants such as Superoxide dismutase (SOD) are among its other confirmed effects (19). As Shirali et al, (2017) have pointed out a significant increase in SOD in response to 6 weeks of aerobic exercise and consumption of silymarin as one of the products of Martighal (19). In the study by Roghani et al, (2012), Martighal extract consumption (100 mg/kg) for 4 weeks decreased hepatic MDA in diabetic rats (20). Despite its effect on the components of oxidative and antioxidant stress that affect cardiovascular function, the direct role of Martighal supplementation on the markers of vascular endothelial function that were mentioned earlier are less reported. Therefore, based on the contradiction in the findings regarding the effect of exercise training on the indicators of vascular endothelial function on the one hand and the lack of direct evidence on the effect of these components to Martighal, especially in patients with metabolic syndrome, the present study was done with the aim of evaluating the effect of interval aerobic training combined with Martighal consumption on vascular endothelial growth factor and homocysteine in sedentary women with metabolic syndrome.

Material and Methods

Subjects

The study employed a clinical trial (Ethic code: IR.IAU.CTB.REC.1401.139), utilizing an experimental framework that incorporated both pretest and posttest assessments. The study sample frame included voluntarily participating women aged 30-45 years who were obese (30 ≤ BMI ≤ 36, height: 163 ± 5.35 cm) and have been diagnosed with metabolic syndrome, selected through a convenience sampling method. Based on similar articles (13, 19, 20), sample size was calculated with a confidence level of 95% and a test power of 80% and according to previous studies (S = 0.6). To reach a significant difference of at least 5%, 12 people were considered in each group. The study sample was 48 adult obese women with metabolic syndrome who were randomly divided into 4 groups: 1) Aerobic exercise group "8 weeks of interval aerobic training every other day", 2) Martighal group "8 weeks of taking Martighal supplement, 280 mg daily", 3) combined group "8 weeks of interval aerobic exercises with Martighal supplement" and 4) control group "no intervention".

Inclusion or Exclusion Criteria

Obesity (BMI ≥ 36 ≥ 30) and the presence of at least 4 criteria of metabolic syndrome (waist circumference greater than 88, fasting glucose greater than 100 mg/dL, systolic blood pressure greater than 120 and diastole greater than 90, HDL lower than 50 mg/dL and triglycerides above 150 mg) were the inclusion criteria for the study. The study participants were non-smokers, non-pregnant, non-alcoholic and non-athletes. They also abstained from any consistent exercise training over the past six months. Furthermore, these participants did not adhere to a specific diet, and their weight has remained relatively stable, with variations of less than one kilogram. The non-inclusion criteria were the absence of any previous renal disorders, cancer, or seizure episodes. The exclusion criteria were lack of routine involvement in exercise programs, as well as the non-use of dietary supplements or the presence of any medical conditions that could influence the outcome variables under investigation

Anthropometric Measurements

The anthropometric indices were assessed before and after the intervention. For the determination of height, a rigid measuring tape was employed, with participant standing in barefoot to the nearest 0.1 cm. Measurements of the hip and waist circumferences were taken after a standard exhale using a non-stretchable measuring tape to the nearest 0.1 cm. The participants' weight was measured using a Secca scale to the nearest 0.5 kg. Body mass index (BMI) was calculated by dividing the individual's weight in kilograms by their squared height in meters. The percentage of body fat was determined using a body composition monitor (OMRON-BF 508, Finland).

Exercise protocol and Martighal consumption

Participants in the Martighal group were administered a daily 280 mg Martighal extract (21) after breakfast (8-9 am) for eight weeks, during which they were instructed to abstain from all exercise training. The aerobic group underwent an interval aerobic training program on alternate days between 9-11 am over an eight-week period with explicit guidance to refrain from any additional exercise programs beyond the specified aerobic exercise. The combined group engaged in an eight-week protocol that incorporated both aerobic exercise (9-11 am) and the daily intake of 280 mg Martighal extract (8-9 am). Meanwhile, the control group continued with their regular daily activities without any involvement in structured physical activity programs for the same eight-week timeframe.

During the aerobic intervention, participants engaged in interval aerobic exercise at intensities between 55% and 75% of their maximum heart rate (HRmax). Each workout commenced with a 10-minute warm-up, followed by 15 to 40 minutes of aerobic exercise, and ended with a 5 to 10-minute cooldown period. The aerobic training involved level-surface running. For the initial week, the exercise regimen was maintained at a lower intensity, which was incrementally escalated to reach 75% of HRmax during the concluding weeks (22, Modified). The Polar heart rate monitor (manufactured in the

USA) was utilized to precisely regulate the intensity of exercise throughout each session.

Laboratory and Clinical Measurements

Blood specimens were obtained from the participants after an overnight fasting for 12 hours at 8 to 9AM in the morning prior to the initiation of training. Participants were instructed to refrain from engaging in strenuous physical activities for a period of two days preceding the collection of blood samples. After the end of the final exercise session, participants underwent a 48-hour recovery period before their fasting blood samples were drawn similar to the initial pre-training procedure. Serum was promptly extracted from each collected blood sample and preserved at a temperature of -80 degrees Celsius until analysis could be conducted. VEGF was measured using a commercial kit (Cusabio Company, China) with a measurement accuracy of 0.8 pg/ml by ELISA method. Serum homocysteine was also measured calorimetrically using a specialized kit (Zelbio Company, Germany).

Statistical Methods

Comparative statistical analyses were conducted using IBM SPSS Software version 22, with a significance level of less than 5 percent. The Kolmogorov-Smirnov test verified the normal distribution. To compare the data between the groups, ANCOVA test was used along with Bonferroni's post hoc test. Additionally, the paired t-test was employed to assess changes within the groups.

Results

Table 2 shows the change of anthropometric indicators in response to aerobic exercises, martighal supplementation, as well as aerobic exercises combined with martighal supplementation.

Table 1. Distribution of exercise intensity while running during the interval aerobic training (22 modified)

weeks	Exercise intensity (%HRmax)	Time of running
First and second	%55 ≤ intensity ≤ %60	3 × 5 minute
Third and fourth	%60 ≤ intensity ≤ %65	2 × 10 minute
Fifth and Sixth	%65 ≤ intensity ≤ %70	2 × 15 minute
Seventh and eighth	%70 ≤ intensity ≤ %75	2 × 20 minute

Table2. Intra-group variations of anthropometric indices in the pre-test and post-test conditions in the studied groups

Group	Time	Control	Exercise	Martighal	Combine
Height (cm)	*Pre-post test*	160 ± 3.28	162 ± 3.18	163 ± 4.64	162 ± 3.06
	Pre-test	*84.18 ± 3.26*	84.44 ± 3.26	84.92 ± 5.41	84.25 ± 4.14
Weight (kg)	*Post-test*	*84.22 ± 3.39*	79.17 ± 2.20	83.21 ± 5.35	79.62 ± 4.40
	Sig	*0.713*	0.001*	0.001*	0.001*
	Pre-test	*113 ± 9.19*	109 ± 5.96	108 ± 8.21	106 ± 5.48
AC (cm)	*Post-test*	*113 ± 8.80*	103 ± 5.11	106 ± 9.91	100 ± 5.38
	Sig	*0.417*	0.001*	0.338	0.001*
	Pre-test	*114 ± 8.73*	108 ± 6.51	108 ± 8.46	105 ± 6.03
HC (cm0	*Post-test*	*114 ± 9.06*	104 ± 5.03	108 ± 8.96	101 ± 5.55
	Sig	*0.615*	0.001*	0.731	0.001*
	Pre-test	*32.77 ± 1.48*	32.35 ± 1.24	32.13 ± 0.86	32.23 ± 1.23
BMI (kg/m2)	*Post-test*	*32.79 ± 1.53*	30.33 ± 1.20	31.49 ± 1.01	30.47 ± 1.64
	Sig	*0.703*	0.001*	0.001*	0.001*
	Pre-test	*41.69 ± 1.43*	40.59 ± 1.43	40.49 ± 1.02	40.33 ± 1.20
Body fat (%)	*Post-test*	*41.61 ± 1.40*	34.50 ± 0.79	38.63 ± 1.03	34.30 ± 1.67
	Sig	*0.470*	0.001	0.001	0.001

*- **AC**; abdominal circumference, **HC**; hip circumference, **BMI**; body mass index*
- Data compared by paired t-test (p < 0.05)
** represent significant difference between groups*

Based on the results of the ANCOVA test, a significant difference was observed in the change of serum VEGF between the studied groups (P=0.001, F = 72.202, df = 3). On the other hand, based on the results of the Benferroni test (Table 3), a significant difference was observed between the control group and the aerobic, martighal and combination groups. In other words, compared to the control group, serum VEGF increased significantly in the aerobic, martighal and combined groups. No significant difference was observed between the exercise and martighal groups. Nevertheless, a significant difference was observed between the combined group and the aerobic and martighal groups. In other words, the aerobic exercises combined with the consumption of martighal led to a significant increase in serum VEGF compared to the application of each of them alone.

Table 3. Bonferroni post hoc test results for VEGF between the studied groups

Group	Group	Average difference	Standard error	sig
Control	**Aerobic**	*- 16.656*	*1.993*	*0.001*
	Martighal	*- 16.579*	*2.071*	*0.001*
	Combined	*- 29.781*	*2.029*	*0.001*
Aerobic	**Martighal**	*0.077*	*2.032*	*0.999*
	Combined	*- 13.126*	*2.003*	*0.001*
Martighal	**Combined**	*- 13.202*	*1.994*	*0.001*

The intra-group changes in serum VEGF were compared using paired t-test. The findings revealed that in all three groups, the intervention led to a significant increase in serum VEGF compared to the pre-test (Table 4, Fig 1).

Table 4. Data of intra group changes of VEGF in the studied groups

Group	Control	Aerobic	Martighal	Combine
Pre test	*160 ± 5.70*	*161 ± 6.62*	*164 ± 3.73*	*163 ± 4.93*
Post test	*159 ± 4.96*	*176 ± 6.23*	*176 ± 3.60*	*189 ± 5.11*
Sig (paired t test)	*0.497*	*0.001*	*0.001*	*0.001*

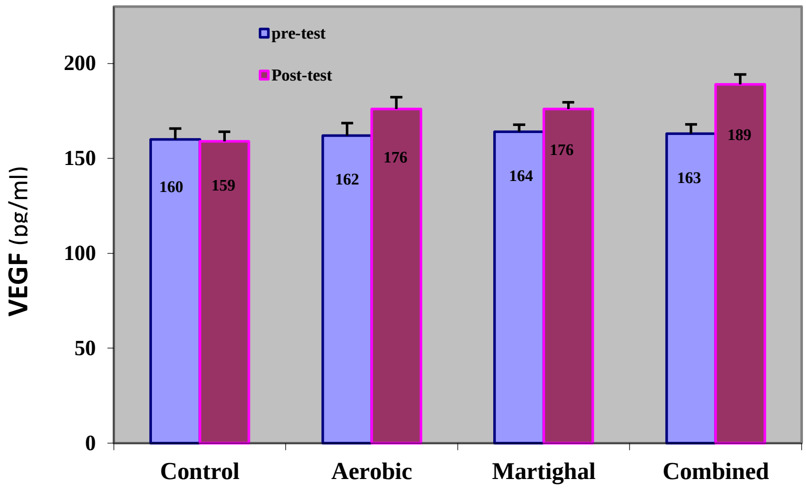

Figure 1. The pattern changes of serum VEGF in the studied groups. Serum VEGF increased significantly in the aerobic, martighal and combined groups compared to the control group.

Based on the results of the ANCOVA test, no significant difference was observed in the changes of serum homocysteine between the studied groups (P=0.919, F = 0.165, df = 3). On the other hand, intra-group changes by the paired t-test revealed no significant difference in serum homocysteine between pre- and post-test in any of the control, exercise and martighal groups, but in the combined group, there was a significant decrease compared to the pre-test (Table 5).

Table 5. Data of intra group changes of *homocysteine* in the studied groups

Group	Control	Aerobic	Martighal	Combine
Pre test	*46.23 ± 10.60*	*42.63 ± 6.29*	*44.62 ± 7.*	*43.11 ± 5.35*
Post test	*45.31 ± 13.85*	*40.96 ± 7.97*	*43.15 ± 8.95*	*40.86 ± 4.90*
Sig (paired t test)	*0.800*	*0.505*	*0.164*	*0.001*

Discussion

The findings of the study showed a significant increase in VEGF in response to aerobic training and Martighal supplementation in obese women with metabolic syndrome compared to the control group. On the other hand, the increase in VEGF in the combined group or in other words in the group that consumed Martighal extract during aerobic training was much higher than each of them alone. Nevertheless, aerobic training and Martighal consumption alone or in combination with each other did not lead to changes in serum homocysteine compared to the control group. Regarding the effect of exercise training or nutritional supplements on the components affecting cardiovascular function, there are consistent and inconsistent findings. In this context, although no study that tested the effect of Martighal was available, but Sahafian et al, (2016) pointed out that exercise activity combined with the consumption of Caffeine and Ginseng led to an increase in VEGF in heart tissue in laboratory rats (23). Mehri Elwar et al, (2016) have pointed out the increase in serum levels of vascular endothelial growth factor in young men in response to 5-week resistance training (24). Toloui Azar et al, (2019) also reported an increase in vascular endothelial growth factor following aerobic and resistance training in inactive women (25). Farzanegi et al, (2014) also reported an increase in VEGF and a decrease in systolic and diastolic blood pressure following aerobic training in postmenopausal women with hypertension (26). On the other hand, despite the fact that so far, no study has been reported on the effect of Martighal consumption during sports training, but Jahangiri et al, (2017) found an increase in VEGF along with a decrease in TNF-α in the heart of diabetic rats after 6 weeks of aerobic swimming training with the use of arbutin (27).

On the other hand, Etemad et al, (2016) mentioned a significant decrease in serum homocysteine in response to 8 weeks of resistance training (28). Bizheh et al, (2011) also cited that short-term exercise training in the form of 3 weeks of aerobic training was associated with a significant decrease in serum homocysteine in sedentary overweight men (29). In addition, a significant decrease has been reported by Soori et al (2016) in serum homocysteine in response to 10 weeks of aerobic training (5 sessions/weekly) in obese or overweight women (15).

Nevertheless, Bahram et al, (2013), Antunes et al, (2015) and Rousseau et al, (2005) reported the lack of effect of exercise training on homocysteine (30, 31, 32). In the study by Subasi et al, (2012), although 3 months of aerobic and resistance training were associated with improvement in body composition, homocysteine levels and lipid profile did not change significantly (33). The lack of change in homocysteine after exercise has also been reported by Nikbakht et al, (2007) (34). Some studies have also pointed to the effectiveness of pharmaceutical or antioxidant supplements on serum homocysteine levels. In this context, although no study evaluated the effect of martighal on homocysteine; however, in the study by Habibian et al, (2016), although 8 weeks of aerobic exercise and vitamin C consumption and their combination led to a decrease in homocysteine and insulin resistance in obese girls, the improvement in the combined group was far greater than the application of each of them alone (35). Despite the aforementioned contradictory evidence, the consumption of Martighal during aerobic exercise in the present study did not lead to a change in homocysteine levels compared to the control group, but the findings of the independent t-test indicated a significant decrease compared to the baseline levels.

In summary, the findings of the present study indicated the cardiovascular effects of consuming Martighal extract during aerobic training in women with metabolic syndrome. Because its consumption during exercises leads to improvement of VEGF compared to the application of each of them alone. On the other hand, unlike other groups that only experienced aerobic training or Martighal supplementation, their combination led to a decrease in homocysteine compared to baseline levels. In this context, although the direct response of homocysteine and VEGF to martighal has not been studied yet, its antioxidant effects have been reported many times. In such a way that it has been introduced as a regenerator of free radicals and maintaining the cell membrane by increasing the levels of cellular glutathione (36, 37). Martighal compounds inhibit or slow down the lipoperoxidation process that causes cell membrane damage (38, 39). Some other studies have also supported the anti-oxidative stress effects of martighal, which have been shown by reducing MDA and H2O2 (40).

The present study points out that the consumption of martighal extract during aerobic training was associated with an increase in VEGF compared to the application of any of the supplement or exercise alone, in patients with metabolic syndrome and this improvement was one of the strengths of the present study. However, based only on this improvement, it is not possible to refer to their cardiovascular effects and vascular endothelial function. So that the lack of measurement of other vascular endothelial indicators such as nitric oxide or endothelial microparticle (EMPs) is one of the limitations of the present study and the need to measure them in order to better understand the mechanisms responsible for the effect of aerobic exercise and martighal extract on vascular endothelial function.

Conclusion

Based on the findings, consumption of martighal and aerobic exercise was associated with an increase in serum VEGF in females with metabolic syndrome but the use of martighal during aerobic exercise led to a significant increase compared to the application of each of them alone. However, homocysteine levels were not affected in response to any of the interventions. However, based only on these findings, it is not possible to interpret the cardiovascular effects of martighal during aerobic exercise, and understanding the mechanisms responsible for it requires more studies in this field.

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Conflict of Interest

No conflict of interest has been declared by the authors.

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Estimating the Cost and Ratio of the Optimal Food Basket by Iranians to the Minimum Wage

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ABSTRACT

Introduction: Estimating the food supply cost is one of the essential applications of optimal food baskets. This study aimed to calculate the cost of an optimal food basket for Iranians and the ratio of the cost of an optimal food basket to the minimum wage in 2020 and 2021.

Methods: This cross-sectional study estimated the costs and ratio of the optimal food basket to the workers' minimum wage from the perspective of economically vulnerable groups. The cost approach was based on the research data, using forms and checklists prepared by the researcher and referring to information published by the Statistics Center, the Central Bank, and the Ministry of Welfare. Excel software was used to calculate and draw graphs.

Results: This study showed the high cost of red meat, fruits, and dairy products. The lowest cost was for pasta, sugar, and potatoes. The per capita cost of an optimal food basket for one day in 2020 and 2021 was 223576 and 306833 Rials, respectively. The ratio of the cost of an optimal food basket of the minimum wage in 2020 and 2021 was 57% and 50%, respectively.

Conclusion: The study's most crucial finding was the importance of purchasing power adequacy in providing the optimal food basket. This finding underscored the urgency of addressing wage issues, particularly in the lower-income and working classes, as a significant basis for determining wages and planning for sustainable development.

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Introduction

Food security stands for people with physical and economic access to sufficient, healthy, and safe food to meet their needs and preferences and have a healthy and active life. Food security is a matter of satisfying the energy requirement and a concern with essential micronutrients to maintain healthy eating to ensure the well-being of individuals (1). Therefore, providing the ability to purchase items appropriate for the household plays a vital and decisive role in achieving sustainable food and nutrition security. Policymakers and decision-makers can plan for better and more sustainable development using the available detailed and in-depth information. Various methods have been used to estimate household food purchase costs, including information on the optimal household food basket (2). An optimal food basket is a translation of sufficient and balanced food, which can be considered a great goal in food security policies (3-5)

The second optimal food basket of the country was compiled by the Faculty of Health of Tehran University of Medical Sciences, the National Nutrition and Food Technology Research Institute of Iran, and the Community Nutrition Improvement Office in 2013 (Table 1) (6). According to the energy needs and key nutrients, the standards and international recommendations were compiled for the entire population of Iran, as well as the separation of different age and gender groups. The optimal food basket designed based on the order of the Deputy Health Minister of the Ministry of Health and Medical Education was used. The average requirement of the Iranian population for energy, protein, and key micronutrients (iron, calcium, vitamins, and riboflavin) was included in the tables of recommended daily amounts of energy and protein and micronutrients recommended by Recommended Dietary Intake (RDI), Recommended Dietary Allowances (RDA) and Word Health Organization (WHO). The key

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items in this optimal food basket were determined based on gender and age groups based on the ability to supply food in Iran and the climate and culture of the Iranian people, including rice, bread, pasta, legumes, vegetables, fruits, potatoes, white and red meat and eggs, dairy products, vegetable oil, and sugar. The compiled basket provides B2 with 100% energy, vitamins, and protein, 80% calcium, and 90% iron. Based on this food basket, the percentage of energy supplied from the carbohydrate group is 63%, protein 13%, and fat 23.8%. According to assumptions, 10% more consumption is acceptable. Currently, the food basket has improved due to increasing household income, easy access to food, improving the culture and nutritional literacy of the people, and enhancing access to health services (7).

The Iranian Ministry of Health and Medical Education (MoHME) reported the optimal food basket for the Iranians (2013).

The importance of maintaining economic stability and, most importantly, price stability, exchange rate stability, and the effect of changes on the general level of domestic prices cannot be overstated. The high share of food in households' consumption basket, especially in low-income groups, adds to the importance of food price stability (8). Increasing prices, which affect consumption differently across income groups, decreases purchasing power, resulting in smaller tables for households. Cost pressure caused by food inflation may change household food consumption habits, causing food insecurity and poverty, which will undoubtedly affect welfare, especially for low-income households. People who face food insecurity suffer many physical, mental, and financial damage. The issue of food security demands more than just filling the stomach or providing calories; it also requires macronutrients. The increase in inflation limits the lower deciles' access to the goods and consumption basket with minimal nutritional value, which should be evaluated. The symptoms can indicate a malnutrition crisis in these households as well as growth disorders, especially in children from lower income levels (9).

The purpose of compiling optimal food baskets is to provide energy and key nutrients, prevent common micronutrient deficiencies, and prevent overeating. However, the main point here is the use of these baskets. Estimating the minimum cost of providing them enables you to determine things such as the minimum salary, obtain the salary, and provide a reasonable estimate (10). The 2017 report of the World Food and Agriculture Organization shows an increase in food-insecure households in the world in 2016 compared to 2015 (10), which should be considered plan by the country's politicians. This study aims to estimate the cost of the optimal food basket for Iranians and the ratio to workers' minimum wage in 2020 and 2021.

Materials and Method

This cross-sectional study estimated the costs and ratio of the optimal food basket to workers' minimum wage from the perspective of economically vulnerable groups with a time horizon of two years using the top-down and prevalence-based costing approach. The data was created using forms made by the researcher and by referring to official information and documents published by official authorities such as the Iran Statistics Center, Central Bank, and the Ministry of Cooperation, Labor, and Social Welfare.

The data is related to 2020 and 2021, and Excel was used to calculate and draw the graph.

Based on the average price of selected food items in urban areas and the cost of providing them for one day and one month per household of one to four people, the cost of an optimal food basket for one individual, the share of each food subgroup, and their price were calculated for each year. The inclusion and exclusion criteria for the study were added in the text of the article as follows:

The selection of food items in each group was based on prices reported by official authorities, such as the National Statistics Center of Iran. Food items without official reports were excluded from the study.

In this study, the optimal food basket of the country compiled was used in 2013 by the Faculty of Health of Tehran University of Medical Sciences, the Institute of Nutritional Research and Food Industries of the country, and the Community Nutrition Improvement Office on the order of the Deputy Health Minister of the Ministry of Health, Treatment and Medical Education (6). The optimal food basket cost and share of each food subgroup for each person is determined by per capita consumption of food items, and then their prices are determined by analyzing the average prices of selected food items in urban areas of the country that are available from the Iran Statistics Center until the end of December 2020 and 2021 (11). The cost of providing breakfast for one day and a month per household of one to four people was calculated each year. In addition, the ratio of each food item's cost to the basket's total cost was calculated. The selection of the two years 2020 and 2021 was due to the completeness of the prices of the food items in the desired food basket on the website of the Statistical Center.

Iran Statistics Center reports that the average is calculated by examining the prices of selected food items in urban areas of the country, based on the average price paid for a product of consistent quality, and then calculating the consumer price index from the collected prices. These prices include a wide range of products of different quality. The food items considered to calculate the costs were as follows: rice of the first-grade foreign type, dairy products including milk, yogurt, and cheese, oil only liquid oil, fruits including pomegranate, banana, apple, orange, watermelon, vegetables including zucchini, cucumber, eggplant, tomato, onion, legumes including peas, pinto beans, red beans, lentils, and cobs. The applied bread was traditional Lavash bread due to its higher consumption, and red meat, including veal and sheep meat, was consumed at prices reported in Rials per kilogram. The average price of the items in the food groups was calculated for each year, and the amounts were calculated in the optimal food basket. The selection of food items in each group was based on prices reported by the Iranian Statistics Center. The food basket cost is calculated based on net food (without considering waste) and the average price of food products in the country's urban areas.

The workers' minimum wage was obtained from the official circulars of the country published by the Ministry of Cooperation, Labor, and Social Welfare. The ratio of the optimal food basket cost to the total wage of the workers per month was calculated for each year. Excel software was used to calculate and draw graphs.

Results

Table 2 reports the cost of optimal food basket items for 2020 and 2021. The highest cost is for red meat, fruit, and dairy products, and the lowest cost is for pasta, sugar, and potatoes based on the weight stated in the optimal food basket. The per capita cost of the optimal food basket for one day in 2020 and 2021 is 0.862 and 1.182 United States dollars (USD), respectively (a 37% increase).

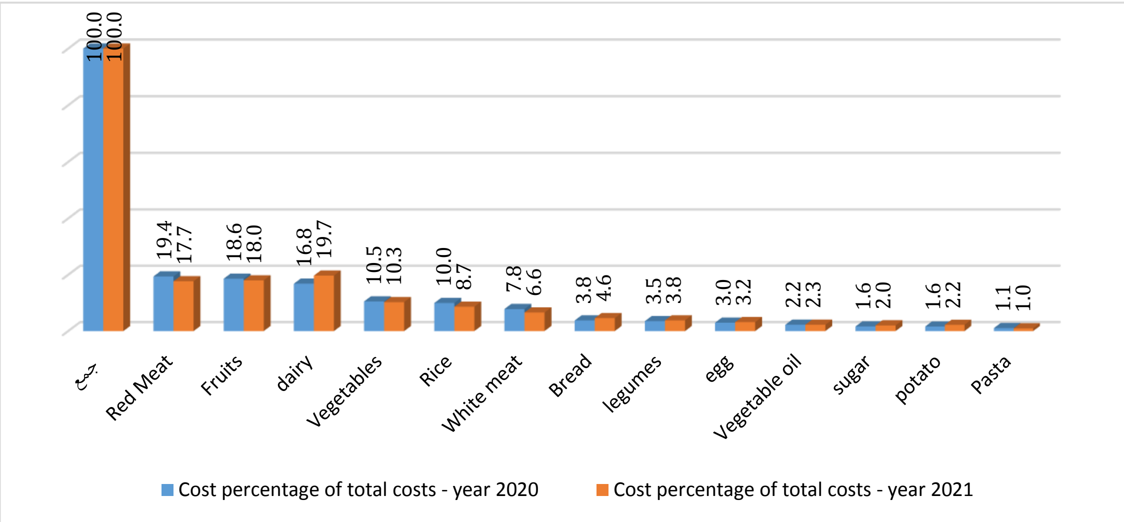

Figure 1. Cost percentage of total costs in 2020 and 2021

Figure 1 shows the cost ratio of each item to the optimal food basket to the total cost. The highest price ratio is for red meat, fruits, dairy, and vegetables. The percentage of cost per item in December 2021 for dairy items, bread, legumes, eggs, sugar, and potatoes increased compared to December 2020 for red meat, fruits, vegetables, rice, and white meat.

Table 1. Suggested optimal food basket per capita and the resulting energy and nutrients in Iran, 2013

Food	Per capita consumption (grams per day)	calories (kilocalories)	protein (grams)	Calcium (mg)	Iron (mg)	Vitamin A	Riboflavin (mg)
Bread	310	879	27.6	160.3	6.6	0.17	0.13
Rice	95	339	6.6	7.2	0.8	0.0009	0.03
pasta	20	72	2.5	5.4	0.3	0	0.01
legumes	26	91	5.9	37.4	1.8	0.47	0.06
potato	70	57	1.7	5.04	0.25	1.3	0.02
vegetable	300	85	4.3	124.3	2.5	514.4	0.18
Fruits	280	141	1.5	63.8	1.5	116.9	0.07
Red Meat	38	106	5.4	5.02	0.5	0.01	0.06
White meat	64	82	11.5	7.7	0.6	47.6	0.08
egg	35	45	3.7	14.7	0.4	57.7	0.1
dairy	250	207	13.1	371.3	0.3	111.1	0.5
Vegetable oil	35	315	0	0	0	0	0
sugar	40	155	0	1.6	0.07	0	0.002
Total	1563	2573	83.9	803.8	15.7	849.8	1.3

Table 2. Per capita consumption of optimal food basket and price and cost percentage of food items in the years 2020 and 2021

Food	Per capita consumption of optimal food basket (grams per day)	for the price of 2020 (USD)	for the price of 2021 (USD)
Bread	310	0.033	0.054
Rice	95	0.086	0.102
pasta	20	0.009	0.011
legumes	26	0.030	0.045
potato	70	0.014	0.026
vegetable	300	0.091	0.122
Fruits	280	0.160	0.213
Red Meat	38	0.167	0.209
White meat	64	0.067	0.078
egg	35	0.026	0.038
dairy	250	0.145	0.233
Vegetable oil	35	0.019	0.027
sugar	35	0.014	0.024
Total	1.563	0.862	1.182

The Statistical Centre of Iran (SCI).

The cost of the optimal food basket per household size is reported in Table 3. This cost for a 30-day month in December 2020 for a family of 1, 2, 3, and 4 people was 25.8, 51.7, 77.5, and 103.4 USD, respectively. In December 2021, it was 33.6, 67.2, 100.8, and 134.4 USD, respectively, which increased by 30% compared to the previous year.

Table 3. Optimal food basket cost per household size in 2020 and 2021

	Cost (USD per day)		Cost (USD per month)		Cost (USD per year)		The percentage of changes in 2020 and 2021
	2020	**2021**	**2020**	**2021**	**2020**	**2021**	
Per capita	0.9	1.1	25.8	33.6	310.2	403.1	30
Family of 2 people	1.7	2.2	51.7	67.2	620.3	806.3	
Family of 3 people	2.6	3.4	77.5	100.8	930.5	1,209.4	
Family of 4 people	3.4	4.5	103.4	134.4	1,240.7	1,612.6	

The Statistical Centre of Iran (SCI).

The optimal food basket cost ratio to the minimum wage in 2020 and 2021 was 57 and 50%, respectively. This ratio is 80 and 70% for workers with one child and 99 and 87% for workers with two children in the same year (Table 4).

Table 4. The ratio of the cost of the optimal food basket to the minimum wage of the worker in the years 2020 and 2021

	2020 (USD)	The cost of the optimal food basket to salary (percentage)	2021 (USD)	The cost of the optimal food basket to salary (percentage)
minimum wage	90.0	57	135.2	50
Minimum wage with one child	97.1	80	144.9	70
Minimum wage with two children	104.1	99	154.6	87

Discussion

The findings indicated that the cost of optimal food basket items for 2020 and 2021 is the highest cost of red meat, fruit, and dairy products and the lowest cost of pasta, sugar, and potatoes. The per capita cost of the optimal food basket for one day in 2020 and 2021 is 223,576 and 306,833 Rials, respectively (a 37% increase).

The cost of the optimal food basket per household size for a 30-day month in December 2020 for a family of one, two, three,, and four people is 6707289, 13414578, 20121566, and 26829155 Rials, respectively. In December 2021, the corresponding values of the the previous year were 18409980, 27614969 and 36819959, and 9204990 Rials, respectively. The corresponding values of the previous year were 18409980, 27614969, and 36819959, which increased by 37% compared to last year.

In addition, the cost of the optimal food basket to the minimum wage in 2020 and 2021 was 57 and 50%, respectively. This ratio is 80 and 70% for workers with one child and 99 and 87% for workers with two children in the same year.

Food security refers to the area where people have access to enough nutritious food according to their culture to lead a healthy and active lifestyle. The four main dimensions of food security include availability, access, the productivity of consumed food (health and nutrition), stability, and sustainability of the previous three dimensions. Access is one of the essential dimensions of establishing food security, along with two physical and economic dimensions. Economic access means that households or individuals can buy quality food items derived from the food price and income. As the price of food increases or household income decreases, the purchasing power of the household decreases, and their table becomes smaller in quantity and quality (elimination of food containing high-quality proteins and other micronutrients (12).

Many studies have shown that price increases cause a decrease in the consumption of some food items. For example, the study of victims, devoted to the study of the effect of price increases on food security in the rural society of Iran, has shown that the rise in food prices decreases food intake. Examples of high protein foods are meat and dairy products instead of "Protein is like meat and dairy products" (13). Ahmadi Javid showed that consuming meat, one of the essential protein sources in the food basket, in low-income households has low purchasing power for this food group (14).

Food items such as red meat, fruits, and dairy products account for a high percentage of expenses in the desired food basket, which is consistent with the findings of Jazayeri et al. (2). Reducing the consumption of this group could lead to a decrease in iron and calcium intake. Food items that contain calories and do not have valuable micronutrients, such as sugar and vegetable oil, have a lower cost than the optimal food basket, which is used in the low-income groups of society for abdominal satiety and does not cause cellular satiety. Therefore, it leads to obesity, overweight, and other non-communicable diseases such as diabetes and cardiovascular disease.

Price policies to provide food items with vitamins, minerals, and quality proteins to prevent non-communicable diseases and the resulting deaths are among the policy priorities in food, nutrition, and public health.

In a study in Brazil to analyze the role of the minimum wage policy in Brazil in ensuring food security among low-income people, the nutritional composition of the national basic food basket that was initially proposed to estimate the minimum wage, the content of calories, sodium, saturated fat and had high added sugar compared to adults' recommendations and insufficient amounts of essential nutrients (calcium, potassium, and vitamins) and food groups (fruits, vegetables, and grains) (15).

In addition to the above issue, policymakers should pay attention when determining the minimum wage, as assessing the level of this minimum wage with inflation can lead to a better quality of life and nutrition for low-income households. A study conducted in the United States in 2021 aimed to examine the extent to which an increase in the minimum wage would improve the quantity and quality of food purchased by low-income households and showed that households likely to receive the minimum wage increase their purchased calories in response to an increase in the minimum wage. In addition, families that used to buy less healthy foods buy more nutritious foods in response to the minimum wage increase (16).

Therefore, policymakers should consider all the individual needs for nutrients and micronutrients to have a healthy workforce when formulating the optimal food basket based on the minimum wage.

Specifically, the increase in prices causes a decrease in the purchasing power and, subsequently, the welfare of households. The current phenomenon becomes more critical in the case of essential goods such as food. In this regard, the calculation of welfare changes caused by the increase in food prices in Gahramanzadeh et al. shows that the rise in food prices reduced the welfare of all Iranian urban households, and the loss of welfare for poor households was far more significant than that of rich households(17). Liani and Esmaili also revealed that the amount of welfare lost by urban households due to the price of imported food, equivalent to the average annual growth in imported food from 1961 to 2011, is 4.20 of the average household income (18).

Based on the findings, the ratio of the optimal basket cost of the minimum wage increased. In other words, the increase in the size of the household caused a rise in food costs, and when the household income does not improve, the household's food security would be at risk. The minimum wage for households suggests that more than an increase in wages would be required to cover costs. Therefore, more support from the government seems necessary for families with more children. Rostami et al. showed a positive and significant relationship between the social and economic status of the household and the food security of the studied households in such a way that there is a significant relationship between the household's food security and the monthly income of the household, the job position of the father, the status of having living facilities and the number, there are household members (19). In a study on households in Shiraz, food insecurity was higher in households with more children and poor socio-economic levels, having children under 18 years old and a female head(20).

The findings showed that the ratio of the cost of the optimal food basket to the minimum wage of workers had not increased logically according to the household size. A family of four workers should spend more than 80% of their income to meet the minimum needs of protein and micronutrients in the year 2021. The household table, which has sufficient quality and quantity for a healthy and active life, spends what is impossible along with other expenses (housing, energy, and clothing) and leads to the elimination of quality foods in terms of nutrients and a vicious circle. Hunger and the lack of micronutrients cause non-communicable diseases, increasing the cost of treatment and poverty. Therefore, low-income households, due to the inability to buy food products with high nutritional value, consume foods with a higher energy content but at the same time have few nutrients and essential substances. Based on the current salaries and wages in the country, it is impossible to provide the minimum of an optimal food basket. Hence, reviewing the state of salaries and wages in the country seems necessary. One of the limitations of this study is the limited scope of the research, which discussed only two parameters (cost of food basket and minimum wage). Researchers interested in solving this problem are encouraged to use other relevant parameters if they can access the needed data. Another limitation of the current study is that it uses cross-sectional rather than panel data.

Conclusion

A household's purchasing power is one of the most critical factors in establishing food security, so paying attention to the extent to which it is sufficient to provide an optimal basket of food can be a necessary basis for determining salaries and wages, especially when the worker comes from lower income deciles. Supporting similar groups is effective in providing them with access to quality protein (red and white meat types) and foods containing micronutrients (fruits, vegetables, and dairy products) and has an influential role in the prevention of health problems and increasing the productivity of the productive workforce through providing nutritional needs. Different strategies can be used to improve the food security of the weaker sections of society, such as allocating subsidies, increasing labor wages, empowering unemployed and unskilled young people and female heads of households to create employment, or providing employment loans with low-interest and repayments long and used to provide facilities for the provision of food to the workers.

Conflicts of Interest

The authors declare no conflicts of interest.

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Does Ramadan Fasting, along with Circuit Resistance Training Affect the Body Composition and Lipid Profiles of Healthy, Inactive Middle-aged Men?

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ABSTRACT

Introduction: Physical activity and healthy nutrition are essential to a healthy lifestyle. Fasting during Ramadan also shows potential health benefits. However, the effect of combining fasting and physical activity on health outcomes in diverse individuals remains relatively unclear. Therefore, this study aimed to evaluate the effect of Ramadan fasting alone or with circuit resistance training on middle-aged men's body composition and lipid profile.

Methods: Thirty inactive and healthy adult males were randomly assigned to three groups in this study: fasting (30 days of Ramadan fasting), circuit resistance training (30 days of Ramadan fasting plus 12 training sessions), and the control group. A day before the commencement of the fasting month of Ramadan and one day after its conclusion, anthropometric and biochemical parameters were assessed utilizing established methodologies. The training program consisted of 12 sessions every other day, lasting between 30 and 45 minutes. This training protocol was performed at 5:00 PM. Data were analyzed with SPSS software and using the analysis of covariance (ANCOVA) test ($P \le 0.05$).

Results: The results showed that compared to the control group, body weight (P=0.001), BMI (P=0.001), body fat % (P=0.001), and muscle mass (P=0.002) decreased significantly in the fasting and fasting combined with exercise groups. In contrast to the control group, the intervention groups exhibited non-significant reductions in total cholesterol (P=0.082), triglyceride (P=0.132), and LDL-C (P=0.123) levels. In addition, the HDL-C level in the fasting (P=0.001) and fasting combined with training (P=0.001) groups was significantly reduced compared to the control group.

Conclusion: Based on the results, integrating circuit resistance training with a one-month fast during Ramadan resulted in changes in body composition, including a reduction in body weight, BMI, body fat percentage, and muscle mass. However, a non-significant decrease in the blood lipid profile accompanied these modifications. HDL was also reduced following the implementation of exercise and fasting.

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Introduction

Behavioral risk factors, such as consuming unhealthy diets and reducing physical activity, have metabolic consequences that result in overweight and obesity. This, in turn, can lead to non-communicable diseases like metabolic abnormalities, cardiovascular diseases (CVD), stroke, type 2 diabetes, and other related conditions [1]. Additionally, imbalances in energy balance and hormones can result from overweight and obesity, which can also cause substantial alterations in adipose profile [2].

Dyslipidemia has been shown as a metabolic abnormality with a decrease in high-density lipoprotein cholesterol (HDL-C) and an increase in low-density lipoprotein cholesterol (LDL-C) and triglyceride (TG) levels [3]. The presence of one or more abnormal blood serum concentrations is the cause of dyslipidemia and is a decisive risk factor for the onset of CVD and other health problems [4]. Research has indicated that interventions associated with a healthy lifestyle, such as increasing physical activity and enhancing dietary habits, may improve lipid profile and decrease the incidence of diseases induced by an improper diet [5, 6]. Dietary intervention is regarded as a significant fitness and lipid profile modifier [7].

Intermittent fasting (IF) has attracted much attention as a method for improving metabolic

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indices. IF refers to an eating pattern that reduces calorie intake for several days, may not be consumed frequently, or consumes very few calories throughout the day [8]. Based on the reports, IF improves body composition, lipid profile, and overall health [9]. Ramadan fasting (RF) is a form of IF practiced by millions of Muslim adults worldwide as a process in which calories and liquids are avoided for 12 to 22 hours [10]. The predominance of carbohydrates characterizes RF in the early morning hours as the primary fuel. However, fat becomes more important in the afternoon hours, and cells recycle unwanted items, which is associated with reducing inflammation and autophagy and improving metabolic indicators [11]. In addition, RF changes the secretion of hormones such as cortisol, insulin, leptin, and ghrelin by changing the sleep pattern and circadian rhythm, improving whole-body metabolic homeostasis, and reducing disease risk [12]. The health benefits of RF have been demonstrated in clinical trials. In this regard, research results have reported that RF improves body composition, improves lipid profile parameters, and reduces the risk of coronary artery disease [13].

On the other hand, exercise is considered a significant modifier in improving lipid profile and body composition along with RF. Lifestyle changes, including nutritional interventions and sports activities, can be effective in controlling body weight and metabolic health by shifting the energy balance from the positive to the negative side by reducing calorie consumption and increasing physical activity [4 ,14 ,15]. Physical activity along with RF increases lipolysis in fat and muscle tissue and can be a good solution for metabolic regulation. Recent studies have supported the positive effects of RF and exercise in improving body composition and health. The results of a recent study showed that RF combined with physical activity significantly improves body composition and cardiovascular health compared to RF alone [16].

Circuit resistance training (CRT) is one of the most popular exercises and has many benefits. Previous systematic reviews and meta-analyses reported that CRT improves body composition, muscle strength and endurance, cardiorespiratory fitness, and neuromuscular function [17, 18]. This approach involves performing one or multiple sets of various exercises consecutively with minimal rest. The specific number of exercises, intensity, weight, rest periods, duration of sessions, and duration of the training phase may differ based on the training goal [19]. However, the results of some studies are conflicting because a wide variety of these types of exercises can be designed and implemented [17, 18, 20].

The development of a healthy lifestyle is critical for individuals' well-being, and authorities place significant emphasis on the identification of appropriate nutritional solutions and physical activity. The results regarding the health effects of fasting alone or in conjunction with exercise are somewhat unknown and obscure. However, CRT exercises appear more appealing than conventional strength and aerobic exercises due to their greater variety and combination. Hence, this study was designed to ascertain the impact of CRT exercises and Ramadan fasting on middle-aged males' body composition indices and blood lipid profiles.

Materials and Method

This experimental and applied study was conducted with a pre-test and post-test design. The statistical population of this research was healthy, inactive middle-aged men (20-45 years old). The statistical sample consisted of 38 people who were randomly divided into the following groups: fasting (RF, n=14), fasting and circuit resistance training (RF+CRT, n=14), and control (C, n=10) groups.

The inclusion criteria were male gender, age 20 to 45 years old, no underlying diseases, no alcohol and tobacco consumption, no supplement use, and no regular exercise in the last six months. The exclusion criteria included not observing the principles of RF (more than two days), not participating in the exercise program (more than one session), and unwillingness to continue the study. According to this, eight subjects from the experimental groups were excluded from the study (five of these people had not fasted for more than two days, and three of the subjects did not participate in the training). Finally, statistical analysis was performed on 30 subjects (ten subjects in each group).

At first, the subjects completed individual and health history questionnaires. Before starting the research, the study's objectives were explained to the subjects, and informed consent was obtained. This study was implemented based on the ethical principles of working with human subjects and under the supervision of the Ethics Committee of Shahrekord University (IR.SKU.REC.1401.013). Blood samples were collected from all subjects two to three days before the commencement of RF and training and one day following the conclusion of the protocol, at 8 to 9 am, after the subjects had fasted for a minimum of eight hours overnight. Then, the samples were subjected to biochemical analysis following the preparation of serum samples by centrifuging them at 3000 rpm for ten minutes.

Anthropometry and body composition were evaluated the day before the study and two days after the RF and exercise protocol completion. Body composition was measured using the body tech device (MAGIC/ J0402/ Guangdong, China). Height, weight, and body mass index (BMI) were calculated separately using standard devices and procedures.

Fasting Protocol

The fasting protocol for the RF and RF+CRT groups consisted of 13:30 to 14:30 hours of fasting (about 5- 4:20 am to 6:30- 6:50 pm) and about 9:30 to 10:30 hours of unfettered access to food and fluids, seven days per week for four weeks. Amidst the investigation, the control group participants conducted their daily routines with unrestricted access to nourishment and water [10].

Circuit Resistance Training Protocol

The RF+CRT group performed the circuit resistance training protocol thrice a week for four weeks. The subjects performed essential warm-up and cool-down at the beginning and end of each session. The main training course consisted of a circular design with eight stations (exercise), 40 seconds of training, 20 seconds of rest, three repetitions, and 30-45 minutes. The activities included integrated resistance exercises (simultaneous upper and lower limb exercises, multi-joint movements, core stability exercises, coordination, and balance exercises). These exercises included Sit-to-stand with elbow flexion, Push-ups, Crunches with rotation, Dumbbell swing, Front pulldown with squat (Elastic bands), Good morning, Side-lying hip abduction, and Airplane. The Borg rating of perceived exertion (RPE) scale (6-20) was used to ensure the appropriate form of exercise and its intensity. The intensity of the exercise was set at the limit of 15-18 on the Borg scale [21].

Lipid Profile Assay

The serum lipid profile was evaluated using special kits for each factor and a photometric method (Byrex Fars kit for measuring TC and TG and Pars Azmoun kit for measuring LDL and HDL).

Statistical Analysis

In the statistical analysis of the data, mean statistics and standard deviation were used to describe the data. The Shapiro-Wilk test was utilized to check the normality of the data distribution. A one-way analysis of variance (ANOVA) was employed to assess differences among groups during the pre-test phase. The changes in the post-test phase were examined using the analysis of covariance (ANCOVA) test, with the pre-test variables being regarded as covariate variables. In addition, the dependent t-test was used to compare the group in the pre- and post-test phases. Statistical analysis was performed with IBM SPSS 25 software at a significance level of $P<0.05$.

Table 1. Between-group differences at baseline

Characteristics	C	RF	RF+CRT	P-value
Age (years)	28.6 ± 6.88	31.5 ± 8.24	26.9 ± 3.83	0.093
Height (cm)	176.3 ± 4.92	176.2 ± 6.12	176.5 ± 6.24	0.99
Weight (kg)	77.29 ± 13.05	78.55 ± 12.18	76.56 ± 9.57	0.93
BMI (kg/m2)	24.82 ± 3.73	25.31 ± 3.98	24.65 ± 2.5	0.886
Body fat (%)	21.62 ± 4.24	23.79 ± 6.22	20.33 ± 3.63	0.286
LBM (kg)	56.81 ± 7.06	55.99 ± 4.92	57.32 ± 5.58	0.88
Chol. (mg/dl)	170.1 ± 23.82	165.9 ± 33.09	156.1 ± 52.79	0.709
TG (mg/dl)	106.1 ± 39.37	98.7 ± 45.97	137.9 ± 84.38	0.315
LDL-C (mg/dl)	99.8 ± 19.92	97.9 ± 32.6	85 ± 34.98	0.494
HDL-C (mg/dl)	49.9 ± 6.66	48.2 ± 6.36	44.7 ± 3.31	0.203

C: Control Group, RF: Fasting Group, RF+CRT: Fasting + Exercise group. BMI: Body Mass Index; LBM: Lean Body Mass, Chol.: Cholesterol, TG: Triglyceride, LDL-C: Low Density Lipoprotein, HDL-C: High Density Lipoprotein. Data are presented as mean ± SD.

Results

The subjects were men aged 20 to 45 years with age 30±7 years, height 176.33±5.5cm, initial weight 77.46±11.32, and BMI 24.9±3.3Kg.m^{-2}. The baseline characteristics data are presented in Table 1. The One-Way ANOVA showed no significant difference between the groups for measured variables at the baseline (Table 1).

The data related to pre-test and post-test and differences between groups and within groups are presented in Table 2.

Table 2. Pre and post treatment characteristics for study participants.

Variable	Time	C (n= 10)	RF (n= 10)	RF+CRT (n= 10)	B-G p. Value
Body Weight (kg)	Pre	77.29 ± 13.05	78.55 ± 12.18	76.56 ± 9.57	F= 31.36
	Post	78.49 ± 13.88	75.94 ± 11.78	74.16 ± 9.05	P= 0.001*
	W-G p. Value	0.013 ǂ	0.001 ǂ	0.001 ǂ	
BMI (kg/m2)	Pre	24.82 ± 3.73	25.31 ± 3.98	24.65 ± 2.5	F=31.77
	Post	25.20 ± 3.98	24.47 ± 3.82	23.81 ± 2.55	P= 0.001*
	W-G p. Value	0.013 ǂ	0.001 ǂ	0.001 ǂ	
Body Fat %	Pre	21.62 ± 4.24	23.79 ± 6.22	20.33 ± 3.63	F= 21.94
	Post	22.23 ± 4.41	22.24 ± 5.6	19.39 ± 3.66	P= 0.001*
	W-G p. Value	0.006 ǂ	0.001 ǂ	0.001 ǂ	
LBM (kg)	Pre	56.81 ± 7.06	55.99 ± 4.92	57.32 ± 5.58	F= 8.16
	Post	57.27 ± 7.38	55.28 ± 5.10	56.31 ± 5.45	P= 0.002*
	W-G p. Value	0.061	0.049 ǂ	0.005 ǂ	
Total Cholesterol (mg/dl)	Pre	170.1 ± 23.82	165.9 ± 33.09	156.1 ± 52.79	F= 4.09
	Post	183.2 ± 31.33	154.2 ± 26.68	147.1 ± 36.96	P= 0.082
	W-G p. Value	0.279	0.179	0.339	
TG (mg/dl)	Pre	106.1 ± 39.37	98.7 ± 45.97	137.9 ± 84.38	F= 2.190
	Post	122 ± 49.31	80.2 ± 31.18	127.9 ± 82.92	P= 0.132
	W-G p. Value	0.042 ǂ	0.047 ǂ	0.627	
LDL-C (mg/dl)	Pre	99.8 ± 19.92	97.9 ± 32.6	85 ± 34.98	F= 2.28
	Post	110.4 ± 29.5	93.6 ± 27.02	82 ± 22.5	P= 0.123
	W-G p. Value	0.239	0.673	0.719	
HDL-C (mg/dl)	Pre	49.9 ± 6.66	48.2 ± 6.36	44.7 ± 3.31	F= 12.21
	Post	49 ± 5.07	36 ± 8.06	34.4 ± 6.55	P= 0.001*
	W-G p. Value	0.764	0.001 ǂ	0.001 ǂ	

C: Control Group, RF: Fasting Group, RF+CRT: Fasting + Exercise group. BMI: Body Mass Index; TG: Triglyceride; HDL: High Density Lipoprotein. W-G: Within-group, B-G: Between-group. *: indicated significant difference between groups. ǂ: indicated significant within group pre-post difference.

The results showed in the between-groups comparison, there was a significant difference in body weight (F (2,26)=31.37, P<0.001, η=0.707), BMI (F (2,26)=31.77, P<0.001, η=0.710), and BF% (F (2,26)=21.94, P<0.001, η=0.628) variables. Post-hoc test showed that compared to the control group, body weight, BMI, and BF% significantly decreased in the RF (P=0.001) and RF+CRT (P=0.001) groups, but no difference was observed between the experimental groups (P=0.99 in body weight and BMI, P=0.68 in BF%). Moreover, the within-group comparison showed that the body weight (P=0.013), BMI (P=0.013), and BF% (P=0.006) increased in the control groups, but these variables significantly decreased in RF (P=0.001) and RF+ CRT (P=0.001) groups.

The result showed that LBM significantly differs between groups (F (2,26)=8.16, P=0.002, η=0.386). In comparison to the control group, LBM significantly decreased in RF (P=0.018) and RF+ CRT (P=0.002) groups, but there was no difference between fasting and RF+ CRT groups (P=0.99). Compared to the pre-test, LBM in the control group did not change (P=0.061), but in the RF (P=0.049) and RF+ CRT (P=0.005) groups, it significantly decreased in the post-test.

The analysis showed the total cholesterol did not change between groups (F (2,26)=4.9, P=0.082, η=0.240), so there were no differences between the control group with RF (P=0.070) and RF+ CRT (P=0.052) groups. Moreover, we did not observe changes within groups (Table 2). Likewise, TG had no differences between groups (F (2,26)=2.19, P=0.132, η=0.144). Nevertheless, within-group analysis showed TG increased in the control group (P=0.042) and decreased in the RF group (P=0.047). In addition, the LDL-C variable did not change significantly (F (2,26)=2.28, P=0.123, η=0.149). The point to consider is the significant change in the HDL-C variable (F (2,26)=12.21, P=0.001, η=0.484), and the posthoc test revealed that compared to the control group, RF (P=0.001) and RF+ CRT (P=0.001) significantly decreased HDL-C. Additionally, HDL significantly decreased in RF (P=0.001) and RF+ CRT (P=0.001) groups in posttest data compared to the pretest.

Discussion

This study aimed to investigate the simultaneous effect of fasting and exercise on the body composition and lipid profile of healthy and sedentary men. According to the present study's findings, a significant reduction in body composition indicators (weight, BMI, body fat, and muscle mass) was observed after 30 days of RF alone or in conjunction with CRT training. The findings corroborated the results of Togba et al. (2019) regarding the impact of fasting on body composition. Specifically, they observed that RF decreased body fat percentage, body weight, and body mass in young men in good health [22]. Similarly, Nachvak et al. (2019) reported a significant decrease in body weight, lean body mass, and body fat percentage after RF in middle-aged men [23]. Some studies have shown that caloric restriction through RF does not significantly reduce the variables related to body composition, which contradicts the present study's results [24]. This finding is inconsistent with the results of Tinsley et al. (2020), who reported significant increases in body weight and lean body mass following eight weeks of caloric restriction [25]. The possible reasons for this contradiction in the findings may be differences in age, sex, eating habits, geographical conditions, and economic status [26].

In the context of the simultaneous effect of fasting and physical activity, the results of the present study were under the findings of Attarzadeh et al. (2013), which showed reduced body weight, BMI, and waist-to-hip ratio following four weeks of RF and aerobic training in women aged 20 to 45 [27]. Similarly, Wilson et al. (2018) reported a significant decrease in the variables of weight, fat mass, and absolute and relative muscle strength after eight weeks of intermittent fasting and intense intermittent exercise in obese rats [28]. The combination of RF, CRT, and RF alone in the present study caused a decrease in muscle mass, which indicates their contradictory effect on muscle mass, possibly due to the failure to adopt a suitable nutritional pattern for fasting people. Therefore, some studies have suggested that nutritional interventions can improve muscle mass during fasting. In this regard, Rodriguez et al. (2022) showed that nutritional planning and taking supplements along with proper rest can lead to the improvement of muscle mass during the fasting period [29].

Fat cells are the source of inflammatory cytokines such as interleukin one and 6. Excessive secretion of these cytokines is associated with inflammation, obesity, and other health problems. The results of the recent study showed that RF, with changes in the time of liquid consumption, reduction in the frequency of meals, and hormonal changes, reduces inflammatory cytokines and is a potential solution for mitochondrial adaptations, autophagy, and, as a result, improving body composition [30]. In addition, RF in the early morning hours is characterized by the predominance of carbohydrates as the primary fuel and is associated with decreased glycogen reserves. In the afternoon hours and approaching Iftar time, with a reduction of glycogen reserves, fat fuel and, finally, protein become more critical and are used as more essential fuels. These cases seem to be the primary mechanism for reducing body weight, body fat, and BMI [16].

Regarding the effect of CRT, some studies have shown that this type of training can improve body composition, muscle strength, and endurance. Of course, the changes depend on the circuit training type and the rest of the duration. The rest interval ≤1 minute had significant improvement in body composition [19]. Another point is that the combination of aerobic and strength in circuit training also affected its results, so it has been observed that this combined training decreased skinfold measurements, waist circumference, and waist-to-hip ratio [19]. Domingo et al. (2021) also reported that CRT reduces fat mass (average of 4.3%) and increases muscle mass (average of 1.9%) in studies on people aged 18 to 65 [18]. On the other hand, another meta-analysis study (Buch et al., 2017) in older and middle-aged adults reported that CRT, despite improving strength, had no apparent effect on LBM and aerobic capacity [17]. Therefore, the difference in the subjects' age and gender is another issue that should be considered when interpreting the results.

The second part of this study showed that 30 days of RF or RF together with CRT led to a non-significant decrease in total cholesterol, TG, and LDL-C levels and a significant reduction in HDL-C levels. The results of different studies about the effect of RF on lipid profile values are inconsistent, and the changes in blood lipids are not constant and are affected by other factors. Prasetya et al. (2021) found a slight decrease in total cholesterol, TG, and LDL-C following RF in non-athletic young men aged 19 to 40 [31]. Akhtar et al. (2020) indicated that RF significantly reduces the levels of total cholesterol, TG, and LDL-C in middle-aged men [32]. The possible reasons for these inconsistencies may be related to the quality and quantity of food consumed, the geographical area, and the degree of weight changes. Therefore, the weight loss of the participants in this study may not be enough to cause significant changes in lipid profile values [33].

The results of different studies regarding the combined effect of RF and exercise training on lipid profile values are different, and various factors are influential. The results of the present study were in line with the findings of Denna et al. (2019), who reported a non-significant decrease in total cholesterol, TG, and LDL-C levels after ten weeks of favorite physical activity and caloric restriction in the diet in healthy men [34]. Contrary to the present study's findings, Khan et al. (2012) showed that 30 days of RF and aerobic exercise increase total cholesterol and LDL-C levels in people over 20 with type 2 diabetes. These researchers stated that drug interventions, sleeping and waking patterns, and diet are of fundamental importance for diabetic patients. The biochemical changes caused by these interventions may have adverse effects [35]. In this regard, Attarzadeh et al. (2014) found a significant reduction in total cholesterol, TG, and LDL-C levels following 30 days of RF and four weeks of physical activity (two wrestling sessions, two wrestling technique review sessions, one aerobic training session, and one weight lifting) [36].

The noteworthy point in the present study was the significant reduction of HDL-C levels in both the RF and RF+CRT groups compared to the control group. The underlying mechanism of this reduction may be related to the type of diet consumed and its biochemical responses. In addition, some studies have stated that the circadian rhythm of RF is not the same as the circadian rhythm in a normal state, and this can lead to different hormonal responses [37]. Contrary to the results of the present study, ten weeks of circuit training using 50–60% of 1RM could increase HDL-C [19]. The duration of the exercise intervention was another influential factor that should be considered.

The reasons for heterogeneity in different studies can be personal approaches, the duration of calorie restriction and the duration of the protocol, age, sex, and fitness level of people. Moreover, some researchers have suggested that nutritional prescriptions should be adjusted based on factors related to the clinical conditions of each person, the amount of daily activity, and nutritional preferences [38]. Thus, intermittent energy restriction for 12 weeks consisting of 16 hours of calorie restriction with free access to only water and no food or caloric drinks, followed by an energy-restrictive diet tailored to each individual, significantly improved lipid profiles. The noteworthy point in this research was the prescription of nutritional patterns according to the individual characteristics of each person and the duration of the nutritional protocol [39]. On the other hand, the fasting duration is one limiting factor. Evidence from a study of animal models exposed to IF showed that IF has the potential to mediate interactions between diet, metabolism, and circadian rhythm. This ability of IF may be due to the 24-hour duration of its calorie restriction. Further, the results of this research stated that an unhealthy eating pattern may overshadow the results of IF. Regarding RF, changes in the sleep-wake cycle and biochemical responses related to it, the duration of fasting, and the amount of calories consumed should be considered [38]. In addition, the difference in the duration of studies and the difference in the type of exercise and nutritional protocol can be considered other essential factors. In this regard, a clinical trial investigated the effect of six months of caloric restriction alone and combined with aerobic exercise. They reported that some risk factors for cardiovascular diseases were significantly reduced. The amount of TG in both groups significantly decreased. The amount of LDL decreased in both groups, although this decrease was more significant in the combined group. In addition, the researchers stated that some of the metabolic benefits of the study occurred after six months [40]. In summary, various factors affect body composition and lipid profile, and more care should be given to interpreting the results and comparing studies.

Conclusion

Based on the results, inactive middle-aged men whose body composition (body weight, BMI, and body fat percentage) was improved and reduced through one month of Ramadan fasting or fasting combined with circuit resistance training. However, the blood lipid profile was not significantly affected. Lean body mass and HDL-C were decreased considerably after fasting and exercise interventions, which is an additional noteworthy finding of this research.

Conflicts of Interest

The authors declared no conflict of interest.

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JOURNAL OF NUTRITION FASTING AND HEALTH

Effect of Modified Atmosphere Packaging Combined with Potato Starch Coating in Improving the Shelf-Life of Fresh Strawberries

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ABSTRACT

Introduction: Strawberry is a highly perishable fruit because of high water content and susceptibility to fungal spoilage, resulting in rapid post-harvest losses with texture softening, easily spoilage, and physiological changes. The objective of the present study was to investigate the effect of modified atmosphere packaging (MAP) combined with potato starch coating in improving the shelf-life of fresh strawberries for 7 days at refrigerated conditions.

Methods: Fresh strawberries at the red-ripe stage were purchased in a regional market, Kermanshah, Iran. The coated samples with potato starch solution were placed in the stomacher bags, gas flashed through a packaging machine, and sealed with a modified atmosphere containing 10% oxygen, 15% carbon dioxide, and 75% nitrogen. Microbial (total viable count, psychrotrophic bacterial count, and yeast/mold count) and chemical (weight loss and pH) properties of samples were investigated during a storage period of one week.

Results: During the seven-day period, strawberries treated with a potato starch + MAP containing 10% oxygen, 15% carbon dioxide, and 75% nitrogen had the best total viable count, psychrotrophic bacterial count, yeast/mold count, weight loss, and pH values, recorded by 3.93 log CFU/g, 2.89 log CFU/g, 2.31 log CFU/g, 2.05%, and 3.91, respectively, at the end of the experiment period (day 7).

Conclusion: Our findings suggest that potato starch coating + MAP treatment is practical for maintaining the microbial and chemical characteristics of fresh strawberries and increasing their shelf-life, which is critical regarding saleability and exporting.

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Introduction

Strawberry (*Fragaria × ananassa*) is considered one of the major popular fruits among consumers worldwide owing to its delicious taste and appealing appearance along with a diverse range of vitamins, fibers, minerals, and antioxidant compounds, which have anti-cancer and anti-inflammatory properties (1). However, strawberry is a highly perishable fruit because of high water content and susceptibility to fungal spoilage, resulting in rapid post-harvest losses with texture softening, easily spoilage, and physiological changes (2). Preservation at chilled conditions (approximately 0–4 °C) and rapid refrigerating to those temperatures are considered to increase the preservation period of strawberries (3). Given that strawberry is a luxury expensive fruit, can be converted into various types of processed products and marketed scientifically to fetch foreign exchange and to maintain the national economy (4). The modified atmosphere packaging (MAP) changes the gas proportions in the package environment by removing oxygen or altering the atmosphere within the package with a combination of carbon dioxide, oxygen, and nitrogen (2). MAP with the combination of 10% oxygen, 15% carbon dioxide, and 75% nitrogen can slow down the growth of spoilage-related bacteria and food-borne microorganisms, decrease the rate of chemical and biochemical changes, and maintain the overall organoleptic characteristics of easily spoiled fruits for a prolonged time storage (5). Previous studies have reported that storage at refrigerated conditions along with carbon dioxide application might enhance the

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postharvest quality and shelf-life of strawberries (6-8). Other approaches that have been utilized to decrease the postharvest decays of fresh strawberries are using coating and films based edible polymers, irradiation along with antimicrobial and antioxidant constituents (9). From this perspective, potato starch is an economical and renewable biopolymer that can be utilized for the shelf-life preservation of strawberries (10). Starch is considered one of the most commonly utilized food packaging polymers owing to its renewability, availability, cost-effectiveness, appropriate film/coating formation, and well-processivity (11). Several studies have reported the potential coating application of starch biopolymer in bananas (12), guavas (13), mandarin oranges (14), mango (15), plums (16), and strawberries (17). Several studies have also been found on the actual application of MAP in combination with aloe vera gel (18), polylactic acid film (10), aqueous chlorine dioxide (19), and chitosan coating (4) for extending the shelf-life of fresh strawberries. Take into consideration the vulnerability of fresh strawberry to decay caused by environmental and microbiological impacts during chilled storage conditions (1, 2), it is crucial to perform more research on its shelf-life extension. However, according to the knowledge of the author, no previous experiment had been performed with the combining MAP and potato starch to investigate their combined efficacy on the shelf-life of fresh strawberries. Therefore, the objective of the present study was to investigate the effect of MAP combined with potato starch coating on microbial and chemical characteristics of fresh strawberries for one week at cooled conditions.

Materials and Methods

Preparation of Potato Starch Coating

2% potato starch (w/v, high purity powder, purchased from Sigma-Aldrich, UK) was prepared by stirring on the heater stirrer (IKA, Germany) at 70 ± 1 °C for 6 h. After that, 0.75 ml/g of glycerol (Merck, Germany) was added as the plasticizer and stirred for 30 min at room temperature (25 ± 1 °C) (20).

Strawberry Packaging

Fresh strawberries at the red-ripe stage were purchased in a regional market, Kermanshah, Iran. Strawberries of similar size in the lack of physical damage and fungal infection were obtained. An amount of 100 g fresh strawberries was soaked in 200 ml potato starch coating solution for 1 h at 24 ± 1 °C on the shaker (Behdad, Iran). The coated strawberries were allowed to drain for 30 min under a biological safety cabinet. The strawberry control was treated similarly in a sterile distilled water solution without potato starch coating solution. After that, coated samples were placed in the stomacher bags, gas flashed through a packaging machine (Fer plast, FPMV 45 1BFG, Italy), and sealed with a modified atmosphere containing 10% oxygen, 15% carbon dioxide, and 75% nitrogen: strawberry volume ratio of 3:1 in sterile stomacher bags (6). All treatments were then kept at chilled temperature (4 ± 1 ºC) and then microbial and chemical properties were investigated during a seven-day period.

Microbial and Chemical Analysis

At each sampling day, 10 g of untreated and treated strawberries were mixed with 90 ml 0.1% buffered peptone water using the stomacher (BagMixer, France) for 1 min, and then the suspensions were cultured on plate count agar (Merck, Germany) for total viable count (TVC: incubated at 37 ± 1 ºC for 48 h), psychrotrophic bacterial count (PTC: incubated at 7 ± 1 ºC for 10 days), and Dicloran Rose-Bengal Cloranfenicol agar (Merck, Germany) for yeast/mold count (incubated at 25 ± 1 ºC for 5-7 days) (21). Moreover, the determination of weight loss and pH of strawberry samples was conducted based on previous approaches published by Velickova et al., (2013) (22). Weight loss was presented as a loss percentage of strawberries in comparison with the initial weight of samples. To determine the weight loss of the samples, 6 strawberries from each treatment were weighed during storage conditions. To measure the pH of samples, 5 g of each sample was mixed with 45 ml distilled water through the stomacher and then the measurement was conducted using a digital pH meter (Farazbin, Iran).

Statistical Analysis

The study was performed three times. Data analysis was performed by employing a Tukey HSD test (SPSS 23, Chicago, IL, USA). The data were presented as mean value ± standard deviation. $P < 0.05$ was presented as the minimal level of statistical significance.

Results & Discussion

Weight loss is considered one of the main quality parameters of fresh strawberries, which can be associated with the freshness and turgidity of the fruits (4). The result of weight loss of fresh strawberries during 7 days of storage at chilled conditions is presented in figure 1a.

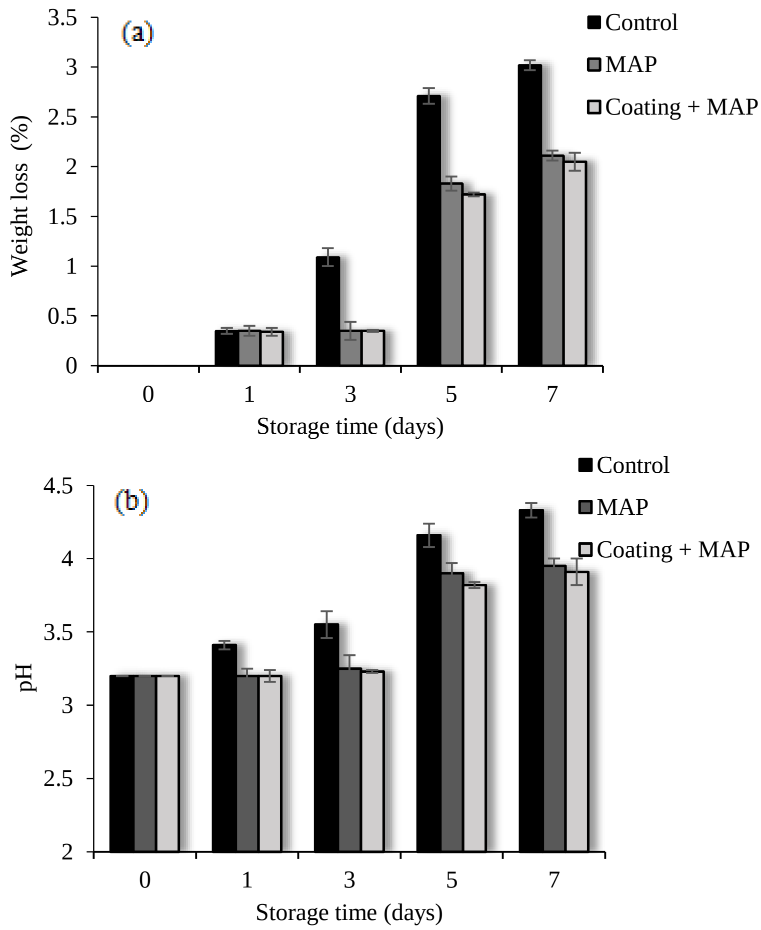

Figure 1. Chemical changes, including weight loss (a) and pH (b), of strawberries during refrigerated storage conditions.

According to our findings, the weight loss percentages of control, uncoated samples treated with MAP, and coated samples treated with MAP reached 3.02%, 2.11%, and 2.05%, respectively after 7 days of cooled storage conditions. The results might be attributed to this fact, the surface coating of strawberries resulted in the reduction of surface pores diameter, respiration, and dehydration (23). The efficacy of chitosan coating on the retarding in weight loss of strawberries comparing with other cellulose derivatives, has been previously found (24). Park et al., (2005) reported that chitosan coating delayed the weight loss of fresh strawberries by approximately 15% in comparison with control samples (25). The utilization of clay nanosilicate packaging has also been reported to retard the weight loss in fresh strawberries (26, 27). The pH was associated with strawberry flavor and maturity (28). According to the results of figure 1b, it can be indicated that in all packaging conditions, the pH changes of coated samples treated with MAP and uncoated samples treated with MAP was lower than the untreated strawberries ($P < 0.05$). In the end of the study period (day 7), the pH values of control, uncoated samples treated with MAP, and coated samples treated with MAP reached 4.33, 3.95, and 3.91, respectively. The chemical changes of treated samples happened lower than untreated samples owing to the decreasing the metabolic processes and reactions as well as microbial activity over post-harvest storage (29).

It has been found that the major spoilage of strawberries was occurred by contamination of yeast/mold and mesophilic bacterial population (9). The results of TVC, PTC, and yeast/mold count of designated strawberry treatments over chilled storage for one week are exhibited in figure 2a-c, respectively.

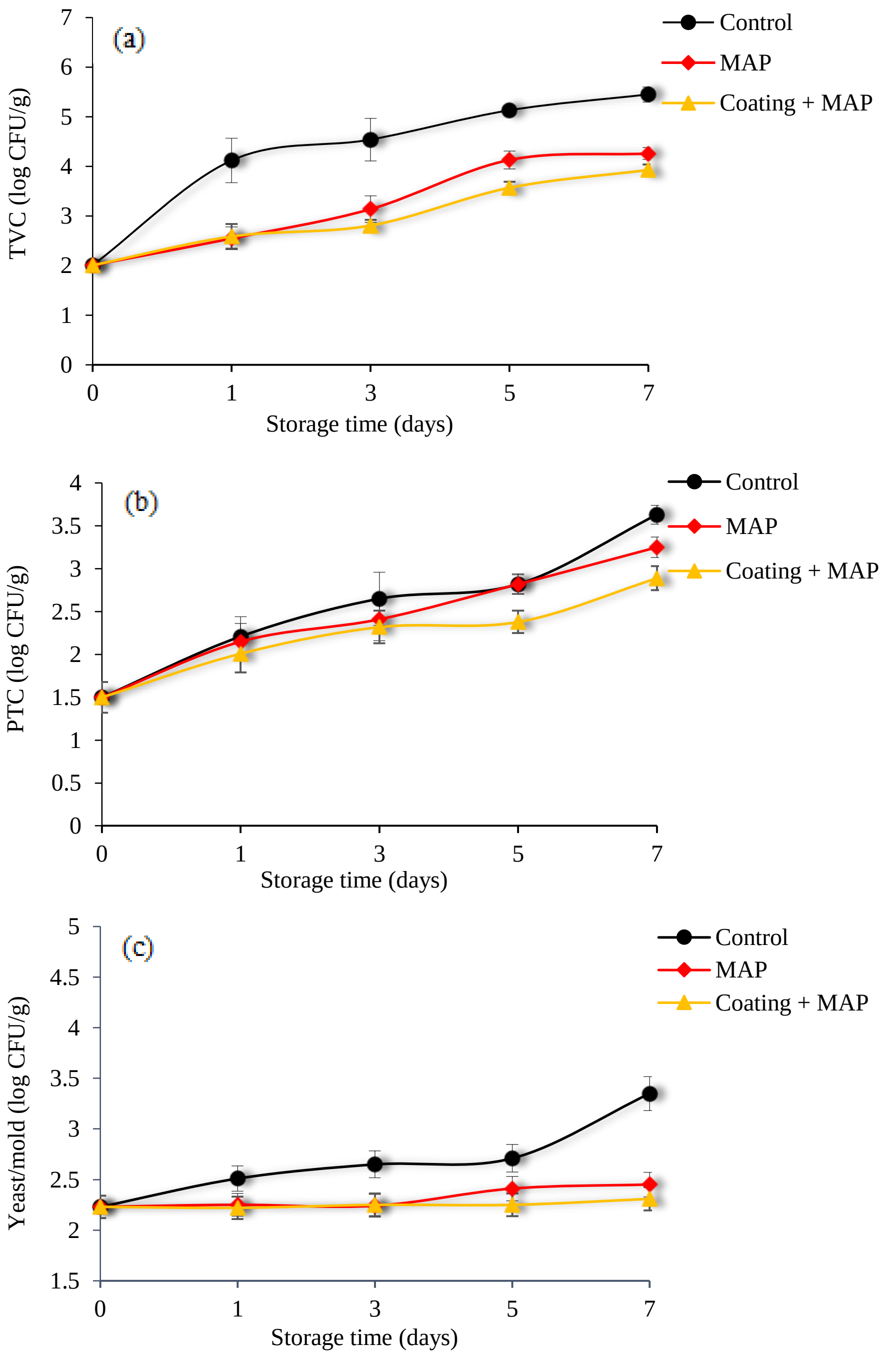

Figure 2. Microbial changes, including TVC (total viable count, a), PTC (psychrotrophic bacterial count, b), and yeast/mold count (c), of strawberries during refrigerated storage conditions.

According to the results of the present study, TVC, PTC, and yeast/mold count of strawberry samples on day 0 were determined to be 2.01, 1.5, and 2.23 log CFU/g, respectively. Our findings presented that TVC, PTC, and yeast/mold count of all strawberries were continuously enhanced with storage time and the microbial population of untreated strawberries was higher than other treatments. Gol et al., (2013) also indicated that strawberries treated with chitosan had remarkably lower TVC, PTC, and yeast/mold count comparing with the uncoated strawberries (27). The lower microbial population of coated samples compared to the control group could be owing to the almost low gas and moisture resistance characteristics of potato starch coating (30). Wu et al., (2023) (28) found that the development of gelatin/zein-based nanofiber film could successfully retard the spoilage process of strawberries stored at room temperature. Li et al., (2021) (9) also reported that poly(lactic acid) nanofibers containing ethyl-dodecanoyl-larginate hydrochloride had antimicrobial activity against *Escherichia coli* O157:H7, *Staphylococcus aureus*, and *Botrytis cinerea* and could also successfully increase the shelf-life of strawberries at room temperature. Moreover, previous studies reported that the application of MAP separately and in combination with biodegradable/edible polymers has no significant adverse effects on the sensory properties of fresh strawberries (27, 31, 32).

Conclusion

In the current experiment, the effect of MAP combined with potato starch coating for 7 days at refrigerated conditions was investigated. Over the experiment period, strawberries treated in a potato starch + MAP containing 10% oxygen, 15% carbon dioxide, and 75% nitrogen had the best TVC, PTC, yeast/mold count, weight loss, and pH values, recorded by 3.93 log CFU/g, 2.89 log CFU/g, 2.31 log CFU/g, 2.05%, and 3.91, respectively after seven days. The results obtained from this experiment hold the potential for extending the shelf-life of fresh strawberries and indicate promising prospects in this field. More studies are essential to investigate the sensory properties and scanning electron microscopy analysis of the coated strawberry surfaces. Further experiments are necessary to improve the productiveness and commercial accessibility of packaged strawberries with MAP.

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Milk Clotting and Proteolytic Activity of *Auricularia Auricula* Fungus and Its Potential in the Production of Feta Cheese

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ABSTRACT

Introduction: This study's objective was to evaluate the *Auricularia auricula* fungus as a coagulant of bovine milk and its potential in feta cheese production. The *Auricularia auricula* fungus displays high levels of milk-clotting activity, presumably due to clotting proteolytic enzymes.

Methods: Feta cheese was manufactured from bovine milk, coagulated with *Auricularia auricula* and the proteolytic properties of the fungus were determined.

Results: on polyacrylamide gel, substrates in the presence of the fungus showed higher proteolysis activity than control samples. In comparison, cheeses made with bovine rennet had more distinct protein bands than those produced with fungi. An initial evaluation of the cheese's flavor indicated a lack of bitterness and yielded positive results.

Conclusion: Due to its properties and availability, the *Auricularia auricula* fungus might be a suitable candidate for replacing calf rennet. More research is needed to evaluate its use as a coagulant in large-scale cheese production in the future.

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Introduction

Cheese is one of the most important dairy products, with its protein rich in essential amino acids. Feta is a type of cheese with a relatively salty, slightly acidic taste and a pleasant flavor that enjoys universal acceptance (1). Feta is white, with a soft texture. It is kept in salt water as it matures. Since ancient times, sheep's milk, goat's milk, or a mixture of both have been used to produce feta cheese (2). One of the main steps in cheese processing is solidifying the casein micelles in milk and converting them into a three-dimensional gel matrix. The enzyme chymosin, which is very active, is used in the preparation of animal rennet. The primary role of chymosin in making cheese is associated with the hydrolysis of the phenylalanine-methionine bond in milk casein, leading to its coagulation (3). Milk coagulation can occur in various ways. Most cheese forms a coagulum through the action of milk-clotting enzymes (4). Various types of milk-clotting enzymes, including animal, microbial, and plant-derived enzymes, have been introduced. Calf rennet, obtained from animal stomachs (rennin), was the first and foremost reported standard milk-clotting enzyme. However, due to the short shelf life of rennin, the incidence of bovine spongiform encephalopathy (BSE), and restrictions for religious reasons (e.g., Judaism and Islam), dietary preferences (e.g., vegetarianism), or objections to genetically engineered foods (e.g., Germany and the Netherlands forbid the use of recombinant calf rennet), the supply and demand for bovine rennet are limited (5). Another type of milk-clotting enzyme is a plant coagulant, which has been used for a long time as a traditional rennet. However, due to their strong proteolytic activity and poor textural characteristics, many plant coagulants are not suitable for industrial applications (3). Therefore, the search for suitable plant proteases is ongoing (6). Other proteases that can be introduced as substitutes for rennet include microbial origin proteases such as *Rhizomucor miehei*, *Rhizomucor pusillus*, and *Endotypalariatica*, which are heavily involved in cheese production. A study of the proteolytic effects in fungal rennet cheeses

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shows that bitter-flavored cheeses were produced with Endothia but not with the mucors. Using the relative staining intensity as a guide, the coagulants' proteolytic activities on the α and β fractions were rated (from least to most) as follows: α-casein - Endothia, Mucor, rennet; and β-casein - rennet, Mucor, Endothia. Rennet mainly attacked α-casein; Endothia predominantly attacked β-casein. The Mucors affected both fractions to about the same degree (7). Another fungal protease with milk coagulation potential is a beneficial fungus with the scientific name (*Auricularia. auricula. Judea*). *Auricularia auricula* fungus belongs to the Basidiomycota phylum and is of the species *Auricularia auricula*-judae. This fungus is known by various names, including wood ears, pork ears, Chinese mushrooms, tree ears, and black and white ears. It has been traditionally used as a medicinal and food supplement in China and Korea. *Auricularia auricula* is high in carbohydrates (nearly 630 g/kg in dried weight), minerals (P, Ca, and Fe), and protein, especially leucine and lysine amino acids. Research has shown that *Auricularia auricula*-judae β-D-glucan has biological activities and exhibits potent inhibition against acinar cell carcinoma (8). The methanol extract of *Auricularia auricula* inhibited lipid peroxidation and decreased liver damage in benzo[a]pyrene-treated mice (9). As a consequence, it is considered to be a healthful food. Researchers are paying more attention to its utilization. Further studies on its medicinal or functional food properties need to be conducted. *Auricularia auricula* fungus has a short harvest period each year and grows in a wet climate. This fungus is not a staple food; nevertheless, it is considered for use in creating new varieties of foods and expanding its role as a healthy food that promotes consumer health. In the 19th century, this fungus was used in traditional medicine. Today, this fungus is used as a food ingredient, especially in soups, and is also used in Chinese medicine. This fungus helps reduce blood sugar and cholesterol. This fungus is more scattered in temperate and tropical regions, but it is found throughout Europe, North America, Asia, Australia, South America, and Africa. *Auricularia auricula* has also been observed to be consumed in countries such as Ghana, Vietnam, Nepal, Mozambique, Indonesia, Poland, and Bolivia. Given the development of the cheese-making industry in recent years, the lack of animal rennet, the disadvantages of other milk-clotting enzymes, and the excellent potential of fungal proteases, research on the use of this fungus in the cheese industry and the descriptions of its characteristics seem necessary. *Auricularia auricula* fungus can be used as an alternative source of milk-clotting enzymes during the Feta cheese-making procedure. Moreover, the enzyme's dependence on pH and temperature and its stability profiles are entirely suitable for the chemical-physical conditions adopted during the cheese-making procedure fungus's can be used as an alternative source of milk clotting enzymes during the feta cheese-making procedure. Moreover, the enzyme's dependence on pH and temperature, along with its stability profiles, are entirely suitable for the chemical-physical conditions adopted during the cheese-making process (10). Considering all the above, the present research was conducted to determine the influences of a novel alternative source of milk-clotting enzymes (*Auricularia auricula*). To our knowledge, there is a gap in the recent literature regarding the investigation of the proteolytic activity of *Auricularia auricula*. This study focuses on determining the milk-clotting activity of this fungus and conducting electrophoretic analysis of the hydrolysis products after treating crude casein and milk, as well as its potential in Feta cheese production.

Materials & Methods

Preparation of Auricularia Auricula Fungus Powder

Five hundred grams of dried *Auricularia auricula* was prepared from northern Iran (Noor city) and authenticated by the Mycology Research Center at the University of Tehran. The mushrooms were then pulverized using a grinder (IKA M20 universal) and used as a replacement protease for rennet.

Milk-Clotting Activity

The milk-clotting activity was determined using the method of Uchikoba and Kaneda (1996). First, 10% solutions of skimmed milk powder in 67 mM NaH_2PO_4 at pH 6.8 and 30°C were prepared as a substrate. Different amounts of the fungal powder preparation (1, 5, 10, and 15 g) were added to a final volume of substrate (3 mL) both in the absence and presence of $CaCl_2$ (5 mM). The endpoint, based on the advent of milk clots, was observed separately in the test tube.

The test was conducted in three replicates, and the average coagulation time was reported (11).

Proteolytic Activity Assay

The reaction mixture contained 2% (w/v) total casein (Sigma-Aldrich, USA), κ-casein (Sigma-Aldrich, USA), and two kinds of milk (pasteurized semi-skimmed and pasteurized whole milk) as substrates dissolved in 67 mM NaH2PO4 at pH 7.2 and 2.5 mM DTT, as explained in Lo Piero et al. (2011). The hydrolysis against different kinds of milk was estimated by incubating the milk with 20 mg of fungal powder, following the method described by Lowry et al. (1951). The assay mixture (1 mL) was incubated for 21 minutes at 55°C. The reaction was then terminated by adding 1.5 mL of 5% (w/v) TCA (trichloroacetic acid). Blanks were prepared by adding the fungal powder at the end of the incubation period, just before the addition of TCA and precipitation. The sample tubes were centrifuged at 9000 g for 10 minutes, and the absorbance of supernatants was evaluated at 280 nm using a Beckman Spectrophotometer, DU® 650 Model, and a Shimadzu UV-VIS 1240 spectrophotometer (Shimadzu Corporation, USA). An arbitrary fungal powder unit was determined as the amount of fungal powder that yields a 0.001 absorbance change at 280 nm per minute under the assay conditions. All tests were repeated three times (12, 10).

Electrophoretic Analysis

Whole casein (10 mg), milk proteins (Sigma-Aldrich, USA), pasteurized semi-skimmed milk, and pasteurized whole milk (10 mg of proteins) were dissolved in 67 mM NaH2PO4 at pH 7.2. Fungal powder (2% w/v) was added to the incubation of each protein solution (final volume 0.3 mL), and hydrolysis was carried out for 20 minutes at 55°C (10). The samples (17 μg) were prepared for SDS-PAGE (15 cm × 20 cm) by adding an equal volume of double-concentrated loading buffer (4 g/100 mL SDS, 30 g/100 mL sucrose, 125 mmol/L Tris-HCl, pH 8.0, 0.042 mmol/L DTT, 2 g/100 mL bromophenol blue). Immediately after finishing electrophoresis, the gels were separated from the plates and stained with 0.25 g/100 mL Coomassie Brilliant Blue G-250 in 50 mL/100 mL methanol containing 7.5 mL/100 mL acetic acid, and left for 8 hours. Destaining was accomplished using a solution of 15 mL/100 mL methanol, 7.5 mL/100 mL acetic acid, and 150 mL/100 mL water (2:1:10). As previously described, protein content analysis of whey and cheese curds was determined by SDS-PAGE (17 μg). The cheese curd was centrifuged at 13,000 g for 10 minutes in a Beckman J2-HS centrifuge, rotor JA-20 (Beckman Instruments, Fullerton, CA, USA) to drain the whey (Refrigerated centrifuge model 5810 R made in Germany). The resulting pellet was dissolved by heating at 55°C in 0.2 g/100 SDS with vigorous shaking for 30 minutes, and the supernatant was discarded. Lowry's method was used to evaluate the protein content of milk, curd, and whey (10).

Cheese Manufacture

Cheese samples were prepared using 5 kg of pasteurized whole bovine milk containing 3.4% protein, 3.70% fat, and 18.28 g/100 g in total solids. The preheated milk (35°C) was inoculated with 0.5% (v/v) starter cultures and supplemented with 0.1 g of CaCl2/kg of milk. The milk was kept at 35°C for approximately half an hour to allow the starter cultures to mature before adding the fungus powder. Then, treatments containing 5% (w/v) fungus powder were added to the milk. After one hour, the curd was sliced crosswise into cubes of 1-2 cm^3. To facilitate draining, the curd was compressed for 6 hours, with pressure gradually increasing to nearly 2000 Pa in the first 2 hours. The clot was then sliced into pieces measuring 6 × 8 × 12 cm and placed in 20% (w/v) sterilized salt for 15 days at 12-14°C. After the initial maturation period (green warehouse), the cheese samples were transferred to 8% brine and refrigerated at 4°C for 6 weeks for the ripening period (4). "A primary cheese tasting was conducted to identify any bitter taste in the recently produced Feta cheese. Tasters were asked to appraise the cheese for flavor and general acceptability using a 5-point hedonic scale, rating from 1 (very undesirable) to 5 (very desirable). This was achieved by comparing the features of Feta cheese produced using *Auricularia auricula* fungus as a coagulant with those of Feta cheese produced with calf rennet. The cheeses were evaluated organoleptically by a group of seven experienced and proficient panelists (four women and three men, aged 20 to 45 years old). Feta cheese samples were divided into seven equal parts, each about 3 cm × 3 cm × 2 cm in size, and placed on white plates. The samples were randomly coded to prevent bias. This test was performed at room temperature (20 ± 2°C) under regular lighting in a single area (14).

Statistical Analysis

Statistical analysis was conducted using Microsoft Excel 2007 (Microsoft, Redmond, WA, USA). The coagulation effect of *Auricularia auricula* fungus in Feta cheese production was assessed using SPSS version 20 software. The investigations were performed in triplicate on individually isolated preparations, and the results are presented as the mean and standard deviation (±SD) of these tests. Quantitative data analysis was done using one-way ANOVA, and the results were presented as mean and standard deviation. The Tukey test was used to compare the means, with significance set at P≤0.05. Sensory characteristics were analyzed using the Kruskal-Wallis statistical test.

Results and Discussion

Milk-Clotting Activity

"The milk-clotting activity of *Auricularia auricula* fungus was tested, and the results of this investigation are shown in Table 1. The milk-clotting activity was associated with fungus powder content. As the amount of fungus increased, the milk-clotting time became notably faster. The first presence of the solid substance in the milk was recognized in 26 minutes using 1% (0.03 g) of fungus powder. The milk-clotting time was measured both in the absence and presence of CaCl2, and there was a significant difference in the milk-clotting time when using 1-10% of fungal powder in the absence and presence of this salt (P < 0.05) (Table 1).

Table 1. Milk clotting activity by *Auricularia. Auricula* in the presence and absence of 5 mM CaCl2. The values are means ± standard deviations (SD) of data from three experiments (*N* = 3). Values with the same letter do not differ significantly (P≤0.05).

Mushroom powder amounts (weight percentage)	Clotting time (CT) (min)	
	−CaCl2	+CaCl2
1	26.17±4.96[a]	22.33±2.51[a]
5	13.83±2.87[b]	10.466±3.09[b]
10	8.91±0.57[bc]	6.18±0.75[bc]
15	5.54±0.39[c]	3.85±0.25[c]

Proteolytic Activity

The proteolytic activity of *Auricularia auricula* fungus powder, assayed toward different substrates, is shown in Table 2. The *Auricularia auricula* fungus powder exhibited the highest degree of proteolysis on total casein, with 129.56 U/mg (Table 2). This amount, slightly higher than the activity obtained against κ-casein (123 U/mg), is presumably due to some lost target sites present in the total casein section but not in the κ-casein (10). However, the *Auricularia auricula* fungus powder's proteolytic activity toward whole casein and κ-casein is almost identical. There was no significant difference between them, presumably due to the fungus's high proteolytic activity affecting different caseins. The tendency toward proteolysis decreased when milk (whole and semi-skimmed) was used as a substrate. In other words, the protease's high proteolytic activity is usually regarded as an unfavorable characteristic of milk coagulants (14). The function of *Auricularia auricula* fungus powder was also measured using commercial milk as substrates (Table 2). Whole milk is the preferred substrate, displaying a specific activity amount equal to 30.9 U/mg. In contrast, the activity of *Auricularia auricula* fungus powder is slightly lower when semi-skimmed milk is used as the enzyme-substrate (25.1 U/mg). This characteristic is probably related to the lipolytic properties of the fungus.

Table 2. Hydrolysis of the total, k-casein, and two types of milk by *Auricularia. Auricula*. The values are means ± standard deviations (SD) of data from three experiments (*N* = 3). Values with the same letter do not differ significantly (P≤0.05).

Substrates	Specific activity (U/mg
Casein	129.56±2.6[a]
k-Casein	123±16.78[a]
Whole milk	30.9±0.1[b]
semi-skimmed milk	25.1±0.34[b]

Hydrolysis of Casein and Commercial Milk by Auricularia Auricula Fungus Powder Treatment

The SDS-PAGE pattern of the proteolytic particles achieved by treating either total casein or milk protein with *Auricularia auricula* fungus powder is displayed in Figure 1. The SDS-PAGE pattern of untreated whole casein shows three prominent bands corresponding to α, β, and κ-caseins, with estimated molecular weights of

33.9 kDa, 27.8 kDa, and 25.1 kDa, respectively (Figure 1, lane 3). The *Auricularia auricula* protease significantly digests total casein into a few large particles, yielding three bands with molecular weights of approximately 32.1 kDa, 21.8 kDa, and 15.8 kDa (Figure 1, lane 4) (10). The same pattern is observed when milk protein is the substrate (Figure 1, lanes 5 and 6).

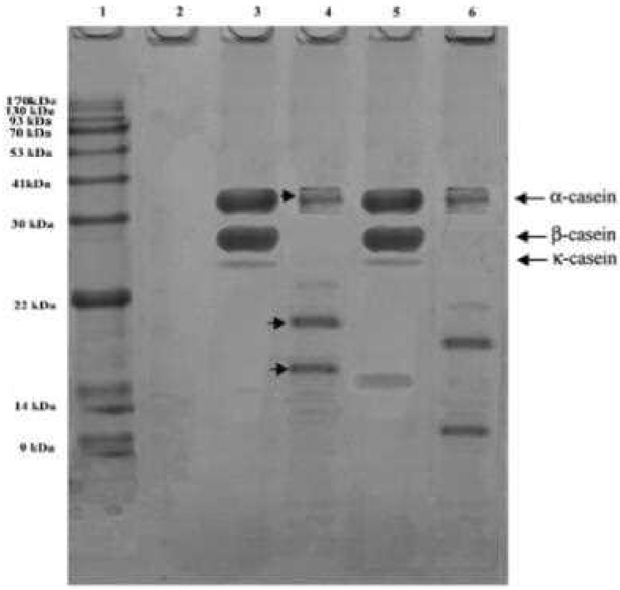

Figure 1. SDS-PAGE patterns of the whole casein and milk protein subjected to the powder of *Auricularia auricula* fungus treatment. 17 µg of each sample was loaded onto 12.5% slab gel. Lane 1: molecular weight standard; lane 2: powder of *Auricularia auricula* fungus (5 µg); lane 3: control total casein; lane 4: treated total casein; lane 5: control whole milk; lane 6: treated whole milk

The SDS-PAGE pattern obtained after fungus treatment of milk with different fat contents (whole milk and semi-skimmed milk) and their hydrolysates is displayed in Figure 2 (lanes 7-10) and reveals another level of hydrolysis on α-casein, β-casein, and κ-casein (Figure 2, lanes 8 and 10). The main digestion products detectable in both types of milk probably correspond to κ-casein hydrolysates with a visible molecular weight of 15.8 kDa (Figure 2, lanes 8 and 10).

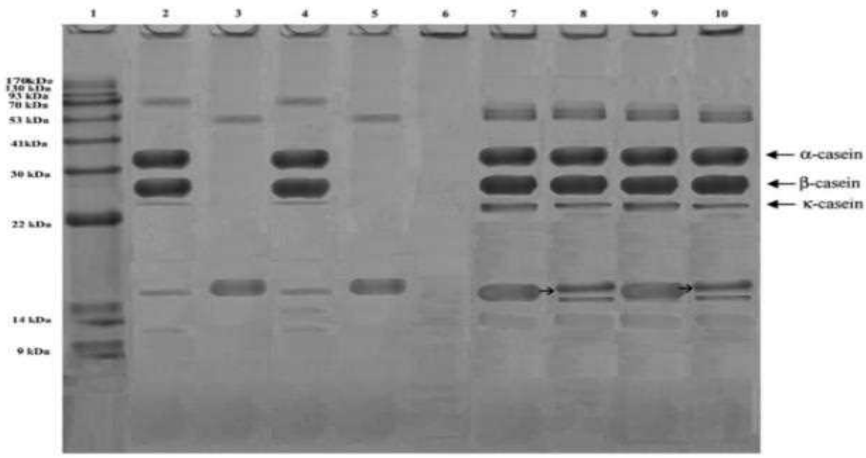

Figure 2. SDS-PAGE pattern of the curd cheese before salting. Samples corresponding to 5% in protein were loaded onto 12.5% slab gel. Lane 1: molecular weight standard, lane 2: treated curd cheese; lane 3: treated whey protein; lane 4: control curd cheese; lane 5: control whey protein; lane 6: powder of *Auricularia auricula* fungus (5 µg); lane 7: control whole milk; lane 8: treated whole milk; lane 9: control semi-skimmed milk; lane 10: treated semi-skimmed milk.

Comparing the SDS-PAGE patterns obtained after treating commercial milk (pasteurized whole milk and pasteurized semi-skimmed milk) with fungus powder to those obtained by treating total casein and milk protein shows significant digestion, likely corresponding to κ-casein hydrolysates, detectable in all samples. Other casein particles are degraded to a lesser extent. Although it was expected that the protective function of the milk fat, which might cover some of the casein target residues, would result in less hydrolysis of milk by the fungus compared to casein and milk protein, the hydrolysis levels were almost the same. (10).These results could be related to the lipolytic properties of this fungus, which can decompose milk fats and reduce their inhibitory effect on casein's target positions.

Feta Cheese Manufacture

This research utilized whole bovine milk (3.40% protein, 3.70% fat, and pH 6.7) for Feta cheese production using *Auricularia auricula* fungus powder as a coagulant. The SDS-PAGE pattern of the proteolytic particles obtained indicates that the coagulation observed from treating whole bovine milk with the fungus powder corresponds to κ-casein coagulum, and the composition of curd cheese before salting was investigated by SDS-PAGE (Figure 2). The main casein fractions reveal their complete digestion into three main particles with apparent molecular weights of 24 kDa and 15 kDa, respectively, and a smaller portion of about 11 kDa (Figure 2, lane 2). The same pattern is also observed when calf rennet is used as a milk coagulant to produce Feta cheese (Figure 2, lane 4). The whey proteins are separated from the casein clot, and the SDS-PAGE pattern is shown in Figure 2. By comparing these outcomes with those collected from the hydrolysis of milk (Figure 2) and data obtained from the SDS-PAGE pattern of calf rennet as a milk coagulant for producing Feta cheese (Figure 2), the aforementioned particles are likely hydrolysis products of caseins. The principal digestion product recognizable in treated milk and curd cheese is the original band, with a molecular weight of 15.8 kDa (Figure 2, lanes 8 and 10; Figure 2, lanes 2 and 4). The results of the preliminary analysis of cheese taste were conducted as described in Section 2.5. All evaluators stated that the cheese produced using *Auricularia auricula* fungus powder as a milk coagulant, compared with cheese production using calf rennet, did not have an undesirable or bitter taste, and no member of the taste panel noted any off-flavor. The appearance parameters (surface, texture, and form) were deemed acceptable by the taste panel. The texture and body were rated as satisfactory by the taste panel for cheeses made with the fungus. The overall acceptability on the hedonic scale was rated as 4 (desirable) and 5 (very desirable). Evaluators highlighted no differences between the sample and control Feta cheese prepared with calf rennet (Figure 3)

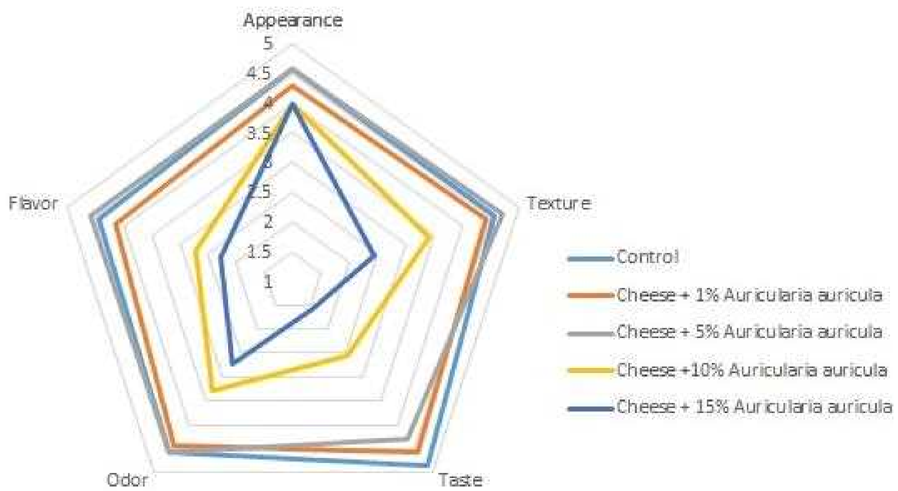

Figure 3. Sensory evaluation of Feta cheese produced using Auricularia auricula fungus as a coagulant.

Conclusion

"This study aimed to measure the applicability of the *Auricularia auricula* fungus as a fungal coagulant in Feta cheese manufacture and to introduce this fungus as a replacement for calf rennet. The focus was on its capability to clot milk and hydrolyze milk proteins and bovine casein. The data demonstrate that the milk-clotting and proteolytic activities of *Auricularia auricula* fungus were not affected by CaCl2, which is typically added to facilitate milk-clotting. During the cheesemaking procedure, the *Auricularia auricula* fungus powder acted as a coagulant, resulting in curd cheese where the casein coagulum was isolated from the whey proteins (Figure 2). The proteolytic activity of *Auricularia auricula* fungus is appropriate in whole and semi-skimmed milk, which is essential for enhancing the cheese's quality and sensory properties. The initial assessment of the Feta cheese's overall acceptability was very high, as considered by all tasters, especially regarding flavor, which was rated between desirable and very desirable. Due to the shortage of calf rennet, resulting from the slaughter of young calves and religious restrictions, the use of *Auricularia auricula* fungus can be an excellent alternative to calf rennet, making it unnecessary to import rennet. Due to the availability of *Auricularia auricula* fungus, its low exploitation costs, and its proper proteolytic activity, it could be used in the dairy industry to design new dietary products and obtain potentially bioactive peptides. The study's limitation was that *Auricularia auricula* is not cultivated in Iran, and we were obligated to gather it in its wild form.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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JOURNAL OF NUTRITION FASTING AND HEALTH

The Effect of High-Protein and Low-Calorie Diets on Sleep Quality in Individuals with Obesity

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ABSTRACT

Obesity, defined as excess body fat, has become a global epidemic, especially in low- and middle-income countries. Sleep problems are among the complications faced by obese individuals, although such problems are not very common. Previous studies indicate that obese individuals have a significantly greater likelihood of developing insomnia and other sleep disorders. Meanwhile, there is growing scientific evidence that diet and sleep may be related, and that weight loss can improve sleep quality and sleep-related indices. In order to improve sleep quality and alleviate sleep disorders, this study examined the effects of weight loss diets on sleep quality in obese individuals. The literature indicates that sleep quality and sleep-related indices are improved by weight loss. Compared with a low-fat diet, a very low-carbohydrate diet (VLCD) does not adversely affect cardiovascular risk factors for short-term weight loss. When weight loss is combined with VLCD, obstructive sleep apnea (OSA) can be improved.

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Introduction

Obesity represents a global health crisis given its rapidly increasing prevalence, especially in low- and middle-income countries. The World Obesity Federation (WOF) predicts that by 2030, nearly one billion people will be living with obesity worldwide. Obesity is an epidemic disease that is defined as excess body fat. According to the definition proposed by WHO, BMIs greater than or equal to 25 and 30 kg/m^2 indicate overweight and obesity, respectively (1).

One of the complications faced by obese individuals is sleep problems, which has received limited attention. Sleep is a basic human needs and is necessary to maintain the energy of appearance, coordination of body rhythm, and physical health (2, 3). Studies have shown that sleep deprivation leads to a decrease in daily performance, impairment of metabolic function, disruption of the immune system and cortisol and insulin levels, and increased mortality. Obese people are more likely to report insomnia or trouble sleeping (4). Also, obesity contributes to the development and severity of obstructive sleep apnea (OSA) through effects on the upper airway (e.g., neuromuscular control of breathing and adipokine production) (5). Insomnia and its underlying pathophysiology may also play a role in predisposing a person to excessive energy consumption. Therefore, nutrition can simultaneously contribute to weight loss and improve sleep in patients with obesity and sleep disorders (5, 6).

Obesity is caused by long-term energy imbalance. Therefore, researchers have sought to identify factors related to positive energy balance (7). Rastrollo et al. showed that increasing the energy density (ED) of the diet over a period of 8 years was significantly associated with weight gain (7). In another study, women who consumed a high-ED diet gained nearly three times as much weight as women whose diets had lower ED. A diet with

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lower ED allows people to eat more food while lowering their energy intake (8). Comparison of the effects of dietary energy density on body mass index (BMI) has confirmed the positive relationship between dietary energy density and BMI in all gender and racial groups (9).

Very low calorie diets (VLCDs) were introduced to help obese people lose weight quickly without losing too much Muscle tissue (10). Further studies have shown that the inclusion of even small amounts of protein in severely calorie-restricted diets results in a significant reduction in the utilization of body nitrogen and protein stores (11). Therefore, due to concerns that low-quality protein intake or inadequate micronutrient supplementation may contribute to early mortality, VLCDs containing high-quality protein along with vitamins, minerals, trace elements, and essential fatty acids were introduced to treat obesity. Widespread use of formulated VLCDs has been repeatedly shown to be safe and highly effective when used for a limited period of time under close medical supervision (11, 12).

Materials & Methods

In this review study, all studies related to keywords study regarding to the effect of high-protein and low-calorie diets on sleep quality in individuals with obesity, were collected from electronic databases. We searched PUBMED-Medline, Scopus, Web of Science Core Collection, and Google Scholar databases to identify the studies based on the Medical Subject Headings (MeSH) with full texts in English. All studies in regarding to the keywords "High-protein", "Low-calorie diet", "Obesity" and "Sleep quality" carefully reviewed and their results were critically discussed according to their impact on dimension of sleep quality.

Results & Discussion

Obesity and Its Complications

There has been a significant increase in obesity rates in the world in the past 50 years. Obesity and overweight are defined as a person having a BMI greater than or equal to 30 and between 25.0 and 29.9, respectively. Overweight and obesity are more strongly associated with increased mortality than underweight and are more common on the global scale, having been reported from all regions of the world (13, 14).

Obesity increases the risk of various diseases and conditions, including type 2 diabetes (T2DM), cardiovascular diseases (CVD), metabolic syndrome (MetS), chronic kidney disease (CKD), hyperlipidemia, hypertension, non-alcoholic fatty liver disease (NAFLD), certain types of cancer, OSA, osteoarthritis, and depression, as well as increased mortality (15). Treating these conditions can place an additional burden on healthcare systems. For example, it is estimated that obese individuals have 30% higher medical costs than individuals with a normal BMI (16).

Increasing Energy Density (ED)

Obesity occurs as a result of long-term energy imbalance. Therefore, researchers have sought to identify factors related to positive energy balance. One of these effective factors is energy density. The energy density of food refers to the amount of energy obtained from each gram of food; foods with high energy density provide more energy in fewer grams. According to WHO, increased consumption of foods with high energy density in the isocaloric state has contributed to the obesity epidemic (7).

Consuming a diet with a lower ED allows individuals to consume more food (in terms of weight) while reducing their energy intake (8). Comparison of the effect of diet ED on BMI among racial and gender groups has confirmed the general positive relationship between diet ED and BMI (9).

Sleep and Obesity

Obese individuals' sleep problems often go unnoticed and untreated. Sleep is a basic human needs and is necessary to maintain the energy of appearance, coordination of body rhythm, and physical health (2). Studies have shown that sleep deprivation leads to a decrease in daily performance, impairment of metabolic function, disruption of the immune system and cortisol and insulin levels, and increased mortality (4).

Sleep disorders are common issues, with 30% of the population suffering from them sometime in their lives and 10% of the population being permanently affected. Sleep disorders are divided into three general categories: 1- problems with falling sleep such as having disturbed and confused thoughts, 2- problems with the continuation phase of sleep such as sleepwalking, nocturnal enuresis, and 3- problems with waking up from sleep such as waking up earlier than the scheduled time (17).Poor sleep causes adverse consequences such as increased overall mortality, increased obesity, type 2 diabetes, blood pressure disorders, and respiratory disorders in adults and children (18).

In general, most physical diseases that cause considerable pain or discomfort or are caused by metabolic disorders can negatively affect the quality and quantity of sleep, including obesity. Various studies have reported a relationship between sleep disturbance and obesity. For example, Gangwisch et al. showed that people who sleep less than 7 hours are more likely to be obese and overweight (19).

Result Category	Measurement/Outcome	Details	Description of Findings
Impact on Obstructive Sleep Apnea (OSA)	Apnea-Hypopnea Index (AHI)	Reduction in AHI scores, a primary measure of OSA severity, tracking frequency and severity of apnea events.	Significant decrease in AHI scores, reflecting fewer breathing interruptions and better oxygenation during sleep. Participants experienced fewer apneic events, improving sleep quality.
Sleep Quality Indices	Sleep Efficiency	Measures the percentage of time spent asleep while in bed, a key indicator of sleep quality.	Improvement in sleep efficiency noted, indicating participants spent more time asleep relative to total time in bed, reducing periods of wakefulness and enhancing restorative sleep.
	Sleep Latency	Duration of time required to fall asleep after going to bed.	Decreased sleep latency, showing faster sleep onset, likely due to increased satiety and stable blood glucose from high-protein intake, supporting more regular sleep patterns.
Blood Pressure & Baroreflex Sensitivity	Blood Pressure	Measurement of arterial pressure levels, often elevated in OSA and obesity, which are risk factors for cardiovascular diseases.	Lower blood pressure levels observed, reducing cardiovascular risks commonly associated with OSA and obesity, and indicating improved autonomic function.
	Baroreflex Sensitivity	Indicator of autonomic nervous system response, assessing body's regulation of blood pressure.	Enhanced baroreflex sensitivity, suggesting improved cardiovascular health, as baroreflex aids in stabilizing blood pressure during sleep and wakefulness.
Metabolic and Hormonal Adjustments	Leptin Levels	Leptin is a hormone regulating satiety and fat storage; higher levels support appetite control.	Increased leptin levels, which help control appetite and reduce late-night eating, minimizing sleep disruptions and enhancing overall sleep quality.
	Ghrelin Levels	Ghrelin, a hormone associated with hunger, typically rises during sleep deprivation and may contribute to night eating.	Reduced ghrelin levels observed, helping to limit nocturnal hunger and cravings, which may lead to a more undisturbed sleep cycle and improved sleep patterns.
Chronic Insomnia Intervention	Insomnia Severity, Sleep Duration	Severity of insomnia symptoms and total sleep duration measured; short sleep duration is often linked with weight gain and poor sleep quality.	Participants with chronic insomnia showed decreased insomnia severity and increased sleep duration, suggesting dietary intervention as a non-invasive treatment for insomnia in obesity.
	Sleep Onset	The time it takes to fall asleep, which is often delayed in individuals with high BMI or metabolic dysfunctions.	Faster sleep onset observed in high-protein diet group, likely due to the thermogenic effect of protein and reduced metabolic disruptions, supporting improved overall sleep health.

Sleep Disorders and Obesity

The International Classification of Sleep Disorders currently lists more than 80 distinct sleep disorders divided into eight categories, including insomnia, sleep-disordered breathing, and sleep-related movement disorders. The relationship between sleep disturbance and obesity is likely an important mediating factor in emerging research linking sleep disturbance and other chronic diseases, including cardiovascular disease (CVD) and diabetes mellitus (20).

Insomnia and Obesity

Previous studies have shown that obese individuals are significantly more likely to report insomnia or difficulty sleeping (21). In addition, obese subjects were significantly more likely to develop chronic insomnia over a median follow-up of 7.5 years. Although this effect was partially negated when controlling for sociodemographic and behavioral factors (22). Finally, in obese individuals, complaints of chronic emotional stress or sleep disturbance have been reported to predict short sleep duration, rather than voluntary sleep reduction as previously thought. On the other hand, Vgontzas et al. showed that in obese people without sleep disorders or emotional stress, sleep duration was similar to the non-obese controls. This may indicate the importance of diagnosing and treating sleep disorders as a potential therapeutic intervention for obesity (23).

Insomnia and its underlying pathophysiology may play a role in predisposing a person to excessive energy consumption and thus lead to weight gain. In a study of over 1000 volunteers from the Wisconsin Sleep Cohort Study, Taheri et al. found that shorter sleep duration (5 hours per night vs. 8 hours per night) was associated with 15.5% lower leptin levels and 14.9% higher ghrelin levels. Reduced sleep duration was independently associated with BMI, which may indicate that chronically short sleep duration can increase appetite and lead to overeating (6).

In addition, Dalman et al. have suggested that chronic elevation of glucocorticoids, such as cortisol, similar to the proposed mechanism for insomnia, may contribute to an individual's increased desire to consume high-fat and high-sugar foods as well as their tendency to store fat. They proposed that chronic elevation of glucocorticoid hormones increases CRF activity in the central nucleus of the amygdala and increases abdominal obesity, which then acts as a means to increase metabolic inhibitory feedback on catecholamines in the brain and CRF. This suggests that the same pathway associated with hyperactivity in insomnia may promote the overconsumption of high-fat and high-sugar foods as well as the deposition of abdominal fat stores in an attempt to counter the hyperactivity occurring in the brain (24).

Obstructive Sleep Apnea (OSA) and Obesity

Knowledge of the fundamental association between OSA and obesity is evolving and involves a bidirectional relationship that affects both the contribution of obesity to OSA and the consequences of OSA for obesity. Obesity contributes to the development and severity of OSA by influencing the upper airway, including respiratory neuromuscular control and adipokine production. Independently, obesity appears to affect upper airway control through several mechanisms, including changes in upper airway structure and function, reduced resting volume, and negative effects on respiratory drive and load compensation (5).

In addition, neuromuscular control of the upper airway is affected by changes in several key cytokines associated with obesity, including leptin, tumor necrosis factor-alpha, and interleukin-6. Independently, leptin can inhibit respiratory drive. Meanwhile, OSA is associated with elevated leptin levels (25). Insulin resistance is also associated with OSA severity independent of body weight and may be related to sleep deprivation or sympathetic activation (26).

Mechanisms Leading to Obesity

The traditional view regarding the cause of obesity is that obesity is mainly caused by the storage of excess energy, exceeding the energy used by the body. Excess energy is stored in fat cells and as a result the pathology of obesity is determined. Pathological enlargement of adipocytes alters the nutritional signals responsible for obesity (27). However, recent research has shown that food sources and quality of nutrients are more important than their amount in the diet for weight control as well as for disease prevention. In addition, genetic factors play an important role in determining a person's tendency to gain weight.(28).

Pathogenesis of Obesity

The pathogenesis of obesity involves the regulation of caloric intake, appetite, and physical activity, but has complex interactions with the availability of health care systems, the role of socioeconomic status, and underlying genetic and environmental factors (29).

Balance of Food and Energy Consumption

The main causes of obesity are somewhat controversial. Current health recommendations for obesity management are based on the underlying physiological reasoning holding that fat accumulation is due to an energy imbalance between absorbed and consumed calories. The obesity epidemic has been fueled in large part by increased energy from the greater availability of highly-nutritious and energy-dense foods. Diet and various social, economic and environmental factors related to food supply have a great effect on a person’s ability to achieve balance (30).

In a 13-year follow-up study of 3,000 young people, it was found that those who ate the most fast food weighed an average of 6 kilograms more and had a larger waistline than those who consumed the least amount of fast food. It was also found that those consuming more fast food had a higher incidence of weight-related health problems such as increased triglycerides and were twice as likely to develop metabolic syndrome (31).

Accumulation of lipid metabolites, inflammatory signaling, or other impaired hypothalamic neuronal mechanisms may also lead to obesity, which may explain increased body fat mass as a form of biological defense (32).

Family History and Lifestyle

Family history, lifestyle and psychological factors all play a role in obesity. The likelihood of becoming obese can be influenced by family genetics (tendency to accumulate fat) or lifestyle (poor diet or exercise)(33). A child with one obese parent has three times the risk of becoming obese as an adult, while when both parents are obese, the child's risk of obesity in the future is 10 times greater. A cross-sectional observational study on 260 children (139 females, 121 males, 2.4-17.2 years old) showed that a family history of cardiometabolic diseases and obesity are important risk factors for severity of childhood obesity (34).

Genetic Factors and Causes

Family and twin studies show that about 40 to 70% of variation in obesity can be explained by genetic factors (35). While environmental changes have increased obesity rates over the past 20 years, genetic factors still play a key role in the development of obesity (36). The low predictive power may be due to conditions where gene-gene, gene-environment, and epigenetic interactions are not fully identified using current methods based on population genetics (35).

The genetic causes of obesity can be generally classified as follows:

1) Monogenic causes resulting from a single gene mutation, mainly in the leptin-melanocortin pathway. Many genes, such as AgRP (agoti-related peptide), PYY (orexogenic), or MC4R (melanocortin-4 receptor) have been identified as monogenic contributors to obesity that disrupt appetite, weight, and hormonal signaling (ghrelin, leptin, and insulin) (37).

2) Syndromic obesity is caused by severe obesity due to neurodevelopmental abnormalities and other organ/system abnormalities. This may result from changes in a single gene or a larger chromosomal region that includes several genes (38).

3) Multigenic obesity is caused by the cumulative contribution of many genes.

In addition, some people develop obesity due to multiple genes (39). These genes affect their appetite (thus their calorie intake), hunger levels, overeating control, satiety, tendency to store body fat, and tendency to be inactive (40).

Epigenetic Modification

Although genes contributing to monogenic forms of obesity have been identified, the pace of change for the human genome is too slow for the genome to be a major player in the current obesity epidemic. However, epigenetics may provide a reasonable explanation for the increased prevalence of obesity in the past few decades without requiring a fundamental change in the genome (41). In multicellular organisms, the genetic code is homogeneous throughout the body, but the expression of the code can vary among cell types. Epigenetic studies have shown that heritable regulatory changes in genetic expression do not require changes in nucleotide sequences (42). Epigenetic modifications can be thought of as differential packaging of DNA that enables or silences the expression of specific genes in tissues (29).

Obesity Treatments

Lifestyle Changes

Due to the lack of specific pharmacological interventions, "lifestyle modification" is the cornerstone of obesity management (16). Obese people are advised to lose at least 10% of their body weight through a combination of diet, physical activity, and behavioral therapy (or lifestyle modification) (43). Significant weight loss can be achieved in the short term by consuming controlled diets. Long-term weight control can be achieved through high levels of physical activity and consistent patient-physician contact. In many cases, lifestyle modification leads to a significant reduction in body weight, leading to a significant reduction in cardiovascular risk (44).

Anti-Obesity Drugs

Pharmacotherapy is recommended for those with a BMI ≥30 (or a BMI ≥27 with comorbidities) who cannot lose weight using lifestyle modification alone(45). The FDA has approved a number of pharmaceuticals for the short-term treatment of obesity, including Naltrexone-Bupropion (Contrave), Orlistat (Xenical, Alli), Liraglutide (Saxenda), and Phentermine-Topiramate (Qsymia) (46, 47). The FDA also approved the MC4R agonist-setmelanotide for use in people with severe obesity due to POMC, PCSK1, or LEPR (leptin receptor) deficiency in 2020. In addition, 11 compounds from 54 plant families have been identified as having anti-obesity potential. These families include Celastraceae, Zingiberaceae, Theaceae, Magnoliaceae and Solanaceae (48). Traditional Chinese medicine also offers unique solutions for the treatment of obesity, such as regulating fat metabolism, increasing hormone levels, and regulating intestinal microflora (49).

Surgery

For people with BMI > 40 or BMI > 35 with comorbidities who cannot lose weight with lifestyle modification or drug therapy, bariatric surgery or weight loss surgery is another option (50). Standard bariatric procedures, including BPD (biliopancreatic diversion), SG (sleeve gastrectomy), RYGB (Roux-en-Y gastric bypass), and AGB (adjustable gastric banding) can alter individuals' metabolic profiles (51). Studies report that the benefits of bariatric surgery go beyond weight loss. Bariatric surgery reduces the chronic inflammation involved in obesity and alters biomarkers, gut microbiota, and long-term T2DM remission (29).

Weight Loss and Energy Consumption

Many studies indicate that lifestyle improvement through following a correct diet and appropriate physical activity is the best and most appropriate way to lose weight. Although medical and surgical treatments have also been offered to treat obesity, there are potential side effects for a number of drugs used in the treatment of obesity (such as amphetamines, phenfluramine-phentermine, sibutramine). The possibility of abuse on the one hand, and weight gain after stopping these drugs on the other hand have raised concerns about this approach and have limited the use of medication for the treatment of obesity. In the case of surgical treatments, side effects such as electrolyte disorders, gallstones, stomach ulcers, arthritis and malabsorption, along with the potential complications of the surgery itself such as anesthesia complications, and laparotomy complications have limited their application to patients with a minimum BMI of 35 who suffer from the complications of obesity and have been unresponsive to other methods of weight loss for at least six months (52, 53).

Considering the above-mentioned cases, it seems that the first step in weight loss is behavioral changes and dietary pattern correction along with increasing energy consumption through increasing physical activity and exercise. The percentage of carbohydrates, protein, and fat in the diet has always been considered as one of the factors influencing weight loss. There is a difference of opinion among experts regarding the most useful prescription regimen. In a balanced diet, about 55% of energy is obtained from carbohydrates, 15% from protein and 30% from fat. While some experts recommend that the percentages remain unchanged in an energy-restricted diet, other combinations such as low-fat, high-protein diets also have their own supporters. The advantages and disadvantages of diets with different ratios are still being discussed (45, 54).

Weight loss diets fall into three general categories:

1. Low-calorie diets with a standard protein percentage

2. Low-calorie diets with an increased protein percentage
3. Very low-calorie diets with an increased protein percentage

Low-calorie diets with a high protein percentage stimulate GLP1, which affects the pancreas and leads to insulin secretion. Insulin secretion plays three important roles:

1. Insulin causes a 2% increases in metabolism, which leads to further increases in thermogenic energy after meals.
2. Insulin acts on fat cells, increases cell volume and leads to more leptin production. Leptin affects neurons and leads to faster and more complete induction of satiety.
3. Incretins prepare the intestine for the next meal and increase chyme production, for this reason, they have an inhibitory effect on the stomach and cause the production of better-quality chyme. Incretins also stimulate the intestine for digestion by directing the food that has already been digested to the anal canal. It seems that high-protein diets may be more accepted for weight loss compared to standard diets (46).

Very Low-Carbohydrate Diets (VLCD)

VLCDs were introduced more than two decades ago to induce rapid weight loss in obese individuals, comparable to starvation but without excessive body tissue loss (10). During their early development, it became apparent that the inclusion of even small amounts of protein in severely calorie-restricted diets resulted in a significant reduction in the utilization of body nitrogen or protein stores (11).

Therefore, to achieve rapid weight loss while preserving lean body mass, these diets are designed to initially provide 300-400 calories of high-quality protein as a regular meal with or without carbohydrates, supplemented with minerals and vitamins (10).

VLCD Composition and Nitrogen Balance

Protein and Carbohydrate Content

A major concern with VLCD therapy is that excessive negative nitrogen (protein) balance and loss of lean body mass may contribute significantly to the development of fatal cardiac arrhythmia. Experts believe that a high-protein, low-carb (or no-carb) diet is needed to maintain full lean body mass throughout the cycle. By restricting carbohydrate intake, hypoinsulinemia is induced, leading to increased lipolysis and ketosis, which in turn provide more fuel for the brain and other tissues while sparing protein stores (47, 55).

Protein Quality and Micronutrient Supplements

Due to concerns that low-quality protein intake or insufficient micronutrient supplementation may contribute to early mortality, VLCDs containing high-quality protein along with vitamins, minerals, trace elements, and essential fatty acids were introduced to treat obesity. Widespread use of these formulated VLCDs has repeatedly shown them to be safe and highly effective when used for a limited period of time under close medical supervision (11).

Low-Calorie Diet and Anthropometric Measurements

In a 1987 study, Barrows et al. examined the effects of a very low-calorie (420 kcal/day), high-protein (70 g/day) diet on body weight and body composition in 15 obese middle-aged women during 4–6 months. According to their results, the average weight loss was 20.5 kg (1.1 kg per week). Fat accounted for 83% and lean mass accounted for 17% of total body weight loss. In this study, the best predictor of body density in obese women before and after weight loss was trunk circumference (48).

In a 2003 study, Brehm et al. designed a randomized controlled trial to determine the effects of a VLCD on body composition and cardiovascular risk factors. Subjects were randomly assigned to either a VLCD or a calorie-restricted diet with a 70% calorie deficit for 6 months. The VLCD group lost more weight and body fat than the low-fat diet group. The mean levels of blood pressure, lipids, fasting glucose, and insulin were in the normal range in both groups at the beginning, but improved during the study with no differences between the two groups at 3 or 6 months. According the researchers, the VLCD was more effective than a low-fat diet for short-term weight loss and was not associated with adverse effects on cardiovascular risk factors in healthy women (49).

In a 2001 study, Djuri et al. examined the effect of low-fat and/or low-energy diets on anthropometric measures in participants in the Women's Diet Study. According to their results, low-fat, low-energy, and combined diets all led to similar and statistically significant reductions in

BMI, body fat percentage, and waist circumference during the 12-week intervention. However, the amount of weight loss varied by baseline weight, and the combination diet was the only intervention that resulted in significant weight loss for women who had been overweight at baseline. This suggests that although there may be an advantage to reducing dietary fat in women who are obese at baseline, any of these counseling strategies can be effective in improving the anthropometric predictors of health risks associated with overweight. In fact, flexibility in food choices may facilitate adherence to dietary recommendations in some individuals (49).

Liao et al. (2007) examined the effectiveness of a soy-based diet compared to a traditional low-calorie diet on weight loss and fat levels in overweight adults. In this study, 30 obese adults (average body mass index: 29-30 kg/m^2) were randomly divided into two groups. The soy-based low-calorie group consumed soy protein as the sole protein source, and the traditional low-calorie group consumed two-thirds animal protein and one-third plant protein on a 1,200 kcal/day diet for 8 weeks. Body weight, BMI, body fat percentage, and waist circumference decreased significantly in both groups. The decrease in body fat percentage was greater in the soy group than in the traditional group. Serum total cholesterol concentration, low-density lipoprotein cholesterol concentration, and liver function parameters decreased in the soy-based group and were significantly different from the measurements in the traditional group. No significant changes were observed in serum triglyceride levels, serum high-density lipoprotein cholesterol levels, and fasting glucose levels in the soy or traditional group. According to the researchers, soy-based low-calorie diets significantly reduced serum total cholesterol and low-density lipoprotein cholesterol concentrations and had a greater effect on reducing body fat percentage than traditional low-calorie diets. Therefore, soy-based diets have health benefits for reducing weight and blood lipids (56).

Low-Calorie Diets and Sleep

In a 1992 study, Suratt et al. investigated the effect of low-calorie diets combined with weight loss on OSA. The subjects consumed a VLCD diet of 420 kcal (67% protein, 4% fat, 29% carbohydrate) or 800 kcal (20% protein, 30% fat, 50% carbohydrate) with 100% of the recommended intake of minerals and vitamins.

The results showed that VLCD and weight loss could improve OSA. However, people who lose a small amount of weight or those who are extremely obese before and after weight loss may not improve (57).

Kansanen et al. (2008) investigated the effect of weight loss through a low-calorie diet on the severity of OSA and autonomic nervous function in obese patients with obstructive sleep apnea syndrome (OSAS). Their results showed that weight loss through a VLCD is an effective treatment for OSAS. Also, weight loss significantly improved sleep apnea and had beneficial effects on blood pressure and baroreflex sensitivity (58).

A study conducted by Martin et al. in 2016 in the United States on 220 adults with an average age of 37 years showed that the participants lost 7.6 kg after 24 months of following a 25% energy-restricted diet. They lost weight and their sleep quality score improved. The study has some limitations however; the subjects were healthy and the number of white women was higher (59).

In 2016, Zhou et al. conducted a study in the US on two groups: 14 healthy individuals with an average age of 56 years (group 1), and 44 healthy individuals with an average age of 52 years (group 2). The first group were given a diet with a 750-kcal deficit and 10, 20, and 30% protein, and the second group were given a 3-week diet with 0.8 gr protein/kg of weight. This study showed that weight loss improves sleep quality regardless of its source. Also, the sleep indices in both groups included secondary measurements, which cannot be directly matched with the inclusion and exclusion criteria. Moreover, self-reported measures by the subjects such as the PSQI may have biased the outcomes (60).

Dobrosielski et al. (2016) conducted a study on 25 obese individuals older than 60 years old in the United States. Their results showed that after 12 weeks of following a restricted diet and following the lifestyle recommendations of the American Heart Association (90 minutes of exercise per week), there was a 9% reduction in weight, 5% in fat, and an 8% decrease in the apnea index among the participants compared to the control group (61).

Hudson et al. (2020) conducted a study in 51 healthy Americans with an average age of 41.5 years. The study was carried out during 12 weeks using a diet with a 750-kcal deficit. According to their results, the BMI of all participants decreased. However, dietary intake did not affect any of the measured objective or subjective sleep quality outcomes. Over time, the objective measures of time spent in bed, time spent falling asleep, sleep onset, and wake time after sleep onset did not change. However, sleep efficiency improved in participants. The subjective measures of global sleep score (GSS) and daytime sleep score improved over time. Based on GSS, sleep classification changed from "poor" to "good". According to the results, losing weight may not improve objective sleep quality, but adults who are overweight or obese may sleep well while eating a healthy or high-fat diet. Among the limitations of this study was objective measurement of sleep quality, which is predictable for the participants beforehand (62).

Conclusion

Obesity is a serious public health problem, and the most common interventions are diet and exercise. Very low-calorie diets (VLCDs) are sometimes used for weight management. Although there are large amounts of evidence for the effect of sleep duration and quality on food choice and consumption in children and adults, less attention has been paid to the effect of food patterns and specific foods on sleep. Studies have shown that certain dietary patterns may improve both daytime alertness and nighttime sleep. The literature also reports that weight loss improves the quality of sleep and the related indices. Compared to a low-fat diet for short-term weight loss, VLCD is not associated with any adverse effects on cardiovascular risk factors. VLCD can improve OSA through weight loss. Furthermore, weight loss reduces sleep apnea and improves baroreflex sensitivity and blood pressure. Research has shown that weight loss diets with more protein, regardless of its source, promotes better sleep. Using a low-calorie diet and following the lifestyle recommendations of the American Heart Association can improve body weight and apnea index.

Declarations

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Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

There is no conflict of interest.

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