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Prevalence and Severity of Chronic Pulmonary Disease and Its Lifestyle Determinants in the Persian Cohort Study Mashhad: A Cohort Study Protocol

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ABSTRACT

Introduction: Asthma and Chronic Obstructive Pulmonary Disease (COPD) are prevalent respiratory disorders with significant societal and healthcare impacts. This study will be conducted on the participants of the Persian Cohort study at Mashhad University of Medical Sciences (POCM) to determine the prevalence of asthma and COPD and to explore their relationship with dietary patterns, urban structure, lifestyle, and the severity in Iran.

Methods: A comprehensive assessment will be employed in this cohort study, including lifestyle factors, dietary patterns, anthropometry, spirometry, depression and stress levels, sleep quality, and physical activity. Individual-level analysis will utilize multivariate logistic regression to assess associations between outcome variables and potential predictors like air quality, smoking, occupation, socioeconomic status, and nutritional status. Adjustments for gender, age, chronic health conditions, and other potential confounders will be incorporated.

Results: This study will investigate the most closely associated factors influencing the prevalence and severity of asthma and chronic obstructive pulmonary disease.

Conclusions: The final outcomes will delineate the relationship between the prevalence of asthma and COPD and various lifestyle factors, including socioeconomic status, air pollution, and physical activity.

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List of Abbreviations:
COPD: Chronic Obstructive Pulmonary Disease
POCM: PERSIAN Organizational Cohort study in Mashhad University of Medical Sciences
DALYs: Disability-Adjusted Life Years
VC: Vital Capacity
FEV1: forced expiratory volume in one second
FVC : forced Vital Capacity
FFQ: Food Frequency Questionnaire
DASS-21: Depression Anxiety and Stress Scale
PSQI: Pittsburgh Sleep Quality Index
BIA: bioelectrical Impedance Analysis
PA: physical Activity
DASH: Dietary Approaches to Stop Hypertension

Introduction

Presently, chronic lower airway diseases such as Chronic Obstructive Pulmonary Disease (COPD) and asthma rank as the third leading cause of mortality in the United States (1). COPD and asthma are aspiratory illnesses that pose a serious open well-being (2). The common characteristic of asthma and COPD is airway obstruction (3). The airflow obstruction in asthma is episodic and reversible and is characterized by symptoms like chest tightness, wheezing, coughing, and breathlessness. On the other hand, airflow obstruction in COPD is chronic and is diagnosed when there is a recurring chronic productive cough for at least three months in two or more consecutive years. Unlike asthma, airflow obstruction in COPD is not entirely reversible (1).

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According to the 2017 Worldwide Burden of Disease Study, the disability-adjusted life years (DALYs) associated with chronic respiratory diseases rose from 97.2 million per year in 1990 to 112.3 million per year in 2017. It was also stated that although DALYs improved over time in patients with asthma, this indicator decreased in COPD. Similarly, global deaths from chronic respiratory diseases increased by 18% over time, reaching 3.91 million in 2017. The average age-standardized yearly death rate (ASMR) for chronic respiratory diseases decreased by 2.41% from 1990 to 2017. In 2017, COPD accounted for 81.7% of all deaths related to chronic respiratory diseases, which represents a 23% increase from 1990 (4). The incidence of asthma has tripled in the last three decades, with the highest rate found in highly developed countries (2). In Iran, Sharifi et al. conducted a cohort study spanning five distinct geographical regions (north, south, west, and east of Iran). The findings disclosed an overall estimated prevalence of COPD at 4.9%. Region-specific prevalence rates were identified as 2.8% in Mashhad, 3.7% in Mazandaran, 8.8% in Ahvaz, 4.1% in Tehran, and 13.9% in Kerman (5). Additionally, the prevalence of asthma in Iran was reported as 8.9% (6). The number of deaths attributed to asthma and COPD in Iran in 2015 was reported to be 6,686 and 8,832, respectively (7).

Asthma and COPD can be detected and distinguished using lung function tests, including post-bronchodilator spirometry, and bronchodilator reversibility testing. Nevertheless, differentiation of chronic asthma with airway alteration from COPD might be challenging. Respiratory functional investigation may include vital capacity (VC), forced expiratory volume in one second (FEV1), and forced vital capacity (FVC). Since airway resistance increases with the severity of the airflow limitation, FEV1 is hypothesized to be able to assess COPD severity. Airflow obstruction is defined as FEV1 below 80% of the predicted value and FEV1:FVC ratio of less than 70% of the predicted value (8). Asthma might be present in more than 40% of patients with COPD (9). Asthma itself has been considered a risk factor for COPD (9). COPD progression and comorbidities increase in patients with consequent asthma (overlap syndrome) (9).

Inherent factors play a role in the development of asthma and COPD in connection with environmental factors. Many environmental factors contribute to asthma and COPD, and some only contribute to asthma or COPD alone. The environmental factors involved in the pathology of asthma and COPD are (10) indoor and outdoor air pollution (for example, exposure to biomass fuel), job-related dangers, infections (10, 11), reduced physical activity, increased psycho-emotional stress (12), low socioeconomic status (13), and unhealthy diet (10, 14). Although previous studies have indicated a correlation between lifestyle behaviors and COPD and asthma, no study has yet evaluated the interactions between modifiable risk factors, including air pollution and lifestyle, and their impact on the risk of developing COPD and asthma. Therefore, this study aims to identify the prevalence of asthma and COPD and to investigate the interaction between modifiable risk factors and COPD and asthma in a cohort study.

Materials and Methods

Study population

This proposed cohort study will be incorporated into the ongoing PERSIAN Cohort study in the Mashhad University of Medical Sciences (POCM) study. The proposal for this study was approved by the Ethics Committee of the Mashhad University of Medical Sciences (Code No: IR.MUMS.MEDICAL.REC.1402.267). The study is based on cohort data, and no new measurements will be collected.

Mashhad, the second-largest city in Iran with a population exceeding 3 million, serves as the initial site within the metropolitan area, showcasing notable distinctions in terms of pathogenic and health determinants. These determinants include migration rates, air pollution, lifestyle diversity, cultural and economic factors; education, and entertainment facilities. As the study aimed to identify the prevalence of asthma and COPD in the POCM population, all participants who were enrolled in the POCM till the initiation of the study will be included taking into account the inclusion and exclusion criteria based on universal sampling. The required data will be obtained from the available POCM data, which includes a total of 12,000 participants. The average age of personnel at the start of the cohort was 39.4±8.9 years, with 44.4% being male.

The inclusion criteria for this study will be being between the ages of 30 and 70 at the time of enrollment, being of Iranian descent, having resided in Mashhad for at least 9 months per year, and not having any plans to relocate within the next 2 years. Informed consent was obtained from the participants prior to their participation in POCM and as this study will be conducted on the recorded data, no informed consent will be obtained from the participants (15).

All assessments and recorded variables in the POCM are presented in Figure 1 and Figure 2, respectively. The questionnaires in this study include demographic parameters, socioeconomic status, lifestyle, sleep and circadian rhythm, and physical activity. The medical questionnaire includes personal and family medical history, and past and current substance abuse (such as smoking, alcohol, and drug use).

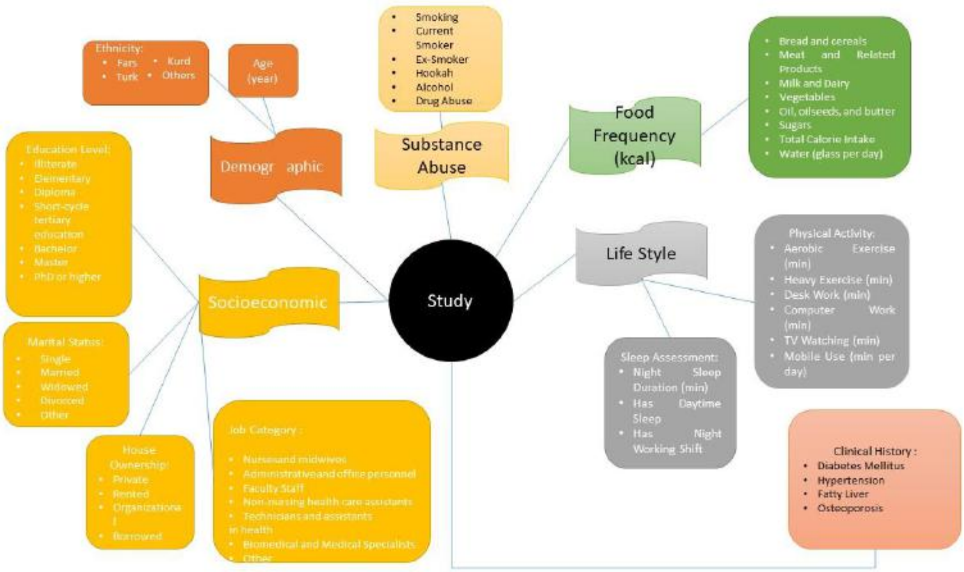

Figure 1. The POCM data collection items

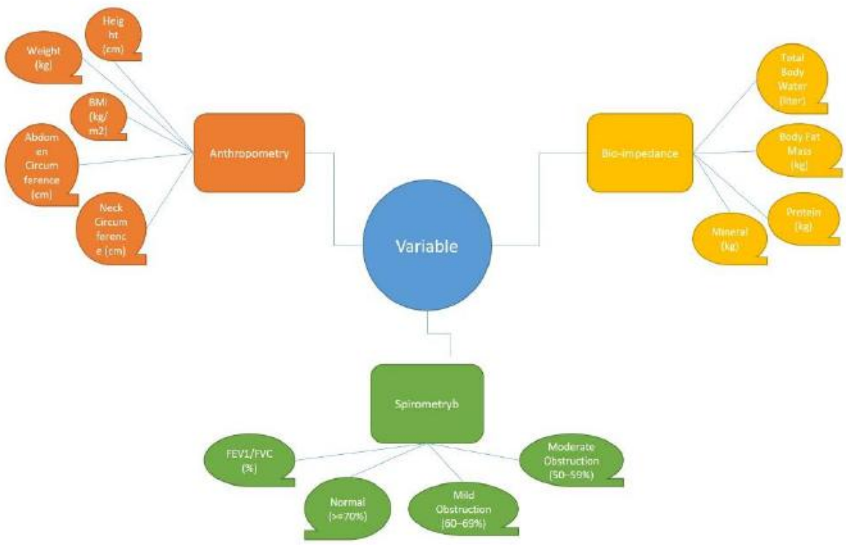

Figure 2. The variables used in the study based on the POCM assessments

Dietary intake information spanning one year will be gathered utilizing the Willett format of the Food Frequency Questionnaire (FFQ). Additionally, data on food preparation and preservation techniques will be collected.

The assessment of depression, anxiety, and stress levels will be conducted through the utilization of the short-form version of the Depression Anxiety and Stress Scale (DASS-21), comprising 21 items (16). The validity of the Persian translation of the questionnaire has been previously examined and endorsed (17).

The Pittsburgh Sleep Quality Index (PSQI) is a self-report questionnaire comprising 19 items, designed to evaluate subjective sleep quality during the preceding month. These 19 items are categorized into seven domains, each assessing distinct aspects of sleep. The aggregate score across these domains classifies respondents as either "good" or "poor" sleepers (18). Farrahi Moghaddam et al. in Iran have verified the validity and reliability of this questionnaire (19).

The short form of the International Physical Activity Questionnaire (IPAQ), comprising seven questions (20), has had its validity and reliability affirmed by Baghiani Moghadam et al. in Iran(٢١).

Bioelectrical impedance assessment is conducted using the Inbody 770 (Inbody Company, Seoul, South Korea). The measurements of body composition encompass intracellular and extracellular water, total body water, skeletal muscle mass, visceral fat level and area, bone mineral content, body cell mass, basal metabolic rate, whole and segmental phase angle, waist-to-hip ratio, degree of obesity, and weight control indices (15).

To differentiate between asthma and COPD and evaluate the participants' FVC using a spirometer (MIR, Rome, Italy). This spirometer has been validated for research purposes, ensuring its reliability. According to the American Thoracic Society/European Respiratory Society (ATS/ERS) standards, FVC is measured following a specific protocol. 1) maximal inhalation, 2) a forceful exhalation, and 3) continued exhalation until the end of the test (15).

In this study, a personal geographic database will be created to store all geo code data files. The living addresses of chronic respiratory patients will be geocoded. The geo codes will be categorized within the geographic database. Due to patient privacy concerns, the personal data of the patients were accidentally exposed in a breach of 500 records (22).

Study Procedure

This study will evaluate the nutrient intakes of the participants of the POCM over the past year, using FFQ. Additionally, the diagnosis and severity of COPD and asthma will be assessed based on spirometry results. Sleep and mental status, as well as any underlying diseases, will also be recorded using the available data at the time of participation in the POCM. The study will evaluate the relationship between study outcomes and exposure to different dietary patterns in the past year.

Primary Objective

This study aims to comprehensively examine chronic pulmonary diseases in the POCM participants. Objectives include assessing disease prevalence and severity, identifying dietary patterns, understanding the relationship between dietary patterns and the disease, exploring the impact of sleep quality, and evaluating the relationship between physical activity and disease severity.

Secondary Objectives

1) To identify individual, behavioral, and environmental factors and other neighborhood characteristics in the POCM participants

2) To examine the spatial variability of chronic respiratory diseases burden in the POCM participants

3) To evaluate the relationship between depression and anxiety and chronic pulmonary diseases and their severity in the POCM participants.

Funding

As this study will be conducted on the recorded data of the POCM, no funding was granted to this study.

Statistical Analysis

The analysis will incorporate spatial analyses and multivariate regression analyses to examine the relative impact of autonomous hazard variables on chronic pulmonary diseases. Spatial analysis is a form of geographic analysis that seeks to elucidate patterns in human behavior and its spatial manifestation through the application of mathematics and geometry, particularly focusing on locational analysis. Statistical investigation will incorporate exploratory, descriptive, spatial examination, and multivariate relapse investigation. The spatial examination will include mapping persons and aggregate results at the neighborhood level. To test for spatial autocorrelation within the result measures, Moran's I (worldwide autocorrelation) and the neighborhood marker of Spatial Autocorrelation (LISA) tests will be used. The latter test allows for the decomposition of the worldwide pointer into the commitment of each person's perception. Spatial relapse models will be assessed employing a range of natural, social, financial, behavioral, and well-being indicators (23).

Results

Drawing from the findings of prior studies, there is a hypothesis positing a connection between air pollution and the onset of COPD (24). Moreover, diet and nutrition are speculated to emerge as potential risk factors for both asthma and COPD. Specific dietary habits, such as the intake of refined grains, cured and red meats, desserts, sweets, French fries, and high-fat dairy products, may be pinpointed as risk factors for asthma and COPD (25).

Discussion

The outcomes of this study will contribute to enhancing our comprehension of the impact of non-medical determinants of health on chronic pulmonary diseases, particularly in the realms of health behaviors, living conditions, personal resources, and environmental factors affecting COPD and asthma. These findings can be used for the implementation of community interventions, policy formulation, and the development of public health strategies aimed at reducing chronic pulmonary diseases by improving living conditions and built environments.

In the study titled "Prevalence, Awareness, and Associated Factors of Airflow Obstruction in Russia: The Ural Eye and Medical Study in 2019" conducted by Bikbov et al., the objective was to evaluate the prevalence of airflow obstruction and asthma among individuals aged 40 years and above in a Russian population. The findings indicated a prevalence of 6.8% for airflow obstruction and 2.6% for asthma. Smoking was identified as the predominant risk factor for chronic pulmonary diseases (26).

In the study titled "Patterns of Medical Care Utilization According to Environmental Factors in Asthma and Chronic Obstructive Pulmonary Disease" conducted by Jo et al., the researchers explored the utilization patterns of medical care in individuals with asthma or COPD during the year 2020, with a specific focus on meteorological factors and air pollution. The results revealed that meteorological factors and air pollutants were associated with an increased utilization of medical care among patients with asthma and COPD, particularly among the elderly population. While the overall impact was more pronounced for COPD, the influence on older individuals was greater for asthma. Additionally, the alterations in medical care utilization patterns due to environmental factors varied by gender (27).

The study conducted by Wen et al., entitled "Associations of Adherence to the Dietary Approaches to Stop Hypertension (DASH) Diet and the Mediterranean Diet with Chronic Obstructive Pulmonary Disease among US Adults in 2023," aimed to investigate the correlation between the risk of COPD and adherence to DASH and Mediterranean diets in American adults. The study revealed that a higher adherence score to the Dietary Approaches to Stop Hypertension (DASH) diet was linked to enhanced COPD prevalence, improved lung function, and a reduction in respiratory symptoms (28).

In the 2023 study conducted by Peftoulidou et al., titled "Physical Activity and Quality of Life in Children with Well-Controlled Asthma," the researchers sought to investigate the relationship between lung function, physical activity, and quality of life in children with well-controlled asthma. The findings indicated that a low level of physical activity exhibited a negative correlation with the quality of life among children with well-controlled asthma (29).

Possible limitations of this study will be related to the sources of bias in cohort studies, namely selection bias, information bias, and confusion bias. In order to reduce the risk of these sources of bias the following strategies were implemented in this study. The universal sampling design of this study will eliminate selection bias. As this study will be a retrospective cohort study, there is a possibility of information bias, which is referred to missing data in follow ups. In order to decrease the risk of information bias, participants with missing data will be contacted and the missing data will be obtained from the participants. However, some data including body composition and laboratory tests may not be retrieved for the past; therefore, there is still the possibility of losing data in this study. Confusion bias might be present in case of the existence of patterns that might mislead the researchers about casualty. This type of bias is mainly seen in terms of age and gender as cohort studies do not randomly include participants of all age groups or equal number of genders. This study will also be at risk for confusion bias. However, the researchers will try to evaluate the relationships using the highest number of possible confounding variables.

Author's Contributions

RR, FDD and AJE designed this study. FDD, AJE and SB conducted the library search, wrote the manuscript, and drafted and edited the manuscript. All authors have read and approved the final manuscript.

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Mind Matters: Examining the Relationship between Mental Lifestyle Determinants and Muscle Mass

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ABSTRACT

Introduction: There was evidence of a relationship between changes in muscle mass and mental health. Depression, stress, anxiety, sleep quality, and smoking are all aspects of one's mental lifestyle. The current study aimed to evaluate the relationship between muscle mass and intangible mental lifestyle variables in a population of Mashhad University of Medical Sciences employees.

Methods: This study used PERSIAN cohort data from 4572 Mashhad University of Medical Sciences employees aged between 30 to 70 who volunteered for the employee health-monitoring plan.

Results: The results showed that factors such as DASS21 ($P=0.002$, $\beta=-0.047$), PSQI ($P<0.001$, $\beta=-0.064$), and smoking ($P<0.001$, $\beta=-0.207$) had a significant negative relationship with skeletal muscle mass index. The structural model of the final hypothesis showed remarkable validity with a high R^2.

Conclusions: The results demonstrated a strong correlation between skeletal muscle mass and mental lifestyle factors, such as smoking, sleep hygiene, and mental health. In the current study, the most significant relationship is related to smoking.

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Introduction

The term "lifestyle" refers to a person's, a group's, or a culture's habits, attitudes, tastes, moral standards, and other components (1). Intangible characteristics are related to an individual's psychological aspects, whereas tangible components are directly related to demographic variables (2-4). Muscular mass and strength are influenced by tangible lifestyle determinants and intangible psychological variables (5). Smoking, diet, and physical activity are examples of lifestyle variables, which significantly affect strength and muscle mass (6). Mental health and lifestyle issues like sleep and smoking are inextricably related. Individuals suffering from mental health issues such as anxiety, depression, and so on frequently have major sleep disturbances, including insomnia, nightmares, and excessive daytime drowsiness (7), which can increase mental illness symptoms and result in lower overall health outcomes (7).

Smoking, a typical coping method for stress and anxiety, might exacerbate these problems by reducing sleep quality and quantity (8). Addressing sleep issues and quitting smoking should be included in complete mental health treatment regimens to promote overall well-being and prevent the perpetuation of this vicious cycle (8).

A robust correlation between mental health disorders such as depression, anxiety, and stress and changes in muscle mass and strength has been considered (9). Individuals with mental health issues frequently have decreased muscle mass as a result of variables such as diminished physical activity, changing hormone levels, and inflammatory processes (10).

Stress, anxiety, and depression have been shown in studies to impair muscle growth, implying mental health can influence muscle mass (11, 12). Stress negatively impacts muscle mass through various mechanisms, including altered gene expression, inflammation, and protein turnover. Daily acute psychological stress is

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associated with myostatin-dependent muscular atrophy and increased expression of atrophic genes, both of which lead to a decrease in body and muscle mass (13). Hypercortisolism, low-grade systemic inflammation, and prolonged stress can potentially have a negative impact on muscle mass (10).

Sleep is essential for both physiological and cognitive functions. Inadequate sleep causes the blood to have more catabolic hormones, such as cortisol, and less anabolic hormones. According to a recent notion, manipulating the sleep-wake cycle may be detrimental to the health of skeletal muscles (14). There are multiple mechanisms via which the quality of sleep affects muscle mass. Lack of sleep can negatively impact muscle mass because stress and stress hormones affect strength and muscle protein turnover. Muscular strength may decrease due to muscle protein catabolism brought on by stress hormones generated during restless nights (15). Long-term stress can increase cortisol levels, which can hinder the body's ability to secrete chemicals necessary for muscular growth, such as testosterone, and slow down the growth and repair of muscle. Muscle mass can be negatively impacted by low-grade systemic inflammation, persistent stress, and the hypercortisolism that results from these factors (10).

There are negative effects of smoking on muscle mass and strength. Research has indicated that smoking is associated with decreased muscle mass and strength and increased discomfort in the muscles (16). Smoking can negatively affect muscle mass by increasing systemic inflammation and oxidative stress, reducing the synthesis of muscle proteins, and speeding up the breakdown of muscle proteins. Additionally, smoking may affect metabolism and muscle performance by reducing the amount of oxygen delivered to muscles through altered vasoconstriction. Therefore, the negative effects of smoking on muscle hypertrophy are mediated by a combination of increased inflammation, reduced muscle protein turnover, and enhanced oxidative stress (10).

Additional studies can yield essential information for creating all-encompassing interventions considering health's psychological and physical components. Most studies have focused on a single factor, and there is a lack of research examining multiple variables simultaneously, particularly in Iran. This study innovatively employs the Partial Least Squares (PLS) statistical model to investigate the simultaneous relationships between muscle mass and essential intangible lifestyle factors like sleep quality, mental health, and smoking among Mashhad University of Medical Sciences employees.

Materials & Methods

Research Design

Data from the PERSIAN cohort—a group of 4572 Mashhad University of Medical Sciences employees aged 30 to 70—were used in this cross-sectional investigation. Study participants were asked to understand the relationship between mental lifestyle determinants and muscle mass by examining the dependent variable, skeletal muscle mass index (SMI), and independent variables, Depression Anxiety Stress Scale-21 (DASS21), Pittsburgh Sleep Quality Index (PSQI), and smoking. Out of the initial cohort (5622), 4572 participants were included after applying inclusion and exclusion criteria. Additionally, the "ten times rule," which states that the minimum sample size should be at least ten times the highest number of pathways in the structural or formative measurement models, was frequently applied in PLS-SEM. Partial Least Squares Structural Equation Modeling (PLS-SEM) has emerged as a widely accepted method for examining intricate relationships between observed and hidden variables. Based on these guidelines, a minimum sample size of 390 was considered appropriate for this investigation (17, 18). A thorough analysis of various variables was made possible by a sample size of 4572, as demonstrated by applying the PLS-SEM method for error inflation compensation. A conceptual model was created, and latent variables were generated after carefully cleaning the data to remove missing values and compute the required indicators.

Measures and Covariates

The data required for this study (PERSIAN Cohort) came from Mashhad University of Medical Sciences' Health Monitoring Centre. This information was collected by skilled workers, including nurses, doctors, nutritionists, and interviewers, and the validity and reliability of the surveys were assessed (19, 20).

The necessary indicators were identified when the data was carefully cleaned using the natural range of the variables. The total score of the SMI, DASS21, and PSQI was then calculated using the information from each person's completed questionnaires.

SMI(m)=ASM/height^2

Theoretical Framework

Reading several studies first created a figurative map of the assumptions. After the latent variables were defined, a conceptual assumptions model was developed using PLS-SEM (partial least squares structural equation modeling) version 3, 2, 8 (Figure 1). Once a non-significant relationship was eliminated, several conceptual models were developed, and each new model was constructed. The final conceptual model was developed based on P-values less than 0.001. The final hypothesis is depicted in Figure 1.

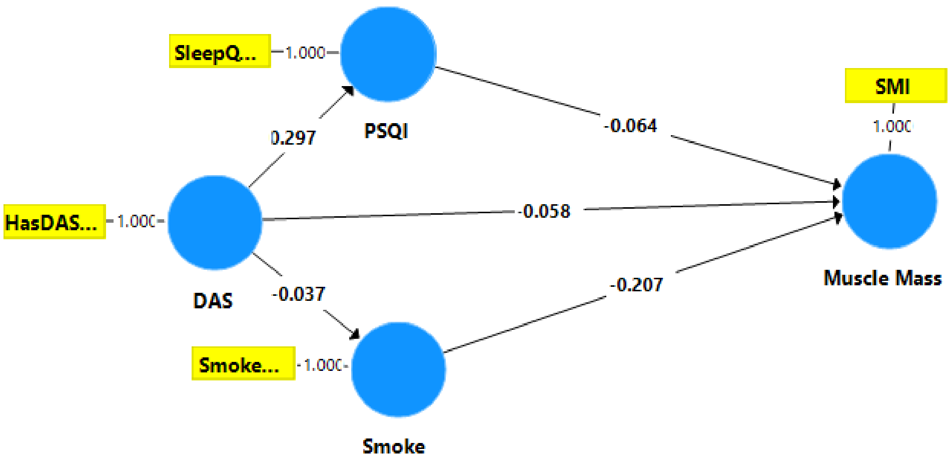

Figure 1. Final partial least squares model (Hfinal).

Analytic Procedure

Index computation was part of the statistical analysis with SPSS version 25. The study utilized partial least squares structural equation modeling (PLS-SEM) to examine the correlation between latent variables. There were several reasons for selecting PLS-SEM, including incremental research (21), the requirement for latent variable scores for further analysis (22), and an assessment of a theoretical framework from a predictive perspective. The data analysis (23), which followed the most recent evaluative PLS-SEM analysis criteria given by Ghasemy, Teeroovengadum et al. (2020) (24), was conducted using SmartPLS 3 (24).

Bootstrapping analyses in PLS-SEM and the algorithm were employed to test hypotheses and evaluate the importance of relationships in the conceptual model. The structural equation model consisted of a measurement model illustrating the links between indicator and latent variables and a structural model showing the relationships between latent variables.

Path coefficient and effect size (f^2) evaluations were part of the internal model quality assessments. Path coefficients ranged from -1 to +1, indicating the strength and direction of the correlations between the variables. The level of influence was evaluated using the effect size (f^2), computed using R^2 and the variance of endogenous variables. Cohen's results showed that values of 0.02, 0.15, and 0.35 indicate weak, medium, and large effects, respectively (25).

Assessment of the Measurement Models

Even though the questionnaires' validity and reliability have previously been assessed (19, 20), it is crucial to reevaluate the questionnaire's psychometric qualities from the perspectives of indicator reliability, internal consistency reliability, convergent validity, and discriminant validity (24-26). The correlations between each item and the construct were assessed by the given standards to determine the reliability of the indicators. The dependability of internal consistency was then evaluated using metrics like Cronbach's alpha, composite reliability (CR), and the recently developed Rho_A metric (27). Convergent validity was then evaluated using the average variance extracted (AVE) measure. Discriminant validity was assessed using the Heterotrait-Monotrait (HTMT) criterion using evaluative criteria to determine the HTMT values (24, 28, 29).

Assessment of the Structural Model

A variety of factors were examined as part of the process of evaluating the study's structural model under the recommendations given, including the significance and relevance of path coefficients and indirect effects, the in-sample and out-of-sample predictive powers, and the f2 effect sizes of endogenous constructs (Table 3). While the variance inflation factor (VIF) values were first analyzed, collinearity was unaffected by any value less than 2 (Table 1). A 5% significance level and 10,000 subsamples were used in the percentile-bootstrapping test of the hypotheses. A summary of the findings is presented in Table 3.

Results

Assessment of the Measurement Models

According to Table 1, the evaluation showed valid loadings over 0.708, meaning that no non-contributory components needed to be removed. Results above 0.7 for reliability estimates and over 0.5 for AVE values were obtained from the computation of the AVE values and the one-tailed 95% percentile confidence intervals of the reliability statistics, indicating acceptable convergent validity and reliability (Table 1).

According to the evaluation, all HTMT values and their upper confidence intervals fell below 0.85, achieving the HTMT0.85 requirement and demonstrating acceptable discriminant validity (Table 2).

Assessment of the Structural Model

The study found empirical evidence for the H-final hypothesis, suggesting that the suggested conceptual model may predict SMI to an extent of 31.7% with an adequate R^2 value of 0.317. The Pittsburgh Sleep Quality Index, smoking with SMI, and the Depression Anxiety Stress Scale-21 were significantly and negatively correlated. On the other hand, favorable correlations between DASS21 and PSQI were discovered. According to the effect size (f^2), the smoke variable strongly correlated with the muscle mass index (Table 3). According to the structural model, there was a relationship (P=0.002) between the skeletal muscle mass index (SMI) and the Depression Anxiety Stress Scale-21 (DASS21). Furthermore, a statistical model study revealed a strong inverse association (P=0.000) between the Pittsburgh sleep quality score and the skeletal muscle mass index. Lastly, the statistical model's results for the current study demonstrated a more potent than previously significant negative relationship between the smoking variable and skeletal muscle mass index (P=0.000, F^2=0.45). Furthermore, a significant relationship was observed between sleep quality (PSQI) and mental health (DASS21) (P=0.000, F^2=0.161).

Table 1. Reliability Estimates, Convergent Validity Statistics, and Outer VIF Values.

	Alpha	rho_A	CR	AVE	VIF
DASS	1.000*	1.000*	1.000*	1.000*	1.000*
SMI	1.000*	1.000*	1.000*	1.000*	1.000*
PSQI	1.000*	1.000*	1.000*	1.000*	1.000*
Smoke	1.000*	1.000*	1.000*	1.000*	1.000*

*Loading>0.7
*Alpha>0.7
*rho_A>0.7
*CR>0.7
*AVE>0.5
*VIF<3
Depression Anxiety Stress Scales (DASS), Pittsburgh Sleep Quality Index (PSQI), Skeletal Muscle Mass Index (SMI), Cronbach's Alpha (ALPHA), Composite Reliability (CR), Average Variance Extracted (AVE)

Table 2. Discriminant Validity Based on HTMT.

	DASS	SMI	PSQI	Smoke
DASS				
SMI	0.058*			
PSQI	0.297*	0.072*		
Smoke	0.037*	0.204*	0.029*	

*HTMT<0.85
Epression Anxiety Stress Scales (DASS), Pittsburgh Sleep Quality Index (PSQI), Skeletal Muscle Mass Index (SMI)

Table 3. Structural Model Evaluation Results (final hypothesis).

	R^2	Path Coefficients (β)	F^2	T Statistics (|O/STDEV|)	P Values	CI95% 2.5%	CI95% 97.5%
DASS -> SMI		-0.047	0.002	3.076	0.002*	-0.074	-0.013
DASS -> PSQI		0.297	0.161*	21.305	0.000**	0.270	0.325
DASS -> Smoke	0.317*	0.037	0.001	2.664	0.008*	0.009	0.062
PSQI -> SMI		-0.064	0.004	4.351	0.000**	-0.096	-0.037
Smoke -> SMI		-0.207	0.45**	14.375	0.000**	-0.235	-0.182

*R^2>0.2
*P-value<0.05
**P-value<0.001
*f^2>0.15
**f^2>0.35
. Depression Anxiety Stress Scales (DASS), Pittsburgh Sleep Quality Index (PSQI), Skeletal Muscle Mass Index (SMI)

Discussion & Conclusion

The fundamental objective of this study was to determine the relationship between muscular mass and the mental lifestyle of Mashhad University of Medical Sciences employees. Therefore, the Pittsburgh Sleep Quality Index, smoking, the Depression Anxiety Stress Scale-21, and the dependent variable, skeletal muscle mass index, were all evaluated. This study's main discovery showed how muscle mass (SMI) and intangible mental lifestyle factors relate.

The results showed a relationship between muscle mass and stress, anxiety, and depression ratings. According to the structural model, there is an average 4.7% drop in muscle mass (SMI) for every unit increase in the Depression Anxiety Stress Scale-21 (DASS21) score. Few research have looked at the relationship between mental health and muscle mass, even though numerous studies have looked at the effects of sarcopenia and muscle wasting on mental health (11, 30). Diabetes patients with a lower SMI score had a significantly lower Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) score, per a study by Low et al. (P = 0.030) (31).

The results of the current study showed that sleep quality (PSQI) was related to muscle mass in the structural model evaluating the relationship between intangible mental lifestyle elements and muscle mass. According to the structural model, there was an average 6.4% drop in muscle mass (SMI) for every unit increase in the Pittsburgh Sleep Quality Index (PSQI). Hayashi et al. discovered that a high PSQI total score significantly correlated with decreased muscle strength (P=0.027), even after confounding variables were considered (P=0.011). The adjusted analysis, however, did not find a significant relationship between the skeletal muscle mass and the PSQI total score (P=0.363). Relationships with PSQI subscores showed that muscle strength was significantly connected with sleep latency, sleep efficiency, and daytime dysfunction scores (9). Buchmann et al. indicated a significant (P<0.03) relationship between parameter adequacy, sleep quality, and muscle mass thickness. The study's findings support the hypothesis that sleep and muscle mass are related but call for more long-term research (32). There are multiple ways in which the quality of sleep affects muscle mass. Lack of sleep can negatively impact muscle mass because stress hormones affect strength and muscle protein turnover. Muscle-derived protein Muscular strength can be lost as a result of catabolism brought on by stress hormones generated during restless nights (15). Long-term stress can elevate cortisol levels, which may hinder the body's production of testosterone and other chemicals necessary for muscle building, thus impeding muscle growth and recovery. Muscle hypertrophy can be negatively impacted by persistent stress, low-grade systemic inflammation, and the ensuing hypercortisolism (10).

The results of the present investigation showed a relationship between muscle mass and smoking. Structural models indicate that muscle mass (SMI) declines by 20.7% for every unit increase in smoking. Xu showed a relationship between smoking and a person's loss of muscle mass (33). Additionally, Buchmann observes that regular smoking and the thickness of one's muscle mass are related (32). In addition, Atkins et al. demonstrated an association between smoking and muscle mass (MAMC and FFMI) (34). According to Rom et al., quitting smoking is associated with increased bone density, muscle mass, and muscle strength (35). Smoking increases the potential for systemic inflammation and oxidative stress, which can lead to the atrophy and malfunctioning of muscles. Moreover, smoking has been related to a reduction in muscle protein synthesis and an increase in muscle protein breakdown, both of which are deleterious to muscle mass (10). Furthermore, smoking's vasoconstrictive effects may worsen muscular performance and metabolism by lowering the quantity of oxygen that reaches the muscles. Consequently, the negative effects of smoking on muscle hypertrophy were mediated by increased inflammation, reduced muscle protein turnover, and enhanced oxidative stress (10).

The final hypothesis's structural model has a high R^2, indicating significant validity. According to the current study, smoking, sleep quality, and mental health are significantly correlated with skeletal muscle mass.

Declarations

Strengths

The significant statistical population in this study makes it possible for various data ranges to be measured. Thus, this study can be received with good validity and reliability. The simultaneous evaluation and analysis of the investigated aspects (muscle mass and intangible mental lifestyle determinants) are drawn based on conceptual models using the Smart PLS software, which is the strength of this study because one of the positive features of this method is to eliminate the inflation of errors.

Limitations and Recommendations

Two of the study's weaknesses are personal recollection and reporting bias in the survey replies. The research team also decided to remove the alcohol use component from the study because of report bias, and more research is required in this field.

Authorship Contribution Statement

Here is the authorship contribution statement:

Sara Telikani: First Author, Main Author, and Data Analyst. Sara Telikani was responsible for designing the study, collecting and analyzing the data, and drafting the manuscript.

Dr. Seyyed Reza Sobhani: Supervisor. Dr. Sobhani supervised the study, providing guidance and oversight throughout the research process.

Dr. Jamshid Jamali: Statistical Supervisor. Dr. Jamali was responsible for designing and implementing the statistical analysis plan, ensuring the accuracy and reliability of the results.

Mohammad Masoumvand: Co-Analyzer. Mohammad Masoumvand assisted in the data analysis and contributed to interpreting the results.

Dr. Reza Rezvani: Article Supervisor. Dr. Rezvani reviewed and edited the manuscript, ensuring its clarity, accuracy, and overall quality.

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Declaration of Conflicting Interests

The authors declare no conflict of interest related to this manuscript.

Ethical Issues

The Mashhad University of Medical Sciences Ethics Committee (IR.MUMS.MEDICAL.RE.C.1401.585) authorized the procedures performed under its ethical guidelines. Before the data collection, the participants also signed an informed consent form, and PERSIAN Cohort protocols were adhered to for data confidentiality and anonymization.

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The Effect of an Aerobic Exercise Session in Fasting Versus Satiety on Fat and Carbohydrate Oxidation in Hypoxia and Normoxia in Overweight Men

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ABSTRACT

Introduction: This study aimed to determine the effect of an aerobic exercise session during fasting versus satiety on fat and carbohydrate oxidation in hypoxia and normoxia in overweight men.

Methods: A total of 16 overweight men with a body mass index (BMI) ranging between 25-29.9 kg/m^2, a mean age of 30.75±6.79 years, and a maximum oxygen consumption of 24.67±4.61 liters per minute voluntarily participated in the study. Each participant underwent four conditions: fasting hypoxia, normal hypoxia, fasting normoxia, and normal normoxia. During the study, the participants completed a 30-minute aerobic test on a CUCLUS2 bike with a heart rate (bp/m) of 60-70% in a hypoxia tent at an altitude of 3000 meters and 14.5% oxygen or in normoxia condition in the laboratory facilities of Razi University of Kermanshah Sports Sciences Faculty. In the satiated state, the participants were given two pieces of toast, one tablespoon of peanut butter, and one glass of milk. The breathing gases were collected for 30 minutes to evaluate fat and carbohydrate oxidation.

Results: The study showed that fat oxidation was significantly higher in the fasted group compared to the satiety group ($p<0.05$) in both hypoxia and normoxia conditions. Moreover, fat oxidation in normoxia was significantly higher than in hypoxia ($p<0.05$). In contrast, carbohydrate oxidation and respiratory exchange ratio were significantly lower in the fasted group compared to the satiety group ($p<0.05$).

Conclusion: Based on the study's findings, training in a hypoxia state, regardless of fasting condition, is a suitable non-invasive method to tackle obesity and overweight.

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Introduction

Overweight and obesity have emerged as a critical public health concern globally (1). Although preventable, obesity is often linked with a host of chronic conditions such as type 2 diabetes, metabolic disorders, high blood pressure, cardiovascular diseases including atherosclerosis, endothelial dysfunction, and even cancer. When left unaddressed, obesity can lead to premature mortality (2). The prevalence of obesity has tripled worldwide since 1975, with 1.9 billion adults classified as overweight and 650 million as obese (3). Obesity is a public health concern that arises from inactivity and unhealthy diets. The prevention and treatment of obesity and overweight conditions require a proper diet and regular exercise. A low-calorie diet and sports activities are essential to achieve energy balance and lose weight (4). The high prevalence of obesity worldwide is attributable to increased calorie consumption and decreased energy expenditure (5). Aerobic activity effectively improves cardiovascular fitness, increases peak oxygen consumption, and prevents and treats obesity and its associated ailments, which enhances cardiovascular fitness without significant changes in physical strength (6). Therefore, it is an effective intervention that can be employed to promote healthy lifestyle changes for individuals dealing with obesity (7).

The human body relies on carbohydrates and fatty acids as fuel sources during low to moderate-intensity activities that last for several hours. The respiratory exchange ratio (RER) decreases gradually during such activities, leading to an increase in fatty acid (FA)

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utilization and a decrease in carbohydrate consumption (8). As the intensity of aerobic activity increases, the body reaches a peak level of fat oxidation and then shifts to prioritizing carbohydrate oxidation (9). The availability of carbohydrates as a substrate for skeletal muscle and brain metabolism is important and controversial in sports performance, particularly for long-term aerobic activities that exceed 90 minutes. Endogenous carbohydrate stores limit the performance of such activities, making them highly dependent on carbohydrate intake (10).

Respiratory rate (RQ) is the ratio of CO_2 production to O_2 consumption, with an RQ of 1 indicating 100% carbohydrate (CHO) oxidation and an RQ of 0.7 indicating 100% fat oxidation (11). Low-carbohydrate (LC) diets shift the body's fuel usage towards fat oxidation. However, maximal and submaximal markers of aerobic activity performance and muscle strength are not negatively impacted by LC diets compared to high-carbohydrate (HC) diets (12).

At the onset of physical activity, signals from within and outside the muscle cell are required to increase carbohydrate and fat fuel for ATP production (13). Fasting activity can increase the amount of fat oxidation at rest for up to 24 hours compared to the same activity performed after a meal (14). Exercise performed in the morning after a night of fasting may be more effective in reducing body fat than exercise performed after eating (15).

Several factors, including activity intensity and duration, training status, nutritional status, and gender, influence fat oxidation during exercise (16). Studies have shown that carbohydrate restriction results in a sustained increase in fat oxidation, whereas fat restriction does not affect fat oxidation significantly (17). The process of fat oxidation during exercise begins with the hydrolysis of triglycerides, releasing transported fatty acids that are transported to the muscle mitochondria for oxidation (18).

Exposure to hypoxia has been documented as a suppressor of appetite perception, leading to a subsequent reduction in energy expenditure compared to normoxia (19). As a result, the practice of training at altitude or hypoxia to enhance aerobic capacity and endurance performance has become increasingly common (20). Hypoxia becomes more prevalent at high altitudes, which decreases inspiratory oxygen, resulting in reduced alveolar oxygen pressure, arterial oxygen pressure, and arterial oxygen content. Conversely, decreasing the amount of oxygen in the blood results in changes in metabolism and the body. Research has shown that exercising in conditions of oxygen deficiency and hypoxia reduces blood pressure, fat mass, and peripheral vascular congestion (21). Hypoxia therapy is currently used as a general medical practice to treat obesity in most developed countries. Therefore, hypoxia therapy has the potential to be a practical new method and therapeutic approach to combat obesity and related diseases (22).

Lippel et al. (2010) examined the effects of passive exposure to an altitude of 2650 meters on obese individuals. The study reported significant reductions in caloric intake and body weight (1). The hypoxic environment resulted in the release of erythropoietin (EPO), a glycoprotein hormone that influences the production of red blood cells and enhances oxygen transfer to active tissues (23). Additionally, the AMPK system, an intracellular energy sensor, responds to metabolic stress by activating in response to an increase in the ATP/AMP ratio (24). The fasting state activates AMPK more than the fed state, and its activation persists for at least 150 minutes post-exercise (25).

Hypoxia has emerged as a new method for combating obesity, considering the high prevalence of obesity and related health conditions and the use of aerobic exercise as a therapeutic agent. Aerobic training is more effective due to the time limitations and physical constraints faced by obese individuals. Moreover, aerobic exercise is beneficial for reducing body fat and improving cardiovascular fitness (26). This research aims to determine the effect of an aerobic exercise session during fasting versus satiety on fat and carbohydrate oxidation in hypoxia and normoxia in overweight men.

Materials & Methods

The participants voluntarily agreed to participate in the study after completing the health and medical questionnaire and providing their written consent by filling out the consent form. None of the subjects had a history of smoking, anabolic steroid use, cardiovascular disease, or respiratory disease.

The present study was conducted after obtaining the necessary approval from the Ethics Committee of Razi University of Kermanshah under the ethics ID (IR.RAZI.REC.1402.013). Body composition factors such as body fat percentage before the commencement of the test and body mass index (BMI) were measured using a body composition analyzer model (ZEUS 9.9) manufactured in South Korea. The subjects' height was measured in centimeters using a height measuring device and model scale (FARS) made in Iran with an accuracy of 0.1cm. Furthermore, the subjects' weight was measured in kilograms with an accuracy of 0.1kg without shoes and with minimum clothes.

Hypoxic conditions were achieved using a height simulator tent manufactured in Germany, equipped with a device built into the tent wall that adjusted oxygen concentration to 14.5% at a simulated height of 3000 meters. The tent door was left open for at least an hour before the test commenced to ensure the homogenization of air. The tent was turned on, and the oxygen percentage was automatically adjusted. The tent door was then closed, and the hose was connected to the generator device on one side and the hypoxia tent on the other. The generator was turned on, and the tent was allowed to reach the desired height.

The subjects' exercise was performed on the CYCLUS2 bike for thirty minutes with a 60-70% heart rate (bp/m). They were first asked to ride the bike without load for 5 minutes to warm up. The test was conducted in hypoxic and normoxic states in the Razi University of Kermanshah Faculty of Sports Sciences laboratory. The test protocol consisted of four conditions: fasting hypoxia, normal hypoxia, fasting normoxia, and normal normoxia. Each subject repeated the research protocol with a one-week break. Each subject went to the laboratory fasting and performed the protocol. Those who were satiated were given two pieces of toast, a spoonful of peanut butter, and a glass of milk. Calorie 350, Fat% 25, Pro% 25, CHO% 50. The test started one hour after breakfast.

In hypoxia mode, the bicycle was taken into the tent, and the hose of the device was connected to the tent. The tent was then placed at the simulated height, and the device was turned on until the desired height was reached (1). The subjects performed the activity for thirty minutes with a 60 to 70% heart rate. A gas analyzer (Meta Max 3B), manufactured in Germany, was used to analyze the respiratory gases, calculate the oxidation of fat and CHO, and measure the subjects' maximum oxygen consumption (VO2max). The device enabled a moment-to-moment breath-by-breath method of breathing analysis and was equipped with a polar heart rate monitor, three masks (of different sizes), and a wireless transmitter and receiver device for portable field use. The device transmitted the data telemetrically to the computer and displayed the values of O_2 and CO_2 and the respiratory exchange ratio (RER) by recording moment-by-moment and breath-by-breath (27).

*Fat oxidation rate (g/min) = 1.695 * VO2 – 1.70 * VCO2*
*Carbohydrate oxidation rate (g/min) = 4.585 * VCO2 – 3.226 * VO2*

Statistical Analysis

The present study employed descriptive statistics to elucidate and interpret the findings. The Shapiro-Wilk test was used to check the normal distribution of the data. Furthermore, the ANOVA test with repeated measurement and the Bonferroni test were deployed to ascertain the location of the difference. The statistical level of $P < 0.05$ was employed to analyze the research results. The sample size was calculated using Cochran's formula, which had an 80% statistical power and 0.05 significance level. Based on the output of Cochran's formula, the number of 16 subjects was considered a sufficient sample size for this research, considering the dropout rate of 15% in the subjects. Finally, 17 subjects participated in this research and were randomly placed in four states.

Results

The study included 16 overweight men with a mean age of 30.75±6.79 years (Table 1).

The results obtained from the repeated measurement test indicate a significant disparity between the measurement times (F=7318.59 and P=0.001). In hypoxia and normoxia, fat oxidation was significantly higher in the fasted group than in the satiety group (p0.05). Furthermore, fat oxidation in normoxia was significantly higher than in hypoxia (p0.05) compared to hypoxia and normoxia conditions (Figure 1).

The repeated measurement test shows a significant difference between measurement times (F=2245.03 and P=0.001). In hypoxia and normoxia, CHO oxidation in the satiety group was significantly higher than in the fasted group ($p<0.05$). In addition, CHO oxidation in normoxia was significantly lower than in hypoxia ($p<0.05$) (Figure 2).

The results of the repeated measurement test showed a significant difference between different measurement times (F=890.38 and P=0.001). In hypoxia and normoxia, RER in the satiety group was significantly higher than in the fasted group ($p<0.05$). In addition, RER in normoxia was significantly lower than in hypoxia($p<0.05$) (Figure 3).

The results of the repeated measurement test revealed a significant difference between different measurement times (F=5096.68 and P=0.001). In hypoxia and normoxia, heart rates in the satiety group were significantly higher than in the fasted group ($p<0.05$). Moreover, heart rates in normoxia were significantly lower than in hypoxia ($p<0.05$) (Figure 4).

Table 1. Descriptive characteristics of subjects as mean ± standard deviation.

attributes	Mean ± SD
Age (year)	30.75 ± 6.79
Height (cm)	177.63 ± 7.38
Weight (kg)	85.59 ± 11.20
Fat percentage	25.09 ± 2.39
BMI (kg/m2)	27.02 ± 1.93
VO2max (L/min)	24.67 ± 4.61

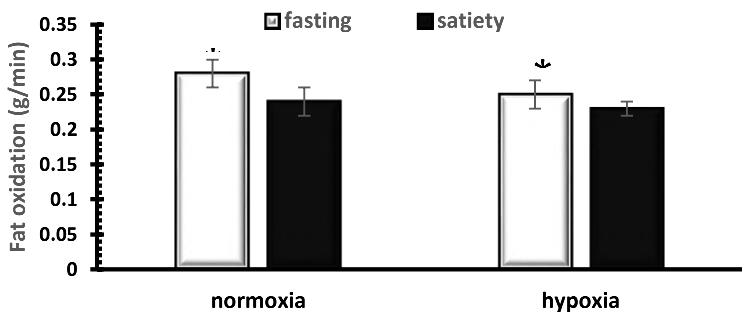

Figure 1. Investigation of fat oxidation changes in the studied groups
*Indicating a significant change compared to the satiety hypoxia group

Figure 2. investigation of carbohydrate oxidation changes in the studied groups
*indicating significant change compared to the satiety hypoxia group,#indicating significant change compared to the satiety normoxia group

Figure 3. Investigation of respiratory exchange ratio changes in the studied groups
*Indicating significant change compared to the fasting normoxia group

Figure 4. Investigation of heart rate changes in the studied groups
*Indicating significant change compared to the satiety normoxia group

Discussion

The present study demonstrated that hypoxia and normoxia conditions significantly change fat oxidation rates during sated and fasting states. Specifically, in the fasting state, the fat oxidation level was higher than that observed during satiety, irrespective of the oxygen availability. Moreover, physical activity was also observed to significantly affect fat oxidation levels in both hypoxia and normoxia conditions. In contrast, the satiety state significantly altered carbohydrate oxidation rates in response to activity in both hypoxia and normoxia conditions. Conversely, satiety or fasting was observed to influence carbohydrate oxidation rates significantly. These findings suggested that oxygen availability and nutritional state significantly influence the body's physiological response to physical activity, highlighting the importance of these factors in regulating energy metabolism.

Basami et al. (2013) explored the impact of satiety and fasting on substrate oxidation. The findings of the study were consistent with the current research, indicating that the amount of fat oxidation increases after a period of fasting and regular aerobic activity (28). Keli and Bassett align with the current research concerning the changes in carbohydrate and fat oxidation in hypoxia and normoxia conditions, while the findings of Lundby, Hall, and Sami et al. did not (29).

Furthermore, the present study revealed that the activity in normoxia and hypoxia conditions impacts fat and carbohydrate oxidation. Lundby and Hall (2002) investigated the effect of training in situations at sea level and 4100 meters altitude. The researchers concluded that acute activity at sea level and 4100 meters altitude does not affect the amount of fat and carbohydrate oxidation in normal-weight men. The difference in height, weight, and intensity of activity could be the reason for the disparity between their findings and the current research (30).

Kelly and Bassett (2017) indicated an increase in fat oxidation induced by cycling activity in normobaric hypoxia compared to normoxia. The type of activity could be a possible reason for the difference in the findings. Sami et al. did not observe a significant difference in carbohydrate oxidation in satiety in normoxia and hypoxia conditions. However, the type of nutrition consumed, such as materials with a high sugar index in Sami's research, and the normal weight of the participants could be factors contributing to the disparity between their findings and the current research (31).

Consuming a high-fat meal the previous evening may explain the changes in fat oxidation since carbohydrates after dinner, when glycogen stores are replenished, affect fat oxidation. (28). During exercise, the body responds to a rapid reduction in carbohydrates by increasing fat mobilization in skeletal muscle while maintaining blood sugar levels in the brain, similar to the effects of fasting (32). Triglyceride movement from fat tissue into the bloodstream results in free fatty acid (FFA) availability to metabolically active tissues, allowing muscle function to continue despite decreased blood glycogen and glucose concentrations (33). Regarding fasting and aerobic exercise, biochemical pathways involved in fat mobilization and burning are activated to increase FFA consumption. Short periods of fasting regularly expose active tissue to increased fat availability and consumption, leading to the adaptation of lipid consumption by tissues such as skeletal muscle (34).

Changes in glucose and insulin due to fasting and satiety may also account for differences in fat and carbohydrate oxidation. Blood glucose levels decrease after a few hours of fasting in healthy individuals, but the continuous decrease in blood glucose is halted by increased gluconeogenesis from the liver. This increase is due to decreased insulin secretion, increased glucagon secretion, and increased sympathetic nerve activity (35). Blood glucose levels have an inverse relationship with fat intake and a positive relationship with carbohydrate intake. The hours of fasting are an essential factor in the variability of blood glucose levels, and the amount of glycogen reserves, physical activity, and dietary patterns (amount and type of food consumed) of subjects can also affect serum glucose levels. Hormonal changes, such as an increase in fatty acids, may also contribute to fat and carbohydrate oxidation changes during fasting and satiety (28).

A contributing factor to the present findings may be the subjects of the study being overweight since recent studies have shown that overweight and obesity increase hepatic glucose and fatty acid production. Exercise in such conditions may increase insulin secretion from the pancreas, decrease insulin resistance, and alter carbohydrate metabolism (21). Some studies have shown a relative increase in glucose oxidation during physical activity following exercise in hypoxic conditions, which is due to the activation of hypoxia-inducible factor-1, which is responsible for the production of erythropoietin, vascular endothelial growth factor-1, and GLUT transporter-1 (36).

The present study's findings indicated a significant difference in the respiratory exchange ratio (RER) between the satiety hypoxia and fasting normoxia groups and between the exercise groups in satiety normoxia and fasting normoxia. The results showed a decline in carbohydrate oxidation in normoxia conditions compared to hypoxia, as evidenced by the decrease in RER between hypoxia and normoxia conditions.

The findings of Ofner et al. (2014) are consistent with those of the present study, which found that the RER was higher in hypoxic conditions after increasing aerobic activity in healthy men (37). However, Hozouri et al. and Hamlin et al. revealed nonaligned results. Hozouri et al. (2022) studied the effect of four weeks of polarized training on professional rowers' aerobic fitness and performance and observed no change in the RER. The disparity may be due to the duration of the exercise protocol, the level of physical fitness of the participants, and their weight (38). In Hamlin et al. (2010), sea dwellers performed cycling in hypoxia conditions. The participants performed the Wingate test for ten consecutive days, and the RER increased two days after the intervention. The causes of disparity may include the implementation protocol, the intensity and duration of the sports activity, and the level of preparation of the participants (39).

The present study examined the effect of fasting and hypoxia on the respiratory exchange ratio (RER), glucose metabolism, and heart rate during moderate-intensity exercise. The findings indicated that a reduction in glucose metabolism leads to an increase in endurance capacity during moderate-intensity exercises (38). Additionally, previous studies have suggested that the hypothalamus-pituitary-adrenal axis becomes stimulated in the presence of obesity and overweight, resulting in increased cortisol levels (40), (41), and (27).

Furthermore, the results indicated that heart rate changes significantly in response to fasting, satiety, and hypoxic conditions. The training group in fasting hypoxia and satiety normoxia conditions experienced more significant differences in heart rate compared to the training group in satiety hypoxia and satiety normoxia. Notably, the intensity of sports protocols, physical activity, and body weight could account for variations in results across studies.

According to Haddad et al. (2012), exposure to altitudes higher than 3000 meters leads to changes in the parasympathetic nervous system, whereas exposure to lower altitudes stimulates the sympathetic nervous system. In addition, the low partial pressure of oxygen (PaO2) at high altitudes may delay heart activity in unhealthy individuals. Chemoreflex activation appears to be a significant determinant of parasympathetic reactivation (42).

In summary, this study highlighted the importance of considering factors such as fasting, hypoxia, and exercise intensity when examining RER, glucose metabolism, and heart rate. The findings could have implications for individuals seeking to enhance their endurance capacity and those living at high altitudes or participating in activities involving hypoxia exposure.

Research limitations include two controllable and uncontrollable parts. The controllable limitations include subjects' nutrition, exercise, altitude, medical health questionnaire, and physical fitness. Uncontrollable limitations include subjects' motivation and anxiety, physiological characteristics, and sleeping and resting conditions. One of the strengths of this research was the comparison of normoxia and hypoxia in fasting and satiety states. Much research has been conducted on obesity and overweight, but less research has been performed on the conditions presented in this study.

Declarations

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Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Author Contributions

RM and MA contributed to the conception and design of the research; RM, MA, and WT contributed to data collection; MA and WT contributed to the acquisition and analysis of the data; MA and WT contributed to the interpretation of the data; RM, MA, and WT contributed to draft the manuscript. All authors have read and approved the final manuscript, ensuring transparency in the research process.

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Feasibility of Mobile Telehealth for Health Literacy Enhancement via Nutritional Counseling during COVID-19

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ABSTRACT

Introduction: The COVID-19 pandemic has created numerous challenges, including widespread misinformation and limited access to reliable health resources. These issues have led to the proliferation of superstitions and impulsive decisions regarding drug and supplement use. This study focused on the development and feasibility evaluation of a nutritional teleconsultation system designed to address these challenges.

Methods: This cross-sectional study was conducted from April to May 2020 at the Mashhad University of Medical Sciences, Mashhad, Iran. After conducting a needs assessment, a nutritional teleconsultation system was developed. The usability and functionality testing led to iterative modifications. The system was piloted on clinical staff for a week, after which it was improved and made available to the public.

Results: Over 1,000 system logins were recorded, with 641 users completing questionnaires. Of these, 344 accessed consultation services. The majority of consultation users were aged 31–40 years, and hypertension was the most commonly reported underlying condition. Approximately 53% of users were Mashhad residents. Nutritional queries dominated during the pandemic, with 49.1% focusing on nutrition in the context of COVID-19, 32.0% addressing COVID-19 directly, and 7.5% solely about nutrition. Among combined nutrition and COVID-19 questions, the most common topics were diet (46.2%), immunity (28.4%), and supplementation (20.7%).

Conclusion: This study highlights the feasibility and public acceptance of telehealth solutions for nutritional consultation during crises. The findings underscore the need for ongoing public education on diet, immunity, and supplementation through diverse media channels to combat misinformation effectively.

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Introduction

In recent years, due to the increase of environmental, social, and health crises, preparation to face the crises is an indispensable part of crisis management strategies. In the last two decades, approximately more than one billion people have been affected by different crises, and more than 700,000 mortalities have been accounted for by natural disasters alone (1). In addition to inducing death and diseases, such crises lead to extremely destructive psychological, social, economic, and political effects that will remain for years. Iran is among countries at the highest risk of accidents in the world and is faced with a significant number of natural or human-made crises every year (2). On the other hand, the transmission of false information has become a challenge, and the current era is described as the era of fake news, which false information may spread rapidly during disease outbreaks and cause problems in the field of health, for example, the nutritional status of people and health affect society(3). Recent studies have shown that false or

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misleading health information may spread more easily than scientific knowledge through social media. The essential role of health misinformation in social media has recently been highlighted by the COVID-19 pandemic, as well as the need for quality and accuracy of health messages. The purpose of public health crisis management and disease caused by misinformation is highlighted. In fact, the lack of control over health information on social media is used as evidence for the current demand to regulate the quality and public availability of online information(4). Studies have shown that telehealth may have a significant impact on the development of health care in the future, improving access to health services, providing access to the right education, especially for citizens in remote or disadvantaged areas. and thereby improve health outcomes(5, 6).

Telehealth involves sending information from one region to another using electronic communication to improve the quality of health and treatment (7). Today, telehealth services are widely used in various health fields through cellphones (8). Employing technology in the health field has allowed users to access reliable information about healthy lifestyles and to use the advice of health experts regarding their problems. It has prevailed more after developing smartphones and their practical applications in recent years (2). The use of smartphones and the Internet has been almost twofold from 2013 to 2018 (9). The increasing use of smartphones has made them popular in accessing information and has led to the growth of practical applications in changing people's lifestyles. One benefit of cellphones in the health domain relates to nutrition, diet, weight control, and access to reliable information on healthy diets (10). Since the new circumstances (the Coronavirus crisis) have been unprecedented, there is no public access to appropriate information sources. This issue is more serious concerning proper nutrition methods. Many of public ambiguities and questions have remained unanswered due to the lacking access to resources and experts, and they seek answers to their questions through the superstitions spread in society.

Telehealth has become an essential resource during emergencies, such as the COVID-19 pandemic, offering several key features that improve healthcare delivery. Here are some of the most advantageous aspects:

✓**Swift Information Sharing**: Telehealth platforms can rapidly distribute crucial health information to patients and healthcare professionals. This encompasses updates on treatment protocols, vaccination details, and guidelines for managing specific conditions during a crisis. Research shows that timely access to information can significantly enhance patient outcomes and minimize misinformation(11).

✓**Access to Specialists from A far**: In times of crisis, especially when physical access to healthcare facilities is restricted, telehealth allows patients to consult with specialists without the need for travel. This is particularly advantageous in rural or underserved regions where expert healthcare providers may not be readily available. Studies indicate that remote consultations can lead to effective diagnosis and management of health issues, ensuring patients receive prompt care(12).

✓**Improved Accessibility**: Telehealth eliminates geographical barriers, enabling individuals with mobility challenges or transportation issues to access healthcare services. This increased accessibility is crucial during crises when traditional healthcare systems may be overwhelmed or inaccessible (13).

✓**Boosted Patient Involvement**: Telehealth fosters patient engagement through intuitive platforms that empower individuals to take an active role in their healthcare. Patients can easily schedule appointments, access their medical records, and communicate with their providers, leading to better adherence to treatment plans and improved health outcomes(11).

In summary, telehealth's capacity for swift information sharing, remote access to specialists, robust communication channels, enhanced accessibility, cost efficiency, and increased patient involvement makes it an invaluable asset during crises. These features not only help mitigate the impact of emergencies on healthcare delivery but also contribute to building more resilient health systems for the future(11-13).

The COVID-19 pandemic has intensified pre-existing nutritional issues, particularly by increasing food insecurity and spreading misinformation regarding dietary choices. Research conducted by Gundersen and Ziliak indicates that the economic repercussions of the pandemic have led to a notable rise in food insecurity, which has had a disproportionate impact on marginalized communities, limiting their access to healthy food options(14). "Nutrition teleconsultation" provides the opportunity for remote patient monitoring in remote areas and supports reducing costs, reduce patientand provider-travel time expanding access to services, continuity of care, and reducing geographic barriers. Gradually, nutrition telecare is increasingly used by professionals, and the creation of a systematic telecare protocol in this field seems necessary(15, 16). Providing nutrition services through telemedicine requires the provision of an infrastructure that does not impose high limits on service quality and allows telehealth to address mobility, usability, interoperability, intelligence, and adaptability in a systematic way(17). Telehealth and nutrition platforms may potentially help users improve household food security, modify child and family eating habits, and improve the effectiveness of nutrition and physical activity intervention(18-20). Physicians and specialists can communicate with patients, interpret specialized test results, and monitor disease progress when access to specialized care is unavailable or limited via telemedicine. Telemedicine can improve the effectiveness of professionals both in daily hospital operations and in critical situations where regional hospital systems and care facilities are pressured by sudden peaks in patient volume. This is while taking care of regular visitors of the health and treatment center at the same time(21).

Strategies in virtual nutrition include synchronous (phone calls or video conferencing) and asynchronous (e-mail, educational videos, or text messages) or combined approaches. Previous studies show that diet quality, increased consumption of fruits and vegetables, reduced consumption of highly processed foods, and weight loss, as well as encouragement to keep appointments and patient satisfaction have improved as a result of using these methods(15). However, it is important and necessary to create a basis for public understanding, awareness and readiness for the successful implementation and sustainable acceptance of this technology(22).Therefore, the purpose of this study was to develop and evaluate a teleconsultation system on nutrition-related questions with a focus on providing consultation to people about the prevention, immunity, and control of Coronavirus disease and also providing access to reliable information sources for public awareness and healthier food alternative during this crisis.

Materials & Methods

The present cross-sectional research was conducted in April-May 2020 at Mashhad University of Medical Sciences.

Need Assessment

In order to design a system that catered to the specific nutritional needs during the COVID-19 pandemic, a thorough needs assessment was conducted. A multidisciplinary team comprising nutritionists (n=2), health informatics specialists (n=2), and software developers (n=4) held several meetings to identify the core user needs. Open discussions were held within the team to identify the most frequently encountered issues during nutritional consultations in the early phases of the pandemic. These discussions revealed that during the COVID-19 pandemic, the public needed accurate, timely information regarding:

- Nutrition to boost immunity and prevent disease,
- Safe use of supplements, and
- Access to reliable nutritional advice through remote means due to quarantine measures.

Based on these needs, the following decisions were made:

➢ **User-driven Design:** A system allowing users to ask questions through text and attach images, which was based on the feedback that visual representation of food products, symptoms, or supplements was often essential for consultations.

➢ **Timeliness:** Users were concerned about getting timely advice, hence the system was designed to allow consultants to respond promptly (within a set timeframe, between 6 am and 12 pm), ensuring rapid consultation.

➢ **Categorization and Filtering of Questions:** Given the volume and nature of inquiries anticipated, consultants highlighted the need for a robust question-labeling system. This was implemented to streamline the categorization into relevant themes such as immunity, diet, supplementation, etc., which further guided the iterative development of the system's database architecture. Several primary labels were used for question labeling. Then, by an iterative and incremental development and examination of the questions, the labels were completed, and the previously labeled questions were reviewed. Two categories of labels were used, one about *the relevance to the topic of interest* and the purpose of using the system, and the other *the topic of the question*. After the iterative process, the relevance label read "relevant", "relevant only to Corona", "relevant only to nutrition" and "irrelevant". The topic label included diet, immunity, supplementation, comorbidity, traditional medicine, stress control, prevention, children, symptoms, treatment, lactation, medication, pregnancy, and the elderly. Table 1 presents the descriptions of the topic labels.

Table 1. Question topic labels and their definition

Topic	Description
Diet	Proper nutrition during the Coronavirus crisis
Immunity	Proper nutrition to improve the immunity system during the Coronavirus crisis
Supplementation	Supplementation to reduce symptoms/ prevent the Coronavirus disease
Comorbidity	Proper nutrition during the Coronavirus crisis for people with a comorbidity
Traditional medicine	Nutrition according to the recommended alternative medicine during the Coronavirus crisis
Stress control	Proper nutrition during the Coronavirus crisis to control stress
Prevention	Proper nutrition to prevent Coronavirus disease
Children	Proper nutrition for children during the Coronavirus crisis
Symptoms	Proper nutrition during the Coronavirus crisis for patients with Coronavirus disease symptoms
Treatment	Proper nutrition for Coronavirus treatment
Lactation	Nutrition during the Coronavirus crisis for lactating women
Medication	Taking medications to relieve/prevent Coronavirus symptoms, drug-food interactions

System Development

The user-centered development method was used to develop the system. An iterative process of answering users' questions and fulfilling their needs was at the core of this approach. In this phase, through cooperating with the software development team, items such as system architecture, programming language, the database, and functions were determined so that the utilized resources and infrastructure could fulfill the needs of users. Therefore, it was decided to implement the system according to the following uses:

HTML, CSS, and JavaScript were used as client-side web programing languages. The programming language used on the server-side was C. The server-side database was MySQL, and also a native memory-based NoSQL was used to preserve the temporal cache of the information. Communication with the server was in JSON, Text, Html, and if necessary, a dedicated textual marked text structure like Markdown was used.

Usability Evaluation

Having developed the preliminary version of the system, the usability test was administered through some of the potential system users who had different educational levels. This test answers the question of how easy it is for users to use the system.

The think-aloud method was employed to do the test. In this way, some scenarios were designed to work with the system provided to users, and they were asked to express their thoughts loudly while working on the system for acting out the scenarios.

The following scenarios were designed to test core system functions:

1. Logging in and completing the demographic questionnaire,
2. Submitting a nutrition-related question along with an optional image attachment, and
3. Viewing the response from a nutritionist.

During the test, an evaluator observed the participants and recorded any usability issues. The feedback was then analyzed according to a predefined rubric focusing on:

- Ease of navigation,
- Clarity of instructions, and
- Efficiency in submitting questions and receiving responses.

Finally, a total of 5 participants were involved in the testing phase. The participants were selected to represent a range of ages, education levels, and technology proficiency, ensuring the system was intuitive for different user groups. This included individuals with varying levels of familiarity with smartphones and teleconsultation services, ranging from novice to experienced users.

The problems users faced while working with the system were recorded by the evaluator. Finally, after summarizing the recorded problems, the system went redesigned.

Functionality Evaluation

The functioning of the system was tested by scenarios and simulated data in a laboratory to do the functionality test facing the significant volume of questions people asked and to evaluate the communication speed, communication quality, weakness, and system capabilities. After ensuring the functioning of the system in the laboratory, it was provided to the clinical staff of the University of Medical Sciences to ask their questions about the nutrition of patients with Coronavirus for a week. At the end of this phase, a survey was conducted of users. The analyzed results were presented to the development team to fix the defects.

Public Accessibility and Recruitment

To increase public awareness and accessibility, the system was primarily promoted through social networks affiliated with Mashhad University of Medical Sciences. Notifications were shared via WhatsApp groups, Telegram channels, and official university communication platforms. Additionally, announcements were made in local hospitals to inform the clinical staff and public about the system.

During this process, one challenge was ensuring access to individuals outside of the Mashhad area. Although the system was developed and initially promoted in Mashhad, it was accessible to users from other provinces. Over 32% of users were from other provinces, indicating a broader uptake beyond Mashhad. However, due to limited initial promotion and infrastructure, system access was not as widespread. In the next phase, more robust recruitment strategies—such as collaborations with other regional health centers—will be necessary to ensure broader coverage.

Security and Privacy Considerations

In this program, the confidentiality of the information provided by individuals was respected. The authorization and authentication of users to access the application were controlled. Patient identification information such as name, surname, and national ID number were neither requested nor registered. The questions were provided to experts in a way that they could not identify the patients. Also, access to information for anonymous people was denied because the data were encrypted during transmission.

Statistical Analysis

The data were analyzed using descriptive statistics using SPSS version 26. Frequencies and percentages were calculated for categorical variables such as age groups, underlying conditions, and the types of questions submitted (e.g., diet, immunity, supplementation). For continuous variables like age, means and standard deviations were used to summarize the data.

Results

User Engagement

The system was used by people from April 3 to May 21, 2020. During this period, nine nutritionists, who were university faculty members, answered users' questions. A total of 1006 logins were recorded, with 743 being new users. 641 users completed the demographic questionnaire upon logging in. Out of these, 344 individuals sought counseling and asked questions, all of which were answered by nutritionists.

The user could access the system through a hyperlink. On the home page, users were asked to enter their phone number, which acted as their username to maintain history for re-access (Figure 1). Once logged in, a questionnaire was provided that included questions about gender, age, underlying diseases, and place of residence (Figure 2). Users could then submit their questions and attach a file if needed (Figure 3). Instructions were provided to users that the system was designed to answer nutrition-related questions during the Coronavirus crisis. Responses were provided by nutritionists within 24 hours (Figure 4).

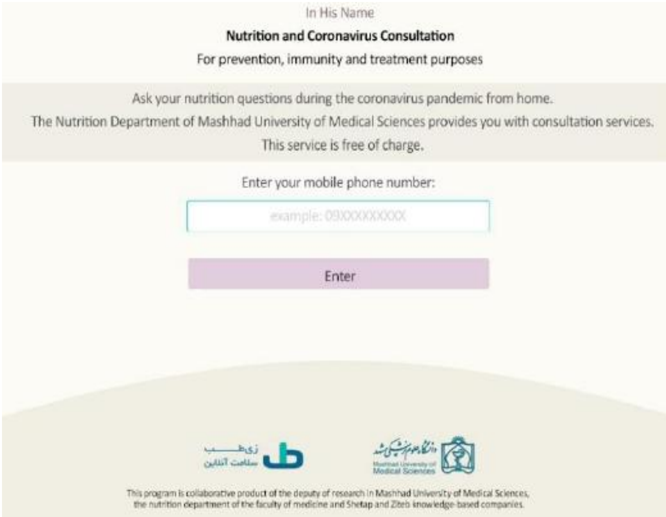

Figure 1. Login page

Figure 2. Patient demographic information questionnaire

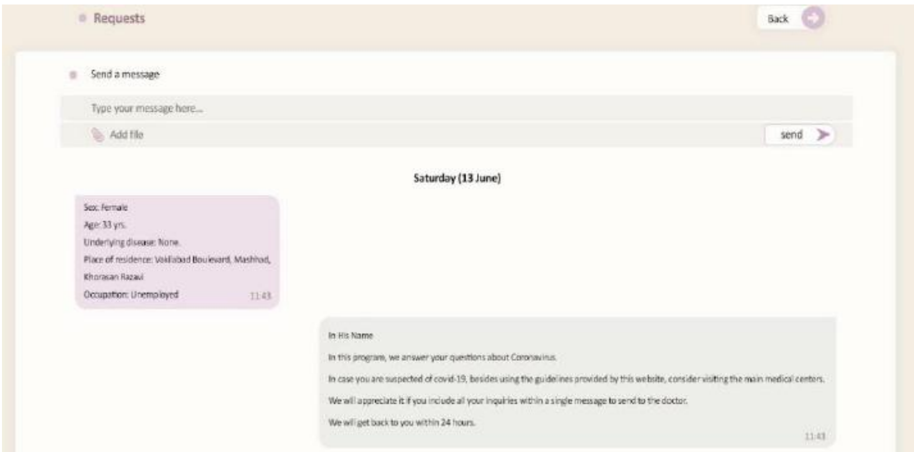

Figure 3. Where to ask questions and attach a file

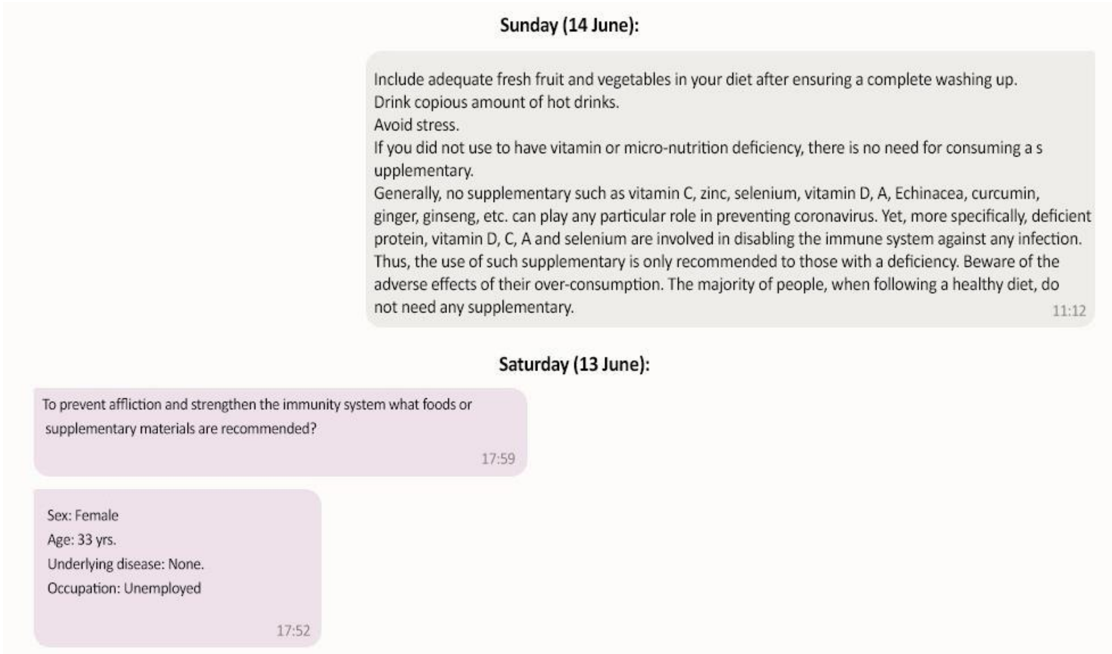

Figure 4. Nutritionist's answer to the user's question

Demographic Data

The average age of the system users was 36.9 ± 11.0 years, while the average age of those who asked questions was 37.9 ± 11.0 years. The most common age group for both users and consultants was 31-40 years (40.6% and 38.4%, respectively). The most common underlying disease in both groups was hypertension (Table 2).

Out of 641 system users, 1 individual (0.15%) mentioned 5 comorbidities, 5 individuals (0.78%) mentioned 3, 17 individuals (2.65%) mentioned 2, and 182 individuals (28.4%) mentioned only one comorbid disease.

A total of 436 users (68.02%) did not report any underlying disease. Among those who asked questions, 63.08% did not report any disease, while the remaining had varying comorbid conditions as follows: 3 individuals (0.78%) mentioned 3, 13 individuals (3.78%) mentioned 2, and 111 individuals (32.27%) mentioned only one comorbid disease.

Among all users who sought consultations, 53% were Mashhad residents (Figure 5).

Question Categories

A total of 344 questions were submitted. 49.1% of the questions were related to nutrition during the Coronavirus crisis, 32.0% focused solely on Coronavirus, and 7.5% pertained only to nutrition (Figure 5).

Figure 6 lists the frequency of question topics labeled by nutritionists. Since a question could cover multiple topics, the total exceeds the number of unique questions. After excluding nonsense questions, 199 questions addressed a single topic, while others covered multiple. The most common topics were Diet (46.2%), Symptoms of the disease (26.2%), and Immunity and supplementation were also frequently mentioned (20.7%).

Among the 169 questions specifically related to both COVID-19 and nutrition, the top three topics were diet (46.2%), immunity (28.4%), and supplementation (20.7%).

Table 2. Demographic characteristics and users' comorbidities

Variables		People who completed the questionnaire (n=641) Frequency (percent)	People who asked for consultation (n=344) Frequency (percent)
Sex	Male	293 (45.71%)	170 (49.41%)
	Female	341 (53.20%)	173 (50.30%)
	Unknown	7 (1.09%)	1 (0.29%)
Age (years)	14-20	19 (3.0%)	13 (3.8%)
	21-30	169 (26.4%)	81 (23.5%)
	31-40	260 (40.6%)	132 (38.4%)
	41-50	129 (20.1%)	78 (22.7%)
	51-60	44 (6.9%)	28 (8.1%)
	61-70	16 (2.5%)	12 (3.5%)
	71-80	2 (0.3%)	0
	81-90	2 (0.3%)	0
Location	Mashhad	339 (53.0%)	183 (53.2%)
	Suburbs of Mashhad	97 (15.0%)	50 (14.5%)
	Other	205 (32.0%)	111 (32.3%)
Cardiovascular disease		13 (2.03%)	10 (2.91%)
Hypertension		48 (7.49%)	28 (8.14%)
Diabetes		30 (4.68%)	17 (4.94%)
Pulmonary disease		19 (2.96%)	11 (3.20%)
Immune deficiency disorders		20 (3.12%)	19 (5.52%)
Chemotherapy		8 (1.24%)	6 (1.74%)
Other diseases		98 (15.29)	55 (16%)

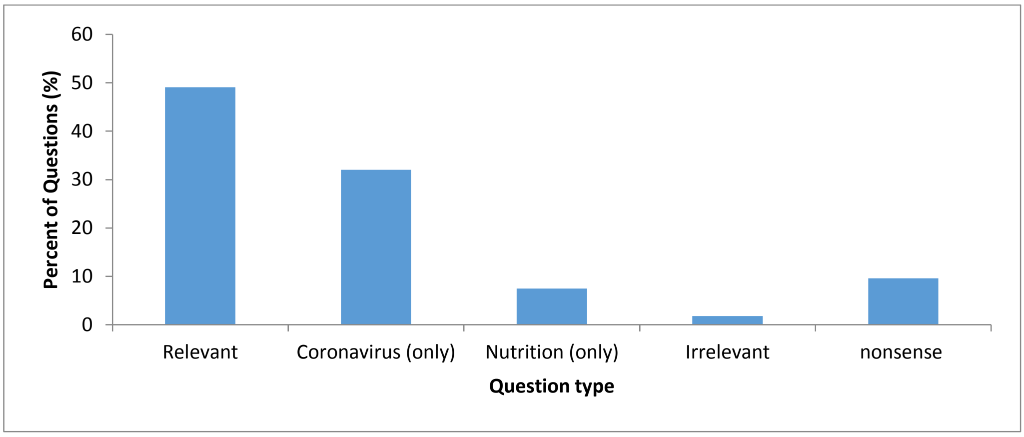

Figure 5. Percentage of questions based on relevance

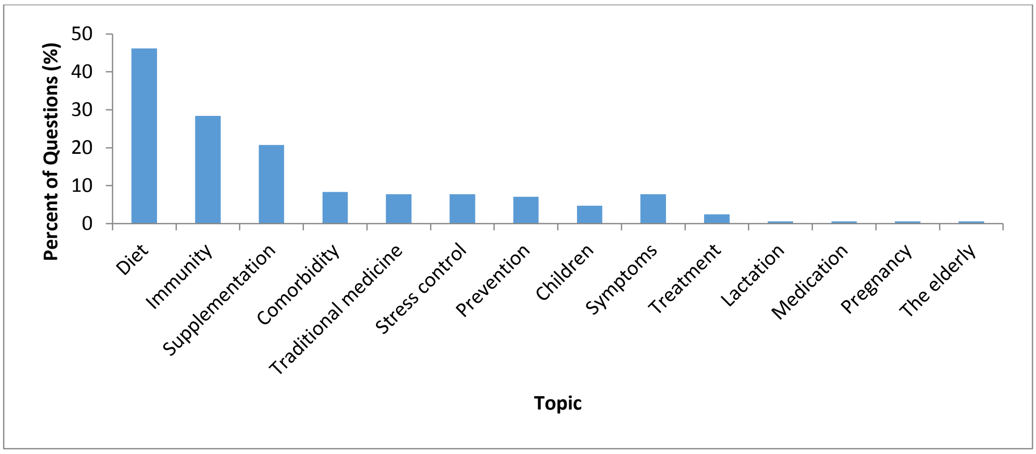

Figure 6. Percentage of questions based on the topic

Discussion

Implications for Telehealth

A major challenge during the Coronavirus crisis was the lack of awareness regarding proper nutrition. The present study provided valuable insights into the use of web-based applications to deliver essential nutritional consultation during the pandemic. This study was conducted when most businesses, private clinics, and counseling centers were closed down, and it was highly recommended to stay at home. The success of this project demonstrates the effectiveness of telemedicine or teleconsultation in similar crises, as it allowed continuous access to health services despite these closures.

The study represents the first phase in the development and feasibility of a system designed to address nutrition questions during the pandemic. In the early stages of the Coronavirus pandemic in Iran, 641 users completed the embedded questionnaire, with half of these users proceeding to ask questions and receive consultations from nutritionists. Despite limited promotion through social networks related to Mashhad University of Medical Sciences, the system received significant engagement, indicating public interest and acceptance of telehealth solutions. These findings suggest that such systems can be expanded with improved infrastructure to reach broader audiences and play a critical role in future health crises.

Implications of Nutrition-Related Inquiries

The fact that 49.1% of the questions submitted were related to nutrition during the pandemic reflects the public's urgent need for reliable nutritional guidance, particularly regarding immunity and the prevention of illness. The widespread misinformation surrounding diet and supplements during this time likely contributed to this demand. The system's ability to address these concerns highlights the importance of telehealth in providing trustworthy, timely advice during health crises. The findings suggest that addressing public health fears through accurate, expert-guided teleconsultation can help prevent misinformation from leading to harmful health behaviors.

User Engagement and Demographics

The system was primarily accessed by users in the 31-40 years age group, representing 40.6% of all users. This age group is typically more technologically adept and engaged with online health resources, which may explain their higher participation rate. The average age of system users was 36.9 ± 11.0 years, and most individuals who asked questions were between 31-40 years. The system also attracted individuals from other provinces, with 32% of users coming from outside Razavi Khorasan province, indicating the potential for national scalability. However, the system’s promotion through social networks affiliated with the university may have limited its reach to certain demographics, particularly older adults or individuals in more rural areas.

Fear, Anxiety, and Nutritional Behavior

Fear and anxiety about the Coronavirus played a significant role in shaping the type of inquiries received. Many of the questions centered around immunity and supplementation (20.7%), driven by the public’s concerns over preventing infection through dietary adjustments. The persistent focus on immunity, even months into the pandemic, highlights the public's belief in nutrition as a preventive measure. Previous studies have shown that during times of uncertainty, individuals tend to gravitate toward dietary and lifestyle changes believed to boost immunity. For example, the rush to take supplements without proper guidance can lead to overuse and related health issues.

These psychological factors influence dietary behaviors, as individuals experiencing fear or anxiety may seek out information on how to protect themselves. The high proportion of questions related to immunity (28.4%) and diet (46.2%) demonstrates this trend. This is consistent with findings from previous health crises where anxiety prompted people to turn to dietary changes and self-prescribed supplementation. Similarly, studies have shown that mobile applications can help monitor patients' physical and psychological conditions during the COVID-19 pandemic, addressing both their mental health and nutritional concerns (7-10).

Statistical Findings and User Understanding

Only 11.4% of the questions were deemed irrelevant or nonsense, indicating that most users understood the system’s purpose and functionality. This low percentage suggests that the system successfully conveyed its intended use to users, who asked questions mostly aligned with its goals. However, the presence of some irrelevant questions also points to a need for clearer communication on the system's capabilities, possibly through more detailed guidelines on the login page or an initial onboarding process that clarifies what types of questions the system can address.

Barriers to Broader Implementation

The mention of consultation costs and the need for insurance coverage highlights a critical barrier to the broader implementation of telehealth systems. As telemedicine becomes more widely used, issues of cost and access must be addressed to ensure equitable access (11). Literature on telemedicine adoption has shown that financial barriers can deter low-income individuals from accessing these services, which could exacerbate health disparities (12-15).

Limitations

Several limitations impacted the study. One significant limitation was the system's restricted promotion through networks tied to Mashhad University of Medical Sciences. This limited promotion likely resulted in a user base that skewed towards the university's affiliates or those already engaged with its health services. This could have influenced both the demographics of users and the types of questions asked. Additionally, while the system successfully catered to urban and tech-savvy populations, its accessibility to older adults and rural populations remains uncertain.

Another limitation was the lack of a fee structure for consultations during the feasibility phase. While the system was offered for free, scaling it for broader use would necessitate financial sustainability, likely through insurance coverage or service fees. Incorporating such a payment structure could present a barrier to some users, and future implementations would need to address these economic challenges.

The lack of personalized data collection, such as height and weight to calculate BMI, also limited the system's ability to provide tailored nutritional advice. More comprehensive data collection, including patients' medical histories, would enable more customized and effective consultations. This issue should be addressed in future iterations of the system.

Recommendations for Future Research

Future research should focus on expanding the system's reach to a more diverse user base by partnering with regional health authorities and launching targeted outreach efforts, particularly in rural and underserved communities. Additionally, integrating telehealth systems with existing public health campaigns could enhance public health literacy and increase engagement. Given the high levels of fear and anxiety influencing dietary behavior, future iterations of the system should include mental health support to help users make more informed decisions about their nutrition and supplement use during crises.

Conclusion

The present study successfully demonstrated the feasibility of a teleconsultation system for nutritional counseling during the Coronavirus pandemic. The system's high engagement rate and the large proportion of nutrition-related inquiries indicate the public's demand for reliable, evidence-based guidance during health crises. Moving forward, telehealth systems like this one could be expanded and integrated into public health initiatives, with the potential to play a vital role in ensuring the continuity of care during future emergencies. By addressing the challenges of accessibility, cost, and tailored consultations, telehealth could become a permanent fixture in public health strategies.

Declarations

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of Mashhad University of Medical Sciences and Medical School (Ethical code: IR.MUMS.REC.1399.006). Informed consent was obtained verbally from participants upon entering the study, ensuring that their participation was voluntary and confidential.

Consent for Publication

Not applicable.

Availability of Data and Materials

The datasets enabling this research are not publicly available due to privacy or ethical restrictions but are available from the corresponding author on reasonable request.

Conflict of Interests

The authors declare that they have no competing interests.

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in study design, data collection, analysis, decision to publish, interpretation, or preparation of the manuscript. The corresponding author will have full access to all the data in the study and will have final responsibility for the decision to submit for publication.

Authors' Contributions

RR and SE conceived the study idea and design. RR, KK and MN designed the plan of study implementation. SA, RR, MN and SE designed and developed the system. NF and MA analyzed the data. SA, NF and MA drafted the manuscript. All authors have been involved in critically revising the manuscript. All authors read and approved the final manuscript.

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Abbreviations

Not applicable.

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The Effect of Fisetin Supplementation and High-Intensity Interval Training on Neurogenesis Markers in Aged Alzheimer's Model Mice

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ABSTRACT

Introduction: Alzheimer's disease (AD) is associated with a marked reduction in brain-derived neurotrophic factor (BDNF) and fibronectin 1 (Fn1). This study investigates the effects of fisetin supplementation combined with high-intensity interval training (HIIT) on these neurogenesis markers in an aged mouse model of AD.

Methods: In this experimental study, 30 aged C57BL/6 mice (weight: 30 g) with AD were randomly assigned to one of the five groups: (1) Control, (2) AD, (3) AD + Fisetin, (4) AD + HIIT, and (5) AD + HIIT + Fisetin. Alzheimer's disease was induced in the AD groups by injecting amyloid-beta (Aβ1-42) into the hippocampus. The HIIT protocol consisted of a 10-minute warm-up at 50-55% VO2 max, followed by seven intervals, each comprising 4 minutes at 80-90% VO2 max and 3 minutes at 65-75% VO2 max. Fisetin was administered at 20 mg/kg for eight weeks. Data were analyzed using one-way ANOVA with a significance level of $P \leq 0.05$.

Results: Significant differences were observed in BDNF, Fn1, and Aβ gene expression levels across the five groups of aged mice ($p < 0.001$). BDNF and Fn1 expression were significantly reduced in the AD groups compared to the healthy controls ($p < 0.001$). However, their expression levels increased significantly in the AD + Training + Fisetin, AD + Training, and AD + Fisetin groups compared to the AD-only group ($p < 0.001$). The AD + Training + Fisetin group exhibited the highest expression levels, followed by the AD + Training and AD + Fisetin groups ($p < 0.001$). Aβ expression was significantly reduced in all intervention groups, with the AD + Training + Fisetin group showing the most substantial decrease ($p < 0.001$).

Conclusion: Combining HIIT and fisetin supplementation may promote cerebral neurogenesis in AD by reducing Aβ levels and enhancing BDNF and Fn1 gene expression. Notably, the combined intervention of HIIT and fisetin exhibits a more significant effect than either HIIT or fisetin alone, with HIIT being more effective than fisetin as a standalone treatment. Thus, the combination of HIIT and fisetin appears to be the most effective complementary approach for managing this disease.

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Introduction

Alzheimer's disease (AD), the most prevalent chronic disease among the elderly, is an irreversible neurodegenerative disorder characterized primarily by memory loss and other cognitive impairments. It is the most common form of dementia (1) and is also recognized as a proteinopathy, a condition where proteins become abnormal (2). The hallmark of AD is the formation of dense amyloid plaques, known as senile plaques, in the brain (3). The primary component of these plaques is beta-amyloid (Aβ). Aβ1-42, a proteolytic product derived from the amyloid precursor protein (APP) (4), is a soluble peptide in the body that can form dense, neurotoxic aggregates through fibrillization and oligomerization (5). AD is a progressive form of dementia, neuropathologically characterized by a significant reduction in the number of neurons, particularly in the hippocampus, along with the deposition of beta-amyloid in blood vessel walls, the spread of neuritic plaques, and the formation of intracellular fibrillar tangles (6). These

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pathological changes affect the cortex, hippocampus, and medial temporal lobes. Additionally, the disease is associated with mitochondrial dysfunction, oxidative neuronal damage, synaptic loss, neuronal degeneration, and cognitive and perceptual impairment (7).

Neurotrophins are a small group of structurally and functionally related growth factors (8). The Brain-Derived Neurotrophic Factor (BDNF) has received considerable attention due to its essential role in synaptic plasticity, memory, and neurogenesis. BDNF is recognized as a key factor positively regulated by exercise (9). Low levels of BDNF are associated with cognitive impairments, depression, neurodegenerative diseases, and increased mortality. BDNF has been shown to mediate the beneficial effects of exercise on synaptic plasticity and cognitive function, potentially through its role in energy metabolism (10). Studies indicate that BDNF mRNA levels significantly increase in rats following voluntary exercise, which correlates with improvements in spatial memory (11). Exercise can enhance the expression of BDNF and CREB genes. BDNF may influence memory by inducing changes in membrane receptor expression and trafficking, as well as activating several pathways (PLC-γ, PI3K, ERK) critical for synaptic plasticity. The exercise-induced increase in BDNF levels, along with improved mitochondrial function, can reduce oxidative stress (12).

Fibronectin (FN) is derived from various cellular sources, including astrocytes, epithelial cells, fibroblasts, and mesenchymal cells, and plays a role in cell adhesion, proliferation, differentiation, epithelial tissue repair, immune regulation, nerve regeneration, and other physiological activities (13). Fibronectin 1 (*FN1*) is an effective vehicle for delivering nerve growth factors (NGF) and provides an ideal matrix for axonal repair. In animal studies, fibronectin has been used to enhance peripheral nerve repair processes (14). It forms cable-like structures that promote cellular connections and facilitate the migration of cells such as fibroblasts, neurons, macrophages, and Schwann cells, thereby contributing to peripheral nerve repair (15).

Results have shown that increasing physical activity and regular exercise, even low-intensity activities such as walking, strongly protect the nervous system and reduce the risk of dementia (16). Regular physical activity plays a positive role in aging-associated brain atrophy reduction and diseases related to cognitive impairment, thereby decreasing the incidence of Alzheimer's disease (17). Physical exercise, particularly aerobic exercise, positively impacts brain health and cognitive function, reducing the detrimental effects of neurological disorders such as Alzheimer's, Parkinson's, and depression (18). The beneficial effects of HIIT on the brain are especially pronounced in the hippocampus and dentate gyrus regions (19). These effects include increased blood flow and hippocampal volume in humans, morphological changes in dendrites and dendritic spines, and enhanced synaptic plasticity and neurogenesis in animal models (19). Recent findings suggest that combined HIIT training and herbal supplementation can produce synergistic benefits in preventing and mitigating AD (20, 21).

Fisetin (3,3',4',7-Tetrahydroxyflavone) is a natural flavonoid in various fruits and vegetables, including strawberries, apples, persimmons, grapes, onions, and cucumbers. It possesses neurotrophic, anticancer, and anti-inflammatory properties, as well as antioxidant effects against cognitive and neurological disorders, such as Alzheimer's disease (AD) (22). Additionally, fisetin has been reported to play a role in preserving neurological function during aging (23). Generally, changes in neurotrophic levels due to aging, genetic factors, and other variables have been observed in neurodegenerative diseases, contributing to neuronal destruction. Given the neuroprotective and nutritional properties of factors such as BDNF and Fn1, which enhance central nervous system function, including memory and learning, and considering the limited studies on the combined effects of exercise and fisetin supplementation on BDNF and Fn1 in neurodegenerative diseases like Alzheimer's, there is a clear need to investigate these impacts. Thus, the present study aimed to examine the effects of fisetin supplementation and high-intensity interval training on specific neurogenesis markers in the brains of aged Alzheimer's model mice.

Materials and Methods

In this experimental study, 30 aged female C57BL/6 mice were obtained from the animal breeding center at the Royan Institute, Isfahan, and were randomly assigned to the following groups: (1) healthy control, (2) AD, (3) AD +

Fisetin, (4) AD + HIIT, and (5) AD + HIIT + Fisetin. Initially, the mice were acclimated to the laboratory environment for one week. The sample size was determined using a previous study and G*Power software. During the study, mice were kept under standard conditions, including a 12-hour light/dark cycle, an ambient temperature of 20 to 22°C, relative humidity of 55%, and free access to food and water. Additionally, the study adhered to the ethical guidelines of the Helsinki Declaration, and the protocol was reviewed and approved by the Ethics Committee of Shahrekord University (approval code: IR.SKU.RERC.1402).

To induce Alzheimer's disease, Aβ1-42 oligomers (Sigma-Aldrich, USA) were prepared. Synthetic Aβ1-42 was first dissolved in cold hexafluoroisopropanol (HFIP) (Sigma-Aldrich, USA) for 20 minutes. The solution was then vortexed for 10 minutes to promote the formation of Aβ1-42 monomers. These monomers were subsequently subjected to vacuum spinning and precipitation and then dissolved in a 10% HFIP solution. The Aβ1-42 solution was incubated with continuous stirring at room temperature for 48 hours, followed by centrifugation at 4°C for 20 minutes. The supernatant was separated and transferred to pre-chilled tubes. After complete evaporation of HFIP, 50 µM Aβ1-42 oligomers were obtained. The oligomer solution was stored at 4°C until use. Female BALB/c mice were anesthetized with an intraperitoneal injection of 0.2% sodium pentobarbital (50 mg/kg) and then positioned in a stereotaxic apparatus (Stoelting, Wood Dale, IL, USA). Aβ1-42 oligomers were injected into the hippocampus's bilateral dentate gyrus (DG) in C57BL/6 mice. The injection was performed with a concentration of 50 µM Aβ1-42 or 0.9% sterile saline at a volume of 1 µL, administered at a rate of 0.2 µL per minute.

High-Intensity Interval Training Protocol

The HIIT program for the rats in the exercise group will be conducted on a specialized animal treadmill (Tajhiz Gostar Iranian, 2016, Tehran, Iran). The protocol consists of a 10-minute warm-up period at 50-55% VO2max, followed by seven intervals, each comprising 4 minutes at 80-90% VO2max and 3 minutes at 65-75% VO2max, and concludes with a 1-minute cool-down period. Notably, no electrical shocks will be used during the exercise program. If needed, the animals will be encouraged to continue the exercise through manual guidance or auditory cues applied to the treadmill cover (24).

3-7- Fisetin Supplement

The fisetin supplement was obtained from Sigma-Aldrich (USA) and administered orally via gavage at a dose of 20 mg/kg for eight weeks (19).

Dissection and Sampling

Forty-eight hours after the final exercise session and following a 12-hour fasting period, the mice were anesthetized with a mixture of 50 mg/mL ketamine and 20 mg/mL xylazine. Laboratory specialists initially performed a pain response test to confirm adequate anesthesia. Once full anesthesia was verified, the animals' brains were exposed, and brain tissue was extracted. The tissue was immediately placed in liquid nitrogen for preservation.

Table 1. Primer Sequences for Research Variables

Genes	Primer Sequences	Temperature	Size (bp)
B2m	Forward: 5'- ACAGTTCCACCCGCCTCACATT -3' Reverse: 5'-TAGAAAGACCAGTCCTTGCTGAAG -3'	60	105
FN1	Forward: 5'- CCCTATCTCTGATACCGTTGTCC-3' Reverse: 5'- TGCCGCAACTACTGTGATTCGG-3'	60	110
BDNF	Forward: 5'- GGCTGACACTTTTGAGCACGTC-3' Reverse: 5'- CTCCAAAGGCACTTGACTGCTG-3'	60	123
AB	Forward: 5'- TCCGTGTGATCTACGAGCGCATC-3' Reverse: 5'GCCAAGACATCGTCGGAGTAGT-3'	59	128

Gene Expression Measurement for BDNF, Fn1, and Aβ1

RNA was extracted from hippocampal brain tissue using TRIzol reagent, following the manufacturer's protocol (Yekta Tajhiz Azma). Subsequently, cDNA synthesis was carried out according to the BIOFACT kit protocol. Gene sequences for BDNF, Fn1, and Aβ1, along with the nucleotide sequences of the forward and reverse primers, were obtained from the NCBI database (Table 1). Primers were designed using Oligo7 and Beacon Designer software. Real-time PCR was performed using SYBR Green kits on the Corbett Rotor-Gene 6000 system, with Beta-2-

microglobulin (B2M) serving as the housekeeping gene. Gene expression was analyzed and evaluated using the 2-ΔΔCT method.

Data Analysis

The Shapiro-Wilk test was used to assess normality, and Levene's test was applied to evaluate the homogeneity of variances. Data analysis was conducted using one-way ANOVA followed by Tukey's post-hoc test, with a significance level set at $p \leq 0.05$ for all tests. All statistical analyses were performed using SPSS software, version 23.

Results

One-way ANOVA demonstrated significant inter-group differences in BDNF gene expression in the brain tissue of aged mice after eight weeks of HIIT (p = 0.001). Tukey's post-hoc test revealed that BDNF expression was significantly lower in the AD group compared to the healthy control group (p = 0.001). Additionally, BDNF expression was significantly higher in the AD + Exercise + Fisetin (p = 0.001), AD + Exercise (p = 0.001), and AD + Fisetin (p = 0.001) groups compared to the AD group. Furthermore, BDNF expression was significantly higher in the AD + Exercise + Fisetin group than in the AD + Exercise (p = 0.001) and AD + Fisetin (p = 0.001) groups. Moreover, BDNF expression in the AD + Exercise group was significantly higher than in the AD + Fisetin group (p = 0.001).

Following eight weeks of HIIT, one-way ANOVA indicated significant differences in Fn1 gene expression levels in the brain tissue of aged mice across the study groups (p = 0.001). Tukey's post-hoc test demonstrated a significant reduction in Fn1 gene expression in the AD group compared to the healthy control group (p = 0.001). However, Fn1 expression was significantly elevated in the AD + Exercise + Fisetin (p = 0.001), AD + Exercise (p = 0.001), and AD + Fisetin (p = 0.001) groups compared to the AD group. Furthermore, Fn1 expression in the AD + Exercise + Fisetin group was significantly higher than in the AD + Exercise (p = 0.001) and AD + Fisetin (p = 0.001) groups. Additionally, Fn1 expression in the AD + Exercise group was significantly higher than in the AD + Fisetin group (p = 0.001).

Similarly, after eight weeks of HIIT, one-way ANOVA indicated significant differences in Aβ gene expression levels in the brain tissue of aged mice across the study groups (p = 0.001). Tukey's post-hoc test demonstrated a significant increase in Aβ gene expression in the AD group compared to the healthy control group (p = 0.001). However, Aβ expression was significantly reduced in the AD + Exercise + Fisetin (p = 0.001), AD + Exercise (p = 0.001), and AD + Fisetin (p = 0.001) groups compared to the AD group. Additionally, Aβ expression in the AD + Exercise + Fisetin group was significantly lower than in the AD + Exercise (p = 0.001) and AD + Fisetin (p = 0.001) groups. Furthermore, Aβ expression in the AD + Exercise group was significantly lower than in the AD + Fisetin group (p = 0.001).

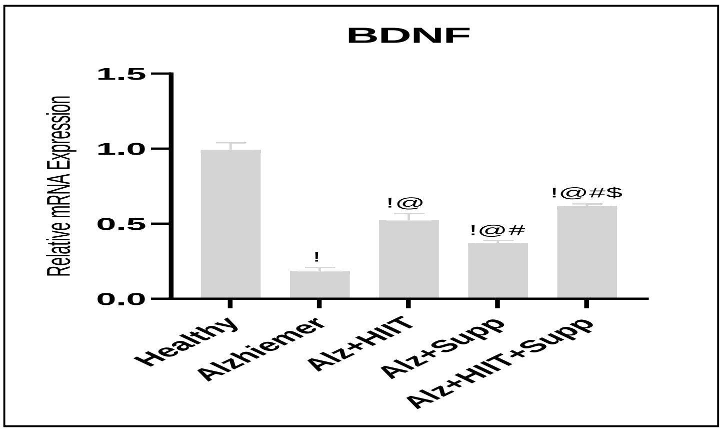

Figure 1: Relative expression changes of BDNF gene in different groups

Con: control, AD: Alzheimer's disease, Fis; Fistin, Exe; exercise

! A significant amount compared to the control group. @A significant amount compared to the Alzheimer's group# Significant level compared to the Alzheimer+HIIT group $ Significant level compared to the Alzheimer+exercise+physitin group

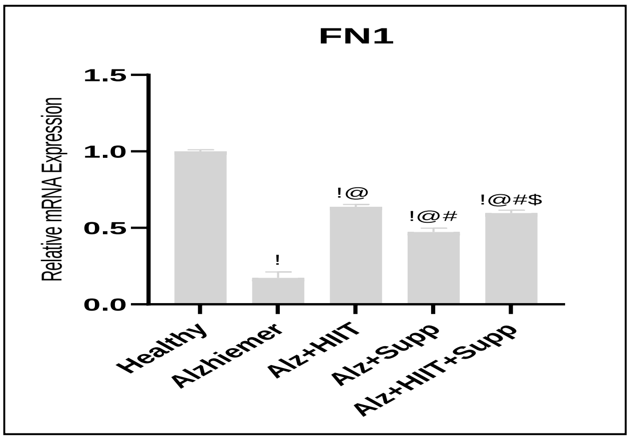

Figure 2: The relative expression changes of Fn1 gene in different groups
Con: control, AD: Alzheimer's disease, Fis; Fistin, Exe; exercise
! A significant amount compared to the control group. @A significant amount compared to the Alzheimer's group# Significant level compared to the Alzheimer+HIIT group $ Significant level compared to the Alzheimer+exercise+physitin group

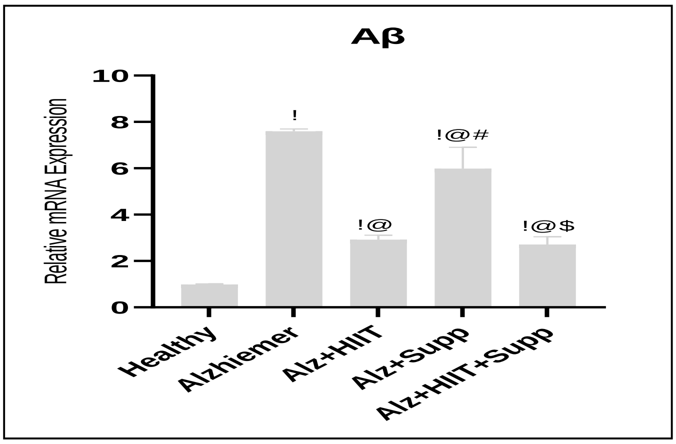

Figure 3: Changes in the relative expression of Aβ gene in different groups
Con: control, AD: Alzheimer's disease, Fis; Fistin, Exe; exercise
! A significant amount compared to the control group. @ A significant amount compared to the Alzheimer's group# Significant level compared to the Alzheimer+HIIT group $ Significant level compared to the Alzheimer+exercise+physitin group

Discussion

Aging and AD are associated with reduced brain angiogenesis and neurogenesis. This study examined the effects of HIIT combined with fisetin supplementation on BDNF, Fn1, and Aβ gene expression in elderly mice with AD. In summary, the combination of HIIT and fisetin supplementation, as well as each intervention individually, led to increased expression of BDNF and Fn1 genes and decreased expression of Aβ following eight weeks of HIIT in elderly mice with AD, compared to the Alzheimer's control group. Additionally, the combined effect of fisetin and HIIT was significantly more effective than either intervention alone, with HIIT demonstrating a more significant impact than fisetin supplementation alone.

Neurotrophic factors, such as *BDNF* and Fn1, play essential roles in supporting and promoting the growth of various brain neurons. Research indicates that exercise can indirectly influence the expression of neurotrophic factor genes by modulating the release of neurotransmitters such as acetylcholine, gamma-aminobutyric acid (GABA), and monoamines, which in turn leads to elevated *BDNF* mRNA levels in the hippocampus (25). High levels of *BDNF* in the hippocampus and cortex reflect its essential role in maintaining proper brain function. *BDNF* has a clear neuroprotective role, and exercise enhances cholinergic activity, which is involved in neuronal plasticity induced by physical activity (26). Luo et al. demonstrated that exercise increases neurogenesis in rats and enhances BDNF mRNA production, contributing to improved brain function, learning, and memory (26). Intense physical activity also increases cerebral blood flow, potentially enhancing neuronal cell formation, angiogenesis, synaptogenesis, and neurotransmitter synthesis in various brain regions (27). In a study by Osali et al., BDNF levels and memory improved after 12 weeks of aerobic exercise (28). Zhang et al. (2023) concluded in a review that exercise, mainly treadmill running, swimming, and voluntary wheel running, significantly increases *BDNF* levels in the hippocampus and cortex of AD models, with swimming being the most effective intervention (29). However, these findings differ from studies by Betio et al. (2020) and Nakhzari et al. (2018), which reported either decreased or unchanged BDNF levels following endurance exercise (30, 31). This discrepancy may stem from differences in subjects or exercise protocols. Another possible mechanism for increased BDNF following HIIT in Alzheimer's mice may involve regulating Trk receptors in brain tissue (32). In the present study, HIIT significantly elevated BDNF levels in the hippocampus of aged Alzheimer's mice.

Fibronectin (FN) is a high-molecular-weight glycoprotein, ranging from 220,000 to 250,000 daltons. As an adhesive molecule, FN was initially isolated from human plasma and fibroblast cell surfaces and is abundantly present in the extracellular matrix (ECM). Fibronectin 1 (FN1) serves as an effective carrier for nerve growth factors (NGFs) and provides a suitable matrix for axonal repair (13). In animal studies, fibronectin has been utilized to enhance peripheral nerve repair processes (33). Research by Ping et al. (2018) highlights FN1's significant role in stimulating the growth, invasion, and survival of gliomas through the activation of the PI3K/AKT signaling pathway (34). Several studies have reported that FN modulates cellular biology via its impact on PI3K/AKT signaling (35, 36). Moreover, research by Lemanska et al. (2009) demonstrated that the molecular status of plasma FN could serve as an additional biomarker for risk assessment (33). Studies show that fibronectin (FN) has a potent angiogenic effect on endothelial cells in the central nervous system. Additionally, fibronectin enhances the survival and proliferation of brain microvascular endothelial cells, mediated by integrins α5β1 and αVβ3 through mitogen-activated protein kinase signaling (37). The mechanisms underlying the increase in FN1 in the brain after chronic HIIT are likely associated with reduced apoptosis and enhanced PI3K/AKT signaling. This exercise-induced increase in *FN1* may also involve the activation of integrins, further supporting the neuroprotective and angiogenic roles of *FN1* (33, 34) .

The results of the current study indicate that a combination of HIIT and fisetin supplementation, as well as each intervention individually, led to a reduction in *Aβ* expression after eight weeks of HIIT in aged mice with AD. Mousakhani et al. (2024) demonstrated that voluntary physical activity in an enriched environment likely improves *PI3K* and *Akt* protein mechanisms and suppresses amyloid beta accumulation through enhanced insulin sensitivity and hormone function, resulting in cognitive benefits and reduced cell death in AD (37). Additionally, Zarine Afshar et al. (2020) showed that moderate-intensity continuous exercise combined with curcumin supplementation can increase plasma soluble LRP1 levels, promoting *Aβ* clearance in the hippocampus and modulating factors affecting AD in laboratory rats (38). In contrast, Fallah et al. (2014) reported increased plasma Aβ levels in diabetic rats following voluntary exercise (39), which is inconsistent with our findings. This discrepancy may stem from differences in subjects and exercise protocols. Interval aerobic exercise may reduce brain *Aβ* plaque levels by regulating amyloid precursor protein processing or enhancing the degradation and clearance of *Aβ* (37).

In a study, fisetin supplementation significantly improved cognitive performance and memory in mice with AD (22). The current study also demonstrated that fisetin enhances BDNF and Fn1 expression in Aβ1-42 model mice, thereby supporting critical neurogenic pathways essential for neuronal survival. Notably, fisetin has been shown to reduce apoptosis and neurodegeneration induced by aluminum in the hippocampus of adult mice (40). These results suggest that fisetin plays an essential role in preventing age-related neurodegenerative disorders, including AD (22). A primary limitation of the present study is the lack of measurement of additional factors related to cognitive performance and brain neurogenesis. Future studies should utilize different measurement techniques, such as Western blotting and ELISA.

Conclusion

Based on the results, HIIT and fisetin supplementation, either alone or in combination, can enhance neurogenesis in elderly individuals with AD by increasing the expression of BDNF and Fn1 while reducing Aβ levels. Notably, the combination of fisetin and aerobic exercise demonstrates a more substantial effect than either intervention alone, with HIIT alone proving more effective than fisetin alone. Therefore, the combined approach of HIIT and fisetin is recommended as the most effective complementary therapeutic strategy for AD. However, further and more comprehensive studies are required to validate and expand upon these findings.

Declarations

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Conflict of Interest

The authors declare that no conflicts of interest are associated with this research.

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Ethical Considerations

This manuscript is derived from a PhD dissertation in Exercise Physiology at Shahrekord University and has been registered with the Shahrekord University Ethics Committee under the code IR.SKU.RERC.1402.064.

Author Contributions

All authors equally contributed to the design, execution, analysis of results, and writing of the manuscript.

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JOURNAL OF NUTRITION FASTING AND HEALTH

Predicting Disordered Eating Behaviors in Women with Overweight and Obesity: Investigating the Role of Stigma, Shame, Guilt, Fear of Negative Evaluation and Self-Efficacy

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ABSTRACT

Introduction: Obesity is a health concern with complex dimensions. This study aimed to investigate the predictive roles of psychological factors, including weight self-stigma, weight- and body-related shame and guilt, eating self-efficacy, and fear of negative appearance evaluation, on disordered eating behaviors in Iranian women with overweight and obesity.

Methods: This descriptive cross-sectional research was carried out in 2020-2021 among 228 Iranian women, aged 18 to 70 years, with overweight and obesity who were selected by purposive sampling method. Data were collected through online standard questionnaires. SPSS-23 was used to analyze the data.

Results: All the predictor variables had significant associations with disordered eating behaviors ($P<0.05$, $P<0.01$). In addition, the results of multiple regression analysis showed that eating self-efficacy was a predictor of emotional eating behavior ($\beta = -0.534$, $P<0.001$). Additionally, fear of negative appearance evaluation and eating self-efficacy were predictors of external eating behavior ($\beta f = -0.416$, $\beta s\text{-}e = -0.416$, $P< 0.001$). Moreover, weight and body related guilt and eating self-efficacy were predictors of restrained eating behavior ($\beta g = 0.442$, $\beta s\text{-}e = 0.300$, $P< 0.001$).

Conclusions: These findings emphasize the importance of psychological variables in obesity. Specifically, self-efficacy, fear of negative evaluation, and guilt were important constructs in predicting disordered eating behaviors. Thus, in clinical weight loss programs, considering psychological treatments based on strengthening self-efficacy and self-compassion in this group is helpful to improve their healthy eating behaviors.

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Introduction

Obesity, a critical global health problem, is prevalent in both high- and low-income countries, with Iran reporting a 70% prevalence rate (1, 2). Women are particularly affected, experiencing higher rates of obesity compared to men (3). Obesity is linked to non-communicable diseases and psychosocial problems such as depression, anxiety, eating disorders, and low self-esteem, alongside societal pressures regarding body image. Additionally, sociocultural norms often favor thinness, leading to negative biases against individuals with overweight and obesity, particularly women (1, 4-6). Therefore, it seems necessary to investigate the relationships between psychosocial variables and obesity among women.

Disordered eating behaviors significantly contribute to the development and persistence of obesity. Beyond its survival purpose, eating is a rewarding activity tied to emotions and

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mood, influenced by physiological, psychological, and social factors (7). These behaviors encompass a range of abnormal patterns, including emotional eating, restrained eating, binge eating, skipping meals, and extreme methods like using diet pills, diuretics, and laxatives or inducing vomiting (5, 8). Disordered eating is strongly linked to psychological factors such as weight stigma, body dissatisfaction, perfectionism, depression, low self-esteem, and anxiety, particularly in individuals with overweight and obesity (9). These factors create a cycle of unhealthy eating habits that perpetuates weight gain and associated health risks, emphasizing the need for targeted interventions to address these behaviors.

Weight stigma, a significant psychosocial factor in obesity, is strongly associated with disordered eating behaviors. Individuals with obesity often face stereotypes labeling them as lazy or lacking willpower, and evidence shows that such stigma, rather than motivating weight loss, exacerbates psychological problems like stress, depression, anxiety, and low self-esteem, along with physical problems such as metabolic syndrome (10, 11). Internalizing weight stigma further degrades self-identity, fostering feelings of worthlessness and contributing to behaviors like restrictive eating, emotional eating, binge eating, and food addiction (6, 10). Understanding the link between weight stigma and eating behaviors, particularly in women with higher weights, is crucial for addressing obesity (11).

Additionally, Shame and guilt, as self-conscious emotions, play a significant role in obesity and eating behaviors. Shame involves global negative self-evaluation, while guilt focuses on specific actions (12-14). Weight-and body-related shame, driven by internalized cultural standards, leads to avoidance and public humiliation, whereas weight- and body-related guilt evokes remorse and reparative actions (15). These emotions often link with unhealthy eating behaviors among individuals with obesity or eating disorders, perpetuate disordered eating, and make effective weight management difficult (16-18). Addressing these emotions is critical for obesity and eating disorder interventions.

Furthermore, fear of negative evaluation, including fear of negative appearance evaluation, plays a critical role in obesity-related eating behaviors. This fear, rooted in anxiety over others' judgments, predicts pressure to be thin, internalized thin ideals, negative emotions, and binge eating symptoms (19–21). Obesity is strongly associated with eating problems and anxiety linked to fear of appearance-based evaluations, which are key risk factors for social anxiety and eating disorders (22, 23). Studies also confirm a connection between fear of negative appearance evaluation and disordered eating in individuals with higher weight, emphasizing the need to address these concerns in obesity interventions (24).

Along with the variety of emotions, self-efficacy, the belief in one's ability to perform behaviors that influence outcomes, is crucial in regulating eating behaviors (25). Eating self-efficacy, a specific aspect of the general concept of self-efficacy, is defined as individuals' beliefs in self-regulation and management of their eating behaviors in challenging situations (26). Research indicates that higher eating self-efficacy improves self-regulation, helps individuals overcome dietary challenges, supports weight management, and promotes healthier food choices. Conversely, low eating self-efficacy predicts obesity and unhealthy eating habits, highlighting its role in enhancing or diminishing self-regulatory behaviors essential for weight control (27-32). Overall, obesity is a multifaceted problem influenced by individual and social factors, with significant implications for individuals and societies, particularly women. Understanding the interplay between obesity-related variables is essential for developing effective interventions. This study examines the relationships between weight self-stigma, weight-and body-related shame and guilt, fear of negative appearance evaluation, and eating self-efficacy with disordered eating behaviors in Iranian women with overweight and obesity, marking the first investigation of these variables in this context.

Materials & Methods

Study Design

This study was descriptive-analytical and cross-sectional.

Participants and Sampling

The participants were Iranian women aged 18 to 70 years with overweight or obesity. The sample size for multiple regression analysis was determined based on guidelines recommending at least 15 cases per predictor variable. To enhance the effect size, 30 items per predictor variable were considered (33), resulting in a sample of 228 women recruited using purposive sampling.

Questionnaire Translation and Validation

The study utilized four questionnaires, which were translated to address weight self-stigma, weight- and body-related shame and guilt, weight efficacy lifestyle (short form), and fear of negative appearance evaluation. A bilingual individual translated these questionnaires from English to Persian. A panel of specialists in psychiatry and psychology reviewed the translations for content and formal validity, suggesting amendments where necessary. After implementing these corrections, a back-translation was performed by another bilingual individual. The original and translated versions were consistent. Preliminary testing was conducted with women visiting a nutrition clinic in Tehran. Internal consistency of the instruments was assessed using Cronbach's alpha and ordinal theta coefficients.

Data Collection Procedures

Due to COVID-19 restrictions and participant reluctance to attend in-person sessions, the research questionnaires were administered online. Inclusion criteria required participants to be female, Iranian nationals, aged 18 to 70 years, with a BMI ≥ 25, internet access, and literacy. Three questionnaires were excluded from the analysis as they were completed by individuals with a normal BMI. Participants completed the questionnaires through a structured Google Docs format. Demographic questions including height (m), weight (kg), age, education, employment and marital status were assessed. Then, the research questionnaires were raised. The questionnaire link sent to various groups whose user identities were known, and volunteers were invited to participate in the research. Moreover, other people also provided this link to other related groups so that people could cooperate if they were satisfied. The questionnaire ensured completeness by logging responses only upon clicking the "Finish" button, eliminating incomplete datasets.

Statistical Analysis

Data were analyzed using SPSS version 23. Descriptive indices (minimum, maximum, mean, standard deviation, skewness, and kurtosis) were calculated. Pearson's correlation coefficients were computed for the research variables. Multiple regression analysis was performed to test the hypotheses. The assumptions of normality (skewness and kurtosis), absence of multicollinearity (VIF and tolerance index), and independence of error sources (Durbin-Watson test) were assessed before conducting the regression analyses.

Measures

The instruments for measuring the predictive variables included four questionnaires:

1. Dutch Eating Behavior Questionnaire (DEBQ) was used to measure disordered eating behaviors, which was the outcome variable of the present study. Van Strien et al. (36) developed it with 33 items and three subscales. The emotional eating subscale, which measures eating in response to emotional disturbance, consists of 13 items ("Do you get the desire to eat when you are anxious, worried, or tense?"). The subscale of external eating, which measures eating in response to external cues of food and not to the body's internal signals of satiety and hunger, has ten items ("If food smells and looks good, do you eat more than usual?"). Restrained eating subscale measures restriction of eating behavior and has ten items ("Do you try to eat less at mealtimes than you would like to eat?"). The items of this questionnaire are scored on a 5-point Likert scale from never = 0 to almost always = 5. Only question 21 of this questionnaire, related to the restrained eating subscale, is scored reversely. According to van Stein et al., the subscales of this questionnaire have high internal consistency and convergent and divergent validity (36). The internal consistency of subscales of emotional, external, and restrained eating in the Iranian study (37) and the present study was 0.89, 0.89, 0.87, 0.91, 0.67, and 0.87, respectively.

2. Weight Self-Stigma Questionnaire (WSSQ) contains 12 items designed to assess self-stigma associated with having obesity or overweight (34). This questionnaire includes two subscales: self-devaluation and fear of enacted stigma. A 5-point Likert scale ranged from completely disagree=1 to completely agree=5. A higher score indicates a higher level

of self-stigma (e.g., "I don't have enough self-control to maintain a healthy weight", "People think that I am to blame for my weight problems"). The overall internal consistency of the original version of this questionnaire has been reported as α = 0.88, with the self-devaluation subscale α = 0.81 and the fear of enacted stigma subscale α = 0.87 (32). In the Persian version, the internal consistency for the self-devaluation subscale is reported as α = 0.91, and the fear of enacted stigma subscale is α = 0.87 (35). In the present study, Cronbach's alpha for the total score of this questionnaire was α = 0.83, for the self-devaluation subscale α = 0.79, and for the fear of enacted stigma subscale α = 0.85.

3. Weight-and Body-Related Shame and Guilt Scale (WEB-SG) designed to measure the extent of shame and guilt associated with weight and body in individuals with obesity (14). It has two subscales for shame and guilt. Each item on this scale is rated on a 5-point Likert scale from never = 0 to always = 4 (e.g., " When I am in a situation where others can see my body (e.g., pool, changing room), I feel ashamed," "When I can't get a grip on my weight, I blame myself."). Conradt et al. (30) reported an internal consistency of α = 0.92 for the shame subscale and α = 0.87 for the guilt subscale. In this research, Cronbach's alpha for the shame subscale was α=0.87, and for the guilt subscale was α = 0.83.

4. Fear of Negative Appearance Evaluation Questionnaire (FNAES) was developed by Lundgren, Anderson & Thompson (21) to measure the level of fear that one's physical appearance is evaluated negatively by others. FNAES questionnaire has six items scored on a 5-point Likert scale from not at all = 1 to extremely = 5 ("I am concerned about what other people think of my appearance"). The internal consistency of the original version of the questionnaire was α=0.94. Cronbach's alpha of this questionnaire was α=0.93 in the present study.

5. Weight Efficacy Lifestyle Questionnaire-Short Form (WEL-SF) was developed from the original form of this instrument contained 20 items and was developed by Clark et al. (27) to measure individuals' belief in their ability to control eating behavior for weight management. The short form consists of eight items that correlate highly with the total score of the original form, explaining 94% of the variance of the total score of the full version. This questionnaire is scored on a 10-point Likert scale, from not confidence at all = 0 to very confidence = 10 (e.g., "I can resist binge eating when I am angry (or irritable)"). The internal consistency of this questionnaire has been reported as α=0.92 (31). A higher score indicates greater eating self-efficacy. In this study, Cronbach's alpha was α=0.84.

Results

Descriptive statistics

This study was conducted on 228 women with overweight and obesity (M_{age}=38.65, SD_{age}=11.06). In addition, 56.5% and 43.5% of participants were with overweight and obesity, respectively. About 10.1% of participants had a diploma and below degree, 6.6% had an associate, 38.2% had a bachelor, 25.9% had a master's, and 19.3% had a Ph.D. Regarding marital status, 36% were single, 55.7% were married, and 8.3% were divorced. The minimum, maximum, mean, standard deviation, skewness and kurtosis of the variables are reported in Table 1.

Table 1. Descriptive indicators of research variables

Variable	Min	Max	Mean	SD
Weight self-stigma	16	55	35.14	7.97
Weight-and body-related guilt	0	24	11.97	5.56
Weight-and body-related shame	0	24	7.50	6.04
Eating self-efficacy	0	80	35.12	17.88
Fear of negative appearance evaluation	6	30	15.75	6.57
Emotional eating behavior	0	48	26.09	10.39
restrained eating behavior	3	39	19.98	6.42
external eating behavior	8	33	21.18	5.07

Table 2 reports the Pearson correlation coefficient matrix of research variables. Among the predictor variables, eating self-efficacy had the strongest association with emotional eating behavior (r = -0.625). Furthermore, eating self-efficacy was strongly associated with external eating behavior (r = -0.498). Additionally, weight- and body-related guilt had the strongest association with restrained eating behavior (r = 0.293).

Table 2. Correlation matrix between research variables

		1	2	3	4	5	6	7	8
1	Weight self-stigma	1							
2	Weight-and body-related guilt	0.608**	1						
3	Weight-and body-related shame	0.653**	0.586**	1					
4	Eating self-efficacy	-0.437**	-0.230**	-0.209**	1				
5	Fear of negative appearance evaluation	0.675**	0.594**	0.725**	-0.211**	1			
6	Emotional eating behavior	0.481**	0.368**	0.370**	-0.625**	0.360**	1		
7	restrained eating behavior	0.019	0.293**	0.077	0.248**	0.126*	-0.071	1	
8	external eating behavior	0.384**	0.218**	0.184**	-0.498**	0.300**	0.551**	-0.254**	1

P<.05* P<.01**

Regression Analysis

Table 3 shows the regression analysis results of emotional eating behavior based on predictor variables. The R2 indicated that predictor variables can explain 46.9% (P < 0.001) of the emotional eating behavior variance. Precisely, with β = -0.534, eating self-efficacy significantly predicted emotional eating behavior. Other predictor variables did not contribute substantially to predict emotional eating behavior.

Table 3. Multiple regression results of emotional eating behavior based on predictor variables

	R	R^2	F(sig)	B	β	t	sig
Weight self-stigma	0.685	0.469	39.16 (.001)	0.100	0.077	0.967	0.335
Weight-and body-related guilt				0.188	0.101	1.52	0.128
Weight-and body-related shame				0.185	0.107	1.40	0.161
Eating self-efficacy				-0.310	-0.534	-9.72	0.001
Fear of negative appearance evaluation				0.092	0.058	0.739	0.460

Results related to the regression analysis of restrained eating behavior based on predictor variables are shown in Table 4. The R2 suggested that predictor variables can account for 20.3% (P < 0.001) of the restrained eating behavior variance. The results demonstrated that weight- and body-related guilt (β= 0.442) and eating self-efficacy (β= 0.300) significantly predicted restrained eating behavior. Other predictor variables did not have significant contributions in explaining restrained eating behavior.

Table 4. Multiple regression results of restrained eating behavior based on predictor variables

	R	R^2	F(sig)	B	β	t	sig
Weight self-stigma	0.450	0.203	11.28 (.001)	-0.077	-0.096	-0.986	0.325
Weight-and body-related guilt				0.510	0.442	5.45	0.001
Weight-and body-related shame				-0.112	-0.106	-1.12	0.261
Eating self-efficacy				0.108	0.300	4.45	0.001
Fear of negative appearance evaluation				0.067	0.068	0.710	0.479

Finally, results of the regression analysis of external eating behavior based on predictor variables are presented in Table 5. The R2 indicated that predictor variables could explain 30.6% (P < 0.001) of the external eating behavior variance. The results indicated that eating self-efficacy (β = -0.416) and fear of negative appearance evaluation (β = 0.235) significantly predicted external eating behavior.

Table 5. Multiple regression results of external eating behavior based on predictor variables

	R	R^2	F(sig)	B	β	t	sig
Weight self-stigma	0.553	0.306	19.60 (.001)	0.106	0.167	1.84	0.066
Weight-and body-related guilt				-0.017	-0.019	-0.246	0.806
Weight-and body-related shame				-0.143	-0.171	-1.95	0.052
Eating self-efficacy				-0.118	-0.416	-6.62	.001
Fear of negative appearance evaluation				0.181	0.235	2.61	0.010

Discussion

This study aimed to evaluate associations, as well as predictions, between five predictive variables, including weight self-stigma, eating self-efficacy, fear of negative appearance evaluation, and weight- and body-related shame and guilt, with three outcome variables including emotional eating behavior, external eating behavior and restrained eating behavior (disordered eating behaviors) in women with overweight and obesity. Considering that obesity is evident in a person's appearance, all of the research predictor variables are psychological variables that emphasize weight and body aspects of women. Results demonstrated correlational and predictive relationships between predictor and outcome variables, which are explained in more detail below. Moreover, considering that, no research was found that examined all the assumed variables of this research simultaneously, some evidence nearly related to this study was considered.

Our findings showed that weight self-stigma, weight- and body-related shame and guilt, and fear of negative appearance evaluation are positively correlated with emotional eating behavior. In addition, eating self-efficacy has a negative association with emotional eating behavior. These results were consistent with the findings of other studies (38, 39). These findings mean that when women with overweight and obesity internalize society's obesity biases and feel more worthless, ashamed and guilty about their body and weight, and more afraid of others' negative evaluations about their appearance, their eating behaviors will be more based on emotions. Meanwhile, when they assume that they cannot manage their eating behaviors in challenging situations and feel less competent, they are still more likely to engage in emotional eating behaviors. Further, according to the escape theory (40), one of the mechanisms people use when experiencing negative emotions is to escape from them and tend to eat food. Therefore, when some people experience negative emotions, instead of paying attention to those emotions and adopting appropriate strategies, they show emotional eating behavior. Emotional eating behavior is one of the unhealthy strategies for dealing with stressful internal or situational states and situations. Therefore, the simultaneous existence of negative emotions towards body size and shape, along with feeling worthless and believing in incompetence in regulating one's eating behaviors, is associated with an unhealthy strategy such as emotional eating. The regression analysis results showed that only eating self-efficacy predicted emotional eating behavior, which explains nearly half of the prediction variance. This finding is also consistent with the findings of previous studies (41). Due to the high importance of self-efficacy in behaviors, despite having other variables, only eating self-efficacy had a predictive role and affected other research variables. Self-efficacy is a person's belief in his ability to influence the environment despite all negative feelings (25). Therefore, if people do not believe in their competence to manage their eating behaviors in such situations, they may act unconstructive (28).

The results also indicated that weight self-stigma, weight- and body-related shame and guilt, and fear of negative appearance evaluation positively correlate with external eating behaviors. In addition, eating self-efficacy had a negative significant relationship with external eating behavior. These findings are entirely consistent with those related to emotional eating behaviors in this study. Evidence has suggested that emotional and external eating behaviors often occur concurrently (42). The escape from self-awareness theory can explain this simultaneous occurrence of these two disordered eating behaviors (40). It postulates that when individuals experience negative emotions, they focus on the immediate environment, such as food cues, to escape from these emotions, displaying external eating behavior. Negative emotions and food cues simultaneously increase binge eating in women with obesity (43). Moreover, results from functional neuroimaging studies among chronic dieters have shown that the same brain regions activated by appealing food cues are involved during the induction of negative emotions (44). Thus, both behaviors are associated with low self-control in individuals. Furthermore, consistent with previous research, the findings of this study showed that eating self-efficacy and fear of negative appearance evaluation are significant predictors of external eating behavior (45, 46). Predicting this type of disordered eating behavior, reliant on external food cues, involves the variable of fear of negative appearance evaluation, which itself depends on the environmental assessment of the appearance of an individual with obesity or overweight. In other words, when an individual is concerned about environmental evaluation and feels incompetent in regulating their dietary behaviors, their behavior is also based on environmental cues related to food rather than on internal hunger and satiety cues. This association has also influenced the predictive role of other variables in the research. Finally, the research findings showed that eating self-efficacy, fear of negative appearance evaluation, and weight and body-related guilt are positively associated with restrained eating behavior. Moreover, eating self-efficacy, weight, and body-related guilt were predictors of restrained eating behavior. These findings align with other research (24, 41). These findings mean that the more women with higher weight are afraid of others' negative evaluations about their appearance, blame themselves for their weight and body condition, and feel competent in managing their eating behaviors in challenging situations, the more likely they restrict their food intake. Simultaneously, this group's high eating self-efficacy and weight-and body-related guilt will predict restrained eating behavior. Restrained eating refers to restricting calorie intake or dieting to maintain or reduce weight. This behavior pays attention to dieting rules rather than internal satiety and hunger cues. Additionally, weight and body-related guilt creates regret and leads people to engage in unhealthy weight and body-related behaviors. This emotion can manifest through increased motivation for intense exercise or restricting caloric intake (47, 48). Also, when individuals believe in their self-efficacy in managing eating behaviors in challenging situations, they may control and reduce their weight by limiting their eating behaviors (28). It should be noted that preventing and restricting eating behavior alone will not be a successful path to weight loss and control, and, in many cases, eating restraint will concurrently be associated with dietary disinhibition (49). The power of these variables in predicting restrained eating behavior also influenced the potential predictability of other research variables.

Overall, eating behaviors in women with overweight and obesity are related to various psychological aspects. Variables related to the women's self (self-stigma and self-efficacy) and emotions (Self-conscious: shame and guilt, basic: fear) can play an essential role in the quality and type of their eating behaviors. Weight self-stigma affects people's social identity and will have multiple psychological and behavioral consequences (6). Self-efficacy is a cognitive structure and one of the determinants of the "self" and is essential in predicting health behaviors (25). The presence of high self-efficacy in women with higher weights can help them manage their eating behaviors despite the presence of various barriers. At the same time, the low self-efficacy belief in this group can provide the basis for behaviors that rely on emotions and the environment. On the other hand, body/weight-related emotions, such as guilt, shame, and fear, are also involved in determining people's unhealthy eating behaviors. Therefore, the internalization of weight stigma and the subsequent feeling of worthlessness in these women, along with the negative emotions of shame, guilt, and fear of others' negative evaluations, change people's beliefs about their abilities to manage their eating behaviors in various situations can create a combination that provides a path to disordered eating behaviors and increase their weight (50). This research had several limitations. Firstly, the study participants only consisted of women, recruited via a purposive sampling method that limited the generalizability of the findings to the entire population. So, a more diverse sample and random sampling methods are recommended for greater generalizability. Additionally, data were collected through online surveys and self-report instruments (including BMI), which may be influenced by social desirability bias. Thus, future research could benefit from in-person assessment, and incorporating methods based on objective criteria or observational data to enhance the validity of findings. Furthermore, the study's cross-sectional design should be cautious about causal inferences. Therefore, longitudinal and/or interventional studies are necessary in the future to determine the sequence and potential causal relationships between variables.

Conclusion

Overall, the findings from this research provide valuable insights into previously unexplored associations between psychological variables and disordered eating behaviors in women with overweight and obesity. The significant correlational and predictive relationships identified in this study highlight the necessity of considering these factors to better understand the eating behaviors of this population. It is recommended that clinical weight management programs focus on enhancing self-efficacy beliefs among these women. Moreover, self-compassion-based therapies could play a crucial role in helping value themselves and adopt healthier behaviors for effective weight management. Finally, further research is recomended to investigate these findings and explore the potential mechanisms underlying these relationships.

Declarations

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Conflicts of Interest

The authors declare that they have no competing interests.

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Ethical Considerations

The study assessed informed consent of participants, mentioned the research purpose, emphasized confidentiality and anonymity of question page, and expressed gratitude for participation. It received ethical approval from the Research Vice-Chancellor of the Islamic Azad University of Karaj, Iran (Code: IR.IAU.K.REC.1398.082).

Authors Contribution

Nazli Tavakoli: writing – original draft, Writing – review & editing. **Mehdi Manouchehri:** Writing – review & editing, Supervision, Conceptualization. **MohammadReza Seyrafi:** Writing –review & editing, Supervision, Conceptualization. **AbdolReza Norouzy:** Writing – review & editing, Supervision, Resources. **GholamReza Sarami:** Supervision.

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A Study of Endurance Exercise Type, Citrus Aurantium (CA) Supplementation and Their Interactive Effects on the Antioxidant Status, CRP, and AngII of Cardiac Tissue in Elderly Rats

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Training
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ABSTRACT

Introduction: The current study was aimed at determining the interactive effects of two training types of HIIT and MICT along with citrus aurantium supplementation on the antioxidant status, CRP and AngII of cardiac tissue in elderly rats.

Methods: Thirty-five elderly female Sprague-Dawley rats with the mean age of 14 months and mean weight of 270-320 g were divided into 7 groups of 5 animals, including High-Intensity Interval Training (HIIT), Moderate-Intensity Continuous Training (MICT), High-Intensity Interval Training and Citrus Aurantium (HIIT+CA), Moderate-Intensity Continuous Training and Citrus Aurantium (MICT+CA), Citrus Aurantium (CA), Sham (Normal Saline), and Control. HIIT was conducted at 85-110%Vo2max and MICT was performed at 65-75%Vo2max. 300 mg/kg/day CA was injected peritoneally into each rat. HIIT, MICT, and CA supplementation protocols were performed before the statistical test. SOD, CAT, GPX activity levels (by ELISA), and CRP and AngII gene expression levels (by Real-time PCR) were measured in cardiac tissue.

Results: Training, CA, and training+CA significantly increased SOD (P≤0.05), and CAT (P≤0.05) activity levels and reduced GPX (P≤0.05) activity levels. Also, training and CA significantly increased AngII gene expression levels (P≤0.05).

Conclusion: The results showed that training and CA supplementation instigated a significant change in SOD, CAT, and GPX activity levels. Thus, it is suggested that HIIT, MICT, and CA should be used in order to improve the antioxidant status in elderly conditions. Also, the interactive combination of Training and CA seems to have a better effect on the antioxidant status in the elderly.

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Introduction

The process of aging comprises a decline in a set of functions. In this process, a set of cellular and molecular mechanisms (including excessive oxidative stress and low-grade chronic inflammation), take place that follow the aging process of cardiovascular function (1). the The strongest risk factors concerning coronary artery disease (CAD) include age (2) and gender (3). Older women are more subject to certain problems related to heart disease. According to the American Heart Association (AHA) (2019), the incidence of cardiovascular diseases (CVD) was 77.2% in men and 78.2% in women from ages 60 to 79 years. On the other hand, in adults above 80 years of age, the incidence of CVD was 89.3% in men and 91.8% in women (2). Nonethless, the CVD risks increase with age in both men and women; these risks correspond to a general decrease in sex hormones, mainly estrogen and testosterone (2).

Estrogen and testosterone are often recognized for their cardioprotective role. In a study, low levels of testosterone were found to be associated with CAD in postmenopausal women (2). In another study, it was reported that men may develop heart disease 10–15 years earlier than women due to the gradual decline in estrogen levels after puberty. On the contrary, men of 70 years of age have lower total cardiovascular risk as compared with women at

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age 50. The average age of menopause in women, which is a strong indicator of estrogen decline, has a greater effect on CVD risks in women than in men. Moreover, the risk for CVD rises vividly by as much as 2–4 times in women at the onset of menopause (2).

Since the Renin Angiotensin System (RAS) is an important regulator of cardiovascular, alterations in this system (RAS) are tightly linked to CVD (4). In many CVDs, the role of the renin-angiotensin-aldosterone system (RAAS) has been recognized. The octapeptide angiotensin (AngII) is the major mediator of the RAAS system. Excessive AngII levels produce heart failure. Studies have revealed that AngII causes an inflammatory phenotype in the heart, resulting in cellular hypertrophy and enhanced deposition of matrix proteins. Besides, it has been shown that AngII inhibition prevents cardiac hypertrophy and fibrosis. Cardiac side effects appear to be facilitated by the AngII receptor type1 (AT1) (5). Activation of the AT1 receptor stimulates vascular contraction, vascular cell hypertrophy and hyperplasia, sodium retention, ROSs production, and induction of inflammatory, thrombotic, and fibrotic processes (4). Exercise interacts with Angiotensin Converting Enzyme (ACE), AngII, AT1, and AngII receptor type2 (AT2) receptors in a varying manner (4). Accordingly, Silva et al., maintained that AngII levels in the renal artery of healthy rats with intrinsic hypertension decreased after 3 months of low-intensity exercise training (6). Also, in the research of Ren et al., it has been reported that sports training reduces the production of AngII through ACE downregulation and ACE2 upregulation (7).

Another marker of vascular inflammation is C-reactive protein (CRP) which is a mediator of atherosclerosis and is linked with an enhanced risk of coronary heart disease (8, 9). CRP levels have been associated with myocarditis, congestive heart failure, atherosclerotic disease, atrial fibrillation, aortic valve disease, and heart transplantation, revealing that it has an active role in the pathophysiology of CVD. Many factors including age, and gender can alter baseline CRP levels. Also, studies have confirmed that the oral sex steroid hormone estrogen may influence CRP levels in older men and women and postmenopausal women (8). Furthermore, in a meta-analysis and systematic review, Fedewa et al. reviewed the effect of exercise training on C reactive protein. It was concluded that engaging in exercise was associated with decreased levels of CRP irrespective of the individuals' age or sex (10). Also, Ihalainen et al., studied the effects of 24 weeks of combined aerobic and resistance training on inflammation markers which were performed on the same day or different days. It was revealed that training significantly rdeclined circulating CRP in the training groups (11).

Increased inflammation and oxidative stress hurt the elderly, so they should be minimized (12). On the other hand, chronic exposure to oxidative stress may result in cardiovascular disorders (13). Glutathione peroxidase (GXP), superoxide dismutase (SOD), and catalase (CAT) are antioxidant enzymes that have been implicated in the prevention of CVD, vascular diseases, atherosclerosis, hypertension, and inflammatory diseases. Their deficiency openly triggers an intensification in vascular oxidative stress along with attendant endothelial dysfunction (14). Exercise affects the production of free radicals and antioxidant capacity, which can upset the balance between the two. A lot of studies on exercise and aerobic metabolism, such as swimming or running, show an increase in the production of free radicals as well as escalated activity of antioxidant enzymes such as GPX, SOD, and CAT (15). Souissi et al., reviewed the effect of different modalities of running exercise (i.e., continuous running exercise (CR); intermittent running exercise (15/15); intermittent running exercise (30/30)) on post-exercise oxidative stress markers in trained athletes. The researchers reported that SOD increased after CR and also remained unchanged after 15/15, and decreased after 30/30. In all experimental sessions, GPX did not change after exercise (15). Also, Accattato et al., reviewed the effects of acute physical exercise on oxidative stress and inflammatory status in young, sedentary obese subjects (normal weight; overweight to moderate obesity; severe obesity). The researchers reported that SOD and GPX levels increased in the normal weight and severe obesity groups, and decreased in the overweight to moderate obesity group, but these changes were not statistically significant (16). In another study, Bouzid et al., conducted a study on four groups (young sedentary (YS), young active (YA), old sedentary (OS), and old active (OA)). Resting SOD activities were higher in YA in comparison with OA. After exercise, a significant increase in

SOD and GPX activities was observed in YS, YA and OA (17). However, Bessa et al., conducted a study on nineteen male athletes who carried out a combination of high-intensity aerobic and anaerobic training exercises. The researchers reported that SOD levels remained fixed across all-time points. In response to exercise, CAT activity increased at 3 and 24 hours after exercise (18). In addition, González-Bartholin et al., in a study of eight male and two female healthy older adults reported that GPX activity increased after all exercises. The subjects of the study performed 30 min of moderate-intensity concentric (CONC-M: 50% maximum power output; POmax) and eccentric cycling (ECC-M: 50% POmax) and high-intensity eccentric cycling (ECC-H: 100% POmax) (12). Chaki et al. reviewed the extent of high-intensity exercise-induced oxidative stress in sedentary pre- and post-pubertal boys. Participants were sixty-four sedentary pre-pubertal (n=32) and post-pubertal (n=32) boys who performed incremental treadmill running exercise at 80 percent of the age-predicted maximum heart rate till volitional exhaustion. SOD and CAT activities increased in both groups following exercise (13). Pal et al., reviewed the high-intensity exercise-induced oxidative stress of 44 sedentary postpubertal boys and girls. The results showed that CAT activity increased significantly in both groups after exercise (19).

Acute exercises increase the level of oxidative stress (12). Such oxidative stress induced by exercise is associated with a parallel increase in the activity of enzymatic antioxidants in the body (13). Dietary supplements with antioxidants may be helpful in this situation. Exogenous and endogenous antioxidants reduce oxidative stress (20). Citrus aurantium Linné (CA), is also known as Seville orange, marmalade orange, or sour orange (21). It is rich in vitamin C, flavonoids, and volatile essential oils (22). Fruits, flowers, essential oils, and plant ingredients of this plant have several biological potentials, such as antimicrobial, antioxidant, anti-cytotoxic, antianxiety, anti-diabetic, anti-obesity, and anti-inflammatory effects (23).

Aerobic exercise training has become an important part of cardiac rehabilitation. Two common exercise strategies are HIIT and MICT training. HIIT versus MICT is more effective in improving the cardiovascular risk profile and requires lower exercise duration. Also, through a single exercise session, a higher amount of work is done at a higher intensity, which is accomplished by alternating HIIT with low-intensity exercise or rest intervals (24-26). Considering the previous research studies conducted by the researchers, in most of the studies, CA and Training have been investigated separately on the variables of the current research. In this vein, the present study attempted to examine the interactive effects of CA and Training. In case either factor (CA or Training) can strengthen another one, either protocol may be used alone provided that there is no CA sensitivity and Training restriction. Accordingly, one purpose of the present study is to fill the gap between the studies that have been conducted so far. In addition, the present study can verify and support the findings of the previously conducted studies. Therefore, the present study aimed to determine the interactive effect of HIIT, MICT, and CA consumption on the antioxidant status, CRP, and AngII of cardiac tissue in elderly rats.

Materials & Methods

In this experimental study, Thirty-five elderly female Sprague-Dawley rats with the mean age of 14 months and mean weight of 270-320 g were divided into 7 groups of 5 animals, including High-Intensity Interval Training (HIIT), Moderate-Intensity Continuous Training (MICT), High-Intensity Interval Training and Citrus Aurantium (HIIT+CA), Moderate-Intensity Continuous Training and Citrus Aurantium (MICT+CA), Citrus Aurantium (CA), Sham (Normal Saline), and Control. HIIT+CA, MICT+CA, and CA groups received 300 mg/kg/day of the hydroalcoholic extract of CA (6 days a week except Fridays/ 10 am) peritoneally one hour after finishing the training (27, 28).

Citrus Aurantium Supplement

For the preparation of the hydroalcoholic extract of Citrus aurantium, first, the Citrus Aurantium plant was prepared from Jahade Daneshgahi Center and was approved by a botanist. Then the plants were milled, and their extracts were prepared by the percolation method. 90% ethanol solvent was used to prepare the extract (29).

Extract Preparation Method

To use CA extract, daily, 2.4 grams of CA extract was dissolved in 7.2 ml of normal saline, and 300 mg/kg/day/weight of CA extract was injected into rats peritoneally. The reason for peritoneal administration was adaptation of this method to previous studies (27, 28).

Endurance Exercise Protocol

First, the maximum velocity was evaluated to determine the maximum oxygen consumption in all rats (to plan for eight weeks of HIIT and MICT training). To determine the maximum oxygen consumption, the standard increasing test of Bedford1 et al., was used (30), which was standardized by Leandro et al., for rats (31). This test consists of 10 stages of three minutes. The speed in the first stage is 0.3 km/h and in the next stages, 0.3 km/h should be added to the speed of the turntable, while the slope is zero in all stages. In each stage of the test when the animal can no longer continue the work, the speed at that stage is considered equivalent to the speed of the animal at the maximum oxygen consumption.

HIIT and MICT training (8 weeks of HIIT and MICT training/ 3 sessions per week) were performed in such a way that at the onset of each session, the warm-up phase was performed, which included running for 3 minutes at an intensity of 10 m/min. Following that, the HIIT groups trained with an intensity of 85 to 90%VO2 max, equal to 7 attempts in 1 minute and a speed of 31 m/min, and active rest between intervals of 6 attempts and a speed of 15 m/min in the first week. With an average increase of 2 m/min per week, the attempts gradually reached 10 1-min attempts at a speed of 55 m/min, intensity 110%VO2max, and finally, active rest reached with 9 1-min attempts (between intervals) at a speed of 25 m/min in the eighth week. In fact, the HIIT protocol consisted of three parts: warm-up (3 min), exercise including 1-min interval repetitions (1x1) (with a 1-min active rest period between each interval), and cool-down (5 min) (Figure1). Meanwhile, the MICT group trained with an intensity of 65%VO2max, which started with a speed of 20 m/min and duration of 15 minutes in the first week, which gradually reached a speed of 25 m/min, at the intensity of 75% VO2max, and the duration of 31 minutes in the eighth week. The training protocol began with warming up for 3 minutes (with an intensity of 10 meters per minute) and 2 minutes (with an intensity of 15 meters) and then cooling up for 1 minute (with an intensity of 15 m/min) and 2 minutes (with an intensity of 10 m/min) (Figure2) (32).

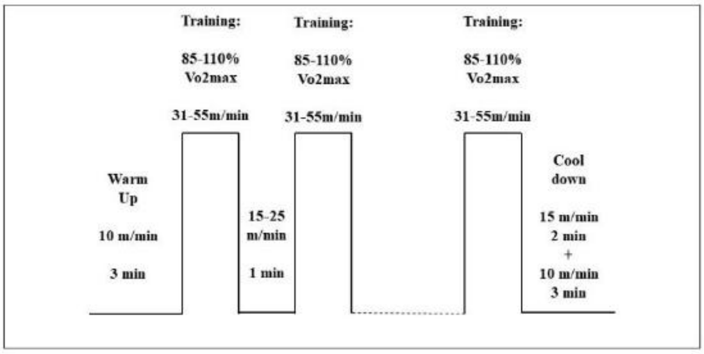

Figure1. Schematic view of the HIIT protocol

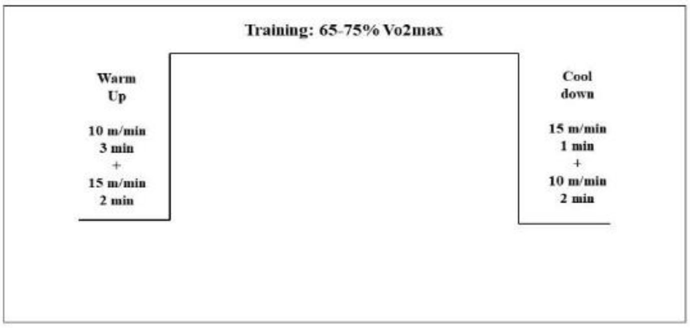

Figure2. Schematic view of the MICT protocol

Dissection and Histology

48 hours after the last training session and after 10 hours of fasting, the rats were anesthetized with ketamine and xylazine (32) (ketamine and xylazine were mixed at a ratio of 3:1 and injected intraperitoneally into the rats). After ensuring the complete anesthesia rats through the pain test with pressure on the soles of the rats' feet and in a state of complete analgesia, the chest of the rats was carefully split open, and the cardiac tissue was extracted by laboratory specialists, and immediately after weighing and washing with normal saline, it was placed in special tissue storage microtubes, and in the shortest possible time after immersion in a nitrogen tank, it was transferred to a temperature of -80 and for further measurements to measure SOD, GPX, CAT, CRP, and AngII were sent to molecular cell laboratory.

The Activity of Antioxidant Enzymes

SOD, GPX, and CAT tissue activity levels were assayed using commercial standard kits (Nasdox™, Nagpix™, and Nactaz™ Navand Salamat Company, Urmia, Iran). The absorbance rates were recorded by an FLUO star OMEGA microplate reader and software (BMG Labtech Ltd., Aylesbury, UK) at 405, 340, and 550 nm for SOD, GPX, and CAT, respectively. The results of SOD, GPX, and CAT are reported in U/mg protein, nmol/mg, and nmol/mg protein, respectively (33). For SOD activity, the reduction of color development at 405 nm was used to determine SOD activity, which is considered an inhibitory activity. An absorbance rate of 550 nm was considered to determine CAT after incubation for 10 min at room temperature. The protein content was assayed based on the Bradford method (34) by a commercial standard kit (Nadford™, Navand Salamat, Iran).

RNA Isolation and Real-Time PCR Analysis

Total RNA was isolated from the cardiac tissue using an RNA extraction kit (FavorPrep™ Tissue Total RNA Mini Kit, Taiwan). The purity, integrity, and concentration of RNA were determined by measuring the optical density 260/280 and agarose gel (1%) electrophoresis. Complementary DNA (cDNA) was synthesized from 1 µg of RNA using RevertAid™ first strand cDNA synthesis kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Real-time PCR was performed according to the protocol of RealQ Plus 2x Master Mix Green (Ampliqon Inc.) in applied Biosystems StepOne™ Instrument (ABI, Step One, USA). Real-time PCR for expression analysis of the primer pairs for AngII, Crp, and β2m was designed, as shown in Table 1. The β2m housekeeping gene was also used as the internal control of real-time PCR reactions. The real-time PCR conditions were set for 10 minutes at 94°C followed by 40 cycles of 15 seconds at 94°C, 60 seconds at 60°C, and extension steps. After each real-time PCR run, melting curve analysis was carried out to confirm the specific amplification of targets. The amplification signals of different samples were normalized to β2m Ct (cycle threshold), and then the delta-delta CT ($2^{-\Delta\Delta CT}$) method (Livak and Schmittgen, 2001) was applied for comparing mRNA levels of test versus control, which represented a fold change in data analysis (35).

Table 1. Sequence of primers used in the present study

Genes	Primer Sequences	Sizes (bp)
B2m	Forward:5- CGTGCTTGCCATTCAGAAA -3 Reverse:5-ATATACATCGGTCTCGGTGG -3	244
AngII	Forward:5- GCTGGAGCTAAAGGACACACA-3 Reverse:5- GATGTATACGCGGTCCCCAG-3	130
Crp	Forward:5- TCAGGCTTTTGGTCATGAAGACAT-3 Reverse:5- GACTCTGCTTCCAGGGACAC-3	90

H&E Staining Analysis

Pathological evaluation was performed to evaluate the heart tissue. The heart tissues were excised, washed with ice-cold phosphate buffer saline (PBS), and fixed in 10 % formol saline for 24 hours, which was followed by dehydration at increasing concentrations of ethanol, clearing with xylene, and embedding with paraffin.

Specimens for 4 µm sections were prepared from paraffin blocks. The sections were stained with a hematoxylin and eosin stain and were examined using Olympus light microscopy at ×400 magnification.

Ethical Considerations

The training protocol was performed by international guidelines and agreements for the care and use of laboratory animals. All the ethical and legal aspects of this research were done with the license number IR.IAU.M.REC.1400.003 of Islamic Azad University, Marvdasht Branch. These animals were maintained in the laboratory for one week to adapt to the environment. The standard conditions, including a standard temperature of 22-24°C, a humidity of 55-60%, a light-dark cycle of 12/12 hours, and free access to water and special food for rats were observed. In compatibility with the Declaration of Helsinki to preserve the life and comfort of laboratory animals, the minimum sample of the study for the implementation and obtaining a reliable conclusion was selected. Also, during the study period, appropriate living conditions and feeding were provided. The conditions of transportation, maintenance, and appropriate feeding for research were met. Besides, to comply with the ethical principles, the minimal appropriate doses of ketamine and xylazine solutions were injected, and the surgery was conducted by an expert in the field.

Statistical Analysis

To determine the normal distribution of the data, the Shapiro-Wilk test was used. Due to the fact that there were two independent variables in the present study and the distribution of data was normal, two-way ANOVA test was used to investigate the effect of training and CA as well as their interactive effect. The independent t-test, two-way analysis of variance (ANOVA), and Bonferroni post-hoc test were used to make between-group comparison. The results were analyzed using SPSS software version 22 at a significant level of $P<0.05$.

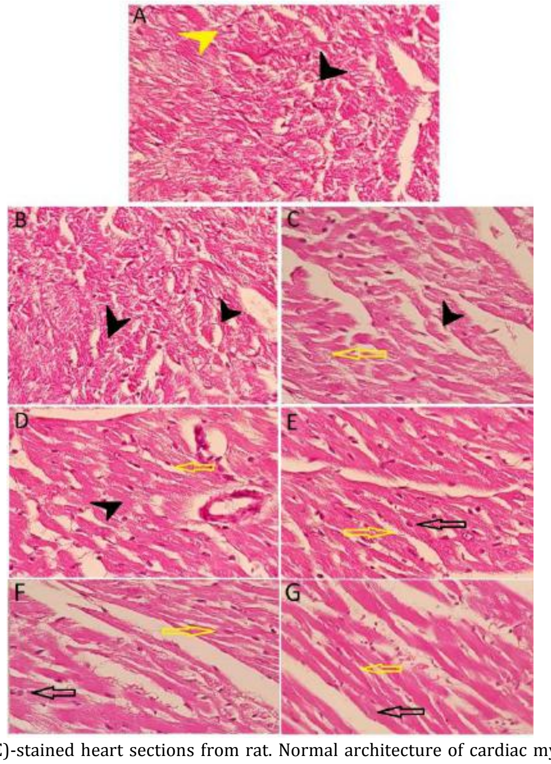

Figure 3. Hematoxylin and eosin (H&E)-stained heart sections from rat. Normal architecture of cardiac myocytes (Yellow arrow) with centrally placed nuclei (Black arrow). Myocyte necrosis with loss of cross striations and loss of nuclei (Black Arrowhead) and the nuclei of cardiac myocytes showed changes in the form of pyknosis (Yellow Arrowhead); (In total, normal cells were indicated with arrows and abnormal cells with arrows) (HF&E, ×400). The results showed that the abnormal cardiac myocytes decreased in training CA groups. This improvement is much greater in the MICT+CA and HIIT+CA groups. (A) Control group. (B) Sham group. (C) CA group. (D) MICT group. (E) HIIT group. (F) MICT+CA group. (G) HIIT+CA group.

Results

H&E staining Analysis

Histological changes were assessed by a pathologist unaware of the type of practice and treatment (Figure 3).

On hematoxylin and eosin staining, the area of myocardial infarction can be easily recognized by viewing the typical areas of necrosis when compared to the surrounding border zone and the healthy myocardium. In detail, classical myocardial morphology loss, necrotic cell death with the loss of the nuclei, as well as the complete loss of entire muscle fibers can be observed. The results showed that the myocardial infarction index decreased in training CA groups. This improvement is much greater in the MICT+CA and HIIT+CA groups.

SOD, GPX, and CAT activity levels as well as CRP and AngII gene expression are shown in Figures 4-8, respectively. The results of two-way ANOVA showed that training (P=0.001), CA (P=0.001), and the interactive effects of training+CA (P=0.001) had a significant effect on SOD activity levels in the elderly female rats.

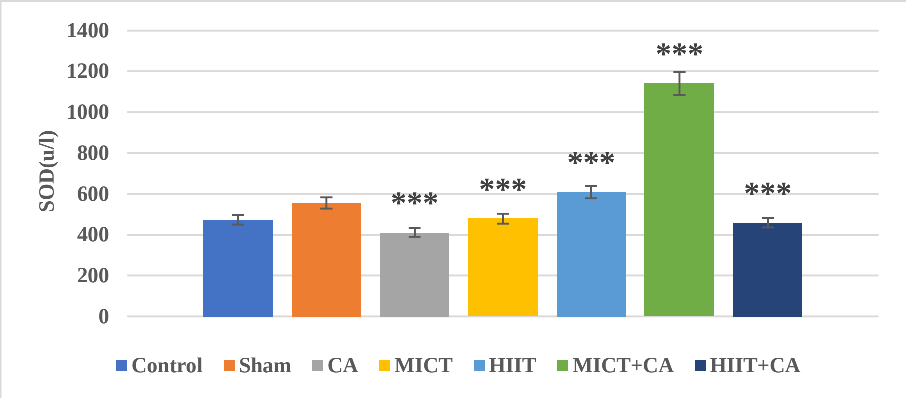

Figure 4. Levels of activity of SOD change in different study groups.
***Significant effect at P≤0.001.

The results also showed that training+CA (Eta=0.896) had more effects than training (Eta=0.853) and CA (Eta=0.547) (Figure 4). The results of Benferroni's post-hoc test showed that there was a significant difference between HIIT and MICT groups on SOD activity levels (P=0.001), and both HIIT (P=0.045) and MICT (P=0.001) triggered a significant increase in SOD activity levels. Also, the results showed that MICT training had more effects on increasing the levels of activity of SOD than HIIT.

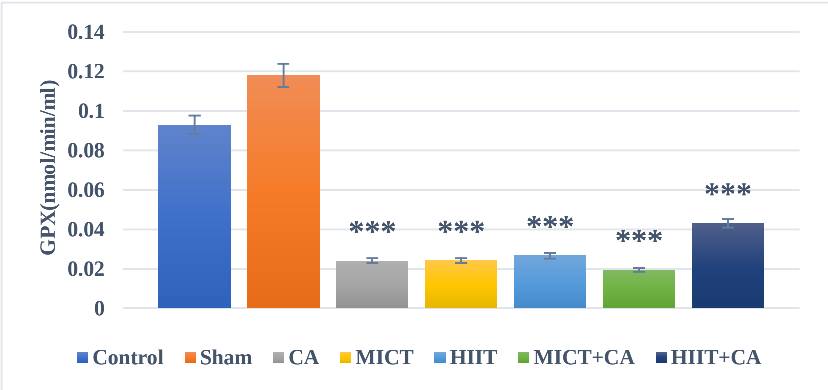

Figure 5. Levels of activity of GPX change in different study groups.
***Significant effect at P≤0.001.

The results of two-way ANOVA showed that training (P=0.001), CA (P=0.001), and also the interactive effects of training+CA (P=0.001) had a significant effect on GPX activity levels in the elderly female rats. Also, it was revealed that training+CA (Eta=0.610) had more effects than training (Eta=0.532) and CA (Eta=0.304) (Figure 5). The results of Benferroni's post-hoc test indicated no significant difference between HIIT and MICT groups on GPX activity levels (P=0.390). Both HIIT (P=0.002) and MICT (P=0.001) brought about a significant decline in GPX activity levels. Also, the results showed that HIIT had more effects on levels of activity of GPX than MICT.

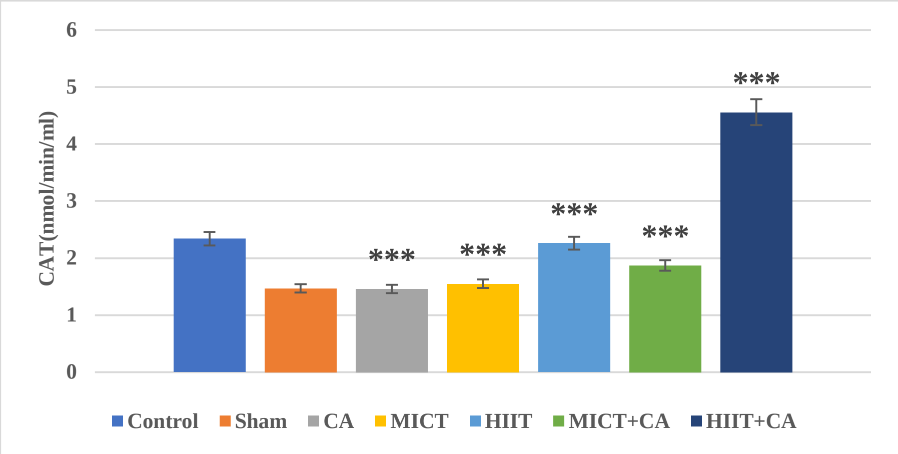

Figure 6. Levels of activity of CAT change in different study groups.
***Significant effect at P≤0.001.

The results of two-way ANOVA showed that training (P=0.001), CA (P=0.001) as well as the interactive effects of training+CA (P=0.001), had a significant effect on CAT activity levels in the elderly female rats. The results also showed that training (Eta=0.668) had more effects than Training+CA (Eta=0.519) and CA (Eta=0.299) (Figure 6). The results of Benferroni's post-hoc test revealed a significant difference between the HIIT and MICT groups on CAT activity levels (P=0.001). While HIIT (P=0.001) caused a significant increase in CAT activity levels, MICT (P=0.001) caused an increase in CAT activity levels which was not statistically significant. The results also showed that HIIT has more effects on increasing CAT CAT activity levels than MIC.

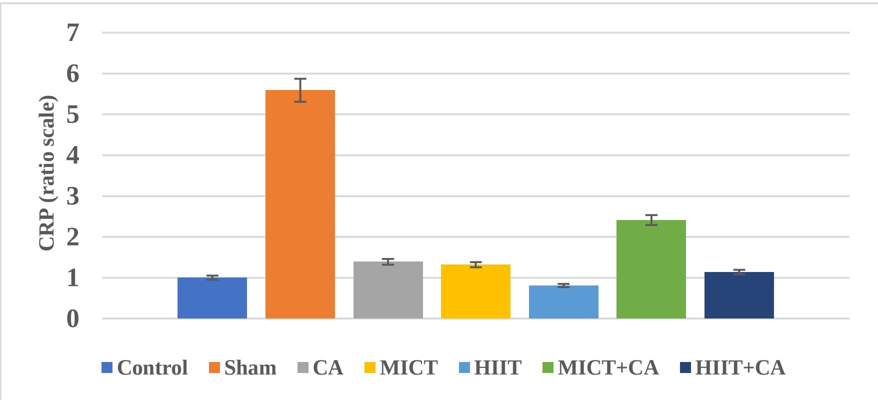

Figure 7. Levels of CRP gene expression change in different study groups.

The results of two-way ANOVA showed that training (P=0.125), CA (P=0.768), as well as the interactive effects of Training+CA (P=0.067) had no significant effect on CRP gene expression in the elderly female rats (Figure 7).
The results of two-way ANOVA showed that training (P=0.001) and CA (P=0.007) had a significant effect on AngII gene expression in the elderly female rats. However, the interactive effects of training+CA (P=0.100) had no effect on AngII gene expression in the elderly rats. In addition, based on the resulrs, training (Eta=0.663) had more effects than CA

(Eta=0.227) and training+CA (Eta=0.147) (Figure 8).

According to Benferroni's post hoc test, there was a significant difference between the HIIT and MICT groups on AngII gene expression levels (P=0.001). While HIIT (P=0.001) triggered a significant increase in AngII gene expression, MICT (P=0.762) triggered an increase in AngII gene expression, though this increase was not statistically significant. The results also showed that MICT had more effects on AngII gene expression than HIIT.

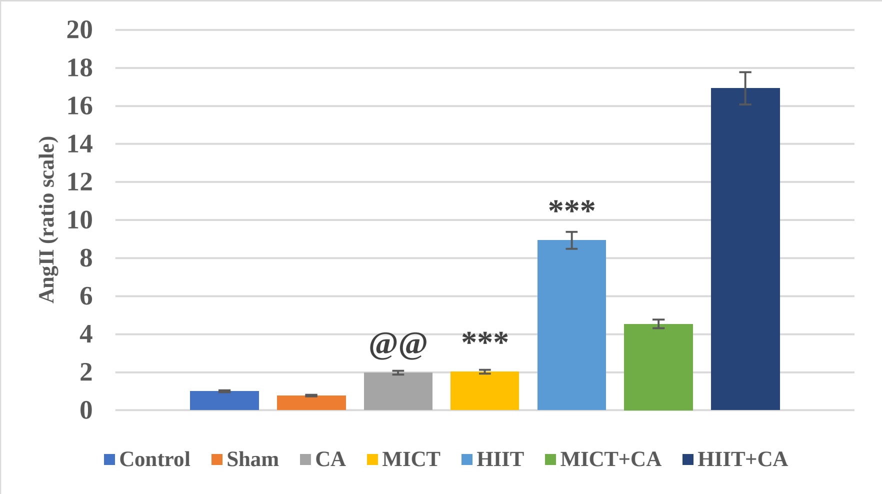

Figure 8. Levels of AngII gene expression change in different study groups.
***Significant effect at P≤0.001.
@@Significant effect at P≤0.001.

Discussion

Based on the findings of this research, Training, CA, and Training+CA significantly increased SOD activity levels. The results showed that Training+CA compared to Training and CA, as well as MICT compared to HIIT had more increasing effects on SOD activity levels. This finding is in line with the results of the research (15-17) that revealed enhanced SOD antioxidant enzyme activity after exercise, nevertheless, it is inconsistent with the results of the studies (16, 18) that reported unchanged or decreased SOD activity levels. Previous studies reported that oxidative stress parameters were associated with various running conditions (aerobic, anaerobic, intermittent, and continuous) and a rise in the activity of antioxidant enzymes such as SOD, GPX, and CAT (12, 15-18). A major finding of this study was that following HIIT, MICT training, and CA supplementation, the oxidative stress parameters (SOD, GPX, CAT) presented different responses depending on the training and supplementation conditions. Continuous running appears to be a prominent inducer of superoxide (-O2) radical production. Consequently, SOD activity levels, as the forefront of antioxidant defense, will be augmented to guard against the damaging effects of O2 radicals. After intermittent exercise, the balance between free radicals and antioxidants appears to be established, and most of the O2 radicals created are unceasingly neutralized by existing antioxidant defenses (15).

Training leads to an increase in AMP, intracellular calcium concentration, and an increase in ROS. These changes trigger the activation of some intracellular signaling pathways, including calmodulin calcium-dependent protein, AMPK, and p38-AMPK, which play an essential role in the upregulation of PGC-1a activity followed by mitochondrial biogenesis; AMPK with a change in NAD^+ to NADH ratio accelerates the process of mitochondrial biogenesis (36, 37). With increasing age, AMPK levels decrease significantly and can play an important role in reducing PGC-1a (38). Increasing access to nitric oxide (NO) caused by shear stress is another important mechanism for explaining the protective role of exercise against endothelial dysfunction (39, 40). With NO production and vasodilation, blood circulation improves during exercise, and this can cause a decrease in FR production and, as a result, a decrease in the oxidative stress marker. NO bioavailability occurs through the balance between the amount of enzymatic and non-enzymatic NO production and on the other hand the elimination of NO, in reaction to ROS. In addition, it depends on the extent of its formation through mitochondria or enzymes such as NAD(P)H oxidase (NOX) and Xanthioxidase (XO) and the extent of ROS elimination through the antioxidant defense system or other reactions (41). However, the effect of exercise depends on the type, intensity, and duration of training, and the mechanism of the type of training on this molecular cellular mechanism is still not well known (42).

Recently, researchers have attracted their attention to the use of natural antioxidants and medicinal plants in addition to physical activities (43). Among the antioxidant plants that have nutritional and medicinal properties, we can mention CA (44). Synephrine-p, ephedrine, salicin, and caffeine are among the main effective substances in CA, which have the most biological effects in this plant. Also, this medicinal plant can have favorable effects on the cardiovascular system by improving the function of prostaglandin F2a. This natural antioxidant can reduce ROS, increase NO enzyme, activate guanylyl cyclase, inhibit extracellular dependent $Ca2^{+}$, adjust K^{+} and its adaptations in calcium-potassium channels, and also improve the function of the ryanodine receptor in the heart (45). It also appears that CA plays a role in the phosphorylation of PGC-1a through the mechanism of AMPK activation, but this mechanism depends on the dose and duration of consumption (46). In the previous research, the beneficial and positive effects of HIIT, MICT and CA supplements on the elderly and cardiovascular patients have been well demonstrated. However, it seems that the combination of training and CA has more synergistic effects than training and CA alone on increasing SOD. In addition, MICT has greater effects on increasing SOD than HIIT.

The present study showed that Training, CA, and Training+CA reduced GPX activity levels significantly. It was also revealed that Training+CA in comparison with Training and CA, as well as HIIT in comparison with MICT had more effects on GPX activity levels. This finding is consistent with Accattato et al.'s, study (16), yet it is inconsistent with Wajedi Souissi et al., and some other researchers, who showed that GPX activity did not respond to exercise stress, remained constant or increased (12, 15-17, 47). In their research, Accattato et al., examined the effect of exercise training on three groups of people with normal weight, moderate obesity, and severe obesity. The results showed that GPX levels decreased after exercise in the group of people with moderate obesity, which is in line with the results of the current research. Also, researchers in their study reported that after exercise, GPX levels increased in the groups of people with normal weight as well as severe obesity, but this increase was not statistically significant, which is inconsistent with our results (16). Wajedi Souissi et al., investigated the effects of different running posture modes on post-exercise oxidative stress markers in skilled athletes. The researchers reported that GPX did not change in all experimental periods (15).

CA leads to the neutralization of ROSs. Ephedrines present in CA mostly affect the AMPK/PGC-1a axis (48), therefore the effect of ephedrine interacts with the molecular cellular adaptations of catecholamines and can yield to augmented capacity of total antioxidant, expression of the electron transport chain family proteins and rate of oxidative phosphorylation in the heart tissue (49). In addition, in line with exercise, flavonoids are able to remove free radicals and have a protective effect against lipid peroxidation; they can also reduce endothelial NO metabolism, which leads to the production of NO radicals, and modulate the activity of NADPH oxidase (50). Thus, antioxidant mechanisms of flavonoids comprise 1) suppressing the formation of ROSs by inhibiting the enzymes involved in their production; 2) eliminating ROSs, and 3) regulating and protecting the antioxidant defense systems (50). Considering the levels of SOD after exercise in the present study, it seems that most of the O2 radicals created after exercise are neutralized by SOD in the forefront of antioxidant defense. The result is that just a small amount of H2O2 radical is created, which explains the decline in GPX activity (15) following HIIT, MICT training, and CA supplementation.

Training, CA, and Training+CA significantly increased CAT activity levels in this study. The results showed that Training compared to Training+CA and CA, as well as HIIT compared to MICT had greater effects on the increase in levels of activity of CAT. This suggests that exercise stress can regulate the activity levels of this antioxidant gene, which copes with high-intensity hydrogen peroxide produced during exercise. These findings could be consistent with previous studies indicating that CAT activity levels respond to exercise stress (13, 19). However, in terms of the percentage of difference in CAT activity levels after exercise, a significant difference was seen between the study groups, so that CAT enzyme activity levels significantly changed in all groups of rats.

CA causes PGC-1a phosphorylation by the mechanisms of improving prostaglandins, increasing antioxidants, regulating ion channels (45), and activating AMPK (46). HIIT is likely to have a more favorable effect than MICT training due to its adaptability, which challenges the oxidative stress system and leads to the activation of the FOXO3a pathway (51). Therefore, it seems that following HIIT along with CA consumption, the FOXO3a cellular redox pathway is simultaneously activated, but MICT with CA consumption contributes to mitochondrial biogenesis by the mechanism of increasing AMPK phosphorylation (45, 46, 52). Considering the positive and antioxidant effects of HIIT, MICT, and CA supplements, some of which were mentioned above, the increase in CAT in HIIT, MICT, and CA supplementation protocols is justified and not far from expected.

Training, CA, and Training+CA significantly reduced CRP gene expression levels, but this reduction was not statistically significant. Our finding was in line with previous studies that showed that CRP gene expression levels respond to exercise in a decreasing manner (10, 11). Many factors, including age, sex, weight, and fat levels, can alter baseline CRP levels (8). CRP is an acute-phase homopentamer inflammatory protein that increases its expression in inflammatory conditions such as some cardiovascular diseases and infections. Based on evidence, CRP is not merely an inflammation or infection marker; rather, it is an important regulator in the process of inflammation. Complement pathway, phagocytosis, apoptosis, NO release, and cytokine production are major areas of inflammation and host response to infection that are modulated by CRP.

Exercise training can improve CRP levels probably through several mechanisms. Performing regular exercise directly by enhancing the secretion of NO from the endothelial tissue leads to the improvement of endothelial function and the increase of antioxidant factors, which results in the reduction of general inflammation and as a result the reduction of inflammatory cytokines of the endothelial system smooth muscles (53). The reduction of the pro-inflammatory CRP marker following training in the current study can lead to a reduction in the release of chemical mediators and a decline in pro-inflammatory transcription factors, such as NF-kß, and so play a role in modulating vascular inflammation (54). In addition, various studies have demonstrated that the increase of ROS activates the NF-kB pathway. Evidence has shown that phenolic extract of CA (TPE-CA) can inhibit MAPK and NF-κB signaling pathways, thereby reducing inflammatory symptoms and acting as an anti-inflammatory (55). Therefore, any intervention that leads to the reduction of this marker can be effective in preventing cardiovascular problems or contributing to the treatment of cardiovascular diseases. Considering the findings of the present research, the implementation of HIIT, MICT, and CA supplementation can reduce CRP levels in elderly rats.

Regarding the results of the present research, training and CA elevated AngII gene expression levels significantly; also, Training+CA increased AngII gene expression levels, but this increase was not statistically significant. In addition, training compared to CA and training+CA, as well as MICT compared to HIIT had more effects on AngII gene expression. These findings contradict previous studies showing that levels of AngII gene expression respond to exercise in a reduced manner (6, 7). Silva et al., reported that AngII levels in the renal artery of healthy rats with intrinsic hypertension decreased after 3 months of low-intensity exercise training (6). In the research of Ren et al., it has been reported that sports training reduces the production of AngII through ACE downregulation and ACE2 upregulation (7). RAS proteins are involved in many types of tissue mechanisms, cellular aging, and longevity. AngII is a determining factor in this process, which increases oxidative stress and inflammation through the AT1 receptor. One of the reasons for this inconsistency is the difference in the type, intensity, and duration of training. Considering the type, intensity, and duration of training, a wide range of changes are created in the body. High and moderate intensity training can increase free radicals production and inflammation, which in turn elevate AngII levels .

Training can have beneficial effects on AngII levels in several ways. The first effect is a decrease in the levels of AT1 and AT2 receptors (56). Inhibition of AngII/AT1 prevents the production of ROS. As stated earlier, ROS contributes to the production of inflammation and deformation of vascular structure through activating transcription factors NF-KB, MCP-1, and IL-1 (57). Also, the inhibition of the AT1 receptor allows for inhibition of Profilin-1 and PKC, therefore controlling and inhibiting the downstream pathways of Profilin-1, namely ERK/MAPK, and PKC, namely, JAK/STAT, respectively, which directly affect the vessel's smooth muscle cells (57).

By performing exercise training in one session, free radicals in the body increase, but gradually by creating adaptation, the body's antioxidant/antioxidant defense is strengthened as well. Therefore, after participating in a rehabilitation training session, the amount of free radicals scavenged by the body's antioxidant enzymes increases. This ability of the body is able to reduce the effects of ROSs in the production of AngII, so the plasma levels of AngII decrease (58). Another adaptation of regular exercise training is the increase in plasma and blood hematocrit levels, and because the secretion of AngII is somewhat influenced by changes in plasma levels, therefore, in a trained body, less fluctuations in plasma levels can lead to less secretion of AngII (59). The possible cellular mechanisms that develop in adaptation to aerobic training may affect the sympatho-stimulatory process by reducing oxidative stress and increasing NO (60). Participation in HIIT or MICT, through triggering shear stress and nitric oxide production (eNOS), oxidative defense enzymes including SOD, improving vascular endothelium functional disorder as well as reducing O2- levels and pro-inflammatory cytokines such as TNF-a and IL-6 can result in declined levels of AngII and reduced levels of vasoconstrictor response induced by this vasoconstrictor (56, 61).

A major limitation of this study is not having access to the western blot tool. Considering that the measurement of the variables of the present study by the western blot method can confirm the results of this research, it is recommended that in the analogous studies, changes in the variables of this study should be investigated by the western blot method. The lack of access to human subjects is another limitation of this study. Still again, another limitation is the lack of measurement of CRP and AngII activity, which can be attributed to the absence of research funding. Moreover, the present study lacks administering a pre-test between groups at the onset of the study to confirm the absence of differences before the training intervention due to financial shortages; hence in citing the absence of differences before the intervention, the researchers sufficed to deal only with the control group. Still, another restriction of this study is the lack of investigation of the effect of long-term activities and follow-ups. So it is suggested that in the following studies, researchers consider the impact of several months of training and follow-up on the indicators discussed in this study. It is also suggested that the comparison of the mentioned training model with resistance training be examined more widely.

Conclusion

Exercise training can bring about beneficial changes in the antioxidant defense system and inflammation of the heart tissue. These outcomes were observed in HIIT and MICT protocols and the combination of both training methods with CA consumption. Also, it seems that the interactive combination of Training+CA in the elderly condition has a better effect on the antioxidant status, even though further studies are required to make generalization of the results to human beings and to understand the mechanism of action of their combination.

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The Effect of Training and Royal Jelly on the Expression of FoxO3a, MAFbx and AMPK of Cardiomyocytes in Obese Rats

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ABSTRACT

Introduction: Overweight and obesity increase the risk of cardiovascular diseases. Obesity is also known to contribute to heart muscle atrophy. This study investigates the effects of aerobic exercise and royal jelly (RJ) supplementation on indices of heart tissue atrophy and hypertrophy in rats fed a high-fat diet.

Methods: Forty-five male Wistar rats were randomly divided into five groups (n=9 per group): normal diet (ND), high-fat diet (HFD), high-fat diet with training (HFDT), high-fat diet with royal jelly (HFDRJ), and high-fat diet with both training and royal jelly (HFDTRJ). The supplement groups received 100 mg of royal jelly per kg of body weight orally every day. The exercise program involved running on a treadmill at an intensity of 50-60% of VO2max, performed five days a week for eight weeks. Data were analyzed using one-way ANOVA and Tukey's post hoc test with a significance level of $p<0.05$.

Results: In the HFD group, the expression of FoxO3a and MAFbx was significantly increased, while AMPK expression was decreased compared to the ND group (P=0.000). There was a significant decrease in FoxO3a and MAFbx expression in the HFDT (P=0.033 and P=0.027), HFDRJ (P=0.049 and P=0.041), and HFDTRJ (P=0.000 and P=0.000) groups compared to the HFD group. Additionally, the HFDTRJ group showed significant reductions in FoxO3a and MAFbx compared to the HFDT (P=0.045 and P=0.041) and HFDRJ (P=0.030 and P=0.027) groups. A significant increase in AMPK expression was observed in the HFDT (P=0.002), HFDRJ (P=0.007), and HFDTRJ (P=0.000) groups compared to the HFD group. Furthermore, the HFDTRJ group exhibited higher AMPK expression compared to the HFDT (P=0.048) and HFDRJ (P=0.015) groups.

Conclusions: Aerobic training, with or without the intake of royal jelly, leads to a reduction in the expression of FoxO3a and MAFbx and an increase in AMPK. These changes may result in decreased atrophy indices and enhanced hypertrophy of cardiomyocytes in rats fed a high-fat diet.

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Introduction

Obesity is an expensive condition that leads to a diminished quality of life and, ultimately, premature death. It is also a significant risk factor for many cardiovascular diseases (CVD) (1). Several mechanisms, including cytokines, biological mediators, beta-adrenergic signals, and nutritional signaling pathways, link obesity to cardiovascular diseases (2, 3). However, one of the most critical contributors to cardiovascular diseases is the breakdown of cellular proteins and the replacement of lost cells with collagen (3). The primary pathway for protein degradation involves the activation of FoxO. FoxO is the key initiator of protein degradation during the atrophy process. The FoxO3 signaling pathway plays an important role in the pathogenesis of skeletal muscle. This role is carried out by regulating E3 ubiquitin ligases and certain autophagy factors (4). FoxO3a acts as a central mediator in atherogenesis; when activated, it translocates from the cytosol to the nucleus, triggering the activation of two proteins that degrade muscle tissue: MAFbx and MuRF1, thus initiating tissue destruction (5). Relling (2006) demonstrated that obesity increases FoxO3a levels in the cardiac cells of obese rats, contributing to cardiac dysfunction and mitochondrial damage (6). Kandola (2016) also showed that the FoxO family plays a significant role in cardiomyopathy (7). In addition, Torabi et al. (2022) found that the induction of obesity in elderly rats is associated with a decrease in the levels of FoxO3a, MAFbx, and MuRF1 in cardiomyocytes (8). Interestingly, the activation of FoxO regulates the genes for ubiquitin ligases Atrogin-1/MAFbx and MuRF1, leading to cardiac wasting. The upregulation of MAFbx gene

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expression in the heart reduces both physiological and pathological hypertrophy. Furthermore, the upregulation of MuRF1 gene expression in cardiac muscles prevents hypertrophy (9). Inhibiting this process may reduce protein degradation and the incidence of cardiovascular diseases. FoxO inhibition occurs through AKT and PGC1α (10). Moreover, AMPK has been identified as a crucial factor in cardiac hypertrophy. Key processes for the initiation and progression of cardiac hypertrophy include the transcription of hypertrophy-related genes, cell growth, strengthening of the cytoskeleton, protein synthesis, and expansion of the sarcomere. These anabolic processes demand substantial energy, all of which is regulated by AMPK (2). In recent decades, exercise training, alongside diet, has been promoted as an effective non-pharmacological method for managing obesity. Exercise training has beneficial effects on the cardiovascular system by enhancing the condition of myocardial cells. It is thought that chronic exercise leads to an increase in PGC-1α in muscle tissue (11). Research has demonstrated that FoxO, a protein responsible for degrading muscle tissue, interacts with PGC1α, an essential cofactor in mitochondrial biogenesis. Elevated levels of PGC1α can inhibit this muscle protein-degrading factor (12). Liu et al. (2018) showed that moderate-intensity exercise mitigates diabetes-induced muscle atrophy in db/db mice by influencing the SIRT1-AMPKα-PGC1α axis (13). Additionally, aerobic exercise downregulates the expression of MuRF1. Nevertheless, the mechanisms by which exercise training affects atrophy and hypertrophy signaling pathways in obesity remain unclear. Produced by honeybees, royal jelly (RJ) serves as the nutritional source for queen bees and is rich in various nutrients. Certain components in RJ may stimulate muscular adaptation. In studies involving rats, RJ consumption increased serum IGF-1 levels and promoted the regeneration of damaged muscle via the IGF1-Akt pathway in satellite cells (14). Furthermore, the combination of RJ administration and endurance training led to mitochondrial adaptations by activating AMPK in the soleus muscles of rats (15). Clinical trials have also demonstrated that RJ enhances handgrip strength, which tends to decline with age (16). Additionally, RJ treatment has influenced muscle fiber size, as well as markers related to satellite cells and catabolic genes (17). One potential target for protein and/or amino acid supplementation is AMPK, a cellular energy sensor and crucial regulator of mitochondrial biogenesis. Previous studies indicated that leucine and casein peptides can activate AMPK in skeletal muscle. In addition to amino acids and protein, 10-HAD, a fatty acid found in RJ, is also capable of activating AMPK in skeletal muscle (18). Therefore, using royal jelly (RJ) to promote adaptation in skeletal muscles and cardiac tissue can be effective. While the beneficial effects of RJ on various bodily systems have been well documented, there remains insufficient research demonstrating its impact on indices of atrophy and hypertrophy in heart tissue. Conversely, obesity and being overweight may be linked to a decline in cardiac function, which can subsequently damage heart structure. As a result, the implementation of non-pharmacological treatment methods, such as exercise, alongside the use of natural supplements (in this case, RJ), could offer new therapeutic avenues due to their positive effects. This study hypothesizes that the combined application of exercise and RJ may have a more pronounced effect on the indicators of atrophy and hypertrophy in obese rats compared to either treatment alone. Consequently, this study seeks to investigate the simultaneous effects of aerobic exercise and royal jelly on specific indicators of atrophy and hypertrophy in cardiomyocytes of rats fed a high-fat diet (HFD).

Materials and Methods

Forty-five male Wistar rats, eight weeks old with an average weight of 187.51 ± 9.37 grams, were used in this study. The animals were maintained under a 12:12-hour light-dark cycle, at an ambient temperature of 22 ± 1.4°C, with humidity levels at 55.6 ± 4%. After one week of acclimatization to the new environment, the rats were divided into two groups: a normal diet group (ND, n=9) and a high-fat diet group (HFD, n=36). The ND group received a standard diet consisting of 23% protein, 65% carbohydrates, and 12% fat for eight weeks, while the HFD group was fed a high-fat diet comprising 40% fat, 43% carbohydrates, and 17% protein. After eight weeks, the rats were further categorized into five groups: ND, HFD, high-fat diet training (HFDT), high-fat diet with royal jelly (HFDRJ), and high-fat diet training with royal jelly (HFDTRJ). Obesity in the rats was assessed using Lee's index, with a value of 310 or higher indicating obesity. Rats with Lee index values exceeding 310 were classified as obese (19).

Training Protocol

Before the main training program began, the rats were given five minutes to familiarize themselves with the treadmill. During this familiarization period, they ran at a speed of 8-10 m/min on a flat surface across five sessions over one week. The aerobic exercise protocol consisted of running on a treadmill with a 0% incline, performed five days a week for eight weeks. In the first week, the rats followed an incremental exercise regimen, starting at an intensity of 15 m/min for 30 minutes. Over the following weeks, the intensity progressively increased from 15 m/min to 25 m/min by the seventh week, while the duration of the exercise also increased, reaching 60 minutes by the end of the training period (Table 1) (20).

Table 1. Training protocol

week	1	2	3	4	5	6	7	8
Intensity (m)	15	16	18	20	21	23	25	25
Time (min)	30	35	40	45	50	55	60	60

Royal Jelly Consumption

Royal jelly powder was obtained from Bulk Supplements Co, Ltd (Henderson, USA). In addition, 100mg/kg body weight of royal jelly was consumed daily by the supplement group (21).

Heart Tissue Sampling Method and Gene Expression Measurement

All samples were anesthetized under identical conditions, following standard protocols, using a combined intraperitoneal injection of ketamine and xylazine. After tissue collection and washing, the desired tissues were frozen and stored at -80°C until further analysis. To minimize the influence of diurnal variations, sampling was conducted between 8:00 AM and 11:30 AM. Table 2 presents the primer sequence.

Table 2. Primer pattern of AMPK, MAFbx, FoxO3a

Genes	Sequence (5' → 3')
F AMPK	ACTATCAAAGACATACGAGAGCA
R AMPK	CTTGAGGGTCACCACTGTATAA
F MAFbx	AGGGCAGGTGGATTGGAAGAAGA
R MAFbx	GTTGGGGTGAAAGTGAGACGGAG
F FoxO3a	GCCTCATCTCAAAGCTGGGT
R FoxO3a	TGCTCTGGAGTAGGGATGCT
F β-Actin	AGGAGTACGATGAGTCCGGC
R β-Actin	CGCAGCTCAGTAACAGTCCG

Statistical Analysis

One-way ANOVA and Tukey's post hoc test were employed for statistical analysis at a significance level of $p \leq 0.05$ after confirming that the data followed a normal distribution.

Results

The average weight of the groups before, during, and after the induction of obesity is presented in Table 3.

Table 3. The average weight of the groups before and during the obesity induction period

	before induction of obesity			Induction of obesity				
	8-week-old Rat	After adaptation	Grouping	First week	Second week	Fourth week	Sixth week	Eighth week
Age (weeks)	8	9		10	11	12	13	14
Groups	187.51±9.37	200.51±16.26	ND (n=9) HFD(n=36)	211.33±19.34 209.61±22.74	216.33±17.66 233.56±13.90	245.22±16.51 271.89±21.20	257.22±22.81 310.58±21.68	270.11±27.55 350.83±41.01

Average weight of the groups after induction of obesity					
Age (weeks)	17	18	20	22	24
ND	270.11 ± 27.55	281.78 ± 24.13	291.55 ± 32.94	297.88 ± 36.06	310.88 ± 38.95
HFD	343.66 ± 50.90	384.33 ± 23.54	410.44 ± 47.65	435.11 ± 81.86	461.11 ± 37.94
HFDT	352.88 ± 42.72	374.22 ± 23.38	389.77 ± 43.33	411.55 ± 37.56	414.00 ± 49.05
HFDRJ	348.44 ± 37.98	377.11 ± 23.12	398.88 ± 51.68	415.88 ± 51.68	423.77 ± 49.11
HFDTRJ	358.33 ± 36.97	368.44 ± 21.48	378.66 ± 45.43	387.22 ± 50.31	390.55 ± 40.12

Data analysis revealed a significant difference in the mean changes of FoxO3a expression in cardiomyocytes (F = 11.553, P = 0.000) (Figure 1). Tukey's test showed a significant increase in FoxO3a expression in the HFD group (P = 0.000) compared to the ND group. Additionally, a significant decrease in FoxO3a levels was observed in the HFDT (P = 0.033), HFDRJ (P = 0.049), and HFDTRJ (P = 0.000) groups compared to the HFD group. Furthermore, a significant reduction was observed in the HFDTRJ group compared to both the HFDT (P = 0.045) and HFDRJ (P = 0.030) groups (Figure 1).

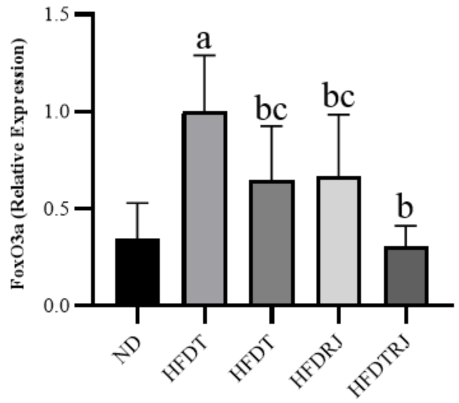

Figure 1. Cardiomyocyte FoxO3a expression by one-way ANOVA test (at P<0.05 level).
a Difference with ND, b Difference with HFD group, c Difference with HFDTRJ group.
ND: normal diet, HFD: high-fat diet, HFDT: high-fat diet-training, HFDRJ: high-fat diet-royal gel, HFDTRJ: high-fat diet-training-royal gel.

Data analysis revealed a significant difference in MAFbx expression changes in cardiomyocytes between the groups (P = 0.000, F = 15.749) (Figure 2). Tukey's test indicated a significant increase in MAFbx expression in the HFD (P = 0.0001), HFDT (P = 0.003), and HFDRJ (P = 0.002) groups compared to the ND group. Furthermore, a significant decrease in MAFbx expression was observed in the HFDT (P = 0.027), HFDRJ (P = 0.041), and HFDTRJ (P = 0.000) groups compared to the HFD group. Additionally, a significant reduction was found in the HFDTRJ group compared to both the HFDT (P = 0.041) and HFDRJ (P = 0.027) groups (Figure 2).

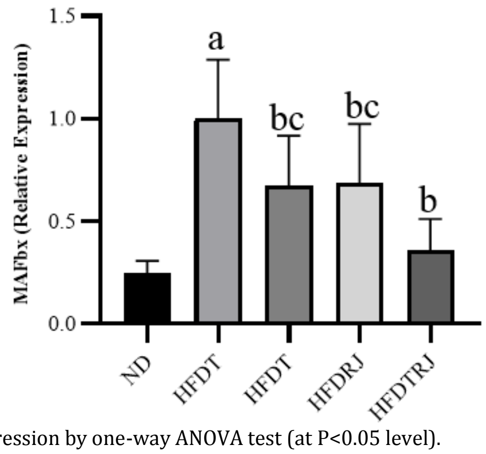

Figure 2. Cardiomyocyte MAFbx expression by one-way ANOVA test (at P<0.05 level).
a Difference with ND, b Difference with HFD group, c Difference with HFDTRJ group.
ND: normal diet, HFD: high-fat diet, HFDT: high-fat diet-training, HFDRJ: high-fat diet-royal gel, HFDTRJ: high-fat diet-training-royal gel.

Finally, data analysis revealed a significant difference in AMPK expression in cardiomyocytes among the different groups (P = 0.0001, F = 18.671) (Figure 3). Tukey's test indicated a significant decrease in AMPK in the HFD (P = 0.000), HFDT (P = 0.007), and HFDRJ (P = 0.002) groups compared to the ND group. Additionally, a significant increase in AMPK expression was observed in the HFDT (P = 0.002), HFDRJ (P = 0.007), and HFDTRJ (P = 0.000) groups compared to the HFD group. Furthermore, a significant increase in AMPK expression was found in the HFDTRJ group compared to both the HFDT (P = 0.048) and HFDRJ (P = 0.015) groups (Figure 3).

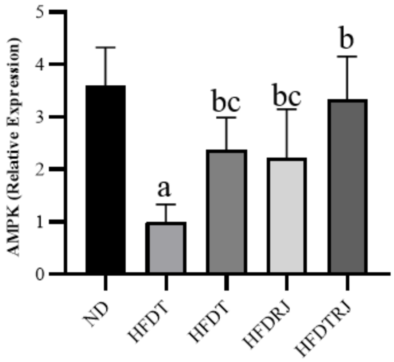

Figure 3. Cardiomyocyte AMPK expression by one-way ANOVA test (at P<0.05 level).
a Difference with ND, b Difference with HFD group, c Difference with HFDTRJ group.
ND: normal diet, HFD: high-fat diet, HFDT: high-fat diet-training, HFDRJ: high-fat diet-royal gel, HFDTRJ: high-fat diet-training-royal gel.

Discussion

In the present study, a high-fat diet (HFD) increased the expression of FoxO3a and MAFbx in cardiomyocytes. This aligns with findings from Hasan et al. (2019), who showed that HFD led to significant increases in FoxO3, Atrogin-1/MAFbx, and MuRF-1, along with a rise in collagen in the soleus muscle of rats (22). Similarly, Abrigo et al. (2016) reported that HFD induced a decrease in muscle weight and muscle fiber diameter, which was attributed to heightened skeletal muscle catabolism (23). Gao et al. (2023) also found that HFD can induce skeletal muscle atrophy by upregulating MuRF1, FbX32, and p53 (24). The elevated expression of FoxO3a has been linked to increased muscle fiber atrophy, with high levels of FoxO3a associated with the upregulation of MAFbx/Atrogin-1 and MuRF-1 (5). Several factors contribute to muscle atrophy, including obesity, excessive caloric intake, insulin resistance, and chronic inflammation (25). Obesity impairs Akt signaling, increases NF-κB and FoxO1, and elevates levels of pro-inflammatory cytokines like TNF-α and IL-6, all of which are associated with muscle atrophy (26). In our study, the increased levels of FoxO3a and MAFbx in HFD rats were accompanied by reduced AMPK expression in cardiomyocytes. AMPK functions as a cellular energy sensor, activated when intracellular AMP levels rise. AMPK activation triggers ATP generation pathways and regulates cell proliferation and biosynthesis. AMPK also plays a role in controlling muscle mass by modulating protein degradation via the ubiquitin-proteasome system and autophagy (27). Previous studies have shown that AMPK activation influences muscle fiber degradation through the FoxO transcription factors. The FoxO family (FoxO1, FoxO3, FoxO4, and FoxO6) regulates cellular processes such as apoptosis, differentiation, metabolism, proliferation, and survival. FoxO transcription factors integrate signals from insulin, growth factors, cytokines, and oxidative stress. AMPK directly phosphorylates FoxO3 (28). Notably, our study showed a decrease in FoxO3a and MAFbx expression, along with an increase in AMPK in the cardiomyocytes of HFD rats following aerobic training. Targeting the inhibition of the FoxO3a pathway may be a promising strategy for preventing muscle atrophy. This is supported by studies showing that aerobic training reduces FoxO3a expression in the cardiomyocytes of diabetic rats (29). Furthermore, Esmaili et al. (2019) demonstrated that aerobic training elevates AMPK expression while decreasing MAFbx expression in cardiac myocytes (30). Rahimi et al. (2023) found that high-intensity interval training (HIIT) downregulates FoxO1 and Atrogin-1 in the muscles of diabetic rats (31). Aghaei et al. (2021) also showed that HIIT reduces cardiac levels of FoxO3a and FoxO1 in diabetic rats, suggesting that inhibiting these proteins could prevent cardiac autophagy (32). However, Holloway et al. (2015) found that HIIT training did not affect FoxO3a protein levels in the cardiac muscle of rats with heart failure (33). Differences in exercise type, training frequency, and subject characteristics may account for these discrepancies; for instance, previous studies focused on rat models with heart issues, whereas our study focused on HFD rats. Additionally, aerobic training and HIIT differ in intensity and the physiological adaptations they induce. Exercise training inhibits FoxO3a and MAFbx expression in cardiomyocytes by increasing AMPK expression, thereby preventing heart atrophy. One potential mechanism behind these changes is the enhanced muscle levels of PGC1α, which can suppress FoxO3a. FoxO3a is a key mediator in atherogenesis and can activate MAFbx (5).

In our study, royal jelly (RJ) increased the expression of AMPK and decreased the expression of FoxO3a and MAFbx. You et al. (2019) reported that 10-HDA, a fatty acid found in RJ, activates the AMPK pathway and its downstream signaling pathway, PI3K/AKT (34). Furthermore, Okumura et al. (2018) observed that oral intake of RJ in aged mice resulted in a reduction of catabolic genes such as MuRF1 and MAFbx (17). These researchers noted that RJ delays muscle apoptosis by inhibiting the activity of catabolic genes. Additionally, another study demonstrated that RJ enhances the AKT signaling pathway by boosting serum levels of IGF-1. Evidence suggests that RJ (specifically 10-HDA) has beneficial effects on inflammation and autophagy by regulating AMPK, which, in turn, modulates NF-κB and IL-1β inflammatory signaling (35). The molecular mechanism by which RJ inhibits proteolysis involves the interaction of various intracellular messenger pathways. RJ activates mTOR and AKT while inhibiting proteolysis (14). AKT, as a key substrate of mTORC2, is part of the PI3K family. In the presence of growth factors, PI3K activates mTORC1, which regulates key factors in protein synthesis, such as S6K and eIF4E. Additionally, mTORC1 interacts with AMPK, leading to the phosphorylation of Unc-51 Like Autophagy Activating Kinase 1 across various tissues (36). RJ has also been shown to influence FoxO transcription by modulating insulin/IGF-1 signaling (37). FoxO is instrumental in activating the AKT pathway, which regulates multiple stress response pathways, including ROS and DNA repair, as well as translation. Furthermore, the FoxO family directly regulates muscle atrophy genes, such as MUSA1 and SMART (38). Among other findings, we observed a decrease in FoxO3a and MAFbx expression, along with an increase in AMPK expression in the cardiomyocytes of HFD rats in the HFDTRJ group compared to the other groups. To the best of our knowledge, no study has concurrently examined the effects of training and RJ on these indicators in HFD rats. However, Takahashi et al. (2018) reported that oral administration of RJ and 10-HDA, alongside endurance exercise, enhanced mitochondrial function in the soleus muscle and induced AMPK activation (15). Research has shown that aerobic exercise can activate PGC1α while inhibiting FoxO3a (12), thereby facilitating the activation of MAFbx (5) and potentially preventing cardiac atrophy (12). Furthermore, RJ may inhibit the degradation of cardiac muscle by influencing the mTOR and AKT pathways (14). The combination of exercise and RJ resulted in more significant changes in FoxO3a, MAFbx, and AMPK expression due to their synergistic effects. RJ has the potential to enhance the benefits of exercise, despite the differing signaling pathways involved. Therefore, exploring these pathways in future studies could yield valuable insights into the combined effects of exercise and RJ on cardiac muscle hypertrophy and atrophy. The limitations of the current study include its short duration and the forced induction of obesity, which may not fully reflect the long-term effects of obesity, exercise, and RJ supplementation. Additionally, examining the cellular changes in heart tissue, particularly morphology, could provide a clearer understanding of the effects of exercise and supplementation.

Conclusion

In the current study, both aerobic training and royal jelly (RJ) positively impacted cardiac function by improving the indices of atrophy and hypertrophy in rats fed a high-fat diet. Notably, the combined effect of aerobic training and RJ on these metrics was found to be more significant. Consequently, it is recommended to use both aerobic training and RJ together as an effective approach to mitigate HFD-induced cardiac atrophy in obese individuals.

Declarations

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Conflict of Interest

There is no conflict of interest.

Funding

Not applicable.

Ethical Consideration

Approval was granted by the Ethics Committee of the Islamic Azad University, Marvdasht (No. IR.IAU.M.REC.1400.020).

Author Contributions

All authors equally contributed to the design, execution, analysis of results, and writing of the manuscript.

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