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Evaluation of Energy and Protein Intakes and Clinical Outcomes in Critically Ill Patients: A Cross-sectional Study

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ABSTRACT

Introduction: Critically ill patients admitted to the intensive care unit are often hyper-metabolic, hyper-catabolic, and at malnutrition risk. This study aimed to evaluate the amount of energy and protein intake and its correlation with the required amount in critically ill patients.

Methods: A total of 70 patients with critical conditions admitted to the ICU were eligible (age≥18 years and over a 3-day stay in ICU). Basic characteristics, medical history, and laboratory test results were extracted from the patient's medical records. Anthropometric indicators and the APACHE II questionnaire were assessed. Patients' energy and protein requirements were 25kcal/kg/day and 1.2g/kg/day, respectively.

Results: The mean age in the target population was 57.69±20.81 years, and 48.6% were men. The mean actual energy intake was significantly lower than the requirement (531.27±365.40 vs. 1583.77±329.36 Kcal/day, P<0.001). The mean actual protein intake was significantly lower than the requirement (14.94±18.33 vs. 74.11±17.89 gr/day, respectively, P<0.001). Energy and protein provision to the patients had a growing trend over time. There was a significant reverse correlation between the age of patients and total lymphocyte count (r= -0.38, P=0.003). In addition, there was a significant reverse correlation between the Glasgow coma scale and mechanical ventilation duration (r=-0.49, P<0.001). The lowest average energy and protein intake were in patients with poisoning.

Conclusion: The energy and protein intake in critically ill patients is significantly less than recommended, requiring routine nutritional assessments.

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Introduction

Critically ill patients admitted to the intensive care unit (ICU) are often hyper-metabolic, hyper-catabolic, and at malnutrition risk (1). Malnutrition is independently associated with poorer clinical outcomes, higher mortality risk, longer ICU, and hospital length of stay (2). Systemic inflammatory responses occur during catabolic stress in critically ill patients (2, 3). An increase in mortality rate is predicted in this state because of physiologic instability (4).

Clinical nutrition can play a crucial role in alleviating and managing the morbidities of patients (5). Critically ill patients are often unable to eat. Therefore, early initiating nutrition support such as enteral nutrition (EN) and parenteral nutrition (PN) is essential (6). The nutritional intakes of many patients are either too many or too few compared to their metabolic

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needs (6, 7). The prevalence of malnutrition in ICU patients in developing and developed countries has been 78.1% and 50.8%, respectively (1). Inflammatory response associated with increased metabolic rate leads to hyperglycemia, lean body mass wasting, and inability to metabolize nutrients (2). Providing an adequate amount of each nutritional substrate may help these patients (8, 9). Generally, EN is superior to PN, and the guideline recommends starting as soon as possible (10, 11). A low calorie and protein intake, usually less than 70% of a patient's needs, has been associated with poor clinical outcomes (2). This study aimed to evaluate the amount of energy and protein intake and its correlation with the amount required for critically ill patients. A few clinical outcomes specialized for critically ill patients were also surveyed.

Methods

This Cross-sectional study was conducted from June 2018 to May 2019 after inspection by the Medical Ethics Committee of the Mashhad University of Medical Sciences (IR.MUMS.MEDICAL.REC.1398.078) about any possible ethical issues. Patients were recruited from two different ICUs (general and medical ICU), Imam Reza Hospital, Mashhad University of Medical Sciences, Mashhad, Iran, using simple sampling from available ICU patients according to the inclusion criteria (21-and 16-bed units).

A total of 70 patients with critical conditions admitted to ICU were screened for eligibility. The Inclusion criteria were age range >18-80 years, ICU admission, being evaluated from the first 24h of admission for three days. The exclusion criteria included death within the first three days of admission, pregnancy and lactation, chronic and acute renal failure and Hepatic encephalopathy, and inability to obtain informed consent.

A specialized nutritionist recorded data on the daily energy and protein intakes from EN and PN during ICU admission. Basic characteristics, medical history, and laboratory test results were extracted from the patient's medical records. Anthropometric and dietary data were measured by the specialized nutritionist or extracted from medical records. A tape measure was used to measure the ulna's length and determine the patients' heights. By calculating the ideal body weight based on height, we calculated the patients' ideal body weight. Mid-arm muscle circumference was assessed by measuring an accuracy of 0.1cm for each patient.

In both groups, initial EN via a nasogastric tube was performed using a hospital gavage formula with a specific amount of energy and protein (measured by Quality TESTA Laboratory Control), and nutrition-related confounders were modified. EN started continuously at the flow rate of 25ml/h, which was increased every 6 hours based on patient tolerance to achieve the desirable energy when the patients displayed no gastrointestinal symptoms (diarrhea, vomiting, abdominal distention, and gastric residual volume >300 mL).

Patients' energy and protein requirements were calculated based on ESPEN (European Society for Parenteral and Enteral Nutrition) guideline recommendations (10). The energy and protein requirements based on patients' condition and underlying disease were 25kcal/kg/day and 1.2g/kg/day, respectively (10). Hand-made formulas' energy and protein content (measured by Quality TESTA Laboratory Control) were compared with the required amount for each patient.

Sample Size

The sample size was calculated by a previous study (12), and the energy supply percentage was used as the primary variable. The total sample size was estimated at 65 patients based on the Type I error of 5% (α =0.05) and Type II error of 20% (β =0.20; the power of the study was 80%) with a 20% probability of drop-out patients during the study.

Statistical Analysis

Statistical analyses were conducted with SPSS software version 19 (SPSS Institute, Chicago, IL, USA). Descriptive statistics were used to examine the characteristics of patients. Independent samples t-tests determined the difference between patients' intake and requirements in each evaluation session. Pearson correlation coefficient was used to show the association of some clinical outcomes with age and GCS (Glasgow coma scale). A value of $p<0.05$ was used as a criterion for statistical significance.

Result

There were 70 eligible patients included in the study. The mean age in the target population was 57.69±20.81 years, of whom 48.6% (34) were men and 51.4% (36) were women. Among these participants, 25.7% (18) were poisoned patients, 21.4% (15) had respiratory problems, 15.7% (11) had sepsis, 11.4% (8) had gastrointestinal disorders, and 5.7% (4) had hematological disorders, 2.9% (2) renal disorders, 1.4% (1) cardiovascular disorders, and 15.7% (11) suffered from other disorders. Among the patients, 15.7% received EN, 22.8% received PPN, and 61.4% received SPN (Supplemental Parenteral Nutrition). The mean height was 157.97±9.26cm, weight was 65.26±7.40kg, and MAC was 26.10±3.47cm. The mean length of ICU and hospital stay was 24.18±25.20 days (range: 3-120 days) and 28.83± 26.81 days (range: 5-134 days), mean duration of mechanical ventilation was 2.43±2.17 days. A total of 61.7% (47) were dependent on mechanical ventilation. The mortality rate during the study was 60% (42 from 70 subjects) (Table 1).

Table 1. Demographic and baseline characteristics of patients (n = 70).

Variable	Value
Age (mean ±SD)	57.69 ± 20.81
Sex (n, percent)	48.6% men (34) 51.4% women (36)
Weight (means ±SD)	65.257± 7.399
Height (means ±SD	167.97± 9.261
MAC (mean ±SD)	26.10±3.475
Diagnosis (n, percent)	
Respiratory disorders	21.4% (15)
Poisoning	25.7% (18)
Gastrointestinal disorders	11.4% (8)
Sepsis	15.7% (11)
Renal disorders	2.9% (2)
Hematologic disorders	5.7% (4)
Cardiovascular disorders	1.4% (1)
Other	15.7% (11)
Type of feeding route (n, percent)	
EN	15.7% (11)
PPN	22.8% (16)
SPN	61.4% (15)
Ventilator Dependence (n, percent)	
Yes	67.1% (47)
No	32.9% (23)
Length of hospital stay (days)	28.833± 26.817(66)
Duration of ICU stay (days)	24.181±25.00(66)
Duration of mechanical ventilation (days)	2.428±2.170
APACHE	15.63± 4.428
Mortality rate (n, percent)	60% (42)

* MAC, Mid Arm Circumference; EN, Enteral Nutrition; PPN, Parenteral Nutrition, SPN, Supplemental Parenteral Nutrition

Table 2. Mean actual energy and protein intakes and requirements

Variable	Mean ± SD
Actual energy intake (Kcal/day)	531.272 ±365.40
Energy requirement (Kcal/day)	1583.77 ± 329.36
P value	P<0.001
Actual protein intake (Kcal/day)	14.94 ±18.33
Protein requirement (Kcal/day)	74.11 ± 17.89
P-value	P<0.001

Note: Quantitative data are reported as mean (SD).

Table 3. Percentage of energy and protein supply in first 3 days of ICU admission

Time (days)	Energy (mean ± SD)	Protein (mean ± SD)
1	28.608± 18.977	12.389± 21.036
2	37.836± 25.464	21.552± 29.952
3	41.565± 27.483	27.056± 33.618
Total	34.708± 22.033	20.332± 14.854

Note: Quantitative data are reported as mean (SD).

The mean actual energy intake for the study population was 531.2726±365.40Kcal/day, while the mean energy requirement was 1583.77± 329.36Kcal/day, based on patients' stress and bedridden period time. A comparison of the means showed a significant difference between actual energy intakes and requirements (P<0.001). Mean essential protein intake and requirement were 14.94±18.33 and 74.11 ±17.89gr/day, respectively, and the analysis for comparing these means for protein showed a significant difference (P<0.001) (Table 2).

The data showed that the percentage of energy and protein supply based on requirements in the total population were 34.70±22.03 and 20.33±14.85, respectively. Over time, patients had a growing trend in providing the required energy and protein (Table 3).

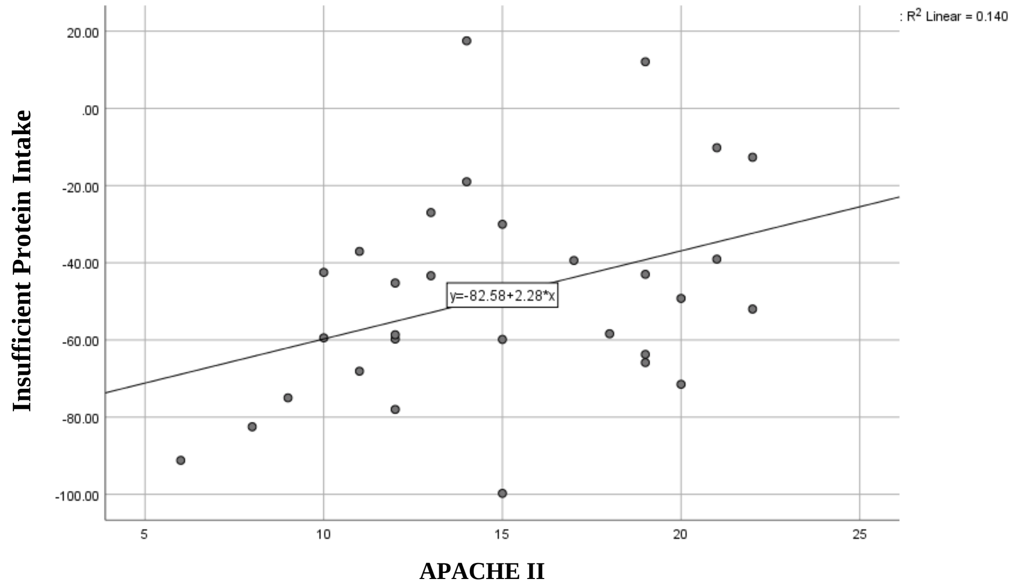

Figure 1. The Association between disease severity (according to the APACHE II score) and insufficient protein intake

There was a significant correlation between disease severity (APACHE II score) and insufficient protein intake (r=0.374, P=0.042) (Figure 1). Despite the correlation between disease severity and inadequate energy intake, no significant correlation exists (r=0.336, P=0.070).

The lowest average energy and protein intake is in patients with poisoning. Insufficient energy intake was associated with underlying diseases (P=0.03) but not with protein intake (P=0.417) (Table 4).

Table 4. The association between underlying diseases and energy intake

Underlying diseases	Mean Energy and Protein intake (MD±SD)		Mean Ratio Energy and Protein intake * (MD±SD)	
Respiratory disorders	652.37 ± 445.41	20.57 ± 22.23	43.51 ± 26.98	30.64 ± 36.05
Poisoning	378.85 ± 186.29	7.60 ± 12.30	24.57 ± 10.19	9.24 ± 13.34
Gastrointestinal disorders	421.66 ± 402.14	13.50 ± 20.11	32.61 ± 31.28	21.89 ± 33.08
Sepsis	766.69 ± 301.52	22.93 ± 14.50	44.03 ± 18.60	27.99 ± 19.99
Other	487.61 ± 377.75	13.33 ± 19.64	32.57 ± 19.88	17.72 ± 24.04
P value	0.03	0.15	0.08	0.15

*Percentage ratio of energy and Protein intake to required energy and Protein, respectively

Note: Quantitative data are reported as mean (SD). The association between underlying diseases and energy intake was compared using one-way ANOVA.

A significant reverse correlation existed between the patient's age and total lymphocyte count (coefficient= -0.38, P=0.003). In addition, the results indicated that the duration of mechanical ventilation decreases as the GCS increases (coefficient=-0.49, P<0.001). Other correlations between age and serum albumin and duration of

ICU stay and association between GCS and ICU and hospital stay were insignificant.

Discussion

The results showed that the actual intake of protein and energy in patients admitted to the ICU is significantly less than the values recommended by the guidelines. According to previous studies, achieving the desired energy level and protein intake is one of the essential points for achieving positive clinical outcomes (13). The study's low protein and energy intake were probably due to the NPO of several patients in the first three days of hospitalization, unstable hemodynamic conditions, gavage intolerance, and a low percentage of protein and energy in hospitalized gavage used to feed patients. These results were consistent with those of Campbell et al. (14). Another similar study showed that patients admitted to the ICU received only 65% of their calorie needs and 61% of their protein requirements (12).

Nutritional evaluation in patients in critical conditions differs from other patients; it is necessary to prepare an efficient nutritional treatment protocol and medical treatment (11, 15). Intermittent and continuous nutrition monitoring are essential components of nutritional assessment (15, 16). Nutritional protocols in ICU patients recommend feeding within the first 24 hours of admission and gradually increasing over time (9, 16).

Acutely ill patients suffer from reduced nutritional intake, muscle protein loss, and poor clinical outcomes due to the inflammatory cascade (17, 18), similar results were obtained in this study, and calorie and protein intake was significantly lower than recommended by guidelines. Therefore, nutrition interventions should begin when the patient's condition stabilizes to improve energy and protein intake and prevent malnutrition.

Increasing the level of consciousness reduces the rate of mechanical ventilation and consequently reduces the need for intubation (19). One study showed that increasing calorie and protein intake in patients admitted to the intensive care unit, especially patients with a body mass index (BMI) greater than 35 or less than 25, was associated with better clinical outcomes, including decreasing mortality rate and increased ventilator-free days (VFDs). This result was in line with that of the present study, which showed that increasing the GCS level reduces the duration of mechanical ventilation (16). The optimal amount of protein and calories in patients admitted to the ICU has not yet been entirely determined, but some studies have shown that a hypocaloric diet for obese patients admitted to the ICU has better clinical outcomes (12, 14, 20). Looijaard et al. showed that increasing protein intake in the acute phase of patients with low skeletal muscle area admitted to the ICU reduces the mortality rate (21). Generally, the results of studies on the administration of high-protein diets in patients admitted to the ICU are very controversial (22). Some observational studies have shown the beneficial effects of high protein intake on clinical outcomes. At the same time, these benefits have not been fully confirmed in clinical trial studies. However, few studies have found that very early protein intake is even harmful (23-28). A retrospective study showed that low protein intake (<0.8g/kg/day) in the first three days of ICU admission, along with high protein intake ((>0.8 g/kg/day)) after the third day of admission, was associated with a reduction in 6-month mortality. In addition, low protein intake throughout the all ICU stay was associated with worst clinical outcomes (29). Weijs et al. reported that high protein intake (>1.2 g/kg/day protein on day 4 of ICU admission) is associated with improved clinical outcomes (23).

Based on the systematic review conducted by Lew et al., malnutrition in patients admitted to the ICU leads to poor clinical outcomes and increased length of hospital stay in critically ill patients (1); therefore, performing nutritional assessments and starting nutritional support can prevent malnutrition or treat existing malnutrition. According to previous studies, nutritional support by a specialist nutrition team can improve calorie intake and reduce clinical complications and mortality in patients admitted to the intensive care unit (8, 30). Therefore, it can be concluded that nutritional support by a nutrition support team (NST) can effectively improve clinical complications, reduce ICU stay length, and ultimately reduce mortality. The medical and nutritional treatment of patients admitted to the ICU is a multidisciplinary task and should involve clinical nutrition specialists, surgeons, nurses, intensivists, and pharmacists, which leads to achieving the desired protein and calorie goals.

According to previous studies, enteral nutrition is the preferred method of feeding in ICU patients to reduce the rate of infections and decrease the length of ICU stay (1, 31). Only 15.7% of patients in our study received antral nutrition, indicating no nutrition support team in the intensive care unit. Therefore, nutritional guidelines in intensive care units of the studied hospitals should be re-evaluated, and nutritional assessments, nutritional care, and nutritional interventions performed by the nutrition support team should be considered.

Similar to other studies, the present work had some limitations. The first limitation was that the patient's weight was not measured but was asked by the patient or their companion. The patients were highly heterogeneous regarding the type of disease, which could affect the outcomes. In this study, even the amount of calories and protein needed was considered the same for all patients. Future studies should select homogeneous patients.

The difference between the energy received and the amount required leads to many problems. The primary use of hospital gavage without enough energy and protein, administration of gavage to all patients with the same volume based on a routine schedule, and failure to administration nutritional advice to adjust the appropriate diet for each patient can be the differences (32).

The reason for the low protein and calorie intake of patients in this study can be the NPO state of several patients in the first three days of hospitalization, unstable hemodynamic conditions and feeding intolerance, exclusion of TPN patients from this study, low protein and calorie percentage in hospital gavage solutions (in one study our hospital gavage contained 0.65kcal per cc and 4.2g of protein per 100cc), the variability of the gavage solution and frequent interruption of the gavage due to the non-implementation of standard protocols in intensive care units of the hospital. Further studies should be conducted after the first week to assess intake status and larger sample sizes.

Conclusion

Based on the results, the energy and protein intake in patients admitted to the ICU was significantly less than the recommended amount. Therefore, nutrition support teams should perform nutritional assessments and determine the feeding route and amount of energy and protein for feeding patients to prevent or treat malnutrition.

Author Contribution

Ahmad Bagheri Moghaddam: Supervision, Conceptualization, Methodology, Writing - Review & Editing, Project administration. Mohaddeseh Badpeyma: Project administration, Writing - Review & Editing, Data Curation, Visualization, Investigation. Mahsa Malekahmadi: Writing - Original Draft, Visualization. Alireza Sedaghat: Methodology, Project administration, Writing - Review & Editing. Andisheh Norouzian Ostad: Investigation. Majid Khadem-Rezaiyan: Formal analysis, Writing - Review & Editing. Naseh Pahlavani: Writing - Original Draft. Fatemeh Ebrahimbay Salami: Investigation

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Conflicts of Interest

The authors declare no conflict of interest.

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Evaluation of the Food Waste Volume in Pediatric Intensive Care Units (PICUs) of Akbar Hospital in Mashhad, Iran

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ABSTRACT

Introduction: Understanding food waste is essential to reduce environmental impact and financial burden and ensuring food security in hospital settings. Research on hospital food waste in Iran, especially in Pediatric Intensive Care Units (PICUs), has been limited despite the importance of reducing food waste. Therefore, this study aims to evaluate the amount of food waste in the Akbar Children's Hospital PICUs department. The findings of these studies can provide evidence to improve food management in hospital settings.

Method: The rate of food waste among patients admitted to the Akbar Hospital's PICUs was measured in this cross-sectional study. Food waste was measured by calculating the difference between the total food delivered to the patient and the total food they consumed. Patients or their parents provided information about the proportion of hospital food intake within the past 24 hours.

Result: Of the 140 patients in the initial sample, 21 received hospital food during the study. The average food waste for the one-week study period was 53.57%, indicating that 90.5% of patients caused varying degrees of food waste, with only 9.5% consuming the entire amount of hospital food they received. No significant association was found between patients' age or gender and food waste.

Conclusion: The results suggested a significant amount of food waste in the hospital setting that needs to be addressed to improve patient nutrition and reduce costs.

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Introduction

Hospital malnutrition is a critical problem affecting between 20 and 50% of patients admitted to the hospital (1). Malnutrition profoundly affects patients' clinical outcomes, increasing mortality rates and length of hospital stay (2). Furthermore, malnourished patients are at higher risk of developing complications during hospitalization. Two-thirds of malnourished patients admitted to the hospital worsen their nutritional status during hospitalization if not treated promptly, while one-third of non-malnourished patients become malnourished (1). Proper nutritional status and healthy feeding of hospitalized patients are paramount. In addition, the hospital nutrition department plays an essential role in ensuring that patients receive appropriate diets and menus to optimize their nutritional status (3-5).

A hospital's nutrition and food service optimize patients' nutritional status by providing appropriate diets and menus (6). However, Simzari et al. revealed that hospital malnutrition is related to the amount of food waste, and the increase in food waste is related to inadequate protein and energy intake (7). According to Practice Green Health, 10-15% of hospital solid waste is food waste (8). Therefore, reducing food waste is a challenge for the nutrition department in every hospital (9).

As Williams and Walton demonstrated, food waste in hospitals is significantly higher than in other food services (10). Plate waste is one of the components of hospital food waste, which refers to the amount of food prepared for the patient

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that is not consumed (11). The causes of plate waste can be stress, anorexia, pain, quality, quantity, and inappropriate feeding time (12). Some strategies can be applied to manage food waste, such as optimizing portion sizes and delivery systems, increasing patient food choices, and employing people to help feed patients (13). In addition to reducing food waste, conducting food waste studies if it leads to increased food intake can reduce costs and financial burden (14), improve patient food access (15), increase food intake (15), prevention of malnutrition (16), and increase patient satisfaction (17).

Despite the importance of reducing food waste, limited research has been conducted on hospital food waste in Iran. To our knowledge, no study has examined the amount of food waste in the Pediatric Intensive Care Units (PICUs). Therefore, this study aims to evaluate the amount of food waste in the PICUs department of Akbar Children's Hospital, considering the significant role of food waste management in reducing the financial burden and improving patient access to food.

Material and Methods

Participants

This observational exploratory research cross-sectional study was conducted in 2022 in the Pediatric Intensive Care Units (PICUs) of Akbar Hospital in Mashhad for one week to gain initial insights regarding food waste in PICUs. In this study, all the available patients in the PICUs were evaluated. The inclusion criteria were participants admitted to the PICUs, ability to receive the food orally, desire to participate in the study and consume the provided hospital food. The exclusion criteria included becoming nil per os (NPO), not desire to contribute to the study, and receiving oral nutrition support (ONS) or parenteral or enteral nutrition that could affect the amount of the daily provided hospital food amount and the condition of breastfeeding.

Table1- Descriptions of the codes used in the food waste checklist.

Code	Code Description
0	**All** the food delivered to the patient by the nutrition department has been eaten
1/4	**3/4 of** the food delivered to the patient by the nutrition department has been eaten
2/4	**2/4 of** the food delivered to the patient by the nutrition department has been eaten
3/4	**1/4 of** the food delivered to the patient by the nutrition department has been eaten
4/4	**All** the food delivered to the patient by the nutrition department **has not been eaten**

Data Collection

In this study, food waste was calculated by comparing the total food delivered to the patient with the total food consumed. The validity and reliability of the checklist used to evaluate food waste were established by Tabibi (5). Table 1 describes each code on the checklist ranging from 0 to 1. Patients or their parents were asked to provide information about the patient's proportion of hospital food intake within the past 24 hours (12). The food intake values reported by children were cross-checked with parents' observations at the patient's bedside.

Statistical Analysis

The data were analyzed using SPSS version 16 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were used to define baseline characteristics and determine the final food waste mean ratio. The data normality was evaluated using the Kolmogorov–Smirnov test. An Independent sample t-test was used to compare the food waste amount between the two sexes and their age. The $p<0.05$ is considered significant.

Results

This study examined the food intake of 140 patients in the PICU Wards of Akbar Hospital, Mashhad, Iran, during a one-week period in which food waste was measured. Among the initial sample, 52 patients were excluded due to eating nothing by mouth or "nil per os" (NPO) diet, seven patients due to exclusive breastfeeding, 42 patients were excluded due to enteral feeding, and 17 patients due to non-hospital foods consumed during the main meals. Finally, food waste for 21 patients receiving hospital food was evaluated. The mean age of the participants was 79.80 ± 4.82 months, and other baseline characteristics are presented in Table 2. The average food waste for the one-week study period is 53.57 ± 29.88%, as shown in Table 3.

Approximately 9.5% of patients consumed all the hospital food they received, while the remaining 90.5% caused varying degrees of food waste. Specifically, 28.6% of patients left 1/4 of their food, 4.8% left ½ of their food, and 47.6% left 3/3 of their food. Additionally, 9.5% of patients left the entire hospital food they received (Table 4).

No significant association was found between patients' age or gender and their food waste (P=0.53 and P=0.48, respectively).

Table 2. Baseline characteristics of PICU patients

Variable	value	mean±SD / N (%)
Number	**n**	21
Age (months)	**months**	79.80± 4.82
Gender	**Male**	14(66.7%)
	Female	7(33.3%)

Table 3. Mean Main Course Waste among PICU patients

Food Waste	N	Mean	SD
Main Course Waste	21	53.57	29.88

Table 4. Food waste grades distribution among PICU patients

Food Waste	Total population	Male	Female
0	2 (9.5%)	1 (7.1%)	1 (14.2%)
1/4	6 (28.6%)	5 (35.7%)	1 (14.2%)
1/2	1 (4.8%)	1 (7.1%)	0 (0.0%)
3/4	10 (47.6)	6 (42.8%)	4 (57.1%)
4/4	2 (9.5%)	1 (7.1%)	1 (14.2%)
Total	21 (100%)	14 (100%)	7 (100%)

Discussion

In recent years, several studies have been conducted with the aim of evaluating the amount of hospital plate waste and the amount of patients' consumption of food received from hospital catering. Dehnavi et al. (2018) found that the average food wastage in lunch at Qaem and Imam Reza hospitals in Mashhad was 41.40 ± 37.95% for the Main Course meal and 27.84 ± 34.47% (18). Furthermore, the study revealed that 44.6% of patients did not waste any food, while 11.6% of patients left 1/4 of their food, 20% of patients left 1/2 of their food, 9.9% of patients left 3/4 of their food, and 13.9% of patients left the entire of their food.

Similarly, Tabibi et al. (2011) evaluated food waste in the lunch of patients hospitalized in Bo Ali Hospital in Tehran, and 58.4% of patients did not consume all the food they received. Of these patients, 5.5%, 30.5%, 9.4%, and 2.7% did not consume the total, 1/4, 1/2, and 3/4 of the received food, respectively. It is noteworthy that only 41.6% of patients consumed their entire received food (19).

Frakes et al. (1986) reported an average food waste of 21.9% for all meals (13), while Hirsch et al. (1979), which examined food waste in 11 UK hospitals, reported that 44.2% of patients consumed all their meals, while the rest produced varying amounts of food waste(20). The present study supports these findings, indicating that the mean food waste produced was 53.57 ± 29.88%. Specifically, 9.5%, 28.6%, 4.8%, and 3.5% of patients did not consume 100%, 25%, 50%, and entire received food, respectively.

The findings indicated that food waste in the hospital setting is notably higher than previous studies have reported. Additionally, a smaller proportion of patients succeeded in consuming all the food they were offered during their hospital stay. These observations could be attributed to the distinctive conditions of patients hospitalized in the intensive care unit, which may affect their appetite and food intake differently than non-ICU patients. Moreover, child patients' physiological and clinical states may differ significantly from adult patients, contributing to food consumption and waste differences.

The findings suggested no significant difference in the amount of food waste between genders, and we did not observe any association between the patient's age and their food waste amount. These observations align with a similar study conducted by Dehnavi et al. (2018), which did not find a significant association between the amount of food waste and patients' age or gender, with similar food waste levels observed across both genders (18). In previous research, Roberto S et al. (2004) (21) found that food waste levels were higher inwards with older patients, while Hamilton K et al. (2001) found that food waste was more common in female patients compared to males (21, 22). The present study focused exclusively on food waste in the PICU ward. Dehnavi et al. (2018) found that food waste varied significantly across different hospital wards, specifically, the amount of waste in the heart ward was significantly more than in other wards. However, the surgical ward reported less food waste than other wards (18). A study in 2011 found that the surgery and rehabilitation wards had the least and most food waste, respectively, compared to other wards (21). Similarly, Barton A. et al. (2000) observed that medical and surgical wards had lower food waste than the geriatric ward, which had more waste than other wards (3, 4).

A notable strength of this study is that, to the best of our knowledge, it is the first to investigate food waste in specialized children's hospitals, particularly in PICUs. Children's food intake values were cross-checked with parents' observations at the patient's bedside. This rigorous approach provides confidence in the accuracy of the findings and underscores the importance of including parent observations in assessing food waste in pediatric hospital settings. A possible limitation of this study is not accounting for food waste caused by appetizers, and separately examining the amount of food waste caused by different main meals could provide a more detailed picture. Moreover, the limited number of participants who met the inclusion criteria for this study might be considered another limitation, as it reduces the generalizability of the findings.

Based on the findings, the researchers recommended the need for future studies with larger sample sizes and extended durations. Additionally, future studies should consider the inherent differences in food waste generated by different meals and variations between patients with different medical conditions. These efforts would contribute to a more comprehensive understanding of food waste management in hospital settings and facilitate the effective development of waste reduction strategies. Future studies should also evaluate the amount of waste of gavage formulas and parenteral nutrition products, as well as the amount of food waste, according to the authors of this study. It is also recommended that the amount of hospital capital wastage and possible cost savings if the amount of food waste is reduced, should be considered as the goal of future studies.

Conclusion:

Food waste management is a critical issue that confronts hospitals and impacts treatment outcomes and financial viability. The first step toward reducing food waste is to understand the amount of waste generated by each department within the hospital. Based on a recent study, PICU wards pose unique challenges regarding food waste management due to the potential risk of malnutrition. Thus, hospitals should conduct comprehensive food waste assessments in the PICU ward to address this issue and optimize patient outcomes.

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Conflict of Interest

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

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Comparison of Nutritional Risk Index with Subjective Global Assessment in Evaluating the Nutritional Status of Liver Transplant Candidates

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ABSTRACT

Introduction: We launched this study to compare subjective global assessment (SGA) and nutrition risk index (NRI) as malnutrition screening tools for nurses to use in the care of End-stage Liver Disease (ESLD) patients.

Methods: This pilot study was conducted on liver transplant patients in two hospitals in Iran from May to September 2021. Sensitivity, specificity, and predictive values of NRI were evaluated compared with SGA in ESLD patients.

Results: Sixty-five cirrhotic patients were assessed. The sensitivity, specificity, positive and negative predictive values for NRI in detecting malnutrition based on SGA were 97.67%, 31.82%, 77.68%, and 87.5%, respectively. However, the agreement between NRI and SGA was low (k=0.349). Changing the NRI cut-off value to 83.7 could yield acceptable sensitivity (72.7%) and specificity (58.1%).

Conclusion: NRI can be used as a screening tool in ESLD patients, but a different cut-off might be required to improve its validity against SGA in ESLD patients.

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Introduction

Cirrhosis is widely prevalent worldwide and can result from various causes, such as non-alcoholic fatty liver disease (NAFLD), obesity, hepatitis B or C infection, autoimmune diseases, excessive alcohol consumption, cholestatic diseases, and copper or iron overload(1). Liver cirrhosis develops after replacing the healthy liver parenchyma with fibrotic tissue and regenerative nodules due to a long period of inflammation(2). Malnutrition is a frequent but often neglected complication in patients with cirrhosis, and it is a significant prognostic factor for morbidity and mortality (3, 4). The reported prevalence of malnutrition in cirrhosis varies from 23–60% (5, 6). This malnutrition is associated with the degree of hepatic dysfunction and increased morbidity before and after liver transplantation (7, 8). So, it is crucial to evaluate the nutritional status of cirrhotic patients before liver transplantation. Significant variability in the prevalence of malnutrition has been observed, depending on the method used for assessment and the severity of the disease, which can change the body composition and analytical parameters(8). Therefore, there is no consensus among authors on the most effective methods to assess the nutritional state of these patients. Also, Studies have consistently revealed the inadequacy of any single assessment method or tool to evaluate the nutrition status of patients.

The European Society for Clinical Nutrition recommends evaluating malnutrition in patients with liver cirrhosis through tests, including subjective global assessment, anthropometry,

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biomedical impedance, and hand-grip strength evaluation(9). Some authors have tried to identify the best tools for evaluating the nutritional status of these patients, considering the recommendations and variety of methods. Subjective global assessment (SGA) is a non-invasive, easy-to-apply, low-cost, validated method to assess malnutrition in patients with cirrhosis (10). It has been widely advocated because it relies on patient history and physical examination, overcoming the drawback of interpreting objective parameters affected by liver disease(11). The nutritional risk index (NRI) is a scale widely used in recent years, which allows us to evaluate nutritional risk in a simplified manner using two basic parameters: weight and albumin(12). Its usefulness has been demonstrated to predict the risk of mortality, survival, and postoperative complications in different scenarios, such as liver transplantation (13).

In this study, we aimed to compare NRI and SGA in liver transplant candidates and to determine the sensitivity, specificity, and predictive values of NRI compared to SGA as a nutritional screening tool in cirrhotic patients who are candidates for liver transplantation.

Methods

We conducted a pilot study in Montaseriyeh Hospital, Mashhad, Iran, and Firoozgar Hospital, Tehran, Iran. During the study period (from May to October 2021), Patients over 18 years who were on the waiting list for liver transplant and signed the informed consent were included in the study based on the convenience sampling method. Exclusion criteria were refusal to participate in the study. The patients were identified with a code in order to keep their data confidential. Clinical records were reviewed to obtain the necessary information for the study, a physical examination was performed, and the patients were interviewed. The anthropometric indices, including height and weight, were measured, and body mass index (BMI) was calculated for all patients. The degree of liver dysfunction was evaluated using the model for end-stage liver disease (MELD) and Child-Pugh Score, in which a higher score indicates greater liver dysfunction. The assessment of nutritional status was performed using SGA and NRI scales. The sample size was estimated based on the area under curve (AUC). We considered a null hypothesis (H0) of AUC=0.6 and an H1 of AUC=0.8. Considering an alpha error of 5%, power of 80%, and a ratio of 0.5 for sample size in negative/positive groups, the estimated sample size was 38 positive cases and 19 negative cases (overall 57 patients). Considering a 10% dropout, the required sample size was increased to 43 positive cases and 22 negative cases (overall 65 patients). The research protocol was approved by the School of Medicine, Mashhad University of Medical Sciences, Biomedical Research Ethics Committee. (IR.MUMS.MEDICAL.REC.1399.815) A trained investigator performed all measurements to reduce errors.

Nutritional Status Assessment

The nutritional status of the patients was assessed based on SGA, NRI, anthropometric measurements, and biochemical tests.

Subjective Global Assessment

SGA includes nutritional data regarding current weight, weight before illness, and weight change in the past 15 days, as well as one and six months; nutritional history (appetite, diet intake, gastrointestinal symptoms), gastrointestinal problems (diarrhea, vomiting, nausea), functional physical capacity and physical assessment (signs and symptoms of fat loss, edema, muscle wasting, and ascites) (11). Patients were classified as well-nourished (SGA-A), moderately malnourished (SGA-B), or severely malnourished (SGA-C) based on the categorical assessment provided by the SGA tool. SGA has been used as the gold standard for nutrition assessment in various studies of patients with cirrhosis; therefore, it was considered as the gold standard for detecting malnutrition in our study(14).

Nutrition Risk Index

NRI was calculated based on the following equation:

$$NRI = 1.519 \times \text{serum albumin (g/L)} + 41.7 \times (\text{present weight/usual weight})$$

Patients with NRI scores greater than 100 were considered as no-risk, patients with NRI between 97.5 and 100 were considered to be at mild nutrition risk, patients with NRI scores between 83.5 and 97.5 were considered to be at moderate nutrition risk, and patients with NRI below 83.5 were considered to be at severe nutrition risk. The usual body weight was defined as the patient’s stable weight for the last six months based on medical records or previous measurements.

Anthropometric Measurements

The weight and height of patients were measured by a stadiometer. Patients' weight was measured in a standard position with minimal clothes and no shoes using a scale to the nearest 100 grams and deducing one kilogram of weight due to patient clothes. Height was measured while standing with the head in the Frankfurt plane. BMI was calculated as body weight(kg)/height (m^2). Patients with a BMI <18.5 kg/m^2 were considered underweight, BMI 18.5 to 24.9 kg/m^2 normal weight, BMI 25 to 29.9 kg/m^2 overweight, and BMI ≥ 30 kg/m^2 obese(15). To overcome the effect of ascites on BMI, 5% of the weight was reduced in the case of mild to moderate ascites, and 15% of the weight was reduced in the case of refractory ascites(16).

Biochemical Tests

Fasting venous blood samples were obtained from all patients for biochemical assessment. Biochemical markers include Albumin, Total Protein, Blood Urea Nitrogen (BUN), Creatinine, Bilirubin, International normalized ratio (INR) and Prothrombin Time (PT), Potassium, Sodium, Liver enzymes including Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), and Alkaline Phosphatase (ALP) were measured using BT3500 autoanalyzer and Pars Azmoun Biochemistry Kit.

Statistical Analysis

Data were collected and statistically analyzed using SPSS software version 16.0. Data are expressed as mean ± standard deviation. The chi-square test was used to compare the proportion between the two groups. A contingency table was used to determine the sensitivity, specificity, predictive values, and accuracy of NRI as a malnutrition screening tool compared to SGA as the gold standard. The Kolmogorov-Smirnov test was used to check the normality distribution of quantitative variables. Pearson correlation analysis was performed to evaluate the agreement between scores. Agreement in classification was studied using the statistical Kappa (K) index. The receiver operating characteristics (ROC) curve was used to evaluate the diagnostic accuracy (area under the curve, AUC) and the cut-off for NRI in detecting malnutrition. All tests were two-sided, and the statistical significance level was considered 0.05 for all tests.

Table 1. Baseline characteristics of the candidate patients for liver transplantation

Characteristics	Group, n = 65
Weight (kg)	67.92±15.26
BMI (kg/m^2)	24.20±4.92
Total protein (g/dl)	6.51±1.07
Albumin (g/dl)	2.99±0.70
Creatinine (mg/dl)	1.16±0.54
BUN (mg/dl)	24.60±15.86
T.Bili (mg/dl)	3.83 (2.29-7.30)
D.Bili (mg/dl)	2.01 (1.22-5.40)
PT	17.08±5.09
INR	1.40 (1.10-1.78)
AST (UL/l)	57.00 (35.00-92.00)
ALT (UL/l)	38 (26.00-63.00)
ALP (UL/l)	359.00 (225.00-591.00)
Sodium (mEq/L)	137 (135.00-140.00)
Potassium (mEq/L)	4.10 (3.80-4.40)
Child-Pugh stage	
A	10 (15.39%)
B	29 (44.61%)
C	26 (40.00%)
Etiology	
Cryptogenic	12 (20.00%)
HBV/HCV	17 (28.33%)
PSC/PBC	9 (15.00%)
AIH	7 (11.67%)
Other	15 (25.00%)

BMI, Body Mass Index; BUN, Blood urea nitrogen; T.Bili, Total Bilirubin; D.Bili, Direct Bilirubin; PT, Prothrombin time; INR, International normalized ratio; AST, Aspartate Aminotransferase; ALT, Alanine Aminotransferase; ALP, Alkaline Phosphatase; HBV, Hepatitis B virus; HCV, Hepatitis C virus; PSC, Primary Sclerosing Cholangitis; PBC, Primary Biliary Cirrhosis; AIH, Autoimmune Hepatitis.

Results

This study included 65 patients with a mean age of 47.32±14.05 years and a mean MELD score of 16.8 ±5.46. Our patients were predominantly male (60.00%). According to BMI classification, 11.6% of our patients were underweight, 46.51% were normal weight, and 41.86% were overweight or obese. In five patients, the underlying etiology was not recorded. Among the patients with documented etiology, the most common underlying disease that eventually led to liver transplantation was cryptogenic (12 patients, 20.00%). The baseline characteristics of the patients are shown in Table 1.

Table 2. Prevalence of malnutrition based on SGA and NRI

	Normal nutritional status (%)	Moderate Malnutrition (%)	Severe Malnutrition (%)	P-Value
SGA	22 (33.85)	20 (30.77)	23 (35.38)	0.008
NRI	8 (12.31)	26 (40.00)	31 (47.69)	

SGA, Subjective Global Assessment; NRI, Nutritional Risk Index

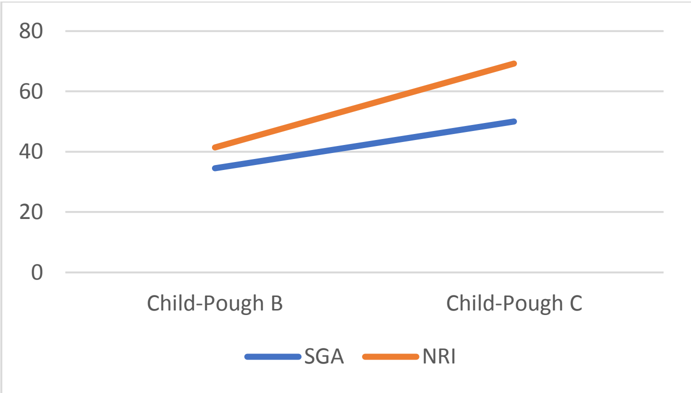

Figure 1. Distribution of severe malnutrition prevalence according to the degree of hepatic dysfunction (Child-Pugh).

The prevalence of malnutrition based on SGA and NRI is shown in Table 2. According to SGA classification, 22/65 (33.85%) were in category A (well-nourished), 20/65 (30.77%) were in category B (moderate malnutrition), and 23/65 (35.38%) were in category C (severe malnutrition). Also, 8/65 (12.31%) were placed in the No risk group, 26/65 (40.00%) were in the moderate risk group, and 31/65 (47.69%) were in the severe risk group based on NRI. Examining the prevalence of severe malnutrition according to the Child-Pugh classification, we found that the higher the hepatic dysfunction, the worse the nutritional state ($p < 0.001$) (Figure 1). None of the patients in the Child-Pugh A group had severe malnutrition.

Table 3. Validity of NRI as a screening tool for malnutrition in ESLD patients as compared to SGA

characteristics	SGA (malnourished)	SGA (normal)
NRI (malnourished)	42 (true positive)	15 (false positive)
NRI (normal)	1 (false negative)	7 (true negative)

SGA, Subjective Global Assessment; NRI, Nutritional Risk Index

The ability of NRI to predict nutrition status is shown in Table 3. Based on the data presented in Table 3, the sensitivity and specificity of NRI in detecting malnutrition were 97.67% and 31.82%, respectively, against SGA. The positive and negative predictive values of NRI were 77.68% and 87.50%, respectively, against SGA. The accuracy of the test was 75.39%. SGA was positively correlated with NRI ($r = -0.334$, $P = 0.007$). The ROC curve was generated for NRI in our patient population using SGA as the gold standard (Figure 2).

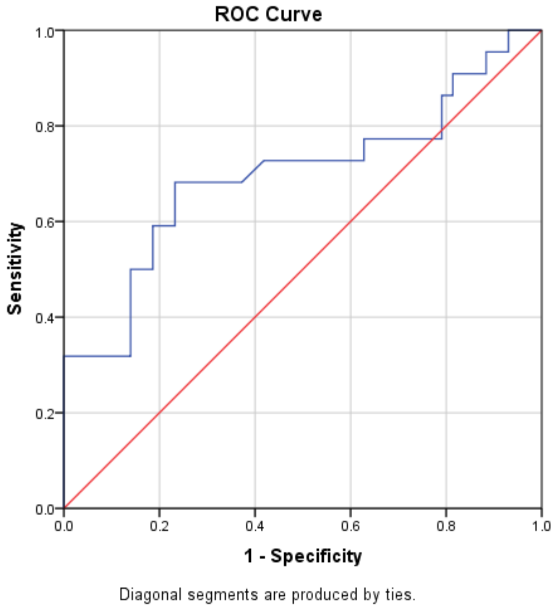

Figure 2. The receiver operating characteristics (ROC) curve of nutrition risk index (NRI) compared to subjective global assessment (SGA) (Area under the curve=0.699, 95% CI: 0.549-0.848, p=0.009)

Considering the difference in the prevalence of malnutrition depending on the method used, we calculated the level of agreement between the two scales. Pairwise agreement between methods was low (K = 0.349). The prevalence of malnutrition based on SGA and NRI and the level of agreement between methods are shown in Table 4.

Table 4. Prevalence of malnutrition and level of agreement between methods

Prevalence of malnutrition	**SGA**	**NRI**
	66.15%	87.69%
Kappa Index among methods		
Methods	**SGA**	**NRI**
SGA	1	0.349
Agreement		P = 0.001

SGA, Subjective Global Assessment; NRI, Nutritional Risk Index

The ROC analysis revealed that the AUC for NRI in predicting malnutrition based on SGA was 0.699 (95% CI: 0.549-0.848). NRI at the cut-off of 83.70 could predict malnutrition with 72.70% sensitivity and 58.1% specificity (Figure 2).

Discussion

Different evaluation methods, including anthropometric parameters, such as BMI, are used to identify the risk of malnutrition in ESLD patients. According to the BMI classification, 11.6% of our patients were underweight, and 41.86% were overweight or obese. This result may be due to the presence of ascites in most patients, which may have confounded the body composition. This finding was similar to other publications, which conclude that BMI underestimates malnutrition and is not a suitable method to evaluate nutritional status in ESLD patients. In the study by Villalobos et al., 5% of hospitalized patients were classified with possible malnutrition according to BMI, which was very low compared to other evaluation methods (17). In conclusion, the assessment of the nutritional status of cirrhotic patients by BMI may not be a reliable method because it can be influenced by water retention. Therefore, it was not included as a nutritional status assessment method in our study.

Subjective global assessment

SGA has been used as the gold standard for nutrition assessment in various other studies for patients with cirrhosis; therefore, we also used SGA as the gold standard in our study(14). Our study showed that according to the SGA questionnaire, the prevalence of malnutrition in patients before liver transplantation was 66.15%, and the prevalence of severe malnutrition was 35.38%. The observed prevalence of malnutrition in our study was similar to most previous studies (18-21). For instance, in Yadav's study, the prevalence of malnutrition using SGA was 86.3%, and the prevalence of severe malnutrition was 35%.(21).

Nutritional Risk Index

Regarding the validity of NRI as a nutrition status screening tool, we observed that using the conventional cut-off for NRI yielded a high sensitivity but low specificity compared to SGA as the gold standard. NRI has been used to define nutritional risk in some recent studies where the effects of undernutrition or nutritional intervention were investigated(22, 23). NRI relies on serum albumin concentration and percentage of usual weight. The formulae-based calculation of NRI provides some objectivity in assessing nutrition status. NRI formula also contains serum albumin level, which is considered an important biochemical parameter to determine the nutrition status of ESLD patients. The prevalence of malnutrition based on NRI was higher (87.69%) than SGA (66.15%) in our study. This can be explained by the pathology of the patients, which may have affected serum albumin concentration. The association between the degree of liver dysfunction and malnutrition was in line with the findings of a previous publication(24).

Concordance of nutritional methods

Our study showed that the agreement between SGA and NRI was low (K = 0.349). This finding was similar to the findings of the study by García-Rodríguez et al. on liver transplant candidates (k=0.041) (24). Similarly, a study on colorectal cancer patients showed that the agreement between NRI and SGA was low (k=0.21) (25). Similarly, Faramarzi et al. evaluated the validity of NRI compared to PG-SGA in colorectal cancer patients and observed that the two scales did not have a statistically significant agreement (k =0.267; P>0.05) (26). There was also a low agreement between SGA and NRI among hospitalized adults (k = 0.24) (27). In contrast to the findings of our study, in a study conducted by Sungurtekin et al.(28) on patients hospitalized in the surgical ward, a good agreement was observed between SGA and NRI (k = 0.57). This finding may be related to the difference in the inclusion criteria because we included patients with liver cirrhosis who had a chronic disease.

Diagnostic validity of the NRI in comparison with the SGA

Although reference bias cannot be ruled out, the results of our study could be helpful in identifying suitable methods of assessing malnutrition in ESLD patients. Using the SGA as the reference method; the NRI showed a high diagnostic validity for malnutrition according to the ROC curve. A cut-off of 83.7 for NRI improved the specificity of the scale compared to the conventional cut-off in our study. In a study by Deniz et al.(29), The optimal cut-off value for NRI to predict malnutrition in hemodialysis patients was 86.0 (64.9% sensitivity and 62.8% specificity). Therefore, it can be hypothesized that different NRI cut-offs might be required to predict malnutrition in different diseases. However, more studies are needed to justify this hypothesis.

NRI can be considered a sensitive scale for identifying malnutrition. Assessing specificity is essential in preventing well-nourished patients from being incorrectly identified as malnourished(30). Accurate identification of malnourished ESLD patients and the resultant timely nutritional intervention will improve transplantation outcomes. Our study indicated that a different cut-off for NRI may increase its specificity while having a still acceptable sensitivity in detecting malnutrition in ESLD patients. This finding adds to the findings of previous studies in a previous study that there might be a need to define different cut-offs for NRI in different diseases, especially diseases with water retention. However, more studies should be conducted to reach a definite conclusion in this regard.

Strengths and limitations

The strength of our study was a multicenter study. However, the sample size was a limitation of our study due to the limited data collection time. External validation of our results in other populations is needed. It is also recommended to test the validity of other nutrition status screening tools against SGA in further studies.

Conclusion

Our study found a high prevalence of malnutrition among patients on the waitlist for a liver transplant and variability in the estimated prevalence of malnutrition depending on the evaluation method. The nutritional risk index can be used as a screening tool for the assessment of the nutritional status of ESLD patients with high sensitivity. However, with the current cut-off, NRI cannot be used as a diagnostic tool because of its low specificity. Modification of the NRI cut-off might be required to improve its validity against SGA in ESLD patients.

Conflict of Interest

The authors have no conflict of interest

Authors' Contributions

Study concept and design: H. B., and M. N.; analysis and interpretation of data: M. A. and A. N.; drafting of the manuscript: H. B.; critical revision of the manuscript for important intellectual content: M. N.; statistical analysis: H. B.

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Evaluating the Dietary Factors Most Closely Associated with Diabetes Mellitus Using a Decision-Making Tree Algorithm

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ABSTRACT

Introduction: The development of type 2 diabetes mellitus (T2DM) is associated with lifestyle factors, including dietary patterns. A diet rich in macro- and micronutrients has been reported to reduce the risk of T2DM. Therefore, this study aimed to identify the dietary factors most closely associated with T2DM in subjects within the MASHAD cohort using a decision tree algorithm.

Methods: This cross-sectional study was conducted on 9704 individuals from the Mashhad Stroke and Heart Atherosclerotic Disorders (MASHAD), of whom 5936 participants completed a 24h dietary recall questionnaire. Macronutrients and micronutrients were estimated using Diet Plan 6 software. A decision tree algorithm was utilized to evaluate the most crucial dietary nutrient intakes concerning T2DM.

Results: The algorithm showed a high specificity (81.34%) but low sensitivity (34.21%), which could predict T2DM with a low-to-moderate diagnostic ability (AUC=0.58). Based on the decision tree, eight features, including dietary potassium, total sugar, sucrose, riboflavin, thiamin, sodium, total nitrogen, and magnesium, were T2DM's most critical dietary components.

Conclusion: Based on the results, consuming sugar, salt, and vitamin B was the most critical related dietary intake to T2DM. Dietary interventions may be a cost-effective strategy for preventing T2DM.

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Introduction

Globally, more than 450 million adults have type 2 diabetes mellitus (T2DM), up from 150 million in 2000, which is estimated to rise to 700 million by 2045 (1). T2DM occurs at various rates in different races and ethnics. Therefore, genetics and lifestyle behaviors, such as a diet that contains high levels of refined sugar and a sedentary lifestyle, might have a predisposing influence (2). A well-balanced and healthy diet and lifestyle have been proven in numerous studies to reduce the risk of diabetes (3, 4). Diabetes type 2 can be delayed by nutritional therapies focusing on weight loss in high-risk

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patients (5, 6). No specific diet is recommended by the American Diabetes Association (ADA) for T2DM prevention (5). However, different eating patterns, including Dietary Approaches to Stop Hypertension (DASH) style, Mediterranean-style, plant-based (vegan or vegetarian), lower-fat, and lower carbohydrate patterns, have been indicated to moderately manage T2DM (7). Hence, using novel algorithms to predict diabetes based on dietary patterns may be a practical approach to risk stratification (8).

Nowadays, machine learning techniques have been widely applied in medicine (9). This technique, as well as healthcare outcomes prediction, could effectively find the associations. Through machine learning techniques, an algorithm will be developed to map input variables to a specific target (10).

Previously different machine learning methods, including decision tree (DT), neural network, random forest, and XGBoost, were applied to investigate the association between various factors and diabetes mellitus (11-13), hypertension (14), metabolic syndrome (15), cardiovascular disease (16-18), vitamin D deficiency (19) and respond to vitamin D supplementation (20, 21). DT models are graphical models designed as trees, with the advantage of being easily interpretable and understandable by clinicians.

Therefore, this study aimed to evaluate the most associated dietary intakes with T2DM using a decision tree algorithm.

Material and Methods

Study population

This study is a part of the Mashhad Stroke and Heart Atherosclerotic Disorder (MASHAD) study which is discussed in detail elsewhere (22). Informed consent was obtained from all MASHAD cohort study participants. The Ethics Committee of Mashhad University of Medical Sciences (IR.MUMS.REC.1386.250) approved the study protocol. The exclusion criteria included participants lacking information regarding T2DM status and those who did not fulfill the 24h dietary recall questionnaire. Hence, a completed questionnaire was available to 5396 participants. Dietary intake was collected using a 24h dietary recall questionnaire, and Diet Plan 6 software (Forestfield Software Ltd., Horsham, West Sussex, UK) was used to analyze the dietary intakes. Each dietary nutrient was adjusted for energy intake, which was previously explained (23-25).

T2DM was defined based on the international diabetes federation (IDF) as an FBG≥126mg/dl or consuming oral glucose-lowering agents or insulin therapy (26).

Analysis

The decision tree algorithm constructs a classification model in a tree-like structure, using if-then rules for classification. Data is incrementally broken down into smaller segments and gradually built into a decision tree. This resulting structure resembles a tree with nodes and leaves, and sequential learning of rules occurs using training data with each rule, leading to removing covered rules. This process persists within the training set until termination criteria are satisfied. Based on a top-down, divide-and-conquer method, the tree's construction unfolds. Decision nodes split into two or more branches, while leaves denote classifications or decisions. The root node atop the tree signifies the best and most important predictor.

Gini index

In a decision tree, node splitting is performed using different methods. The Gini index is one of them, also known as the Gini impurity, and calculates the probability of a specific variable classified incorrectly when selected randomly. The Gini index varies between 0 and 1, and 0 expresses all the elements linked with a single class, showing purity. In other words, all the input variables belong to a specified target. Equation 1 indicates the random distribution of elements across various courses.

$$Gini = 1 - \sum_{i=1}^{n} (p_i)^2$$

Where P_i is the probability of an object being classified to a particular class. The features possessing the least value of the Gini Index would be determined by designing decision tree designation.

Imbalanced classification

Imbalanced classification refers to the uneven distribution of classes within the dependent variable, which occurs when one class significantly outweighs the other in a dataset. This imbalance can hinder accurate classification accuracy. The under-sampling method is one of the methods to deal with imbalanced data sets, which works with the majority class and reduces the number of observations from the majority class to balance the data set.

Table 1. Data table before balancing the target variable

	Training dataset	Testing dataset
Number of dataset	4316 (80%)	1080 (20%)
Number of patients	864 (20%)	228 (21%)

Table 2. Data table after balancing the target variable

	Training dataset	Testing dataset
Number of dataset	1728 (62%)	1080 (38%)
Number of patients	864 (50%)	228 (21%)

In this study, 29 adjusted dietary components were used as input factors, including total nitrogen, protein, carbohydrates, starch, total sugar, glucose, fructose, sucrose, maltose, lactose, non-starch polysaccharides, saturated fatty acid, mono-unsaturated fatty acid, poly-unsaturated fatty acid, cholesterol, sodium, potassium, magnesium, iron, manganese, retinol, carotene, vitamin D, thiamin, riboflavin, niacin, tryptophan, vitamin B12, and vitamin C. The research target was defined as having diabetes based on IDF criteria. Since 20% of cases were positive for diabetes, the under-sampling method was used to access the balanced classification. Then, the decision tree classification method was utilized, in which 1728 cases were used for dataset training. Table 1 presents the imbalanced data with 80% of the cases in the training set, of which 864 are diabetic patients. Similarly, 20% of the included population was put in the testing set, of whom 228 patients had diabetes. On the other hand, Table 2 represents the balanced data, with 62% in the training set (864 patients with diabetes) and 38% in the testing set (228 patients with diabetes).

Table 3. Confusion matrix of testing dataset

Actual outcome	Predicted outcome	
	Positive	**Negative**
Positive	78 (7%)	150 (14%)
Negative	159 (15%)	693 (64%)

Table 4. Confusion matrix of balanced dataset

Actual outcome	Predicted outcome	
	Positive	**Negative**
Positive	341 (20%)	113 (7%)
Negative	113 (7%)	751 (43%)

Results

After balancing the target variable (i.e., having diabetes), 1728 participants were included in the training dataset, of whom 50% (n=864) had T2DM. The testing dataset included 1080 cases, and 21.11% (n=228) were positive for T2DM (Tables 1 and 2). The algorithm showed a high specificity of 81.34%, while the sensitivity was 34.21%. The positive and negative predictive values were 32.91% and 82.21%, respectively. The algorithm showed an accuracy of 71.39% and a low-to-moderate diagnostic ability for diabetes (AUC=0.58). The confusion matrix of testing and balanced training datasets are illustrated in Tables 3 and 4.

Table 5. Performance indexes of the applied algorithm

Variables	Testing
Sensitivity	34.21%
Specificity	81.34%
Positive Likelihood Ratio	1.83
Negative Likelihood Ratio	0.81
Disease Prevalence	21.11%
Positive Predicted Value	32.91%
Negative Predicted Value	82.21%
Accuracy	71.39%
AUC (Area Under Curve)	0.58

Based on a decision tree, eight features (Potassium, total sugar, sucrose, riboflavin, thiamin, sodium, total nitrogen, and magnesium) were found to be the most critically associated dietary intakes with diabetes according to the MASHAD cohort study population. The importance of each variable is given by percentage in the decision tree illustrated in Figure 1. DT performance indicators are shown in Table 5.

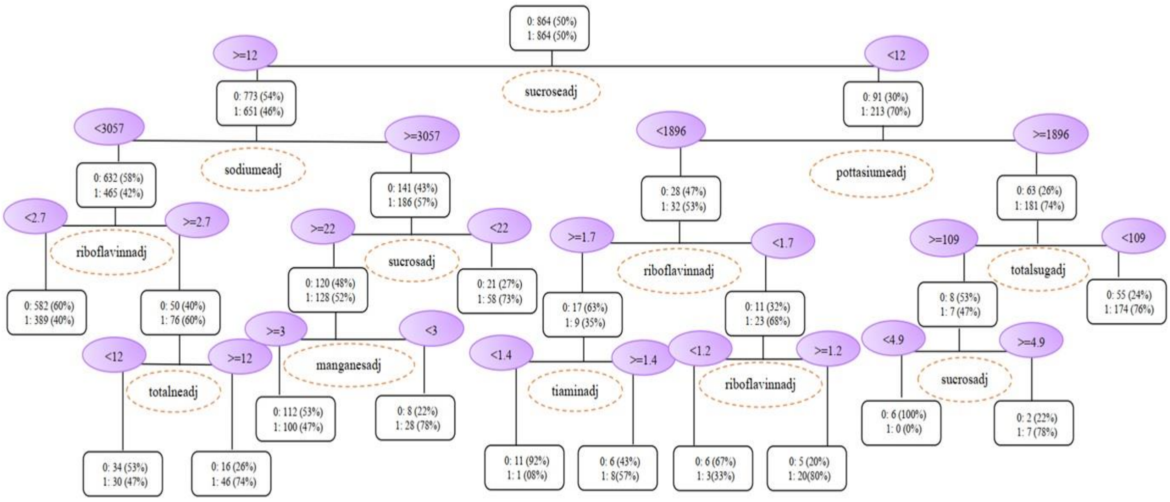

Figure 1. Decision Tree for diabetes mellitus and the most associated dietary components.

Discussion

Individuals at high risk of developing T2DM benefit from nutritional interventions, including lifestyle changes that reduce weight loss (5, 6). There is no prominent difference between the effect of different types of healthy eating patterns on the occurrence of T2DM. Findings from 10 prospective studies on 19,663 cases indicated that the Mediterranean diet reduces the risk of T2DM by 23% (27). More limited evidence exists for the DASH (Dietary Approaches to Stop Hypertension). Results from 3 cohorts on 3415 cases indicated that DASH reduces the risk of T2DM by 27% (28).

Diabetes incidence does not seem to be affected much by different healthy eating patterns. Dietary behaviors could attenuate various cardiometabolic risk factors, and nutrition was a modifiable risk factor for DM. Whole grains, fruits, vegetables, legumes, nuts, fish, chicken, moderate dairy, and heart-healthy vegetable oil intake are the best dietary patterns to reduce cardiovascular risk by around one-third (29). The importance of solutions centered on the globalized food system for food consumption and its impact on cardiovascular disease. The World Heart Federation conducted a workshop, which resulted in this report (27).

Several studies are available on the association between glucose metabolism and blood potassium, but there have been fewer studies looking at the association between dietary potassium and glucose metabolism and the risk of T2DM. According to studies using hyperglycemic clamps, experimentally induced potassium depletion was linked to a decreased sensitivity of pancreatic beta cells to hyperglycemia and a reduction in insulin release (30). The data from epidemiological studies and secondary analyses of hypertension trials have revealed that thiazide use is linked to an elevated risk of diabetes (31, 32) and that potassium depletion caused by thiazide use may be a mediator of this risk (33, 34). Since homeostatic mechanisms maintain serum potassium levels tightly to preserve cellular function, the link between dietary potassium and serum potassium needs to be clarified. On the other hand, dietary potassium has some effect on total body potassium and, presumably, serum potassium (35). The mechanism by which dietary potassium affects glucose metabolism and diabetes risk has not been investigated, but it may be related to serum potassium's effects on glucose metabolism (36).

A sugar avoidance strategy reduces the risk of type 2 diabetes associated with total sugars, fructose, or sucrose consumption. Another possibility can be increased consumption of sugar-rich foods other than sugar-sweetened beverages, which have no protective links with type 2 diabetes (37). Although sugar-sweetened beverages are the primary source of fructose-containing sugars in Canadian and American diets, other sources (such as grains and grain products, fruit and fruit products, and dairy and dairy products) are essential to overall intake (38, 39). Many of these other sugar-sweetened foods have demonstrated either no association with type 2 diabetes (such as cookies, cakes, and Sherbert) or a protective association, such as fruit, yogurt, whole-grain cereals, and even ice cream. Whole-grain cereals, fruit, and yogurt have all been reported to have an inverse dose–response gradient, similar to sucrose (40, 41). The lack of an unfavorable relationship between total sugars, fructose, or sucrose intake and diabetes may be due to considerable contributions from these other dietary sources when taken collectively (37).

Institute of Medicine recently published a review of the effects of salt reduction on direct health outcomes in populations, including those with diabetes. The study found that the available evidence supports a direct relationship between increased salt intake and CVD risk in general people and diabetics. Lowering sodium intake to a goal of 2,300mg/day will likely improve the outcomes of CVD. In those with diabetes, CKD, or prior CVD. However, there was no evidence of benefit from decreasing sodium consumption to less than 1,500mg per day (42). Therefore, dietary sodium intake and HBA1c levels have synergistically influenced CVD development. This finding suggested that a long-term reduction in dietary salt intake is especially significant in people with poorly managed blood glucose (27). Salted food has been shown to promote overeating and weight gain (28). Some interventional studies have proven that A sodium-restricted diet reduces total energy intake (29, 30). Furthermore, sugar-sweetened beverage consumption increased by 17g per day, with each additional 0.4g per day of salt intake (31).

Data mining can be used to identify preventive activities specific to individuals and the effects of each variable on the examined association, but it has severe limitations. A sophisticated procedure requires specialized knowledge and abilities. Furthermore, each application generates many rules, and selecting the most important ones takes practice.

Limitations

The most prominent strength of this study was the number of participants. However, the dietary intakes were not available for all participants of the MASHAD study. Lack of serum nutrient levels measurement and adjustment for smoking, drug use, body mass index (BMI), family history of diabetes, and gender were the main limitations of this study. Moreover, using 24h dietary recall for obtaining dietary intakes could influence the results as 24h dietary recall questionnaire indicating the recent dietary intakes.

Conclusion

Based on the results, the most important dietary components associated with diabetes were potassium, total sugar, sucrose, riboflavin, thiamin, sodium, total nitrogen, and magnesium. Sugar, salt, and vitamin B family members were the most critical dietary intakes associated with T2DM. Nutritional interventions are a relatively low-cost strategy for preventing T2DM.

Declarations

Ethics Approval and Consent of Participants

The study protocol was approved by the Ethics Committee of Mashhad University of Medical Sciences, and written informed consent was obtained from participants.

Consent of Publication

Not applicable.

Availability of Data and Materials

The data that support the findings of this study are available from [Mashhad University of medical sciences], but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. However, data are available from the authors upon reasonable request and with permission from [Mashhad University of medical sciences].

Conflict of Interest

There is no competing interest.

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Author Contributions

All authors have read and approved the manuscript. Study concept and design: MGM, MG, ME; data collection: NV, NA, AG, EH, SG, SD, IAA; Analysis and interpretation of data: SGH, BHR, SE, SSS; Drafting of the manuscript: MB, SSS, MR, NV; Critical revision of the manuscript for important intellectual content: RAD, GAF.

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JOURNAL OF NUTRITION FASTING AND HEALTH

The Effect of 8 TRX on Myeloperoxidase and Total Antioxidant Capacity as Indicators of Vascular Endothelial Function in Obese Women

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ABSTRACT

Introduction: Vascular endothelial dysfunction is one of the consequences of obesity or increased body fat mass. This study aimed to determining the effect of TRX training on myeloperoxidase (MPO) and total antioxidant capacity (TAC) as markers of vascular endothelial function in inactive obese women.

Methods: 28 inactive middle-aged obese women aged 42±3 years of old (30 ≤ BMI ≤ 36) were randomly divided into of TRX (8 weeks, 3days/weekly, n= 14) and control (no training, n = 14) groups. Fasting levels of TAC and MPO activity and anthropometric indices were measured before and 48 hours after lasting exercise session of groups. Independent and paired t -test use to compare inter and intra-group change of variables (P< 0.05).

Results: No significant difference was found in TAC (P= 0.356) and MPO (P= 0.268) between groups at baseline (P> 0.05). TRX led to a significant increase in TAC activity compared (P= 0.004) but MPO remained no change by TRX (P= 0.459). None of these variables were changed in the control group (P > 0.05).

Conclusion: Emphasizing the increase in TAC, it is concluded that TRX training are associated with improved vascular endothelial function in inactive obese women.

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Introduction

Obesity is a global epidemic whose impact on public health has become a major concern, and the prevalence of obesity is expanding in different societies. In the country of Iran, inactivity has been reported to be significantly higher than the average of world statistics, and this issue has a significant effect on causing obesity and its complications (1). Clinical studies have revealed that the decrease in cardiovascular function, which often occurs in response to the decrease or dysfunction of vascular endothelial function, is one of the most important complications caused by obesity, especially in sedentary people (2). So that the increase in body fat is associated with an increase in inflammatory factors, the production of reactive oxygen species (ROS) and the disruption of hormonal and enzyme mediators effective in vascular endothelial function (3).

On the other hand, apart from homocysteine, nitric oxide and vascular endothelial growth factor, myeloperoxidase (MPO) has also been introduced as one of the most important effective mediators in vascular endothelial function. MPO is a hemoprotein and a member of the large peroxidase family, which is a derivative of leukocytes and is often found in neutrophils, monocytes and tissue macrophages, and as a result of their activation, it is released as a

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response to various stimuli. (4) The role of MPO in inflammatory processes and oxidative stress is through catalyzing the respiratory burst reaction causing the conversion of hydrogen peroxide to hypochlorous acid (HOCL) (5, 6). This enzyme has been identified in arterial plaques and its proatherogenic effects have been confirmed. Also, MPO causes peroxidation of low-density lipoprotein (LDL), oxidative changes in high-density lipoprotein (HDL), and decreases the ability of reverse cholesterol transfer by HDL (7). This enzyme reduces the biological production of nitric oxide, and as a result, it causes disruption in the function of vascular endothelium (6).

On the other hand, the reduction of antioxidant capacity leads to the strengthening of reactive oxygen species, which leads to cell wall damage, mitochondria, DNA and functional proteins, dysfunction and even cell death (8). In this context, it has been pointed out that the reduction of TAC and the increase of MPO derived from activated neutrophils and monocytes as one of the agents of oxidative stress due to the reduction of NO leads to dysfunction of the vascular endothelium (9).

In summary, obesity leads to a decrease in vascular endothelial function by increasing MPO levels and decreasing TAC. Under these conditions, physical activity and regular exercise training help to reduce cardiovascular risk factors, especially in healthy or sick obese individual, by improving body composition and regulating endothelial indices (10). Some researchers have pointed out that exercise training, by reducing MPO and increasing TAC, leads to a reduction in inflammatory processes and oxidative stress and prevents LDL oxidation, which in turn is associated with the prevention of atherosclerosis and vascular endothelial dysfunction (11). In Hijazi et al's study (2014), 12 weeks of aerobic training led to a decrease in MDA and an increase in TAC in obese women (12). Nevertheless, Shemshahi et al (2011) reported no change in MPO after 8 weeks of stationary training (13). TRX or total body resistance training, has recently received a lot of attention, so that this unique training method, which uses tools such as two straps and handles, uses body weight as resistance, and its implementation in any place and environment from its distinctive features are (14). Sports science researchers have pointed to the reduction of body fat levels in parallel with the increase in muscle mass in response to TRX (15). For example, in Hosseini et al.'s study (2020), despite a significant increase in glutathione peroxidase in response to 8 weeks of TRX training in obese women, hydrogen peroxide levels did not change (16). In Gaedtke study (2016), 8 weeks of resistance training and TRX led to increased balance and functional ability and strength in elderly people (17). Despite the mentioned evidence, the effect of TRX on TAC and MPO as indicators of vascular endothelial function in obese women has been less studied. Therefore, based on the contradiction regarding the effect of different exercise training and also the lack of a study on the effect of TRX on these variables, the present study aims to determine the effect of TRX on MPO and TAC as two effective indicators on vascular endothelial function in inactive obese women.

Materials and Methods

The current study is semi-experimental and has a pre-test and post-test design with a control group. The statistical population of the current research consisted of 28 inactive obese women with a body mass index higher than 30 kg/m2 and an age range of 40-50 years. Statistical samples were randomly divided into TRX group and control groups. All the subjects were informed by the researcher about the objectives of the study and possible injuries caused by sports exercises, then they completed the consent form.

Inclusion and Exclusion Criteria

The study subjects were non-athletes and non-smokers. Also, their weight fluctuation in the last 6 months was less than one kilogram and they did not have a defined diet. The studied women were not pregnant and did not intend to become pregnant during the study. The absence of history of chronic diseases such as diabetes, cardiovascular, respiratory and kidney diseases, epilepsy, convulsions, as well as any orthopedic abnormalities that make it difficult to perform exercise training are among the criteria for entering the study. Not taking medicine continuously before or during the training program is one of the criteria for inclusion and exclusion from the study. Lack of proper attendance at training sessions, suffering from metabolic diseases, use of pharmaceutical or food supplements to reduce weight or increase physical performance during the study, as well as supplements that disrupt metabolism are among the exclusion criteria.

Anthropometric Measurements

Before and after TRX protocol, anthropometric indices were measured in both groups. The weight and height were measured without shoes and with minimal clothing. So that height was measured using a wall-mounted caliper with an accuracy of 0.1 cm. Weight, percentage of body fat as well as visceral fat were measured by body composition analyzer (OMRON 508, Finland). Body mass index was calculated by dividing weight (kilograms) by height (square meters) (18). Abdominal circumference after a normal exhalation in the thickest area was measured by an inflexible tape measure with an error accuracy of less than 0.1 cm (18).

TRX Protocol

Training program in the form of 8 weeks of TRX with three 20-minute sessions repeated in the first two weeks, which reached 50 minutes in the last two weeks. The initial training sessions started with the lowest intensity of this range. The step-by-step loading method (slope measurement and markings on the ground) was done every two weeks. In the next sessions, the training intensity was gradually increased by increasing the number of exercises and activity time in the session. In this way, before starting the exercises, the subjects were familiarized with this scale and its range, and in order to unify the way of performing the exercises, the speed of the movements in all the subjects was standardized by a metronome of one beat per second (19).

Blood Sampling and Assay

All subjects were requested to avoid any physical activity 48 hours before blood sampling. A fasting blood sample was taken after 10-12 hours of overnight starvation from the study subjects in both control and TRX groups in order to measure the activity of TAC and MPO. So that 5 cc of blood was taken from the brachial vein of the left hand in a sitting position while fasting, and at the end of the training program. All measurements of weight and body mass index were repeated in the same conditions as before the implementation of the study, and also 48 hours after blood sampling was done again from the last training session. All blood samples were centrifuged immediately after sampling to separate the serum. The serum level of TAC and MPO was measured by a specialized kit of Navnad Salamat Company (Iran) by calorimetric method.

Statistical Methods

SPSS version 22 statistical software was used for statistical analysis. Shapiro-Wilk Test was used to ensure the normal distribution of the data. Independent t-test was used to compare data in pre-test and post-test conditions between two groups. The paired t-test was used to determine intragroup changes in each group. The significance level of the tests was considered as ρ>0.05.

Ethical Considerations

This study was approved by the Ethics Committee of Islamic Azad University, Islamshahr Branch (Code: IR.IAU.PIAU.R.1401.001).

Table 1. Pre and post-training of anthropometrical variables of the subjects

Variables	TRX group			Control group		
	Pre-training	post-training	Sig	Pre-training	post-training	Sig
Weight (kg)	82.9 ± 8.68	80.2 ± 8.13	0.001	85.8 ± 5.88	85.7 ± 6	0.449
AC (cm)	114 ± 8.40	105.5 ± 7.77	0.001	119 ± 6.20	119 ± 6.26	0.998
Body fat (%)	46 ± 2.58	43.11 ± 2.31	0.001	47.8 ± 2.01	47.6 ± 7.54	0.323
BMI (kg/m2)	33.24 ± 3.25	32.16 ± 3.08	0.001	33.89 ± 2.11	33.83 ± 2.14	0.454
Visceral fat	9.21 ± 1.05	8.71 ± 0.83	0.013	9.71 ± 0.61	9.79 ± 0.70	0.583

***AC**, abdominal circumference; **BMI:** body mass index*

Results

Anthropometric indices before and after TRX are shown in Table 1. The findings of the independent t-test revealed that there is no significant difference in the baseline levels (pre-test) of anthropometric indices between the two groups (*P > 0.05*). Examining intra-group changes by paired t-test revealed that TRX lead to a significant reduction in body weight, body mass index, abdominal circumference, body fat percentage and visceral fat. But in the control group, there was no significant difference between the pre-test and post-test levels of any of these indicators (*P > 0.05*). The significant values of changes in each of the variables are shown in Table 1.

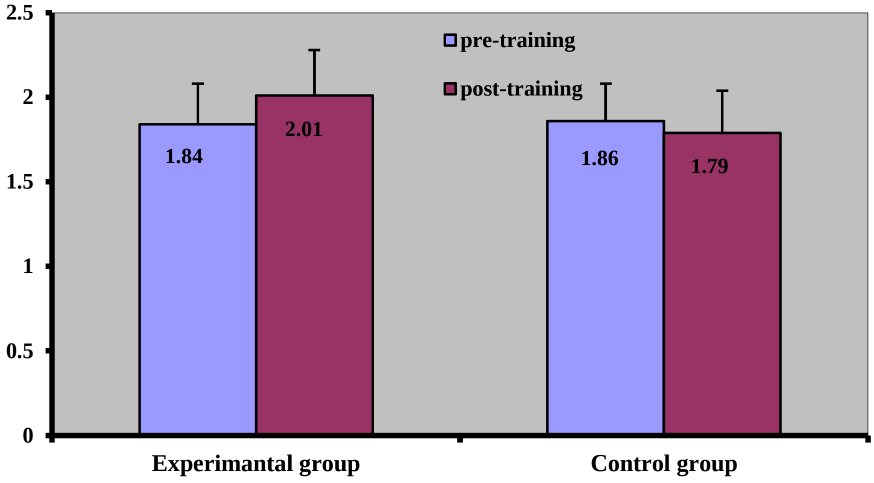

Figure 1. The pattern of total antioxidant capacity changes in the conditions before and after resistance intervention in the studied groups

Determining the effect of TRX on TAC and MPO activity is the main objectives of the study. The mean and standard deviation and the significance level related to the pre- and post-test of these variables are shown in Table 2. Comparison of pre-tests by independent t-test revealed that there is no significant difference in the baseline levels of TAC and MPO between the two groups. On the other hand, despite the lack of MPO difference (P= 0.326), a significant difference in TAC activity was observed in the post-test conditions between the two control and TRX groups (P= 0.013, Fig 1). Thus, TAC levels in the TRX group are significantly higher than the control group.

Table 2. Mean and standard deviation of TAC and MPO of studied groups.

Variables	TRX group			Control group			Pre-test difference	Post-test difference
	Pre-test	post-test	*p-value**	Pre-test	post-test	*p-value**	(*p-value*) †	(*p-value*)†
TAC (ng/ml)	1.84 ± 0.24	2.01 ± 0.27	0.004*	1.86 ± 0.22	1.79 ± 0.25	0.246	0.356	0.013†
MPO (U/mL)	27.91 ± 2.71	27.69 ± 2.41	0.459	29.61 ± 2.61	28.71 ± 3.12	0.652	0.268	0.326

* represent significant level between pre and posttest (intra-group change: data by paired t test)
† represent significant level of post-test between groups (inter-group change: data by independent t test)

Also, comparing pre-post training in each group (intra-group changes) by paired t-test showed that TRX lead to a significant increase in TAC compared to pre-test levels. However, there was no significant difference in MPO between the pre-post training conditions in the TRX group. On the other hand, no significant difference was observed in these variables in the control group. The significant values of changes in each of the variables are shown in Table 2.

Discussion

The main finding of the present study is the increase in TAC in response to TRX. In other words, 8 weeks of TRX, 3 sessions per week led to a significant increase in TAC in obese women who previously had an inactive lifestyle. However MPO serum levels did not change significantly in response to TRX intervention compared to the control group.

In this regard, in line with the present study, Carlsohn et al (2010) showed in a research that TAC values increase following regular sports activities (20). In the study of Sari-Sarraf et al (2016) after 8 weeks of progressive aerobic training and one session of sedentary activity in young inactive men, they reported an increase in TAC in the training group (21). The researcher has pointed out the increase in TAC due to the increase in glutathione levels and the effect of this increase on the change in TAC levels. Also, in the study of Pashazadeh et al (2019), who investigated the role of aerobic exercise on oxidative stress indicators in the heart tissue of bisphenol A (BPA)-poisoned rats, TAC levels decreased in the poisoned groups, but it showed a significant increase in the aerobic exercise group (22). In other words, aerobic training could reduce the intensity of oxidative stress caused by poisoning. The researcher has stated that the increase in TAC after 8 weeks of aerobic training is due to the increase in the production of antioxidant enzymes inside cardiac myocytes (22). Studies show that regular exercise plays a role in reducing the production and secretion of adipocytokines and inflammatory cytokines from skeletal muscles, endothelial cells and the immune system, as well as improving the state of antioxidants as a kind of intervention to reduce systemic inflammation. In the meta-analysis study of De Sousa et al (2017), it was also revealed that depending on the intensity, volume and type of exercise and the studied population, antioxidant indices tend to increase and oxidant indices tend to decrease (23).

In explaining the main mechanisms responsible for the development of TAC, it can be pointed out that performing regular sports exercises, including TRX exercises, through regulating and modulating the synthesis of both enzyme antioxidants glutathione peroxidase (GPX), superoxide desmutase (SOD), catalase and non-enzymatic (uric acid, albumin and ceruloplasmin) in different cells of the body including muscle cells, heart and other organs improves the total antioxidant capacity (24). It also seems that the increase in the levels of nitric oxide, ascorbic acid, bilirubin and indicators such as plasma glutathione can be effective in improving TAC caused by exercise (25). It seems that an increase in glutathione (GSH) levels following TRX training can lead to an increase in TAC levels (26). It should be noted that although the measurement of TAC as a result of enzymatic antioxidants is one of the strengths of the present study, the lack of measurement of oxidative stress markers such as MDA is one of the weaknesses of this study.

Regarding the effect of training methods on MPO, Rahimi Moghadam et al (2020) have mentioned that in the subjects who regularly participated in public sports activities, MPO levels did not change significantly compared to the control group 24 hours after the Bruce sports test. However, its value increased in the control group. These researchers believe that the subjects' active and continuous lifestyle is the cause of lower myeloperoxidase levels in the training group than in the control group (27). On the other hand, Shemshahi et al (2001) reported no change in MPO after a period of stationary exercise in inactive women (13). Nevertheless, the study of Ojaghi et al (2021) has pointed to the decrease of MPO along with the increase in the activity of antioxidant enzymes and the decrease of myeloperoxidase in the heart tissue of rats suffering from cardiac ischemia following increasing endurance exercises (28). These researchers have concluded that adapting to exercise increases antioxidant enzymes against oxidative stress damage and by positively regulating the antioxidant defense system by chaperone proteins, it protects the heart against ischemia (29).

Despite no change in MPO in response to TRX training in the present study, the enzyme MPO is a highly cationic protein that binds to endothelial cells, leukocytes, and LDL. The association of MPO with LDL leads to an increase in the oxidation of this lipoprotein (30). An increase in MPO leads to an increase in the production of some reactive oxidant species, such as hypochloric, chloramine, and tyrosine radicals, which oxidize proteins, lipids, and HDL (31). MPO enzyme is stored in the azerophilic granules of primary neutrophil cells

(30), which plays a very important role in inflammatory processes and oxidative stress, and by catalyzing the respiratory burst reaction, it causes the conversion of hydrogen peroxide into hypochlorous acid (32).

In a summary, despite no change in MPO, but relying on the increase in TAC, it can be concluded that TRX exercises are associated with improving vascular endothelial function in obese women. The increase in TAC activity may be attributed to their weight loss and reduction in body fat mass following the training period. Because obesity and high levels of body fat mass are associated with an increase in ROS and lipid peroxidation due to an increase in the availability of fat substrate (33, 34). Scientific documents show that TRX training or total body resistance training leads to a reduction in body fat percentage. In some cases, this article indicates the reduction of fat mass (35, 36,37). In the end, it is pointed out that only measuring the changes of MPO and TAC does not indicate the vascular endothelial effects of TRX exercise in obese women, but determining the changes of other antioxidant or oxidative stress markers such as nitric oxide or vascular endothelial growth factor to determine the endothelial effects of exercise is needed and lack of measurement of these variables is one of the limitations of the present study and their measurement is suggested in future studies

Conclusion

TRX training is associated with increased vascular endothelial function in obese women. Based on the findings of the present study, relying on the significant increase in TAC even in the absence of MPO change, it is possible to refer to the cardiovascular and endothelial effects of this training method. Understanding the mechanisms responsible for this training method on cardiovascular function requires more studies in this field.

Declarations

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Ethical Considerations

This study was approved by the Ethics Committee of Islamic Azad University, Islamshahr Branch (Code: IR.IAU.PIAU.R.1401.001).

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Authors' Contributions

All authors equally contributed to preparing this article.

Conflict of Interest

The authors declared no conflict of interest.

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The Effect of Eight Weeks of Yoga Practice with Weight along with Spirulina Supplement on Some Indicators of Metabolic Syndrome in Obese and Overweight Older Women

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Introduction: With the increase in the elderly population and their comorbidities, the need for appropriate prevention of their metabolic comorbidities is felt. This study aimed to evaluate the effect of yoga practice with weight and Spirulina supplement on some indicators of metabolic syndrome (Lipid profile) in older women.

Methods: This quasi-experimental study was conducted on 40 elderly volunteer women with a body mass index of 25-30kg/m^2 living in Bushehr who were randomly divided into four groups of 10 people (control, supplement (Spirulina), exercise, and exercise/supplement). The training protocol consisted of three 60-minute exercise sessions per week for eight weeks. Subjects took three 500g Spirulina supplement capsules thrice daily for eight weeks. Lipid profiles (total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL)) were measured at pretest and posttest phases. Student t-test and one-way analysis of covariance (ANCOVA) were used for statistical analysis with a significance level of $P \geq 0.05$.

Results: Yoga with weights, along with Spirulina supplementation, led to a significant decrease in total cholesterol (F=10.22), triglycerides (F=12.20), and LDL (F=16.47). In contrast, HDL increased significantly after eight weeks of exercise and supplement consumption (F=36.02).

Conclusion: The results have shown that practicing yoga with weights for eight weeks has good effects on metabolic indicators, especially with the supplement. Data proved that using Spirulina supplements for eight weeks could not produce a desirable result.

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Introduction

The proportion of elderly worldwide will increase from 12 to 22% between 2015 and 2050, showing a faster increase than other age groups [1]. The body's metabolic conditions play a leading role in the development and progression of some diseases in older adults, such as metabolic syndrome, which affects a quarter of older people [2-4]. Metabolic syndrome has well-known cardiovascular risk factors, including insulin resistance, physical inactivity, atherogenic dyslipidemia, obesity, and hypertension [5-7]. Weight loss and cardiovascular risk management through lifestyle modification are the main goals when metabolic syndrome includes lipid abnormalities [8].

Although aging is an irreversible and inevitable stage of life, physical activity is a practical tool for dealing with age-related problems [7, 9, 10]. Therefore, lifestyle changes, such as increasing physical activity and a low-calorie diet, are recommended as the first intervention to reduce blood lipids and metabolic complications [11, 12].

Regarding exercise interventions, regular exercise in older women with metabolic syndrome reduced fat percentage [13]. The metabolic benefits of resistance training are similar to those of aerobic training, and it can improve the physical performance of older adults at the same time [14]. Resistance training can increase muscle strength and size, reduce body fat, neutralize insulin resistance, and associate metabolic changes in middle-aged and older adults [15]. Strength training programs are not

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the same and do not have common goals because of the relationship of training goals and individual needs [16]. No research was found on the effect of yoga on patients with metabolic syndromes, but a recent comprehensive review found that yoga improves metabolic risk factors, including blood pressure, lipid profile, and body mass index (BMI) [17]. Asanas alone may not be effective for full-body strength training, but adding resistance equipment and other forms of movement can compensate [18, 19].

Various studies have shown the role of supplements in aging and age-related metabolic disorders [20, 21]. The researchers believe that aging exposes cells and tissues to different types of intracellular and extracellular stresses, which leads to chronic degenerative disorders and requires supplements to treat age-related disorders [20]. Spirulina, an herbal supplement, has an antioxidative role, is rich in essential fatty acids, and contains nutrients such as phycocyanin, chlorophyll, polysaccharides, and sulfolipids, which increase the body's energy [22, 23]. Studies have focused on the role of Spirulina on lipid profile [24-26]. Using Spirulina in diabetic patients, for example, showed lipid-lowering potential in a double-blind study by Rostami et al. These results and other studies have illustrated the role of Spirulina as a functional food for older adults, suggesting that Spirulina would be very efficient in regulating metabolic abnormalities in older adults, especially postmenopausal [27, 28].

The popularity of yoga as a form of fitness and physical exercise added this new dimension of combining light dumbbell exercises with powerful yoga moves. Yoga with weight (Iron Yoga) is an incredibly intense, challenging, full-body workout because of a series of upper-body exercises while balancing on one leg, such as pressing above the shoulder, pressing the chest, and biceps [29]. Yoga exercise with weight and Spirulina supplementation has been studied on some biomarkers of metabolic syndrome and lipid profile in elderly obese and overweight women. Thus, this study evaluates the possibility of this goal by practicing yoga with weights.

Materials and Methods

Participants and Study Design

This semi-experimental and practical study was conducted with pretest and posttest. In this research, 40 older women were selected according to the inclusion criteria after announcing and selecting the volunteer subjects. The inclusion criteria were residency of Bushehr, women 60-65 years old, body mass index 25-35kg/m^2, sedentary lifestyle, and lack of regular training history in the last six months. The exclusion criteria included patients with autoimmune diseases and diabetes, irregular participation in the training program, any injury or physical problem, and inability to continue training. Changes in the treatment and medication program, sports exercises except for the research protocol, and lack of consent to continue the research caused the participant's omission. The participants filled out an informed consent and completed the Demographic and Physical Activity Readiness Questionnaire for Everyone (PAR-Q+) [30]. According to the PAR-Q+, older adults with a high-risk level who did not participate had severe diseases or consumed many medications. Participants were randomly divided into four groups according to age and BMI (n=10): yoga, supplement, training and supplementation, and control groups. The participants' blood lipids were measured before and after the test, and their weight was also measured.

The experiment protocol of yoga exercise with weights was started 24 hours after the initial blood sampling (pretest phase) and performed for eight weeks [31]. The first four weeks consisted of two sessions per week, and the second four weeks consisted of three sessions per week. Each training session was based on full-body movements, starting with a warm-up and ending with a cool-down. Therefore, a combination of standing, balancing, sitting, and lying movements was included in each session, and at least six to eight movements were performed. However, the load intensity gradually increased due to time constraints, and the sessions started from 40 minutes and progressively reached 75 minutes in the final weeks. The actions were adjusted according to the principles and rules of yoga, physical fitness, and the principles of gradual overload. As initial sessions focused on structural adaptation and correct movement training, the exercise was started with 450g dumbbells and gradually increased the overload [32]. Consuming groups of Spirulina supplement, a knowledge-based product (Persian Gulf Algae Technology

Development), consumed three capsules (500g) daily for eight weeks [33].

The control group participants had no exercise intervention, and the volunteers continued their usual lifestyle. The exercise and placebo groups received the identical placebo capsules as the Spirulina capsules filled with starch and the same daily dose [34].

Each subject took a 5cc blood sample in two pretest and posttest stages after 12 hours of fasting between seven and nine in the morning. Post-test sampling was done 48 hours after the last training session. Lipid profile parameters, including triglyceride (sensitivity 1mg/dL), total cholesterol (sensitivity 2mg/dL), HDL (sensitivity 1mg/dL), and LDL (sensitivity 1mg/dL) were measured using enzymatic calorimetry method (Pars Azmoun kits, Iran).

The mean and standard deviation of the data were calculated in descriptive statistics. The Shapiro-Wilk test was used to check the normality, and a T-test was used to compare groups based on the significance level. A one-way analysis of covariance (ANCOVA) test was performed to compare between groups. Finally, an LSD post hoc test was also used to determine which group had more effects. All statistical analyses were performed using the SPSS statistical software (version 22) (significant level is $\alpha \leq 0.05$).

Table 1. Demographic data

		Control	Exercise	Supplement	Exercise + Supplement
Age (Year)		62.22 ± 2.13	62.02 ± 2.70	63.50 ± 2.48	61.64 ± 2.88
BMI (kg/m²)	**Pretest**	29.24 ± 1.34	29.25 ± 0.94	29.21 ± 0.87	29.82 ± 1.16
	Posttest	29.37 ± 0.99	28.18 ± 0.96	29.21 ± 0.90	27.65 ± 0.98

Table 2. Descriptive and analytical measurements data

variables	group	The mean and standard deviation: Pre-test	Post-test	Intragroup significance level	Intergroup significance level
HDL	**exercise**	40.96 ± 2.75	44.42 ± 2.71	0.04	0.0001
	supplement	39.53 ± 3.05	39.51 ± 2.00	0.99	
	exercise/supplement	42.04 ± 3.42	48.88 ± 2.73	0.001	
	control	40.11 ± 3.22	39.40 ± 1.47	0.54	
LDL	**exercise**	45.06 ± 3.71	40.52 ± 3.97	0.004	0.0001
	supplement	43.72 ± 2.38	43.12 ± 3.30	0.65	
	exercise/supplement	44.35 ± 3.14	35.07 ± 3.08	0.0001	
	control	43.51 ± 2.03	44.06 ± 3.01	0.58	
TC	**exercise**	225.06 ± 5.36	215.62 ± 7.83	0.01	0.0001
	supplement	219.61 ± 9.35	218.31 ± 11.97	0.74	
	exercise/supplement	225.53 ±11.17	200.43 ± 9.85	0.0001	
	control	222.79 ± 8.75	220.06 ± 11.05	0.41	
TG	**exercise**	159.03 ± 9.49	152.26 ± 9.16	0.03	0.0001
	supplement	160.32 ± 5.04	158.76 ± 6.65	0.40	
	exercise/supplement	157.29 ± 6.97	139.10 ± 9.52	0.0001	
	control	157.86 ± 8.25	158.68 ± 9.63	0.82	

Results

Participants were randomly divided into four groups (n=10). Table 1 shows the participants' ages and BMI in the pre- and post-test. No significant difference is detected in groups or between pre- and post-test data in Table 2.

The independent variable was the type of exercise, and the dependent variables consisted of low-density lipoprotein, high-density lipoprotein, triglyceride, and total cholesterol levels after exercise. Therefore, the LDL, HDL, TG, and TC levels of the participants before the intervention (exercises) were used as covariates in this analysis. Therefore, preliminary investigations were conducted to ensure that the assumptions of normality, linearity, variances, and regression slope homogeneity were not violated. The amount of changes in HDL level between groups after taking measurements and exercises were (F= 36.02), ($p<0.0001$), (Es=0.76), LDL level with (F=16.47), ($p<0.0001$), (Es=0.5), TC level with (F=10.22), ($p<0.0001$), (Es=0.46) and the TG values (F=12.20), ($p<0.0001$), (Es=0.51) showed a significant difference.

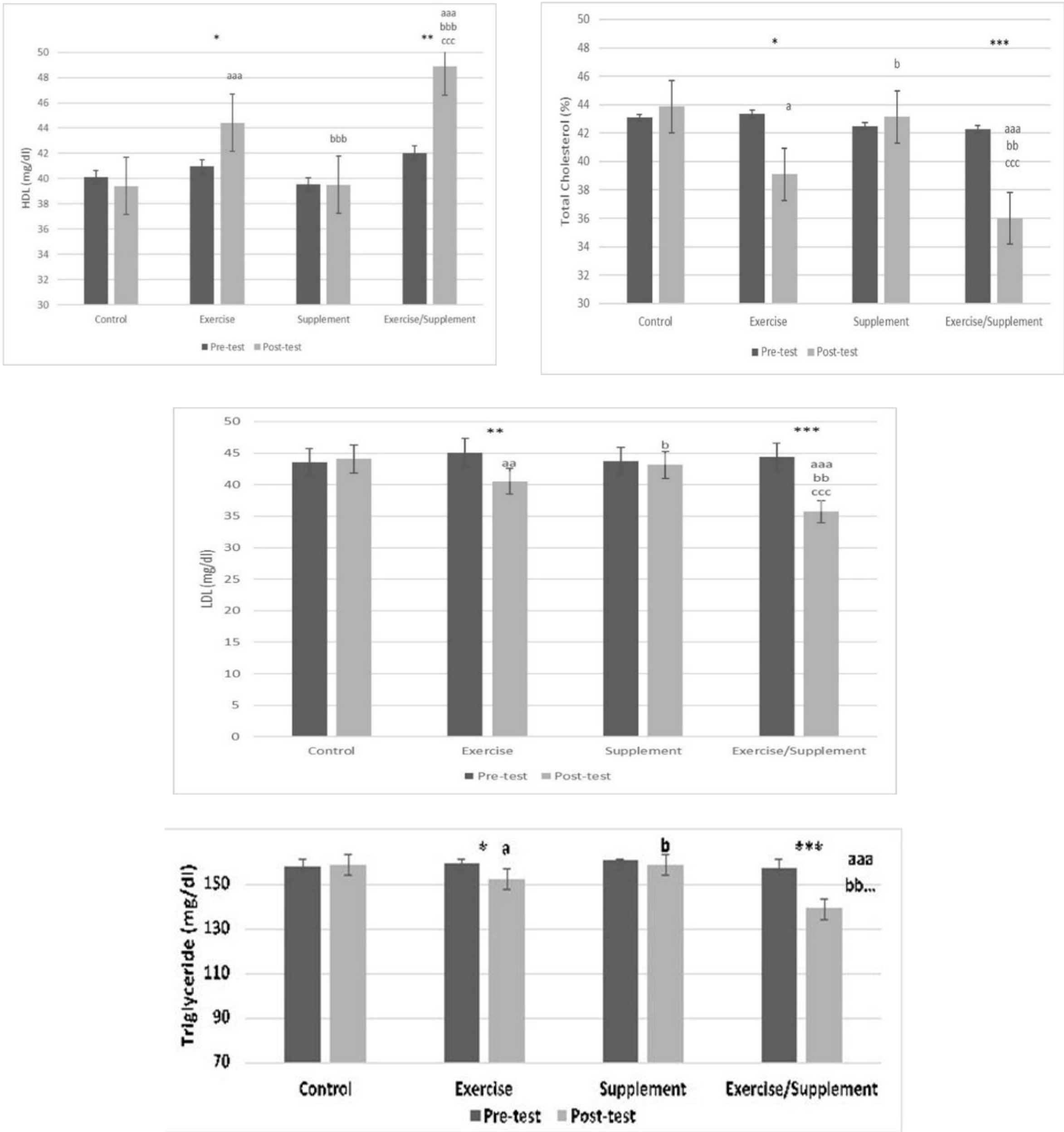

Figure1. The effect of 8 weeks of yoga practice with weight along with Spirulina supplement on Total Cholesterol, TG (triglyceride), HDL (high density lipoprotein), and LDL (low-density lipoprotein) Values before and after the examination

* Significant difference between pre and post-test groups (P≤0.05)
* *Significant difference between pre and post-test groups (P≤0.001)
*** Significant difference between pre and post-test groups (P≤0.0001)
a significant difference with control group (P≤0.05)
aa significant difference with control group (P≤0.001)
aaa significant difference with control group (P≤0.0001)
b significant difference with exercise group (P≤0.05)
bb significant difference with exercise group (P≤0.001)
bbb significant difference with exercise group (P≤0.0001)
c significant difference with supplement group (P≤0.05)
cc significant difference with supplement group (P≤0.001)
ccc significant difference with supplement group (P≤0.0001)

In the final evaluation of the LSD post hoc test and the inter-group comparison of post-test groups, the HDL variable increased significantly. The LDL, cholesterol, and triglyceride groups decreased significantly in exercise and exercise/supplement compared to the control group. In other words, consuming Spirulina supplement with exercise alone causes a significant increase in HDL concentration. Spirulina supplementation with exercise significantly reduced LDL, cholesterol, and triglycerides.

Discussion

The benefits of using Spirulina supplement and exercise showed that Spirulina supplement and exercise alone affected the lipid profile, while Spirulina alone could not be helpful. On the other hand, LDL, Triglyceride, and total cholesterol decreased significantly in the exercise and exercise/supplement groups, while HDL increased. Spirulina supplements and exercise significantly increased HDL compared to exercise, but their simultaneous benefit on other factors was not significant.

Different studies have shown that Spirulina can manage metabolic syndrome and reduce the risk of cardiovascular events. Spirulina supplementation in healthy elderly significantly reduced plasma triglyceride, total cholesterol, and LDL as a helpful food for older adults [27]. In another study, plasma levels of triglyceride, total cholesterol, and LDL were significantly reduced in older adults after Spirulina supplementation for 24 weeks [35], or consumption of Spirulina supplements in type 2 diabetic patients led to a significant reduction in the concentration of triglycerides, total cholesterol, and LDL in plasma [36]. Although various studies have shown the benefits of Spirulina supplementation on lipid profile [37, 38], the researchers believe that the optimal dose, the optimal dose, and duration of use are controversial and require future research. This uncertainty can justify the lack of Spirulina effectiveness in the present study and can be considered one of this study’s limitations.

In one study, a systematic physical exercise program and Spirulina supplementation reduced the BMI and blood lipid profile (TC, TAG, and LDL-C) of obese dyslipidemic men [39]. Hernández et al. demonstrated that Spirulina Maxima supplementation can act synergistically with exercise due to its enhanced effects on body composition and blood lipid profile in diabetic Wistar rats [14]. Another study on mice stated that Spirulina alone or exercise in diabetic mice significantly reduces total LDL, cholesterol, and liver fats [40]. The results of all these articles were consistent with those of the present research. However, the use of yoga can make the present study more practical because yoga is a complementary therapy that helps prevent and treat certain medical conditions despite the benefits of yoga exercise in controlling balance, managing pain, and preventing falls in older adults [41, 42]. Yoga effectively treats cardiometabolic risk factors, such as blood pressure, lipid levels, glucose levels, and body weight, by improving physiological stress [43]. A study reported a better lipid profile in long and medium-term meditators when compared to non-meditators [44]. Despite these claims, some evidence shows that high-intensity yoga has no significant effects on cardiovascular outcomes or blood parameters [45]. Therefore, yoga interventions significantly affected lipid profiles. However, more qualified trials or cohort studies are needed to conclude precisely [46].

Conclusions

Based on the results, yoga practice with weights had good metabolic effects, especially with the supplement. One strength of current research was the significant reduction of cholesterol and triglycerides following yoga exercises with weights, which was more effective than the Spirulina supplement. Thus, finding the best dose and the best period of administration is debated, and future investigation is required.

Declarations

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Declaration of Competing Interest

No conflict of interest can be declared.

Data Availability

Data would be available by emailing the authors.

Authors' Contribution

S.Sh., as the thesis supervisor, did the project administration. M.B. did the investigation and the software analysis; R.R. advised the project methodology. M.B. wrote the paper draft, and other authors reviewed it. All authors read and approved the final version of the manuscript.

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The Simultaneous Effect of Curcumin Extract Supplementation and Aerobic Exercise on Leptin and Adiponectin Gene Expression in Visceral Adipose Tissue of Rats Receiving A High-Fat Diet

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ABSTRACT

Introduction: Herbal remedies can be helpful in the prevention and treatment of obesity, overweight, and related disorders. However, how aerobic exercise and curcumin supplementation interact with adipose tissue is still unclear. This study aimed to determine how aerobic exercise and supplementation with curcumin extract would affect leptin and adiponectin gene expression in visceral adipose tissue of rats fed a high-fat diet.

Methods: In this study, 50 male rats were divided into five groups of equal size: control, high-fat diet, curcumin and high-fat diet, aerobic exercise and high-fat diet, and aerobic exercise and curcumin and high-fat diet. Five sessions per week of aerobic exercise were performed for six weeks at a speed of 25 to 29m/min for 20 to 45 minutes. Rats were given high-fat food emulsion of 0.5mg/kg of body weight per day for six weeks to simulate high-fat food groups. One-way analysis of variance (ANOVA) and Tukey's post hoc test were employed in SPSS software version 22 to analyze the results.

Results: There was no difference between the short-term effects of aerobic exercise, a high-fat diet, and curcumin extract on adiponectin gene expression in male rats (P=0.05). The short-term effects of aerobic exercise, a high-fat diet, and curcumin extract on the leptin gene expression in adipose tissue in male rats were comparable and not significantly different. Consuming curcumin, an antioxidant, and brief exercise affected the fat gene expression.

Conclusions: Based on the results, both agent alone decreases or increases the expression of the adiponectin and leptin genes in fat, and when both agents are consumed simultaneously, the expression increases.

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Introduction

Chronic low-grade inflammation associated with obesity can lead to metabolic dysfunction and coronary artery disease (CAD) [1,2]. Adipokines are made by adipose tissue and have the potential to either promote or inhibit inflammation [3]. Disorders linked to obesity may be exacerbated by impaired adipokine release or synthesis [1,4].

A sedentary lifestyle and changes in diet toward high-fat, high-energy-dense foods are related to the growing westernization, urbanization, and mechanization in most of the world's nations [5]. Developing nations are experiencing a nutritional transition that causes them to consume more energy-dense foods and fewer micronutrients, which may contribute to overweight and obesity [6].

Additionally, there is a direct relationship between the degree of obesity and the amount of dietary fat. Animal studies [7] have shown that high-fat diets lead to greater food intake and weight gain than high-carbohydrate diets. Caloric density, satiety characteristics, and post-absorptive processing all play a significant part.

The brain, liver, pancreas, muscles, immune system, and adipose tissue are just a few ofthe numerous organ's leptin and adiponectin have

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been shown to affect. Inflammation and immune response are affected by them; adiponectin is anti-inflammatory, whereas leptin is pro-inflammatory [8]. Low plasma levels of adiponectin and high plasma levels of leptin are characteristics of obese people [9].
The peptide leptin, released by white adipose tissue, has pro-inflammatory properties and is essential for the central regulation of metabolism and body weight. Higher leptin levels in obese subjects than in lean subjects can explain the inflammatory conditions associated with obesity and possibly the disease development [10]. Leptin regulates, synthesizes, secretes, and functions inflammatory cytokines like TNF-a, IL-6, and CRP. However, leptin is involved in the occurrence of cardiovascular diseases by influencing processes such as angiogenesis and increasing oxidative stress and calcium deposition in endothelial cells and blood vessels, proliferation of smooth muscle cells, and increasing cell adhesion molecules such as ICAM-1 [11]. In contrast to other cytokines derived from adipose tissue, adiponectin has anti-inflammatory, anti-atherogenic, and anti-diabetic properties [13]. Adiponectin is the most prevalent plasma protein in fat and glucose metabolism, vascular biology, and energy balance [12]. Adiponectin levels are decreased in individuals with insulin resistance, obesity, and coronary artery disease [14]. Adiponectin acts by inhibiting the synthesis and action of TNF-a and IL-6. Reducing the production of CRP inhibits inflammatory responses, and lowering ICAM-1 gene expression and improving endothelial function slow the progression of atherosclerosis [15]. Physical activities is one of the effective behavioral interventions in modulating inflammatory mediators [16]. Aerobic exercises independently or by modulating general risk factors such as obesity and associated abnormalities reduce the probability of cardiovascular diseases in older people [18].
Polyphenols and flavonoids are among the herbal and natural compounds that significantly impact leptin and adiponectin [18]. Curcumin is the primary natural polyphenol found in the rhizome of Curcuma longa (turmeric) [18]. It has been known for thousands of years that curcumin has therapeutic characteristics and may benefit one's health [19]. In addition, curcumin is used worldwide in various ways, such as a spice, antibiotic, anti-inflammatory, preservative, or coloring agent, as well as a supplement in the form of capsules or powder [20]. Numerous disorders, such as inflammatory and degenerative ailments, cancer, dyslipidemia, metabolic syndrome (MetS), and obesity, have been related to curcumin's positive effects [21–24]. Additionally, numerous studies have demonstrated that curcumin's antioxidant and anti-inflammatory properties account for most of its advantages [22]. Research shows curcumin exhibits anti-inflammatory, cell-protective, apoptotic, and antioxidant properties. Further, curcumin promotes the production of PGC-1 and further suppresses Adipo-R2 via activating AMPK. Curcumin also offers additional cardiovascular preventive benefits that help patients and people at risk of obesity from (high-fat) diets improve their heart health. Curcumin has been shown to have many positive effects, but there are not enough papers in this regard. Moreover, curcumin inhibits the protein breakdown pathway and affects leptin and adiponectin levels in the visceral fat tissue of obese rats [23]. Adipose tissue inflammation contributes to the emergence of eralsev diseases connected to obesity. According to the research included in this review, supplementing with curcumin causes obese and overweight people to produce much less inflammatory cytokines and have higher plasma levels of adiponectin. Curcumin can also control several molecular targets in adipose tissue, such as signaling pathways, transcription factors (NF-kB, NLP3), and other intricate regulatory systems, suppressing or lessening chronic low-grade inflammation. In vitro studies are needed to better understand curcumin's mechanisms of action and clinical trials in people, given its widespread use as a supplement for its health-promoting effects. A gender-based controlled experiment is conducted to determine genuine efficacy. For specific recommendations on curcumin intake, it is necessary to obtain consensus on the effects of curcumin and identify potential disparities between men and women when it comes to curcumin treatment.
Several human studies have demonstrated that curcumin decreases inflammation in obesity and illnesses by harmonizing anti-inflammatory and pro-inflammatory variables due to curcumin's interactions with a wide range of biomolecules, including transcription [25, 26]. Additionally, several studies have shown that curcumin may

help overweight people with metabolic syndrome lose weight when diet and lifestyle changes [27, 28]. In Vari (2021), a lipophilic polyphenol called curcumin was purified from the turmeric plant Curcuma longa. Since turmeric has numerous health benefits, it has historically been used in traditional Asian medicine. Spices are often used in new dishes around the world. Curcumin may have anti-inflammatory properties, according to numerous research. Obesity is a significant contributor to the chance of developing lsevera chronic illnesses, such as type 2 diabetes, cardiovascular disease, and several cancers. Obesity is expected to have a significant role in the pathogenesis of chronic diseases by causing the systemic and localized development of low-grade chronic inflammation in adipose tissue. Different signaling pathways are involved in the molecular mechanisms that start the inflammation generated by obesity instead of the conventional inflammatory response from infections. The inflammatory process in obese persons is brought on by inadequate nutritional intake, resulting in alterations in fat in adipose tissue on both a quantitative and qualitative level and by various chemicals working as endogenous ligands to stimulate immune cells. Defective adipocytes release adipocytokines, inflammatory cytokines, and chemokines to draw immune cells to adipose tissue and intensify the inflammatory response throughout the body [29].

Less attention has been paid to leptin and adiponectin changes that persist after exercise, and the results of current studies are conflicting. The effect of exercise on preventing nonalcoholic fatty liver disease onset showed that 16 weeks of optional running exercise at 50–75% of maximal oxygen uptake on a treadmill could express the hepatic nonalcoholic fat leptin gene in rats. Therefore, the correlation between energy expenditure and physical and sports activity is one of the critical reasons for the efficacy of exercise in treating fatty liver disease [30]. According to Sirico et al. (2020), new information on adipose tissue physiologic and inflammatory status associated with obesity may be relevant for the long-term prevention of obesity-associated chronic disease by modulating the adiponectin level. A systematic review was conducted with meta-analyses of electronically identified randomized controlled trials. A database search was conducted to evaluate the effect of physical exercise on leptin and other inflammatory markers without concomitant dietary intervention in children aged <18 years with a BMI >95th percentile by age and sex. The results showed that physical exercise decreased the amount of leptin compared to a control group that did not receive any lifestyle modification. The authors concluded that whether physical exercise reduces inflammation in obese children remains to be determined. Sarmi (2016) concluded that exercise improves endurance resistance, and combined exercises minimize insulin resistance. Combination exercise reduces insulin resistance. No significant differences in leptin levels were observed between the groups. As defined by Sarmi et al. (2016), exercise improved the fat-to-leptin ratio in mice, and combined exercise enhanced insulin resistance.

Based on the literature mentioned above on the correlation between exercise, diet, and intake of Curcumin extract supplements, this study looks at the correlation between curcumin supplement intake, aerobic exercise, and the expression of Leptin and Adiponectin genes in the visceral adipose tissues of rats, which were fed with a high-fat diet.

Materials and Methods

This developmental research uses research results to improve and refine human society's behavior, method, tool, device, product, structure, and pattern and, ultimately, to meet a need.

This randomized clinical trial design was conducted under the code of ethics no. IR.IAU.M.REC.1400.031 from Islamic Azad University. Animal subjects (males) were employed because human subjects could not be accessed due to space limitations, ethical considerations, and time limitations. First, permits were obtained, and then male and female rats were housed separately under the Iran Society for Protection of Laboratory Animals (SPLA) guidelines. The sampling method was random, and the study volume included 50 2-month-old male rats. The sample size was calculated using G POWER software based on the statistical analysis of variance with alpha error level (0.05) and power (0.85) equal to 50 rats. The sample was randomly distributed into five groups: control, high-fat, curcumin, and high-fat. The group distribution was as follows: Exercise + HFD + Curcumin + HFD + HFD.

This study selected 50 two-month-old Sprague-Dawley male field rats weighing 200 and 250g (taken from Zanjan University of Medical Sciences) as statistical samples. The rats were kept in controlled conditions for two weeks to familiarize themselves and adjust to the living, nutritional, and training conditions. Then, the rats were divided into five groups and matched according to body weight and categorized equally in weight. The control group served as a reference group and was compared with the experimental group to determine the independent variable effect. Samples were stored for two weeks under novel conditions (temperature =22±2°C, ambient humidity =50±5%) and a light-dark cycle =12:12 hours) to avoid stress and changes in physiological conditions. All subjects ate standard food and drank standard water. During these two weeks, the samples went through a familiarization program in which they were shown how to use the animal electric treadmills (ST008) (designed and manufactured in Tabriz University) (this smart animal treadmill has five separate channels). The smart program controls all related parameters, such as positive and negative slope, speed, and time. During these two weeks, the level of electric shock remained stable at a value of 0.1mV. The treadmill incline was 0% during the familiarization period, and the treadmill speed was 10 to 15m/min. The training duration was 5 to 10min/day. The tested rats were housed in a polycarbonate cage manufactured by the exclusive company of Razi Rad. The cage size was approximately 21×34×54cm. The wood chips were replaced every two days, and the cage was washed and cleaned weekly. Five rats were housed in each cage during this (compatibility with environment) period. The rats are susceptible to respiratory diseases. Therefore, dust and ammonia from the rat urine should not build up in the breeding/maintenance hall. The airflow should be changed 10 to 15 times per hour in the hall to avoid this. In this study, an ordinary ventilated animal house was used, which was kept 24 hours a day, seven days a week. After the study, the rats were randomly assigned to five groups after a weight match [33].

High-Fat Diets

In all the groups consuming high-fat diets, high-fat food emulsions containing 1.5mg/kg body weight daily were used. The composition of the diet was intended for rate in addition to the standard rodent diet [34].

Aerobic Exercise

The group completed an aerobic exercise program on an animal-smart electronic treadmill five days per week (Saturdays, Sundays, Mondays, Tuesdays, Thursdays, and Fridays) for six weeks. The intensity of aerobic activities in human subjects was determined by different ratios of maximum oxygen consumption, maximum heart rate, reserve heart rate, and speed of exercise performance. The activity intensity was controlled through the speed of running on the treadmill and its slope because of using an animal subject and the unavailability of the necessary facilities to accurately determine the intensity of the activity. The training protocol was designed based on Nashio et al. (2001), who determined the intensity of activity using the speed and slope of the treadmill for each training week after estimating the maximum oxygen consumption of rats and access to the necessary facilities. Thus, the strain, sex, age, and approximate weight of the present study subjects were also matched based on the mentioned study. Rats in each group ran on a treadmill at 11 meters per minute for five sessions a week, lasting 30 minutes each. A treadmill endurance training study was conducted on rats for one week at an average 6m/min speed and an 11-degree incline. Subsequently, the rats were subjected to endurance training at an 11m/min speed for five weeks, lasting thirty minutes daily for five weeks (33).

Curcumin Supplementation

Curcumin extract was prepared by ordering from the Medicinal Plants Research Center. The consumption of 3mg of curcumin per body weight of rats per day was considered. The daily consumption for a 70kg rat was 2.4g, fed to the rats by gavage to obtain this amount of curcumin, considering the content of 5% of curcumin [33].

RNA Extraction, cDNA Synthesis, and Gene Expression

The PCR master mix for preparing cDNA was first mixed in a microtube with a sampler proportionally to the kit protocol. Subsequently, 0.5ml of the required RNA was added to the master mixture. Finally, the ingredients were pooled in an ice pool. The real-time PCR (RT-PCR) method was employed to determine whether the gene(s) or mRNA(s) expressing the desired proteins were present. CDNA was then used by the recommended protocol for PCR using Viragen's Mix Red. The primers were taken from the vials and mixed with TE buffer according to their proportions. Then, 180ml of the buffer, 10ml of the forward primer, and 10ml of the reverse primer were poured into the chosen tubes. The applied primer was designed for two different genes, Leptin and Adiponectin, with the following sequence, length, and type:

Shapiro-Wilk was used to check the normal distribution of the variables, and Levene's test was used to examine the homogeneity of variables. The ANCOA test was used to compare the means of the research variables between the groups. The extra Bonferroni was used to determine how significant the difference between the groups was. The significance level for all the tests was $p<0.05$, and all the statistical procedures were conducted via SPSS software version 22.

Table 1. High-fat food composition table

Ingredients	Amount (grams)
Corn oil	400
Sucrose	150
Whole milk powder	80
Cholesterol	100
Multivitamin	2.5
Twin 80	36.5
Propylene glycol	31
Salt	10
Distilled water (ml)	300

Table 2. Endurance training protocol

	Warming up	the speed of endurance training on the treadmill	cooling down	the entire training time
The first week	5min, 50-60% VO2max	The speed is 25 meters per minute with a 15% slope	5 min 50-60% VO2max	20 Min
The Second week	5min, 50-60% VO2max	The speed is 26 meters per minute with a 15% slope	5 min 50-60% VO2max	20 Min
The Third week	5min, 50-60% VO2max	The speed is 27 meters per minute with a 15% slope	5 min 50-60% VO2max	30 Min
The Fourth week	5min, 50-60% VO2max	The speed is 28 meters per minute with a 15% slope	5 min 50-60% VO2max	40 Min
The Fifth week	5min, 50-60% VO2max	The speed is 29 meters per minute with a 15% slope	5 min 50-60% VO2max	45 Min

Table 3. Specifications of the primers used in the Real Time PCR process

Gene name	Primer sequence	Product length (open pair)
leptin	Forward: 5'- CGATGAGGAGCAATCCAGTCC-3' Reverse: 5'- CTCAATTTCAGCCAGACGGC-3'	228
Adiponectin	Forward: 5'- CGTGCTTGCCATTCAGAAA -3' Reverse: 5'-ATATACATCGGTCTCGGTGG -3'	244

Results

There was a normal distribution of data across all variables, as demonstrated by the Shapiro-Wilk test. Consequently, a one-way analysis of variance test was conducted to evaluate the data. The results of the descriptive characteristics of subjects (i.e., pre-test, post-test weight, and leptin gene expression in mice) in the groups are presented in Table 4.

Regarding the expression of this gene, there is a big difference between the control and high-fat diet groups, the control and high-fat diet + exercise groups, the high-fat and high-fat + exercise groups, and the high-fat and curcumin groups. Regarding Leptin gene expression, there was a significant difference in the control group, high-fat diet, control, high-fat diet + exercise, high-fat diet, and curcumin groups.

Table 4. Findings related to the descriptive characteristics of the subjects

group	Pre-test weight		Post-test weight		Leptin gene expression		Leptin gene expression	
	Mean	standard deviation	Mean	standard deviation	Mean	standard deviation	Mean	standard deviation
Control	2.06	3.02	2.25	3.11	1.38	0.043	0.48	0.052
high-fat diet	2.06	3.62	2.87	3.32	2.52	0.047	0.52	0.029
Curcumin and high-fat diet	2.06	3.11	2.51	2.09	0.83	0.045	1.64	0.039
Aerobic exercise and high-fat diet	2.06	2.89	2.75	2.11	2.24	0.072	0.83	0.038
Aerobic exercise and curcumin and high-fat diet	2.06	3.18	2.38	3.32	1.91	0.037	0.80	0.064

Table 5. Results of analysis of variance between groups of leptin and adiponectin gene expression in five groups

	leptin gene expression		adiponectin gene expression	
group	P	F	P	F
Control **high-fat diet** **Curcumin and high-fat diet** **Aerobic exercise and high-fat diet** **Aerobic exercise and curcumin and high-fat diet**	⁕<0.001	6.54	⁕<0.001	3.84

Table 6. Bonferroni follow-up test results

The variable	groups	significant level
Adiponectin gene expression	Control group and high-fat diet group	0.001
	Control group and high-fat diet group + exercise	0.003
	high-fat diet group and high-fat diet group + exercise	0.001
	High-fat diet group and high-fat diet group, exercise + curcumin	0.001
Leptin gene expression	Control group and high-fat diet group	0.012
	Control group and high-fat diet group + exercise	0.003
	High-fat diet group and high-fat diet group, exercise + curcumin	0.001

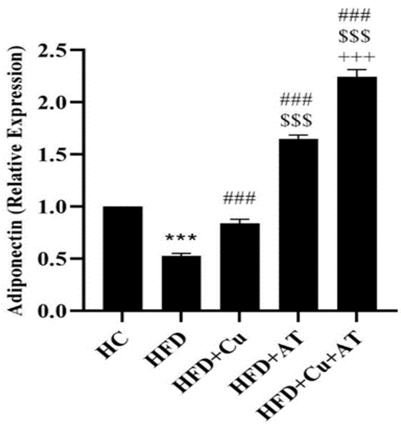

Figure 1. Adiponectin gene expression values in visceral fat tissue of rats in research groups
healthy control group: HC, obese with high-fat diet: HFD; Fattened with high-fat food+curcumin: HFD+Cu; fattened with high-fat food + exercise: HFD+AT; Fat with high fat food+exercise+curcumin ***(P=0.001) significant decrease compared to HC group
###(P=0.001) significant increase compared to HFD group
$$$ (P=0.001) significant increase compared to HFD+Cu group
+++(P=0.001) significant increase compared to HFD+AT group

Discussion

As a result of the difference between the means of the control group and the other groups, it appears that the expression of adiponectin genes decreased in the high-fat diet group and the group with curcumin. Increasing obesity and metabolic syndrome demand a cost-effective way to slow them down. Adipose tissues are active endocrine organs that secrete certain substances known as adipokines. Adipokines such as Leptin and Abdominocetin work both autocrine (paracrine) and endocrine (endocrine) (35). Adipokines regulate glucose and lipid metabolism, energy balance, feeding behavior, insulin resistance, inflammation, the immune system, adipose tissue production, and blood coagulation [36]. Based on studies, the hormone leptin is responsible for sensing the amount of fat in the blood, and its concentration is proportional to the amount of fat stored in the body, increasing adipose tissue production in obesity. On the other hand, adipokines are not inversely related to body fat mass and are involved in regulating glucose and lipid balance [37]. Studies have shown that decreased plasma adiponectin levels are associated with metabolic syndrome, insulin resistance, cardiovascular disease, and hypertension [38]. During fat storage, the metabolic activity of fat cells decreases, resulting in a less responsive response to insulin. The low ability of adipose tissue to quickly respond to insulin and other hormones is considered a factor causing insulin resistance, and the incidence of chronic diseases through these metabolic disorders is significantly increased. Exercise-induced physiological pressure, systemic hormone concentrations, and calorie intake affect plasma adiponectin concentration. Some researchers have stated that adiponectin has an inverse relationship with fat percentage and accumulation in adipose tissue [39]. Dutil et al. showed that six months of aerobic training and a daily reduction of 500 calories through sports activities cause a significant increase in blood adiponectin. In addition, adiponectin had a significant relationship with duration, intensity, and volume of exercise [40].

Curcumin may inhibit the 11 βHSD1 enzyme responsible for activating cortisol [41]. Elevated cortisol levels in adipocytes lead to central obesity. Additionally, curcumin has been suggested to reduce obesity by inhibiting premature adipocyte differentiation by inhibiting the transcription factor, PPAR-c, and increasing monophosphate-activated protein kinase activity, followed by lipolysis [41]. Previous meta-analyses have suggested that curcumin may reduce energy expenditure [42]. Another potential mechanism of action for curcumin/turmeric in obesity is hormonal. According to meta-analyses, curcumin consumption may reduce leptin levels and increase adiponectin levels, altering appetite and energy regulation. In contrast, overexpressing adiponectin hormones through activation of AMPK can increase glucose utilization and oxidation of fatty acids, promoting weight reduction. However, curcumin supplementation over six weeks did not significantly influence weight loss and BMI, which may be attributed to the short duration of supplementation. In addition to the lipid profile indicators improvement, there was a decrease in total cholesterol, triglyceride, and LDL levels and an increase in HDL and CRP levels. The changes were not massive and aligned with Sahai et al. regarding curcumin supplementation changing lipid profile levels in women with PCOS for six weeks [43]. Boam et al. also found no change in the lipid profile of older people by curcumin supplementation for six months and detected a relationship between cholesterol levels and curcumin intake [44]. In addition, a meta-analysis and a systematic study did not indicate any alterations in lipid profile levels following curcumin supplementation. However, some studies have suggested that curcumin supplementation may improve lipid profiles. A cross-over study showed a significant decrease in serum triglycerides in obese participants after 30 days of supplementation with curcumin with no alterations in other components of lipid profiles [45]. Tabrizi et al. also found that taking curcumin significantly reduced triglycerides and overall cholesterol but did not affect HDL or LDL levels [46]. Curcumin has been shown to stop free radicals from forming, and its antioxidant properties could prevent inflammation and complications from hyperlipidemia. In addition, curcumin has been shown to inhibit the activity of FAS (Fatty Acid Synthesis) and increase the oxidation of β-fatty acids with the potential to decrease fat stores effectively. Consequently, curcumin can regulate lipid metabolism through this mechanism [47]. Li et al. concluded that a high dose of turmeric reduces the food intake of rats, probably due to its strong taste or because of the fibers in turmeric that cause the release of digestive hormones, which can stimulate satiety [34]. Rostami et al. revealed that interval training with turmeric supplementation affected visceral and subcutaneous fat in obese women [33]. Curcumin increases lipolysis by increasing the enzymes in fat oxidation, such as carnitine palmitoyl transferase-1 and hormone-sensitive lipase, and decreasing the activity of fatty acid synthesizing enzymes, such as acetyl decarboxylase and the enzyme that creates triglyceride reserves, such as glycerol triphosphate acyltransferase-1 (GPAT-1), inhibit lipogenesis [49].

Previous studies have demonstrated a marked rise in the amount of Leptin after consuming high-fat meals. Leptin levels vary depending on the diet and dosage used. This significant rise in Leptin levels may indicate inflammatory conditions after consuming a high-fat diet. The precise mechanism of the Leptin response to a high-fat diet is yet to be elucidated. The induction of neutrophils may explain the Leptin response after consuming high-fat foods. Leptin resistance is associated with the disruption of leptin transfer from the blood-brain barrier. Therefore, leptin's JAK/STAT signaling is reduced, and the cytokine effect-3 (SOCS-3) suppressor is induced. Weakening of leptin sensitivity in the brain causes additional accumulation of triglycerides in the tissue, fat, muscle, liver, and pancreas, leading to impaired insulin sensitivity. Therefore, leptin contributes to insulin resistance, and exercise enhances insulin sensitivity. Exercise inhibits insulin resistance in tissues sensitive to leptin in obese individuals because leptin plays a significant role in insulin resistance [50]. Akbarpour et al. (2013) investigated the effect of twelve weeks of aerobic exercise on BMI, fat profile, body fat percentage, and some adipokines. A decrease was found in leptin, fat percentage, and BMI, and an increase was detected in adiponectin. The reduction in leptin was consistent with the decline in body fat, associated with the observed changes in energy balance, improved insulin sensitivity, and changes in fat metabolism. Since leptin levels correlate strongly with body fat percentage, after adolescence, girls' leptin levels increase and boys' decrease [51]. A limitation of this study is the lack of measurement for adiponectin, leptin, and other genes that regulate appetite. Therefore, measuring more of these related adiponectin/leptin variables is recommended in future studies. Another limitation is that exercise plays a different role in obesity and its impact on fat tissue. Therefore, measuring these variables in future studies is recommended.

Conclusion

Based on the results, the expression of the fat gene is mediated by short-term exercise and consuming antioxidant curcumin. Thus, each agent decreases or increases the expression of adiponectin in fat, and both agents increase adiponectin expression and reduce leptin expression in these cells in adipose tissue. The intake of curcumin was consistent across research groups, suggesting that the effect of short-term, aerobic exercise depends on its intensity level and that cells' gene expression changes with that intensity level.

Conflict of Interest

The authors declare no conflicts of interest in the current study, which was conducted at the author's expense.

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JOURNAL OF NUTRITION FASTING AND HEALTH

The Effect of 12 Weeks of Dry and Steam Sauna on Fasting Glucose, Sleep Quality, and Cortisol Hormone in Middle-Aged Men

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ABSTRACT

Introduction: This study aimed to evaluate the impact of 12 weeks of sauna (both dry and steam) on fasting glucose levels, sleep quality, and cortisol hormone levels in middle-aged men residing in Kermanshah, Iran.

Methods: This quasi-experimental study employed a pre-test-post-test design with two training intervention groups and one control group and examined all middle-aged men between 45 to 60 years old. Finally, based on the research objectives, 36 men in the age group of 45 to 60 years were chosen as the subjects of the purposive and convenient sampling method. At the end of the familiarization session, the pre-test values of the tested variables (glucose, hunger, sleep quality, cortisol hormone) were measured and then the subjects were randomly divided into three groups including 1) dry sauna 2) steam sauna and 3) control group. Then, the experimental group performed the dry and steam sauna program for 12 weeks. The post-test values in the variables were measured in the same conditions as the pre-test 48 hours after the last training session. One-way analysis of variance and Bonferroni tests were used to analyze the data.

Results: A significant difference was observed between the mean values before and after implementing the dry sauna and steam sauna program in fasting glucose, sleep quality, and cortisol hormone ($p \leq 0.05$).

Conclusion: Based on the results, the program involving both dry and steam sauna positively affected glucose. The exercises of the dry sauna program had less impact on sleep quality compared to the steam sauna. The dry sauna program was more influential on cortisol compared to the steam sauna.

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Introduction

Midlife and aging research has been rapidly evolving in recent decades. The traditional perspective of aging as an unalterable reality has been replaced by the hopeful possibility of increased longevity and better quality of life. According to a widely accepted definition, longevity refers to the duration of a healthy life, free from chronic illnesses and physical disabilities that are commonly associated with aging (1).

The process of aging in middle age shows itself more than ever. Some people may get sick or feel a decrease in their physical abilities. Therefore, it is essential to minimize the crises of this period. Physical activity and sports training have been widely recognized as effective strategies for preventing various health problems and diseases associated with this stage of life (2). As per the current health and exercise guidelines, it is recommended that middle-aged adults engage in moderate-intensity physical activity for 150 to 300 minutes, three to five times per week. Furthermore, it is recommended that resistance exercises be carried out at least twice per week (3). There is a comprehensive understanding of the benefits of exercise and how it can enhance cardiovascular health during this stage of life. Despite its increasing use worldwide, heat therapy and the health benefits of sauna bathing for the prevention of midlife problems are still not well known, unlike exercise(4), even though studies conducted around the world by researchers (5, 6) have found that regular sauna use is positively associated with multiple cardiovascular outcomes in middle age. Sauna

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facilities often claim health benefits that include detoxification, increased metabolism, weight loss, increased circulation, pain relief, anti-aging, skin rejuvenation, improved cardiovascular and immune function, sleep, stress management, and relaxation. However, rigorous medical evidence to support these claims is scant and incomplete, as highlighted in a recent multidisciplinary review of sauna studies (7). Healthy adults generally consider sauna bathing safe, and specific populations may also be able to enjoy this practice under medical supervision. Heat stress through sauna use induces hormetic responses mediated by molecular mechanisms that protect the body from injury, similar to those caused by moderate-to-vigorous exercise, and maybe a means of preventing the effects of aging, and increasing life expectancy.

Saunas have been used for cleansing and healing for thousands of years in many cultures. This practice can be observed in Russian Banyas, American Indian sweat lodges, and Finnish saunas. A sauna bath involves short-term passive exposure to high temperatures, which usually range from 45° to 100°C (113 to 212 F). Sauna baths cause mild hyperthermia, increasing in core body temperature and triggering a thermoregulatory response involving neuroendocrine, cardiovascular, and cellular protective mechanisms. As result of these mechanisms homeostasis is restore and the body is prepared to deal with for stressors. Observational, interventional, and mechanistic studies have provided compelling data to support the claim that sauna use can increase lifespan. Several recent reviews have described sauna use's cardiovascular, neurological, and metabolic benefits (8, 9, 10, and 11). Vandana et al. (2018); examined the effects of steam sauna baths on fasting blood glucose levels (FBGLs). In Vandana et al (2018), 80 subjects, 40 males and 40 females, between the ages of 30 and 50 were exposed to seven steam baths on alternate days.

During the study by Vandana et al (2018), the temperature of the steam sauna was maintained at 50°C and each bath was taken for 15 minutes. Blood samples for FBGL were taken before the first steam bath, and again after the seventh steam sauna bath, to estimate FBGL. The results indicated that the FBGLs significantly decreased after seven steam souna baths compared to pre-team sauna FBGLs. The researchers concluded steam saunas may help prevent hyperglycemia diabetes mellitus (12). Several other studies have also documented the positive effects of sauna on the human body (12, 13, 14, 15, and 16). Sauna bathing promotes more effective rehabilitation after injuries and relieve pain syndromes (17). There is considerable evidence that sauna bathing can produce profound physiological effects (18, 19). Most studies investigating the effects of sauna bathing have been either acute (0-30 minutes post-sauna) or short-term (2-4 weeks) (20). As pointed out by a recent review, there is a need for long-term experimental evidence in heat therapy (8), based saunas. The effectiveness of regular sauna use when combined with exercise has been shown in trained cyclists (21) and runners (22), as well as in patients with heart failure (23) and other diseases (24). A combination of sauna use and exercise has shown to be beneficial for specific populations, such as cyclists, runners, and those with heart failure. However, these studies are not availablefor the general population. Therefore, adding sauna bathing could benefit more than regular exercise alone. Previous research has shown promising results in acute responses using a sauna (25) and a combination of training followed by a sauna (25). However, it is currently unclear whether steam saunas produce the same degree of physiological responses as dry saunas (26). Reacting, more humidity leads to water condensation on the skin and reduced sweat evaporation (27).Therefore, this reasearch aimed to expand these findings and discover the possibility of sugar and hormonal compatibility and raising immunity as primary outcomes. Valuable information should be provided for using the sauna and its potential as an intervention in lifestyle along with exercise in this sensitive period. Therefore, the imposition of material and spiritual costs on the economy of families and society can be avoided mainly by preventing the problems of the middle age period. To date, few studies have been conducted on the effect of sauna bathing on sleep quality in older adults. However, the results of several studies have shown improvement in sleep quality regardless of duration (28).So far, research on the effects of saunas and their aftermath on fasting glucose, sleep quality, and cortisol hormone levels of middle-aged men has been limited to a few foreign studies, and no research has been conducted. Therefore, investigating whether sauna bath affects fasting glucose, sleep quality, and cortisol hormone or not and whether the effect of dry sauna and steam bath is different between middle-aged people. Hence, the researcher has decided to conduct a study to investigate the effects of 12 weeks of sauna use on fasting glucose, sleep quality, and cortisol hormone in middle-aged men in Kermanshah.

Material and Methods

This experimental study was conducted using an applied design with a pre-test-post-test design and a control group. The research objective was to investigate the effect of 12 weeks of sauna use on fasting glucose, sleep quality, and cortisol hormone in middle-aged men from Kermanshah. The statistical population of the present study included all middle-aged men between 45 and 60 years old in Kermanshah, Iran, of whom 36 men between 45 and 60 were selected by targeted and convenient sampling methods. The subjects' average age, weight, height, and body mass index were 52.4±0.7 years, 76.7±3.5kg, 176.5±4.58cm, and 25.9±2.8kg/m^2, respectively. First, all the subjects attended the study protocol familiarization program. The pre-test values of the tested variables (glucose hunger, sleep quality, cortisol hormone) were measured at the end of the familiarization session, and then the subjects were randomly divided into three groups, including 1) dry sauna for 45 minutes; several 12 people 2) steam sauna for 45 minutes; several 12 people and 3) the control group was divided into 12 people. Then, the experimental group performed the dry sauna and steam program for 12 weeks. The post-test values of the desired variables (glucose hunger, sleep quality, and cortisol hormone) were measured in the same conditions as the pre-test 48 hours after the last training session. The inclusion criteria were no history of illness, surgery, medication, or smoking.

Measuring Tools

The subjects' height was measured using a wall-mounted height meter model 44440 of Kaveh Company, Iran, with an accuracy of 0.0±0.1cm. Subjects' weight was measured using a Sairan PDS200 brand digital scale, France Immunotech Kit. Cortisol hormone levels are calculated using the Pars test kit made in Iran using the photometric method to measure blood glucose. The self-report Pittsburgh Sleep Quality Index, developed by Bise et al. (29) and measuring good and bad sleep quality, was used as a measurement tool to assess sleep quality. Pittsburgh index has nine general questions and measures seven components, which include subjective sleep quality, sleep delay (time from when a person goes to bed to the time of sleep onset), sleep duration, sleep adequacy (ratio of actual sleep to the time spent in bed), sleep disturbance, use of sleeping pills and daytime dysfunction. Most of the questions are of the four-choice type (never during the last month=0, less than once a week=1, 1-2 times a week=2, and three or more times a week=3) and are scored from 0 to 3. The total sleep quality index score ranges from 0 to 21, with high scores indicating poor sleep quality. Scores above five also indicate the existence of sleep disorder. The reliability of this scale was calculated as 0.83, and its validity was reported as 89.6 in the patient subjects compared to the control group (29). The validity of this index has also been confirmed in internal studies, and its reliability has been reported as 0.89 (30). The consent form was used to ensure the complete consent of the subjects to cooperate with the researcher during the implementation of the research.

Exercise Protocol

The sauna program lasted 12 weeks, with three weekly sessions, each lasting 60 minutes. The total duration of the sauna program was divided into 30 minutes of steam sauna and 30 minutes of dry sauna (31).

Research Limitations

The research limitations included the lack of complete knowledge of the subject's health, nutrition, and lifestyle, the inability to fully control the level of attention, concentration, and motivation of the participants during the tests, lack of control over the individual differences of the participants such as stress, anxiety, and excitement during the tests, lack of control over the hereditary backgrounds of the people in Physical and movement tests.

Statistical Method

Smirnov-Kolmogorov and Levene's tests were used for the normality of the data distribution and homogeneity of the groups, respectively. One-way analysis of the variance model was used to analyze intragroup changes. The Bonferroni's test was utilized to determine the difference between training methods. All statistical analyses were performed using SPSS software version 20, with a significance level of P≤0.05.

Results

The results of the first question showed that 12 weeks of dry sauna significantly affected glucose fasting in middle-aged men. A significant difference between the average values before and after the implementation of the dry sauna program can be seen in the subjects' glucose, and the averages also show that the glucose level has decreased, which was significant ($p \le 0.05$) (Figure 1).

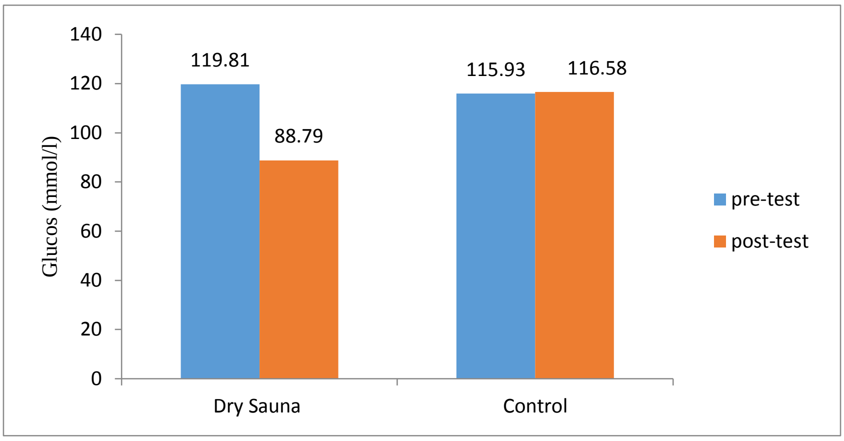

Figure 1. Glucose changes before and after the dry sauna program

The results of the second research question showed that 12 weeks of steam sauna significantly affected fasting glucose in middle-aged men. Significant differences can be seen between the average values before and after the implementation of the steam sauna program in the glucose level of middle-aged men, and the averages also showed that the glucose level decreased, which was significant ($p \le 0.05$) (Figure 2).

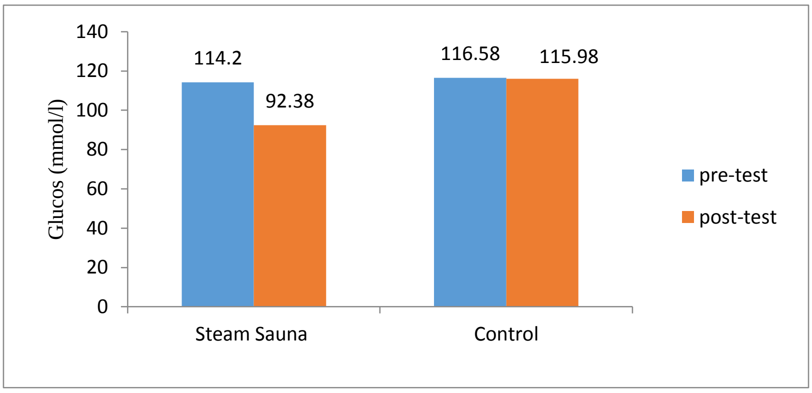

Figure 2. Glucose changes before and after steam sauna program

According to the third question of the research, 12 weeks of dry sauna use significantly affected the quality of sleep in middle-aged men. The mean values before and after the sauna program showed a significant difference in sleep quality among the participants. Additionally, the averages indicate that the sleep quality increased, which was significant ($p \le 0.05$), as shown in Figure 3.

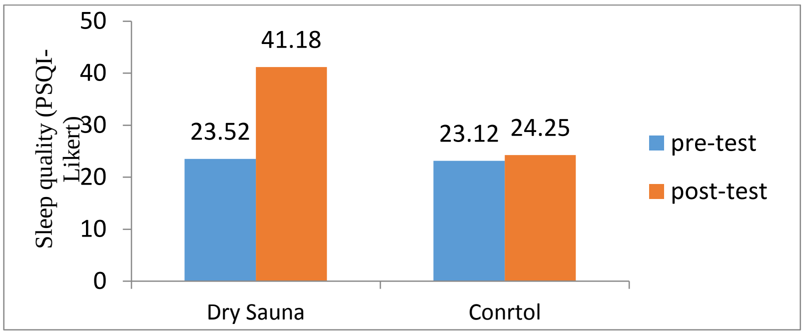

Figure 3. Changes in sleep quality before and after dry sauna

According to the fourth research question, 12 weeks of steam sauna use significantly affected sleep quality in middle-aged men. A significant difference was observed between the average values before and after the sauna program in the sleep quality among the participants. Additionally, the averages indicated that the sleep quality increased, which was significant ($p \le 0.05$), as shown in Figure 4.

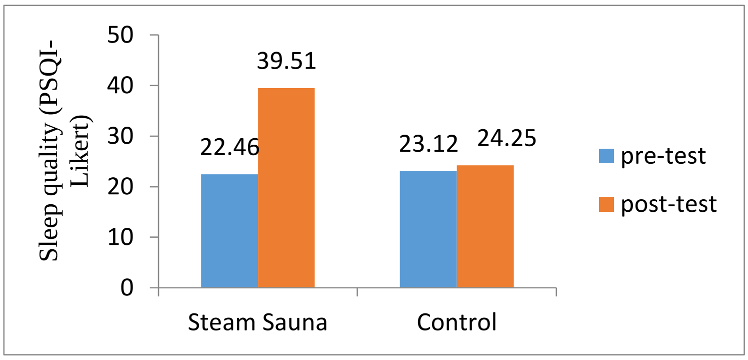

Figure 4. Changes in sleep quality before and after steam sauna

According to the fifth research question results, 12 weeks of dry sauna use significantly affected the concentration of cortisol hormone in middle-aged men. A significant difference was observed between the mean values before and after the sauna program on cortisol hormone concentration among the participants. Additionally, the averages indicated that the concentration of cortisol hormone decreased, which was significant ($p \le 0.05$), as shown in Figure 5.

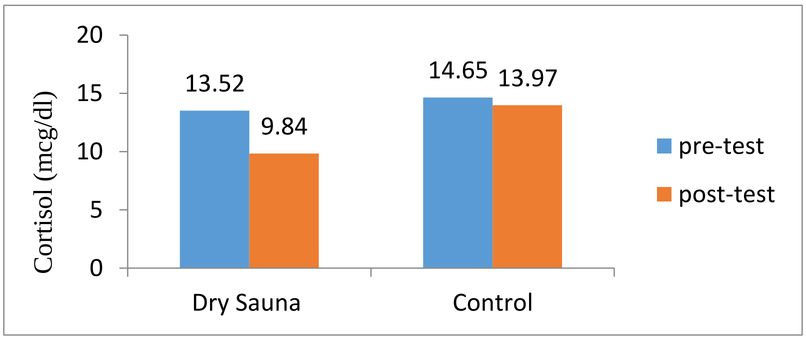

Figure 5. Cortisol hormone changes before and after the dry sauna program

Based on the results of the sixth research question, 12 weeks of steam sauna significantly affected the cortisol hormone in middle-aged men. A significant difference can be observed between the average values before exercise and after steam sauna in cortisol hormone in middle-aged men. In addition, the averages show that the amount of cortisol hormone has decreased, and this increase is significant (p≤0.05) (Figure 6).

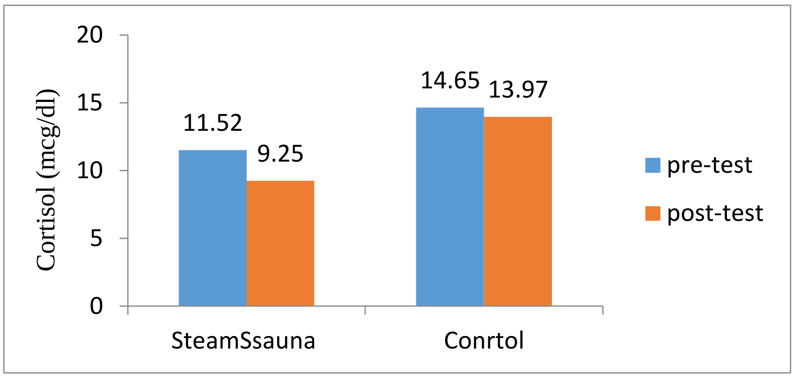

Figure 6. Cortisol hormone changes before and after the steam sauna program

The results of the seventh question showed a significant difference between 12 weeks of dry sauna and steam on fasting glucose in middle-aged men. Bonferroni's post hoc test measured the difference between groups and compared the means pairwise. Based on the results, the dry sauna and steam sauna were influential on the glucose variable of middle-aged men in the post-test stage (p≤0.05). In addition, the results indicated no significant difference in the glucose variable between the effectiveness of these two training methods in the post-test stage (P<0.05) (Figure 7).

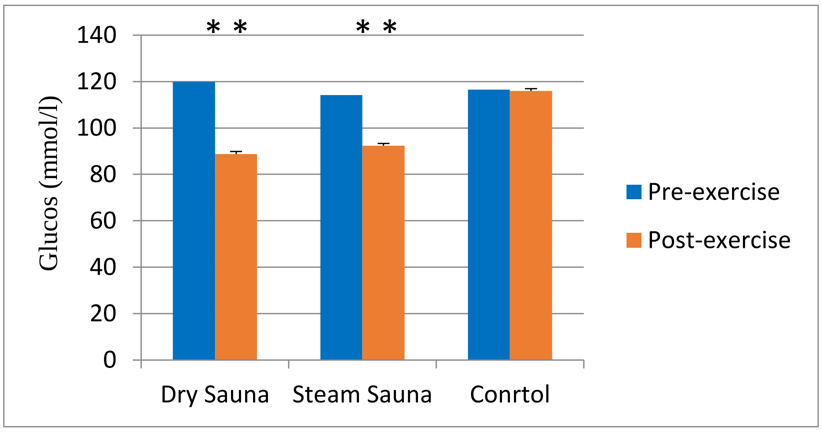

Figure7. Comparison of the average fasting glucose after exercise compared to before the sauna in the three subject groups

The results of the eighth question showed a significant difference between twelve weeks of dry and steam sauna on the sleep quality in middle-aged men. Bonferroni's 12 hoc test measured the difference between groups and compared the means pairwise. According to the results, the dry and steam sauna programs influenced the variable of sleep quality in middle-aged men in the post-test stage (p≤0.05). The results showed a significant difference in the sleep quality variable between the effectiveness of the dry sauna program and the steam sauna program in the post-test stage (P<0.05). The exercises of the dry sauna program had less effect on the variable of sleep quality than the steam sauna program (P<0.05) (Figure 8).

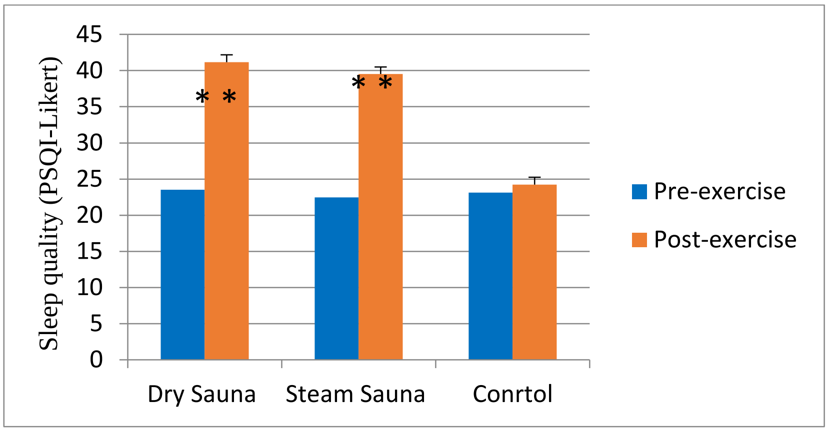

Figure 8. Comparison of the average sleep quality after the exercise compared to before the sauna in the three subject groups

The results of the ninth question indicated a significant difference between 12 weeks of dry and steam sauna on the cortisol of middle-aged men. Bonferroni's post hoc test measured the difference between groups and compared the means pairwise. Based on these results, the dry and steam sauna program influenced the cortisol variable of middle-aged men in the post-test phase ($p \leq 0.05$). Moreover, the results indicated a significant difference in cortisol between the effectiveness of the dry and steam sauna programs in the post-test stage ($P<0.05$). In other words, the dry sauna program was more influential on cortisol than the steam sauna ($P<0.05$) (Figure 9).

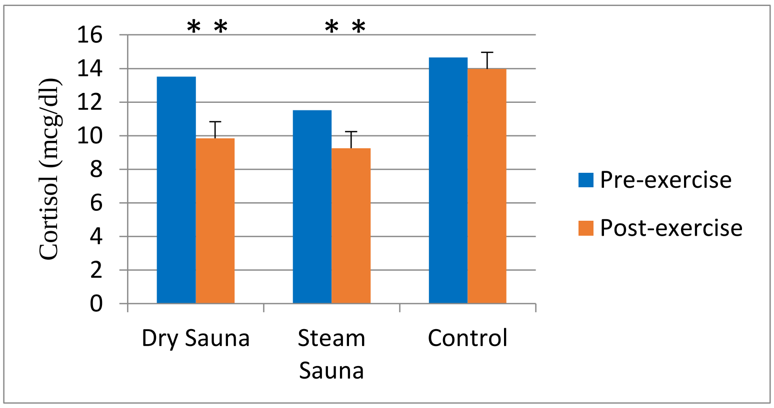

Figure 9. Comparison of the average cortisol after exercise compared to before the sauna in the three subject groups

Discussion

Recent studies have shown that sauna, also known as sauna bathing, involves short-term passive exposure to high temperatures ranging from 45 to 100°C (113 to 212°F), inducing mild hyperthermia. This exposure triggers a thermoregulatory response involving neuroendocrine, cardiovascular, and cytoprotective mechanisms working together to maintain homeostasis. The body becomes acclimated to heat with repeated sauna use and optimizes its response to future exposures, mostly due to the phenomenon of hormesis. Sauna bathing has emerged as a probable means to extend healthspan in recent decades, with strong evidence from observational, interventional, and mechanistic studies. Large, prospective, population-based cohort studies have reported strong dose-dependent relationships between sauna use and reduced morbidity and mortality rates, which are particularly interesting. During this review, the body's physiological response to heat stress is explained, as well as the molecular mechanisms involved; the numerous health benefits of sauna use are discussed, as well as concerns about saunas. The present study investigated the effect of sauna programs (dry and steam) on glucose metabolism in middle-aged men. Based on the test results in two groups, dry and steam sauna positively and significantly affected glucose depletion. In addition, the test results showed a more significant effect of the dry sauna selected in the present study than the steam group, and the fasting glucose of the dry sauna group was significant compared to the control group. Therefore, the fasting glucose of the steam group is significant compared to the control group. These results are independent of the implementation time of the sauna program, in line with those of Arena et al. (2015) (34), Stanley et al. (22), and Pleich et al. (2013) (36). Stanley et al. (2015) noted an average increase in axillary body temperature of 2.6°C after the first sauna versus an average increase of only 1.9°C after completing five months. The researchers also found that the sauna bath increased the average venous pH after ultrasound, decreased the average baseline excess, and increased the average venous 2O and hemoglobin concentration. Lubin pointed in the blood was 5.2%, and the change of the oxygen-hemoglobin dissociation curve, the reduction of affinity, and the release of 2O to the tissues after the first sauna returned with similar changes in the specified parameters were observed after the final sauna five months later (22). There is considerable evidence that sauna bathing can produce profound physiological effects (19, 20). When exposed to short-term extreme heat, skin and core body temperatures increase, and thermoregulatory pathways through the hypothalamus (37) and CNS (central nervous system) are activated. As a result, the autonomic nervous system is activated. The sympathetic nervous system, the hypothalamic-pituitary-adrenal hormonal axis, and the renin-angiotensin-aldosterone system are all activated, resulting in established cardiovascular effects such as an increase in heart rate, skin blood flow, cardiac output, and sweating (16). The sweat from the sauna evaporates from the skin's surface, producing a cooling effect that facilitates temperature homeostasis. Sauna therapy capitalizes on the thermoregulatory property of homeothermy as the physiological ability of mammals and birds to maintain a relatively constant core body temperature with minimal deviation from a set point (38). The degree of these reactions is affected by the humidity level, as increased humidity can lead to water condensation and reduced sweat evaporation. At the cellular level, both wet and dry forms of thermotherapy can produce metabolic changes such as the production of heat shock proteins, reduction of reactive oxygen species and oxidative stress, reduction in inflammatory pathway activities, increased availability of nitric oxide and insulin sensitivity, and changes in endothelial-dependent vasodilation metabolic pathways (39). Hoekstra et al. (2020) demonstrated that exercise coupled with a passive elevation of body temperature can lead to acute increases and chronic reductions in inflammatory markers while positively impacting glycaemic control markers (40). Additionally, the study revealed that dry sauna significantly affected the sleep quality of middle-aged men, with the sleep quality of the dry sauna group being significantly higher than that of the steam sauna group. Furthermore, an increase in sleep quality was reported after a session of both dry and steam sauna. However, the dry sauna group improved sleep quality more than the steam sauna group. The results showed that a dry sauna bath improves sleep quality more than a steam sauna. The results of the research are consistent with those of Ainye and Vardani (2021) and Hayasaka et al. (2010) (28), who determined the effect of spa and sauna on sleep quality and blood glucose levels in people with type 2 diabetes. This study showed that the diabetic foot spa bath and sauna affected sleep quality and blood glucose levels. Ainye and Vardani (2021) indicated the effect of saunas and diabetic foot spas on the sleep quality of people with type 2 diabetes. The average sleep quality decreased after the intervention. Diabetic foot spa is a massage on the soles of the feet to improve blood circulation and increase insulin. In comparison, a sauna bath is a heat or temperature therapy using heat so people can sweat. Sauna bathing can increase blood circulation, detoxify the body, improve cardiovascular function, and improve sleep quality (41). Previous research has reported on the impact of diabetic foot spas on the sleep quality of individuals with type 2 diabetes (42). A study assessed the effect of diabetic foot spa and sauna bathing on sleep quality and blood glucose levels in individuals with type 2 diabetes. The participants underwent three consecutive sauna bathing sessions for 15 minutes each, accompanied by a diabetic foot spa. The intervention reduced the physiological response to stress and promoted relaxation, thereby improving sleep quality. Additionally, a significant decrease was observed in the average blood sugar level after the intervention. The results suggested that diabetic foot spa and sauna bathing may have a positive effect on sleep quality and blood glucose levels in individuals with type 2 diabetes. According to previous research, a high blood glucose level before intervention may be influenced by gender. However, bathing in a sauna has increased body temperature, glucose metabolism, and consumption. In addition, more capillaries are opened, allowing insulin receptors to become more active. Running a spa and sauna bath can accelerate the reduction of blood glucose levels by increasing glucose consumption while improving blood circulation. During a session, individuals often feel relaxed, which can improve the quality of sleep. The hot temperature in the sauna causes the body to sweat, dilate blood vessels, stimulate blood circulation, and release endorphins, ultimately promoting relaxation. Diabetic foot spa and sauna baths may increase sleep quality. (43). Other research has shown that hot baths can have the same health benefits as exercise, including reducing inflammation, improving blood sugar, and lowering blood pressure. Still, other studies have indicated that exposure to extreme cold can help people burn fat, improve the immune system, and counteract some of the effects of type 2 diabetes. Exposure to high temperatures significantly strains the system and regulates heat and blood circulation. The human body actively responds to thermoregulatory mechanisms to maintain thermal homeostasis. Sweating on the body's surface efficiently eliminates excess heat that reaches the body from a hot environment. However, intense sweating is often associated with water loss from the body, which can lead to a decrease in blood volume and blood plasma volume and loss of body mass (36). Hayasaka et al. (2010) investigated the effects of such bathing on health status and quality in research using a population-based cross-sectional study including 617 Japanese participants who underwent routine medical examinations. Bathing times were classified into two levels: "less than seven times a week" (less bathing group) and "seven times or more a week" (frequent bathing group). The characteristics of age, body mass index, blood pressure, blood chemical findings, health, self-assessment, and sleep quality were compared between the two groups. The group that bathed frequently had better health and sleep quality with an adjusted odds ratio based on age and gender for self-rated and reported health. Besides sleep quality, other survey items were similar between the two groups. Bathing in a bathtub every day or more was associated with good health status and sleep quality (44), consistent with the present study. Another result of this research was the positive and significant effect of selected sauna (dry-steam) on cortisol in middle-aged men. The results showed that the dry sauna program had a greater and more significant effect on the hormone levels of the subjects than the steam, which is in contradiction with the research of Lee et al., 2019 (45) due to the type and amount of training and even the subjects' age. The alternating hot and cold conditions used in the sauna bath are considered to accelerate biomedical sports recovery and are often used as therapy in sports, recreation, and rehabilitation. During a sauna session, the human body is exposed to cold and hot stimuli. The hot air in the sauna room affected the skin and respiratory system, increasing core body temperature to 39°C, while skin surface temperature may even rise to 42°C (46). A body unable to release heat will likely overheat (15, 16). The increase in central temperature caused by the sauna session causes the release of psychotropic adrenochor, cortisol, and catecholamines (36). Elevated cortisol concentrations after a sauna session provide a highly sensitive indicator of heat stress in response to excessive heat during sauna bathing. In Folnius et al., the level of peripheral blood cortisol increased in both groups of men in this experiment, but the strongest secretion of this hormone was observed in untrained men compared to athletes. Thus, sauna bathing induces more thermal stress in untrained individuals than athletes. Adaptation to heat stress in the sauna is manifested by a lower increase in cortisol levels. The findings of different researchers about the changes in cortisol levels caused by hyperthermia in the sauna are not the same. Most studies investigating this issue show that cortisol levels are not increased or decreased (36). Subjects who had never been to a sauna or who had adapted to heat stress in a sauna after several exposures were especially affected. According to other authors, a significant cortisol secretion was observed in people not adapted to a warm environment, both in men and women (36). Although Fulnius et al. (1982) argued that cortisol is a sensitive indicator of heat stress, the rectal temperature threshold at which cortisol secretion increases is 38°C. In the present experiment, the rectal temperature exceeded this threshold at the end of the sauna session. In contrast, in the above studies (15), the rectal temperature did not exceed the level of 38°C, and no increase in peripheral blood cortisol concentration was observed. The results of Fatoros et al. (2002) stated that the day time does not affect aerobic performance, which is contradictory. One of the other reasons why the evening program helps the subjects perform better is that the body is under a type of stress in the morning due to the release of cortisol and hormone cortisol in stressful situations (environmental effects, emotional pressure, activity sports, injury, infection, etc.). This hormone is called adaptation or stress hormone, and stress also causes changes in the immune system and the level of performance. The mechanisms of this difference in the increase in cortisol hormone after a dry sauna and steam bath are unclear. This difference seems to be due to the thermoregulatory and circulatory systems. Exposure to high temperatures imposes a significant burden on the thermoregulatory and circulatory systems. the body actively responds to thermoregulatory mechanisms to maintain thermal homeostasis. Sweating on the body's surface is an efficient way for the human body to get rid of excess heat that reaches it from a hot environment. However, when intense sweating occurs, water loss from the body can result in a decrease in blood and plasma volume and loss of body mass. (36).

Conclusion

According to these results, physiological variables, especially the hot temperature in the sauna, cause the body to sweat, dilate blood vessels, start blood circulation, stimulate endorphins, and affect blood glucose levels. As a result of increasing sleep quality, different groups of society are recommended to use saunas, especially dry saunas, to improve sleep quality and reduce blood sugar. Therefore, this implementation can help prevent diabetes and blood pressure complications and improve sleep quality in middle-aged people. Based on the results, the dry and steam sauna program reduced middle-aged men's cortisol variable. The dry sauna program was more influential on cortisol than the steam sauna.

Declarations

Acknowledgments

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Authors' Contributions

All the authors have the same contributions in the execution and authorship of the study.

Conflict of Interest

The authors declare no conflict of interest in this study.

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JOURNAL OF NUTRITION FASTING AND HEALTH

Effects of Green Tea Extract on Ox-LDL and Homocysteine Levels after Resistance Exercise in Obese Men

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ABSTRACT

Introduction: Green tea is a well-known source of polyphenol catechins, which possess strong antioxidant properties. However, the impact of green tea polyphenol catechins on biological markers of atherosclerosis, namely oxidized low-density lipoprotein (ox-LDL) and homocysteine (Hcy), following resistance exercise (RE), has not been studied in obese individuals.

Methods: In this study, ten obese untrained men (age 43-45 y, BMI 32-33) participated voluntarily. They were randomly assigned to receive either green tea extract (GTE) capsules (two capsules of 500 mg per day) or placebo (PL) capsules (two capsules of 500 mg per day maltodextrin) in a double-blind, placebo-controlled crossover design. The supplementation period lasted for two weeks, followed by a two-week washout period. Afterward, the participants performed a RE protocol at 75% of their one-repetition maximum (1RM). Blood samples were collected before and after the RE session to measure the serum concentrations of Hcy and ox-LDL.

Results: In the placebo condition, there was a significant increase in serum Hcy and ox-LDL levels from pre- to post-RE. However, GTE supplementation mitigated the exercise-induced rise in serum Hcy and ox-LDL concentrations in obese men.

Conclusion: These findings suggest that a two-week supplementation of GTE may offer protection against exercise-induced elevation of Hcy and ox-LDL levels in obese men.

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Introduction

In today's world, obesity has become a significant health concern due to physical inactivity and excessive energy intake, leading to abnormal accumulation of fat in adipose tissue. This condition poses a major threat to human health [1]. Obesity can result in oxidative stress, characterized by an increase in the production of reactive oxygen and nitrogen species (ROS, RNS) and a decrease in antioxidant levels. Oxidative stress induced by obesity is primarily attributed to the oxidation of low-density lipoprotein (LDL) [2]. This oxidation is associated with endothelial dysfunction, atherosclerosis, and cardiovascular disease (CVD) [3]. Oxidized low-density lipoprotein (ox-LDL) serves as a reliable clinical biomarker for oxidative stress [4] and is considered a risk factor for CVD [5]. Previous studies have demonstrated that ox-LDL plays a crucial role in the initiation and progression of atherosclerosis [6]. Macrophages' scavenger receptors detect the produced ox-LDL, leading to the uptake of a large amount of cholesterol by these cells. Consequently, macrophages transform into foam cells as a result of this process [7, 8]. Therefore, preventing LDL oxidation may be the initial step in inhibiting their binding and accumulation by macrophages, thereby preventing their transformation into foam cells.

Apart from ox-LDL, homocysteine (Hcy) is another risk factor for the development of atherosclerosis. Hcy is an amino acid containing a thiol group and is produced during methionine metabolism in the liver [9]. Increased Hcy levels can be influenced by various physiological, genetic, and nutritional factors [10]. Hcy has been implicated in endothelial dysfunction through several mechanisms, including increased production of pro-inflammatory cytokines, impaired vasodilation, platelet accumulation, and elevated oxidative stress [11]. Additionally, Hcy has been shown to contribute to the oxidation of LDL and the production of ox-LDL [12]. Elevated Hcy levels have also been associated with reduced physical performance in

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adults and may play a role in morphological changes in skeletal muscle [13].

In recent years, numerous studies have indicated that plasma levels of Hcy increase following acute exercise [14, 15]. Previous research has observed an elevation in Hcy levels after acute circuit resistance exercise (RE) at 40% of one repetition maximum (1RM) in overweight women [16] as well as after acute circuit RE at 35% of 1RM in untrained men [17]. The mechanism proposed for the exercise-induced increase in Hcy production is likely related to the enhanced methionine methylation and subsequent Hcy synthesis triggered by exercise. While there is currently no research on the impact of acute RE on ox-LDL concentration, recent findings have demonstrated higher circulating levels of ox-LDL in overweight and obese individuals compared to those with normal weight [18]. Furthermore, these findings have shown an increase in LDL oxidation due to the generation of free radicals [19]. Therefore, it is evident that the most effective strategy to counteract LDL oxidation and Hcy production involves incorporating an antioxidant-rich diet to reinforce antioxidant systems and prevent the production of reactive oxygen species (ROS).

Previous research has highlighted the positive impact of beta-carotene, alpha-tocopherol, and green tea catechins on LDL oxidation and Hcy production [20-23]. Green tea, in particular, has gained significant attention as a herbal antioxidant [24]. It contains polyphenol catechins such as (−)-epigallocatechin gallate (EGCG), (−)-epicatechingallate (ECG), (−)-epigallocatechin (EGC), (−)-epicatechin (EC), and epigallocatecanine (EGCG), which exhibit potent antioxidant properties and have been shown to inhibit LDL oxidation [23, 25, 26]. Intense exercise can lead to an increase in free radicals like reactive oxygen species (ROS) and reactive nitrogen species (RNS), surpassing the body's antioxidant capacity and resulting in oxidative stress that can damage cellular structures such as proteins, lipids, and DNA. Earlier studies have demonstrated that green tea extract (GTE) containing 250 mg of catechins enhances antioxidant capacity and prevents oxidative damage in healthy individuals [27]. However, there is currently no research on the effects of GTE on atherosclerosis biomarkers following RE in obese individuals. Therefore, the present study aimed to investigate the impact of GTE on Hcy and ox-LDL levels, which serve as biological markers for atherosclerosis, after RE at 75% of one repetition maximum (1RM) in obese men.

Material and Methods

Study Design and Participants

Ten obese men (BMI above 30 kg/m^2) who were apparently healthy volunteered to participate in this study (Table 1). The participants were non-smokers, free from any diseases, not taking any medications or antioxidant supplements, and had not consumed any polyphenolic-rich foods for the past four months. Additionally, none of them had engaged in regular exercise training within the previous six months. The protocol followed the guidelines set by the Human Ethics Committee of the institutional review board, and all participants provided written informed consent before participating in the study.

Table 1. Physical characteristics of the subjects (n=10) in GTE and PL conditions.

Variables	Group	Mean±SD
Age (y)	GTE	43.45±3.2
	PL	45.80±4.12
Height (m)	GTE	1.75±0.02
	PL	1.78±0.01
Weight (kg)	GTE	110.25±7.5
	PL	112.5±5.5
BMI (kg.m^{-2})	GTE	32.42±1.70
	PL	33.06±2.25

Experimental Procedure

The study employed a randomized, double-blind, placebo-controlled, crossover design, which included two 14-day supplementation periods followed by a RE protocol and a subsequent 14-day washout period [28]. One week prior to the supplementation, participants visited the human performance laboratory for familiarization, completion of the Par-Q Health History questionnaire, and measurement of their one-repetition maximum (1RM) in various exercises such as bench press, lat pull down, biceps curl, leg flexion, leg extension, and leg press. Throughout the study, participants were instructed to maintain their regular diets and refrain from engaging in any additional physical activity. Before each testing session, participants recorded their food intake for three consecutive days (Saturday, Monday, and Wednesday) to ensure consistency in their dietary patterns during the two RE protocols, based on the information provided in their dietary record sheets.

In a randomized, double-blind, crossover design, participants ingested either green tea extract (GTE) or a placebo (PL) for 14-day periods, with each consisting of 2 capsules per day. Both the GTE (250 mg GTE gelatin capsules, Olimp Labs, Poland) and PL (250 mg Maltodextrin) capsules were identical in shape, size, and color. The participants took the GTE and PL capsules twice a day, during breakfast and dinner, with an adequate amount of water. Each GTE capsule (55% EGCG) contained 249 mg of polyphenols, including 200 mg of catechins (137.5 mg EGCG). On the 15th day of each supplementation period, participants returned to the human performance laboratory. They took one capsule of either GTE or PL in the morning and an additional capsule one hour before the RE protocol [28]. The RE protocol consisted of three sets to exhaustion for exercises such as bench press, lat pull down, biceps curl, leg flexion, leg extension, and leg press, with a weight equivalent to 75% of their one-repetition maximum (1RM) and a 2-minute rest between sets and exercises [28]. Prior to the RE protocols, all subjects performed a warm-up, which included a 3-minute run, 5-10 repetitions at 50% of their perceived maximum, and a stretching period (Figure 1).

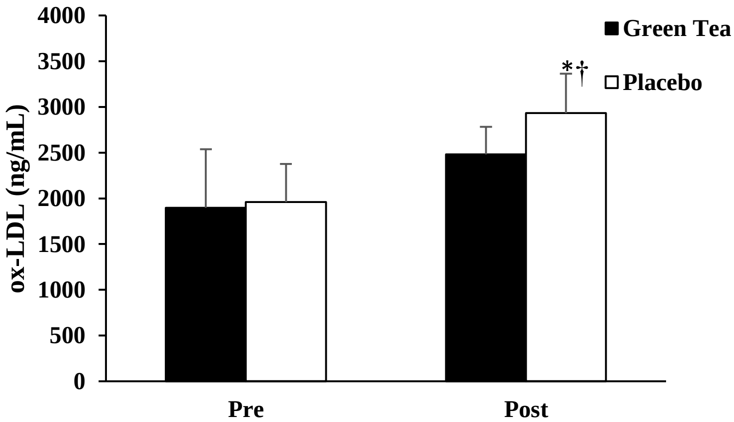

Figure 1. Serum ox-LDL concentrations in pre- and post-RE in GTE and PL condition in obses men (Mean±SD).
* Significant difrences with pre-RE (p<0.05).
† Significant difrences with GTE (p<0.05).

Biochemical Analysis

Forearm vein blood samples were collected from the participants before (pre) and immediately after (post) performing the resistance exercise (RE) to determine the serum concentrations of Hcy and ox-LDL. The serum was obtained by centrifuging the blood samples at 3000 rpm for 10 minutes at 4ºC and then stored at -80ºC for later analysis. The serum concentrations of Hcy (Cat.No. FHCY100, Axis-Shield, UK) and ox-LDL (Cat.No: CK-E10869, HANGZHOU EASTBIOPHARM CO.,LTD) were measured using commercially available ELISA kits.

Statistical Methods

The data are presented as Mean ± SD. Statistical analysis was conducted using SPSS 21 (SPSS, Chicago, IL) for Windows. The changes in serum levels of ox-LDL and Hcy were analyzed using a repeated-measures analysis of variance (ANOVA) with a 2 (treatments) × 2 (times) design. Post hoc analysis was performed using the Bonferroni test. In cases where significant interaction effects were observed ($p \leq 0.05$), independent and paired t-tests were used to assess simple main effects. The significance level was set at $p < 0.05$.

Results

The serum concentrations of ox-LDL and Hcy were analyzed using a 2-way ANOVA. The supplementation (green tea vs. placebo) was considered as the between-subjects factor, and the time of measurement (pre and post) was considered as the within-subject factor.

The results showed a significant main effect of time (F=19.40, η^2=0.58, p=0.001) and treatment (F=5.95, η^2=0.48, P=0.029) on serum ox-LDL concentration. However, there was no significant interaction between treatment and time (F=1.28, η^2=0.08, p=0.28).

After the RE, the post-RE ox-LDL concentrations significantly increased in the placebo condition (p=0.003), but there was no significant change in the GTE condition (P=0.078). The post-RE serum ox-LDL concentration was significantly higher in the placebo condition compared to the GTE condition (p=0.029) (Figure 1).

The results regarding Hcy concentrations were presented in Figure 2. The analysis showed a significant main effect of time (F=10.02, η^2=0.41, p=0.007), indicating that there was a difference in Hcy concentrations between pre and post measurements. However, there was no significant main effect of treatment (F=2.91, η^2=0.17, p=0.11) or interaction between treatment and time (F=1.85, η^2=0.11, P=0.19; Figure 2).

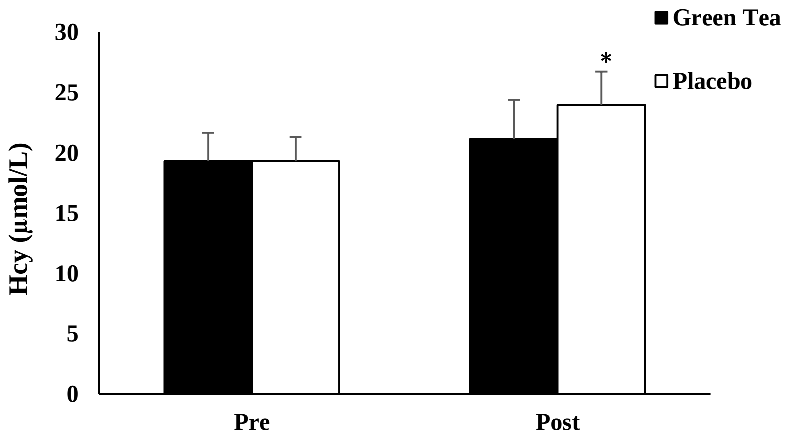

Figure 2. Serum Hcy concentrations in pre- and post-RE in GTE and PL condition in obses men (Mean±SD).
* Significant difrences with pre-RE (P<0.05).

Specifically, the serum concentrations of Hcy were significantly higher in the post-RE measurement compared to the pre-RE measurement in the placebo condition (p=0.014), but there was no significant change in the green tea extract (GTE) condition (p=0.247).

Discussion

The primary result of this study showed that consuming a short-term dose of 500 mg of GTE significantly reduced ox-LDL levels after a resistance exercise session at 75% of 1RM in untrained men with obesity. Obesity is widely acknowledged as a condition linked to elevated levels of inflammatory markers, ROS, RNS, and a decrease in antioxidant capacity. These factors are considered significant contributors to the development of various diseases, including atherosclerosis, type 2 diabetes, hypertension, and certain types of cancer [3]. Additionally, research has shown that obese individuals experience higher levels of exercise-induced oxidative stress compared to those with normal weight [29]. Therefore, it is reasonable to suggest that obese individuals should consider implementing dietary interventions, including the consumption of antioxidant-rich foods, before engaging in exercise training programs to counteract the detrimental effects associated with obesity. In this study, obese untrained men were given a daily dose of 500 mg of green tea extract (GTE) for two weeks prior to participating in a resistance exercise (RE) session at 75% of their one-repetition maximum (1RM). The results showed no significant differences in Hcy levels between the GTE and PL conditions following the short-term supplementation period. However, there was a notable trend indicating a greater percentage change in Hcy concentration in the PL condition (26.14%) compared to the GTE condition (11.53%) in response to RE. This suggests that GTE led to a

14.61% reduction in the Hcy response to RE in obese men. To the best of our knowledge, no previous studies have investigated the effect of GTE on serum Hcy levels after exercise, not only in individuals with normal weight but also in the obese population.

Hcy is widely recognized as an independent risk factor for cardiovascular diseases (CVD) [13]. A meta-analysis conducted by Boushey et al. (1995) concluded that Hcy is an independent risk factor for atherosclerosis in coronary, cerebral, and peripheral arteries. They found that every 5 μmol/L increase in total plasma Hcy level increases the risk of coronary artery disease by 60% in males and 80% in females [13]. Furthermore, a decrease of 3 μmol/L in Hcy has been reported to reduce the risk of ischemic heart disease by 16% [30]. Elevated levels of circulating Hcy pose a risk for CVD through various mechanisms. These mechanisms include inhibiting anticoagulant reactions associated with the endothelium, promoting platelet accumulation and thrombosis through oxidative stress, and activating signal transmission pathways that lead to inflammation and apoptosis [6]. These factors contribute to the increased risk of CVD associated with elevated Hcy levels.

A previous study reported that consuming green tea for one month resulted in a notable decrease in Hcy levels and a significant increase in antioxidant levels among patients with coronary artery disease [22]. However, our findings are inconsistent with this study [22]. The disparity in results could be attributed to variations in the duration of supplementation (one month vs. two weeks) and differences in the participant populations (CVD patients vs. obese individuals). It appears that the beneficial effects of green tea supplements may require a longer duration of use to be observed effectively.

Previous studies have reported an increase in Hcy levels following acute circuit resistance exercise (RE) with 40% and 35% of one-repetition maximum (1RM) in overweight and untrained men and women [16, 17]. In our present study, we observed increases of 11.53% and 26.14% in Hcy density after a resistance exercise session in obese males who consumed green tea and a placebo for 14 days, respectively. These findings indicate that resistance exercise has an impact on increasing Hcy levels, which is consistent with previous research [16, 17]. In a study conducted by Iglesias et al. (2012), an increase of 25.7% in Hcy levels was observed in 8 untrained males after cycling at 85% of their VO_2Peak. This increase is somewhat similar to the Hcy response (26.14%) observed in our study after resistance exercise under placebo conditions [31]. Other studies examining the acute effects of exercise on Hcy levels in trained participants have shown diverse results. Some studies found no effects [32-34], while others observed significant increases [11, 31, 35, 36] or decreases in circulating Hcy levels after intense exercise [37].

The increase in Hcy levels after exercise has been attributed to various mechanisms [38, 39]. One proposed mechanism suggests that the temporary decrease in renal blood flow during exercise is associated with an elevation in Hcy concentration [39]. Another potential mechanism is related to energy metabolism and substrate utilization, which can vary based on the intensity and duration of exercise [38]. Additionally, several studies have suggested a role for Hcy in energy metabolism [38, 40]. During intense exercise, such as resistance exercise (RE), there is a high demand for creatine to support energy production. This process of creatine synthesis in the liver may contribute to the formation of Hcy. It has been previously reported that approximately 75% of daily Hcy production is attributed to creatine synthesis in the liver [41].

In our current study, supplementation with GTE resulted in a 14.61% reduction in the circulating Hcy response to RE compared to a PL in obese men. This finding suggests a beneficial effect of GTE on this risk factor for cardiovascular disease. The exact mechanism by which GTE decreases Hcy levels is not fully understood. However, several mechanisms have been suggested, including the presence of folacin (folic acid) [42] and the antioxidant properties of catechin (EGCG) found in green tea [43].

Elevated levels of Hcy in the bloodstream have been linked to the oxidation of LDL and the formation of ox-LDL, which contributes to the early development of atherosclerosis lesions [44]. Previous studies have demonstrated that antioxidant supplements can inhibit LDL oxidation, reduce LDL sensitivity to oxidation, and decrease the production of ox-LDL [45, 46].

In our current study, the main finding was that obese participants who consumed 500 mg of GTE daily for two weeks experienced a reduction in LDL oxidation. This reduction occurred in individuals who engaged in acute resistance exercise at 75% of their 1RM. While there are no specific studies on the effect of GTE on ox-LDL levels after intense exercise, previous research has shown that EGCG in green tea can inhibit LDL oxidation induced by Cu+2 ions, leading to a decrease in ox-LDL and an improvement in cardiovascular function [25] [26].

Furthermore, various studies have confirmed the role of lipoxygenase in LDL oxidation caused by endothelial cells and macrophages [47, 48]. The catechins present in green tea can inhibit lipoxygenase enzymes and act as scavengers of free radicals, functioning as chain-breaking antioxidants [49, 50]. Additionally, a study involving 12 healthy untrained men who consumed 600 ml of green tea daily for four weeks demonstrated a significant decrease in ox-LDL levels [51].

Anaerobic activities, such as RE, can trigger the production of ROS through various mechanisms, including the xanthine-xanthine oxidase pathway, neutrophilic respiratory burst, self-oxidation of catecholamines, hypoxia-ischemia, and conversion of superoxide to hydroxyl radicals [52]. The production of ROS through these pathways can lead to the oxidation of LDL. In our current study, we observed that LDL oxidation increased to a greater extent in the PL condition compared to the GTE condition during RE. This finding is consistent with previous studies that examined the effect of intense exercise on LDL sensitivity to oxidation, which also demonstrated an increase in LDL oxidation after acute exercise [53, 54]. Ox-LDL is taken up by Lectin-Like Ox-LDL Receptor 1, which is expressed on the endothelial cells of blood vessels. This uptake leads to an increase in intracellular ROS production, activating the NF- κ B pathway [55]. Ox-LDL also enhances the expression of monocyte-chemoattractant protein-1 (MCP-1) in macrophages [56] and endothelial cells [57]. The expression of MCP-1 on endothelial cells plays a crucial role in the migration of monocytes to the subendothelial space [57]. During this process, monocytes transform into macrophages, and a significant amount of LDL-cholesterol is absorbed by macrophages, leading to the formation of foam cells.

Conclusion

In summary, the short-term supplementation of GTE containing 498 mg of polyphenols, including 275 mg of EGCG, in obese men resulted in a reduction in LDL oxidation following acute RE. This reduction in LDL oxidation has the potential to delay the progression of atherosclerosis and decrease the risk of coronary disease by inhibiting LDL oxidation and foam cell formation. Additionally, GTE supplementation showed a tendency to improve circulating Hcy levels in response to acute RE, with a reduction of 2.81 μmol/L (14.61%). This reduction in Hcy level is approximately equivalent to a 10% decrease in the risk factor for cardiovascular disease [30]. Therefore, it is recommended that the obese population, before engaging in intense acute exercise, consider consuming GTE to reduce cardiovascular disease risk factors. Overall, our findings, combined with previous studies, suggest the beneficial effects of GTE on circulating ox-LDL and Hcy levels in obese individuals participating in acute RE. However, further research is needed to determine the long-term effects of GTE supplementation and higher doses on cardiovascular disease risk factors in the obese population.

Declarations

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Author Contributions

M.R.R. contributed to the conceptualization, methodology, formal analysis, investigation, and writing of the original draft. A.J. was involved in the design, research, and data collection for the project. All authors have read the manuscript and agree with its content.

Data Availability

All data used in this manuscript will be made available upon reasonable request.

Submission Statement

This manuscript is not being submitted for review or publication elsewhere while it is under review for this journal.

Ethical Approval

The protocol followed the standards set by the Human Ethics Committee of the institutional review board. All participants provided written informed consent to participate in the study.

Conflict of Interest

The author declares no conflicts of interest in relation to this work.

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Simultaneous Effect of Atorvastatin and Combined Training on lipid and hepatic enzymes in Rats induced Non-Alcoholic Fatty Liver Disease

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ABSTRACT

Introduction: Non-alcoholic fatty liver disease (NAFLD) is one of the most common liver diseases, the prevalence of which is increasing. This study aimed to evaluate the effect of atorvastatin and combined training in NAFLD-induced rats.

Methods: This study was conducted on 21 male Wistar rats, divided into two groups: 1) HFFD + combined training + atorvastatin and 2) HFFD + atorvastatin. The groups received HFFD for 15 weeks to induce NAFLD. Atorvastatin was administrated at the dose of 2mg/kg/day. The interventions (atorvastatin and combined training) were performed for eight weeks.

Results: Alanine transaminase (ALT) was significantly reduced, and high-density lipoprotein (HDL) was increased in the HFFD + atorvastatin group. Low-density lipoprotein (LDL) decreased significantly in the HFFD + combined training + atorvastatin. There was no significant difference in the aspartate transaminase (AST), triglyceride (TG), alkaline phosphatase (ALP), and weight between the groups.

Conclusion: Based on the findings, Atorvastatin, along with combined training, improved NAFLD. Therefore, they can be used to reduce the complications of NAFLD. However, more studies are needed to confirm the results.

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is an exceedingly prevalent chronic liver condition that encompasses a broad range of hepatic steatosis leading to cirrhosis (1). The incidence of this ailment is on the rise, with approximately 2.9 to 7.1% of the general population in Iran grappling with NAFLD (2). Multiple investigations have demonstrated a significant relationship between NAFLD and obesity, type 2 diabetes, cardiovascular disease, as well as sedentary behavior (3). Aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), and other markers of hepatic diseases may be helpful to surrogate measures of NAFLD (4). Accordingly, measurement of aminotransferases, blood lipids (Triglyceride (TG), Total Cholesterol (TC), low-density lipoproteins (LDL), and high-density lipoproteins (HDL), and insulin resistance (IR) are often used in clinical settings to detect NAFLD (5). Currently, there are no FDA-approved pharmacological interventions for treating NAFLD (6). Consequently, the primary action in addressing this affliction involves altering one's lifestyle by reducing the consumption of a diet high in fat and carbohydrates while increasing physical activity (7). Mainly, physical activity exerts its beneficial effects through the enhancement of lipolysis and secretion of pro-inflammatory cytokines, thereby playing a pivotal role in the regulation and control of inflammatory conditions such as NAFLD (8). Lack of physical activity, which encompasses a dearth of bodily movement and exercise, stands as a fundamental component within the realm of cardiovascular risk factors, manifesting as an influential determinant for the emergence of various debilitating conditions

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such as cardiovascular diseases, diabetes, and other causes of death. The absence of regular physical exertion can serve as a remarkable indicator in predicting the occurrence of these health issues. Regardless of whether an individual possesses exceptional stamina or formidable muscular power, athletes, owing to their consistent engagement in rigorous athletic endeavors, exhibit significantly diminished levels of TG, TC, and LDL while concurrently indicating elevated levels of HDL, which confers an enhanced ability to ward off the aforementioned cardiovascular ailments (9).

Resistance training (RT), which emphasizes muscle mass and fortitude, has been recognized as an effective modality for eliciting beneficial effects on the human body. Especially, RT has yielded analogous metabolic advantages compared to aerobic activities while imposing a lesser burden on the cardiorespiratory system (10). Simultaneously, a novel form of training, denoted as intense interval training (HIIT), has garnered considerable attention due to its time-saving nature and heightened efficacy. This revolutionary training approach was instrumental in improving body composition and effectively inducing weight reduction through intermittent and high-intensity exertions, unveiling its potential as a promising intervention in physical fitness and well-being (11). HIIT is characterized by the repetition of brief and intense training sessions, followed by periods of rest or low-intensity exercise. HIIT (12) increases the transfer of free fatty acids (FFAs) from adipose tissue, ultimately resulting in β-oxidation. This biochemical pathway involves the breakdown of FFAs, resulting in the generation of energy for bodily functions (13). On the other hand, combined training in middle-aged women does not have a positive effect on inflammatory factors and adipokines (14).

Among the widely utilized medications are cholesterol-lowering drugs, particularly statins, which operate by exerting control over the production of cholesterol by inhibiting a critical regulatory enzyme within the cholesterol synthesis pathway (15). Furthermore, atorvastatin, a type of statin, has been shown to possess other beneficial therapeutic effects, even when administered at low doses (16). These additional effects encompass anti-inflammatory and antioxidant properties identified in various studies (17). For instance, atorvastatin has been shown to protect cellular structure from damage caused by ischemia and insufficient blood supply to tissues and prevent the loss of antioxidant enzyme activity (18, 19). Considering the information above, the objective of the present study revolves around evaluating the concurrent impact of atorvastatin administration and combined training in male rats with NAFLD. The purpose of this investigation is to examine the effects of atorvastatin, both alone and in combination with exercise training, on the pathology and potential amelioration of NAFLD in male rats. Incorporating exercise training into the study design is particularly important as it permits the study to examine the synergistic effects that may result from both atorvastatin therapy and combined exercise (endurance and resistance). This study can provide a deeper understanding of the interplay between pharmacological interventions and lifestyle modifications in managing NAFLD, thereby contributing to developing more effective treatment strategies for this prevalent liver disorder.

Material and Method

Animal and Design

In this experimental study, 21 male Wistar rats (270-370g) were obtained from Shahid Mirghani Research Institute (Golestan, Iran) and subjected to a 12:12h dark/light cycle at 20-24°C with unrestricted access to food and water. NAFLD was induced in the animals following the protocol of Eslami et al. after a week of acclimation to the environment (20). Blood and liver samples were collected after 15 weeks from five randomly selected rats to assess alanine aminotransferase (ALT) levels and liver tissue changes, which revealed the presence of NAFLD. Subsequently, the rats were divided into two groups: high-fat-fructose diet (HFFD) + atorvastatin (n=8) and HFFD + combined training (CT) + atorvastatin 2mg/kg (dissolved in 6% DMSO, gavage) (Raha Pharmaceutical co, Iran) (n=8), with the interventions being administered for eight weeks. HFFD consists of 45% fructose and 35% olive oil consumed by gavage.

Measurement of Biochemical Indices

The rats were anesthetized through intraperitoneal injection of ketamine (50mg/kg) and xylazine (5mg/kg, Merck, Germany) (21), with the levels of aminotransferases and alkaline phosphatase (ALP) determined through standard enzymatic techniques, and TG, LDL, and HDL levels evaluated using an auto-analyzer (BT-3500, Biotecnica Instruments, Italy) (22).

Combined Training Protocol

Tables 1 and 2 contain the protocol for resistance training and HIIT, respectively (23).

Table 1. Protocol of resistance training

	Session	1st	2nd	3rd	4th	5th	6th	7th	8th	9th
1st week	Repetition	1	1	1	1	1	1	1	-	-
	%	0%	20%	30%	40%	30%	20%	0%	-	-
2nd week	Repetition	0	1	2	2	2	1	0	-	-
	%	0%	20%	30%	40%	30%	20%	20%	-	-
3rd week	Repetition	0	1	1	1	1	1	1	1	-
	%	20%	30%	40%	50%	40%	30%	20%	0%	-
4th week	Repetition	0	1	1	1	1	1	1	1	1
	%	0%	20%	30%	40%	50%	40%	30%	20%0	0
5th week	Repetition	0	1	1	1	2	1	1	1	1
	%	0%	20%	30%	40%	50%	40%	30%	20%	0%
6th week	Repetition	0	1	1	1	1	2	1	1	1
	%	0%	30%	40%	50%	60%	50%	40%	30%	0%
7th week	Repetition	1	1	1	2	2	1	1	-	-
	%	0%	40%	50%	60%	50%	40%	0%	-	-
8th week	Repetition	1	1	2	3	1	1	1	-	-
	%	0%	40%	50%	60%	50%	40%	0%	-	-

Table 2. Protocol of HIIT

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

Statistical Analysis

The distribution of data was determined using the Shapiro-Wilk test. At the same time, the homogeneity of variances was checked using Levene's test, and the mean of the desired variables was compared using One-way analysis of variance (ANOVA), all analyzed using SPSS software version 16 at a significance level of P≤0.05.

Ethical Statement

The research followed the guidelines outlined in the publication "Guide for the Care and Use of Laboratory Animals" by the US National Institutes of Health (NIH publication No. 85–23, revised 1996). The study protocol was approved by the ethics committee in the local jurisdiction (IR.SSRC.REC.1402.119). Every endeavor was undertaken to mitigate animal distress and limit the number of animals employed.

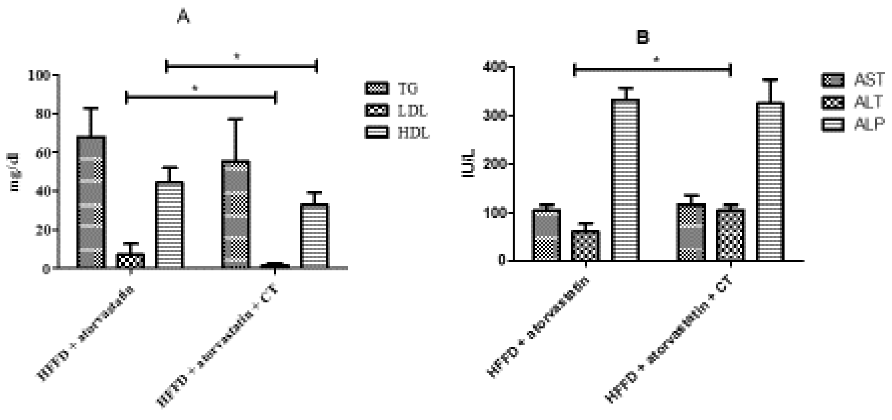

Figure 1. Mean and SD of biochemical parameters in eight weeks of interventions. A: lipid profile, B: liver enzymes

Results

There is no notable disparity in the magnitude of serum TG (P= 0.293), AST (P= 0.251), and ALP (0.749) between the two groups. Consequently, the administration of atorvastatin in isolation yielded for eight weeks a marginal decline in ALT

(P=0.000) and elevation in HDL (P= 0.019) levels. Conversely, co-administration of atorvastatin and CT caused a significant reduction in LDL (P=0.024) level in HFFD + CT + atorvastatin compared to HFFD + atorvastatin (Table 3). The average weight over eight consecutive weeks did not exhibit any noteworthy variance between the two groups. The outcomes of the ANOVA were employed to compare weight in eight consecutive weeks, presented in Table 4 and Figure 2.

Table 3. Average of biochemical parameters in eight weeks of interventions

Group	TG	LDL	HDL	LDL/HDL	ALT	AST	ALP
HFFD + Atorvastatin	62.75 ± 10.81	8.62 ± 5.15	47.02 ± 4.91	0.11 ± 0.10	66.85 ± 9.99	101.78 ± 7.38	342.50 ± 14.10
HFFD + CT + Atorvastatin	66.60 ± 13.14	1.01 ± 0.26	31.46 ± 5.79	0.03 ± 0.007	103.27 ± 7.96	112.53 ± 17.34	314.67 ± 44.44
P	0.293	0.024*	0.019*	0.053	0.000*	0.251	0.749
F	1.230	7.016	7.816	4.798	34.575	1.485	0.108

*Significant: P≤0.05

Table 4. Average of weight in eight weeks of interventions

Group	W1	W2	W3	W4	W5	W6	W7	W8
HFFD + Atorvastatin	332.74 ± 33.92	329.85 ± 34.19	326.93 ± 38.82	336.45 ± 43.10	369.02 ± 42.10	359.12 ± 43.08	360.48 ± 40.37	370.23 ± 38.93
HFFD + CT + Atorvastatin	375.15 ± 46.57	360.65 ± 33.22	368.66 ± 37.94	375.92 ± 40.56	365.06 ± 21.20	372.08 ± 19.73	382.26 ± 19.92	383.85 ± 19.78
P	0.115	0.149	0.092	0.136	0.833	0.495	0.233	0.441
F	2.977	2.448	3.464	2.625	0.047	0.502	1.613	0.643

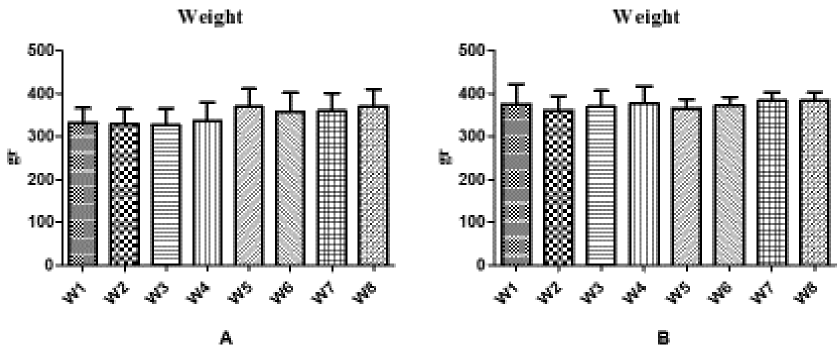

Figure 2. Mean and SD of weight in study groups during intervention. A: HFFD + atorvastatin, B: HFFD + atorvastatin + CT

Discussion

The findings indicate that the administration of atorvastatin for eight weeks significantly reduced ALT levels and elevation of HDL levels. Conversely, engaging in CT concurrently with atorvastatin administration decreases serum LDL levels. Other biochemical parameters did not show significant changes. Various studies exploring the impact of training, including different types, intensities, and durations, on liver enzymes in individuals with NAFLD have yielded conflicting results. For instance, a study demonstrated that 12 weeks of HIIT decreased liver fat and liver enzymes such as ALT and AST in NAFLD patients (24). Another investigation revealed that both HIIT and resistance training led to a reduction in liver fat content in individuals with NAFLD. Furthermore, the plasma concentration of AST did not experience a significant alteration following either type of training, whereas the concentration of ALT exhibited a significant decrease in both groups (25). There have been a limited number of trials that have examined the effects of combined training in patients with NAFLD. Houghton et al. demonstrated that a 12-week intervention involving combined training resulted in a significant reduction in liver fat content, regardless of any accompanying weight loss. However, this intervention did not impact the liver enzymes AST and ALT (26). Another study showed that 12 weeks of HIIT training improved obesity indices more effectively than moderate-intensity training (MIT) (27). Pekkala et al. showed that HIIT and MIT are equally effective in combating obesity (28). The results of the present study indicated that combined training is associated with a decrease in HDL levels, which was in line with the results of Mirghani et al. (29). Piano et al. examined the effects of endurance training alone or in conjunction with resistance training on patients with NAFLD to ascertain the impact of exercise training on individuals with NAFLD. The findings indicated that combined training, encompassing both endurance and resistance exercises, yielded greater efficacy in reducing the prevalence of NAFLD than endurance training alone. Additionally, Hallsworth et al. discovered no significant decrease in ALT levels after an eight-week training period. However, Baba et al. demonstrated that a three-month regimen of aerobic training coupled with a dietary intervention led to a decrease in aminotransferase levels, although no significant alterations were observed in the lipid profile. The inconsistency in the findings about the lipid profile may be ascribed to the variances in the type and duration of the training sessions.

Hepatic enzyme activity is affected by various factors, including the duration, intensity, type, and mode of exercise. Training background, subject characteristics, physical fitness level, and exercise type should also be considered, as each can contribute to these discrepancies. Ebrahimi et al. conducted a study demonstrating that an eight-week period of moderate and vigorous aerobic exercise did not yield any discernible impact on the liver enzymes of rats fed a diet high in fat. Conversely, Mirdar et al. observed that levels of aminotransferases increased post-exercise, which contradicts the investigation findings mentioned above. The elevation of circulating aminotransferase levels can potentially be attributed to muscular damage resulting from exercise. Haghigi et al. affirmed that pharmacotherapy, physical activity, and dietary interventions exhibit similar efficacy in mitigating the severity of liver disease as determined by ultrasound.

Atorvastatin reduces cholesterol by inhibiting the enzyme HMG-CoA reductase in the liver and is rapidly absorbed through the gastrointestinal tract. Most therapeutic drugs for treating various ailments undergo metabolism within the liver. Consequently, these metabolic transformations have the potential to generate harmful metabolites, resulting in the possibility of acute and chronic liver damage in the event of enzyme system disruption. The current investigation demonstrated that atorvastatin alone reduced ALT levels and increased HDL levels. Conversely, Eslami et al. revealed that the administration of atorvastatin at a dosage of 10mg/kg over eight weeks was associated with a decrease in aminotransferase and triglyceride levels, as well as an increase in LDL levels (22). In this particular context, applying atorvastatin at 10 mg/kg/day in rats with NAFLD for eight weeks yielded positive enhancement in the quantities of TG, cholesterol, and liver enzymes (30). Mirghani et al. showed that concurrent training is the most suitable type of training to improve cardiovascular factors by preventing the reduction of HDL levels by comparing eight weeks of strength training and concurrent training on men (31). In a study, the effect of aerobic exercise was investigated on the expression of genes related to lipid metabolism (MAPK P38 and UCP-1). The results indicated that 12 weeks of aerobic training (five sessions per week) is associated with decreased MAPK P38 gene expression in subcutaneous adipose tissue (32). The results of this study indicated that CT does not make a significant difference in the weight of rats fed HFD. In contrast, another study shows that endurance exercise improves anthropometric indices (33).

Conclusion

Based on the results, administration of atorvastatin in conjunction with combined training leads to a reduction in ALT and LDL levels and an increase in HDL levels. Consequently, a regimen combining training and atorvastatin administration seems feasible for treating patients with NAFLD by taking advantage of both approaches.

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Conflict of Interest

The authors declare no conflict of interest regarding the publication of this article.

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