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Leveraging Advanced Technologies to Support Fasting During Disasters

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Dear Editor

In recent years, the role of advanced technologies in various fields has expanded dramatically, offering innovative solutions to longstanding challenges (1). One area where these technologies hold significant potential is in supporting individuals who observe fasting during disasters (2). Disasters, which have been increasing in frequency and intensity globally, often lead to disrupted food and water availability, forcing many individuals into involuntary fasting. In such circumstances, people may also engage in intentional fasting for religious, cultural, or health reasons, further complicating their nutritional needs during emergencies (3). These scenarios highlight the importance of addressing not only fasting practices but also broader nutritional issues, including food availability, water sustainability, and associated health hazards, in the context of disasters. This letter aims to discuss how advanced technologies, such as telemedicine and mobile applications, can provide crucial support to those who fast in these critical times, ensuring their well-being and adherence to fasting practices.

The Role of Advanced Technologies

Telemedicine

Telemedicine has revolutionized healthcare delivery by providing remote consultations and medical advice (4). For individuals observing fasting during disasters, telemedicine can offer immediate access to healthcare professionals who can provide guidance on managing fasting in the context of their health conditions and the current emergency (2, 4). This ensures that individuals receive timely and personalized medical advice without the need for physical visits to healthcare facilities, which may be inaccessible or overwhelmed during disasters (1). Additionally, telemedicine platforms can address nutritional hazards faced during disasters, such as dehydration and malnutrition, by offering tailored advice for maintaining health despite food and water limitations.

Mobile Applications

Mobile applications designed for health monitoring and management can be invaluable tools for those fasting during disasters (4). These apps can offer features such as reminders for hydration and medication, tracking of vital signs, and access to educational resources on maintaining health while fasting (1). Importantly, these applications can also integrate real-time updates on food and water availability in disaster-stricken areas, enabling users to plan their nutritional intake accordingly and mitigate sustainability challenges. Additionally, apps can provide real-time updates on the disaster situation, helping individuals make informed decisions about their fasting practices (4).

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Wearable Devices

Wearable devices, such as smartwatches and fitness trackers, can monitor various health parameters, including heart rate, blood pressure, and hydration levels (4). For individuals fasting during disasters, these devices can provide crucial insights into their health status, alerting them to potential issues that may require medical attention (2). This proactive approach to health monitoring can prevent complications and ensure that fasting practices do not compromise individuals' well-being (4). Wearables can also play a role in detecting early warning signs of nutritional deficiencies or dehydration, which are common risks in disaster settings.

Communication Platforms

Advanced communication platforms, including social media and instant messaging services, can facilitate the dissemination of important information related to fasting and health during disasters (5). Healthcare professionals and community leaders can use these platforms to share guidelines, tips, and updates, ensuring that individuals have access to accurate and relevant information (2). For example, these platforms can be used to circulate information about safe water sources, available food supplies, and precautions to minimize nutritional risks during disasters.This can help alleviate anxiety and confusion, promoting a sense of community and support (4).

Virtual Support Groups

Virtual support groups can provide a platform for individuals fasting during disasters to connect with others who are experiencing similar challenges (5). These groups can offer emotional support, share practical advice, and create a sense of solidarity (2). Advanced technologies enable the creation and management of these groups, making it easier for individuals to find and join communities that align with their needs (1). Furthermore, these groups can serve as forums to discuss the complexities of fasting during disasters, offering tailored advice for managing both intentional and forced fasting due to food scarcity.

Conclusion

Incorporating the discussed advanced technologies into disaster preparedness and response frameworks is crucial for addressing the multifaceted challenges of fasting and nutritional needs during emergencies. This includes considering the prevalence of disasters, forced fasting due to limited food and water availability, and the sustainability of nutritional practices. By utilizing tools like telemedicine, mobile apps, and communication platforms, individuals can better manage their health, adhere to fasting practices, and mitigate the risks associated with nutritional hazards.

These technologies, when combined with public health initiatives, can play a pivotal role in ensuring the well-being of vulnerable populations during disasters. I strongly encourage further research and policy initiatives to integrate such technologies into disaster management strategies.

References

1.Kedia T RJ, O'Connor M, Oluic S, Rose M, Freeman J, et al. Technologies enabling situational awareness during disaster response: a systematic review. Disaster Med Public Health Prep. 2022;16(1):341-59.

2.Müller CC LL, Elzomor M. Leveraging disruptive technologies for faster and more efficient disaster response management. Sustainability. 2024;16(23):10730.

3.Shirdeli M, Yaghoubi F, Sadeghi-Nodoushan F, Marzban A. A Review of the Effect of Synbiotic Foods on Reducing and Treating Constipation in Fasted People during the Month of Ramadan. Journal of Nutrition and Food Security. 2024;9(1):144-51.

4.Vermiglio C NG, Rodríguez Bolívar MP, Zarone V. Disaster management and emerging technologies: a performance-based perspective. Meditari Accountancy Res. 2022 Jul;30(4):1093-117.

5.Ogbaka LC EJ, Chieke JC. Technological innovations in disaster events and management. Int J Humanit Soc Sci Res. 2024;10(3):13-16.

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Investigating the Effects of Ziziphus Jujuba Extract, Metformin, and Myoinositol on Pregnancy Outcomes and Metabolic Parameters in PCOS Women Undergoing Ovulation Induction: A Randomized Controlled Trial Protocol

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ABSTRACT

Introduction: Ziziphus jujuba, a flavonoids rich plant and is renowned for its potent antioxidant properties with diverse health benefits across various conditions. This study aims to evaluate the efficacy of Ziziphus jujuba as an adjunct therapy for improving pregnancy outcomes in infertile women diagnosed with normogonadotropic normoestrogenic Polycystic Ovary Syndrome (PCOS) undergoing ovulation induction with letrozole.

Methods: A total of 196 participants diagnosed with PCOS and infertility will be recruited from the Milad Infertility Center in Mashhad, Iran. Participants will be randomly assigned to one of four groups: Ziziphus jujuba, Myoinositol, Metformin, or Placebo, with each group consisting of 49 individuals. Over 12 weeks, participants will receive their allocated intervention in conjunction with letrozole for ovulation induction. Clinical and biochemical parameters associated with pregnancy outcomes, including biochemical and clinical pregnancy rates, will be assessed.

Results: A total of 196 participants will be included in this study, with 49 participants assigned to each group. It is hypothesized that the Ziziphus jujuba group will exhibit improved glucose metabolism and reduced insulin resistance, as measured by fasting blood glucose (FBG) and triglyceride-glucose (TyG) indices, along with enhanced lipid profiles and reduced inflammatory markers, compared to the other groups. These anticipated metabolic improvements are expected to lead to a higher pregnancy rate in the Ziziphus jujuba group than in the different study groups.

Conclusion: This study aims to investigate the potential of Ziziphus jujuba as an adjunctive therapy to improve pregnancy outcomes in infertile women diagnosed with PCOS undergoing letrozole-induced ovulation induction. The findings are expected to provide valuable insights into the role of herbal medicine in addressing fertility challenges associated with PCOS, potentially presenting a cost-effective and accessible alternative to conventional pharmaceutical treatments.

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Introduction

Infertility is defined as the inability to achieve pregnancy after 12 months of consistent, unprotected sexual intercourse (1). It is a significant global health challenge with profound social, economic, and medical implications (2). Despite its widespread prevalence, the exact global burden of infertility remains uncertain, with estimates ranging from 48.5 million couples worldwide to approximately 186 million ever-married women in developing countries,

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representing about 17.5%, according to recent meta-analyses (3, 4). Among infertility cases, 20–35% are attributed to female factors, with ovulatory dysfunction being the most common cause, characterized by irregular or absent menstrual cycles (5). Polycystic Ovary Syndrome (PCOS) is the leading cause of anovulatory infertility, affecting an estimated 70–80% of women with this condition (6).

Polycystic Ovary Syndrome (PCOS) is a common endocrine disorder affecting a significant proportion of the female population. A range of clinical features, including hyperandrogenism, irregular menstrual cycles, and insulin resistance, characterize it (7). The primary approach to managing PCOS involves lifestyle modifications, with a focus on smoking cessation, increased physical activity, and weight loss for those who are overweight or obese (8). Various pharmacological options are available for PCOS treatment, with metformin being widely used due to its ability to improve insulin sensitivity. While studies suggest that metformin may enhance ovulation, its impact on live birth rates remains inconclusive (9-11). Like many medications, metformin is associated with side effects, including gastrointestinal discomfort and, with prolonged use, an increased risk of vitamin B12 deficiency. Its impact on pregnancy outcomes also remains uncertain (12, 13).

In recent years, there has been growing interest in inositol as a potential alternative to metformin for treating PCOS. Classified as part of the vitamin B complex group, inositol functions as an insulin sensitizer, influencing key components of insulin signaling pathways (14). A primary mechanism is its role in facilitating glucose uptake by activating glucose transporter 4 (GLUT4) and reducing fatty acid release from adipose tissue (15). Myoinositol, in particular, helps regulate glucose utilization and uptake. Studies suggest that inositol supplementation may improve ovulation rates and menstrual cycle regularity, although its impact on live birth rates remains uncertain (16). While generally well-tolerated, inositol can cause mild side effects, including digestive discomfort, insomnia, and occasional allergic reactions. Additionally, cost variations may limit accessibility for some individuals (17, 18).

Herbal medicine has become an alternative to conventional pharmaceuticals due to its diverse bioactive compounds with antioxidant, phytoestrogenic, and nutritional properties (19). Among these, jujube (Ziziphus jujuba Mill.) stands out for its significant nutritional value and potential health benefits. Widely cultivated, particularly in China and Iran, jujube has garnered attention for its effects on blood sugar regulation, cholesterol levels, body composition, and antioxidant activity (20-23). Its key bioactive compounds include vitamin C, phenolics, flavonoids, triterpenic acids, and polysaccharides, all contributing to its health-promoting properties (24, 25). Despite its promising benefits, the precise mechanisms by which jujube influences metabolic factors and enhances fertility potential remain unclear, underscoring the need for further research in this area (26, 27).

This study aims to investigate the potential effects of *Ziziphus jujuba* on pregnancy outcomes in women with Polycystic Ovary Syndrome (PCOS). Specifically, the objectives are to i) evaluate the impact of *Ziziphus jujuba* hydroalcoholic extract on biochemical and clinical pregnancy rates, ii) examine its effects on anthropometric indices, blood glucose levels, lipid profiles, and inflammatory markers, and iii) compare its efficacy with metformin and myoinositol in PCOS patients undergoing ovulation induction with letrozole.

Materials and Methods

This research study is officially registered with the Iranian Registry of Clinical Trials under the code IR.MUMS.MEDICAL.REC.1402.191. Designed as a double-blind, randomized controlled trial, the study will be conducted at the Milad Infertility Treatment Center in Mashhad, Iran. Infertile patients seeking treatment at this facility will undergo an initial evaluation by a gynecologist. Patients diagnosed with Polycystic Ovary Syndrome (PCOS) and recommended for ovulation induction with letrozole will be invited to participate in the study.

The study will include multiple interventions. Initially, all participants will provide informed consent and undergo baseline anthropometric and biochemical assessments. Participants will then be randomly assigned to one of four groups: Ziziphus hydroalcoholic extract, metformin, myoinositol, or placebo. Following a 12-week intervention, blood analyses and anthropometric measurements will be repeated and compared to baseline values across the groups.

In the subsequent phase, letrozole will be prescribed for ovulation induction, administered on days 3–7 of the menstrual cycle for five days. Participants will use luteinizing hormone (LH) test kits to monitor ovulation. If ovulation occurs, they will be advised to attempt conception. After 14–16 days, a Beta hCG test will be conducted to confirm biochemical pregnancy. For participants with a positive test result, ultrasound sonography will be performed at 6–7 weeks to confirm clinical pregnancy.

To further enhance the efficacy of the study, the research team will include a nutritionist, a midwifery team, and a pharmacologist, who will actively contribute to the research process and its implementation. The study design and procedures are summarized in a flow diagram, presented in Figure 1.

Sample size

Based on the study conducted by Agrawal et al. in 2019, which reported a pregnancy rate of 33.3% in the control group and 63.3% in the intervention group, we utilized a formula for calculating sample sizes in studies with qualitative traits across two populations (28). To achieve a statistical power of 80%, the required sample size for each group was calculated to be 44 participants. Accounting for a 10% allowance for potential dropouts, each group will include 49 participants, resulting in a total study population of 196.

Participants selection

All participants will provide written informed consent before enrollment in the study, facilitated by the research team. The study objectives will be thoroughly explained to each participant, along with detailed guidelines on maintaining a healthy lifestyle and personalized medical interventions throughout the research period.

Inclusion criteria will consist of infertility confirmed by a qualified gynecologist, age between 18 and 45 years, and a diagnosis of Polycystic Ovary Syndrome (PCOS) based on the Rotterdam criteria. Exclusion criteria will apply to participants currently using hormonal medications or those with thyroid dysfunction, liver disease, renal failure, a history of cancer, or who engage in smoking or alcohol consumption before or during the study.

Participants will be withdrawn from the study if they become pregnant within the first 12 weeks of the intervention or fail to comply with their assigned intervention protocol.

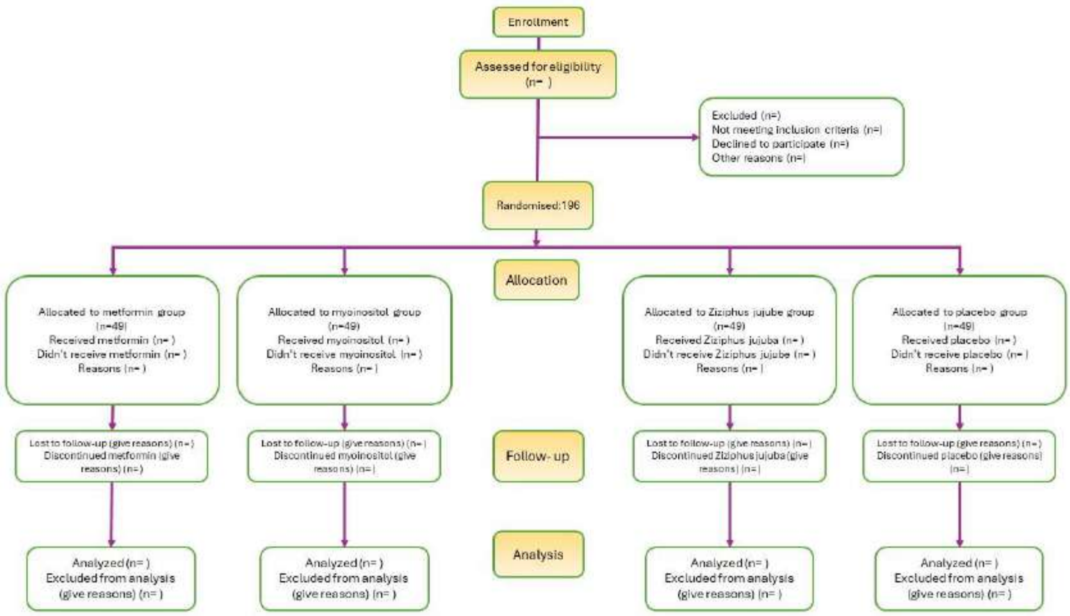

Figure 1. Particant flow diagram according to the Consolidated Standards of Reporting Trials (CONSORT)

Intervention

A multifaceted intervention will be implemented, comprising metformin, myoinositol, hydroalcoholic extract of Ziziphus jujuba, and placebo. While metformin and myoinositol are readily available, the Ziziphus jujuba extract will be prepared specifically for the study. Based on the findings of Mostafa et al.'s 2013 study, the recommended effective dose of Ziziphus jujuba is 30 grams per day over 12 weeks (29).

The following steps will be undertaken to prepare the Ziziphus jujuba extract: Jujube fruits will be collected from a garden in Birjand City, Iran, and authenticated at the School of Pharmacy, Mashhad University of Medical Sciences. The fruits will be dried in a dark environment to prevent degradation. A total of 108 kilograms of dried jujube fruits from a single source, validated by botanists and pharmacologists, will be used. A milling machine will grind the dried fruits into a fine powder. A 50% hydroalcoholic extract will be prepared using equal parts of ethanol (96%) and distilled water, ensuring the retention of various flavonoid compounds. The solvent will be removed under low pressure using a rotary evaporator at a controlled temperature of 35°C to preserve the active ingredients of the extract.

The concentrated extract will be blended with Avicel at a 50% ratio to prepare the sachets, converting them into powdered. Each sachet will contain 15 grams of the prepared powder, ensuring a high concentration of *Ziziphus jujube's* active components. Sachets will be prepared monthly to maintain the extract's antioxidant capacity. For standardization, the total flavonoid content of the extract will be quantified using the Specter method, incorporating High-Performance Liquid Chromatography (HPLC) and Mass Spectrometry (MS).

Randomization Process

Following participant selection based on informed consent and the specified inclusion and exclusion criteria, each participant will be randomly assigned to one of the intervention groups. This double-blind, randomized controlled trial is designed to ensure that both participants and investigators remain blinded. To achieve this, the physical appearance of all interventions will be standardized to maintain indistinguishability.

Intervention Description

1. **Group One:** Participants will receive a daily 500 mg metformin pill and a placebo sachet.
2. **Group Two:** Participants will receive a daily myoinositol sachet and a placebo pill.
3. **Group Three:** Participants will receive a daily *Ziziphus jujuba* sachet and a placebo pill.
4. **Group Four:** Participants will receive a daily placebo pill and a placebo sachet.

The randomization process will be conducted using SealedEnvelope.com, an online platform designed to ensure robust and unbiased randomization. An independent statistician, not involved in the study's execution, will generate the randomization schedule using this platform. This independent party will oversee the allocation of participants to intervention groups, which will occur only after the completion of baseline assessments.

To ensure blinding is maintained, unique codes will be assigned to the packaging of each pill and sachet. These codes will be designed to be unrecognizable to both participants and researchers, preventing potential bias or influence on treatment adherence or outcomes. The intervention groups will be designated numerically (Group 1, Group 2, Group 3, and Group 4) to ensure that the specific treatment each participant receives remains concealed throughout the study.

Regular audits ensure strict adherence to the randomization protocol, with any deviations from the assigned groups documented and promptly addressed. This rigorous approach will enhance the trial outcomes' reliability and reinforce the findings' robustness.

Body weight will be measured using a SECA digital scale (Germany) with a precision of 0.1 kg, while height will be measured without shoes using a wall-mounted instrument. Body Mass Index (BMI) will be calculated using the formula: weight (kg) / height (m^2).

Biochemical evaluation will involve collecting a 10 mL blood sample from each participant following a 12-hour fasting period. Serum levels of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG) will be measured using enzymatic colorimetric techniques. The glucose oxidase method will determine fasting blood glucose (FBG) levels. C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) will be assessed through an immunoturbidimetry approach. Insulin resistance will be calculated using the Triglyceride-Glucose Index (TyG), defined as Ln [fasting triglycerides (mg/dL) × fasting plasma glucose (mg/dL) / 2].

Ultrasound Evaluation

Before and after the intervention, all participants will undergo uterine and ovarian sonography performed by a gynecologist using a Philips sonography device with transvaginal affinity at 70 MHz. The sonography will evaluate ovarian morphology to differentiate between Polycystic Ovary Syndrome (PCOS) and normal ovarian morphology.

Pregnancy Assessment

After 12 weeks of intervention, participants will undergo ovulation induction with letrozole at a dosage of 2.5 mg, administered twice daily for 5 days (days 3 to 7 of the menstrual cycle). A follow-up ultrasound will be conducted on the ninth day of the cycle. Participants will be advised to attempt conception if a dominant follicle measuring 18–20 mm is detected. Within 14–16 days, a blood test for beta-hCG will be performed to confirm or rule out pregnancy. Additionally, during the 6th or 7th week of pregnancy, an ultrasound will be conducted to assess the fetal heart rate and confirm clinical pregnancy.

Data Collection

Data will be collected systematically through pre- and post-intervention visits overseen by a multidisciplinary team. Weekly phone calls will monitor participants' well-being and identify potential adverse events. Participants will also undergo monthly evaluations by a fertility specialist to assess their condition and address emerging concerns. If adverse effects become unmanageable, the intervention for that individual will be discontinued. Monthly research team meetings will be conducted to review the study's progress, evaluate patient outcomes, and address any reported side effects. Supplementary data collection tools include a Food Frequency Questionnaire (FFQ) and a Physical Activity Questionnaire, which utilizes the Metabolic Equivalent of Task (MET) concept.

Data Management

Data collection forms will be identified using participant numbers to ensure anonymity. Participants' contact information and the code linking participant numbers to their names will be securely stored in password-protected files. Anonymized data will then be entered into the data repository of Mashhad University of Medical Sciences.

The trial procedure flowchart is presented in Figure 2.

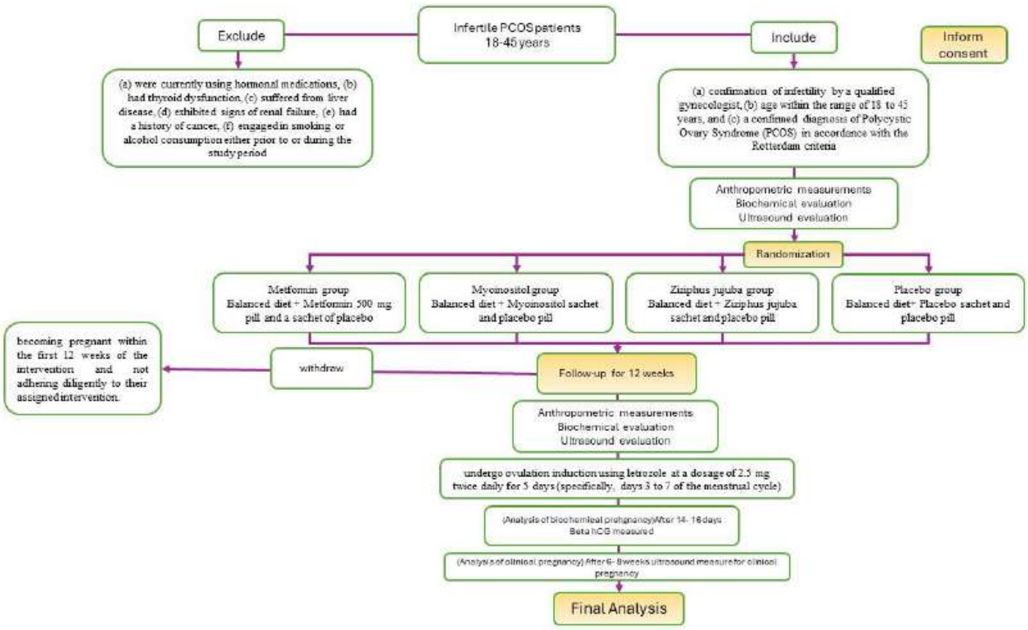

Figure 2. Trial procedure flow chat

Role and Responsibilities

The research team will comprise specialists in gynecology and infertility, nutritionists, and midwives, working collaboratively to ensure the effective implementation of the trial protocol.

At the initial consultation with the gynecologist, a diagnosis of infertility due to polycystic ovarian syndrome (PCOS) will be confirmed. After providing informed consent, patients will be enrolled in the study protocol and placed under the direct supervision of a gynecologist, nutritionist, and midwife. The midwife will conduct weekly monitoring and will provide ongoing support and assessments. Patients will also receive a contact number for the nutrition team to address any questions or concerns that may arise.

The research team will convene bi-weekly to review the project's progress and resolve any potential issues related to protocol implementation. Additionally, the university's ethics committee will oversee the study by reviewing monthly reports submitted for evaluation.

With the active involvement of the treatment team and regular monitoring, no disruptions to the patient's primary treatment are anticipated. However, if any disturbances or adverse outcomes occur during the study, the research will be paused, and blinding measures will be lifted as necessary.

Statistical Analysis

Statistical analysis will be performed using SPSS version 19 (SPSS Inc.), with a significance level set at $P < .05$. Continuous variables will be reported as mean ± standard deviation (SD), and categorical variables will be presented as frequency (%). Normality will be assessed using the Kolmogorov-Smirnov test, as parametric tests require normally distributed data. Non-normal data for non-parametric tests, including the Mann-Whitney U and Wilcoxon signed-rank tests will be employed.

Associations between categorical variables will be evaluated using Chi-squared tests. Independent t-tests compare group means at baseline and follow-up, while paired t-tests assess within-group changes. A covariance (ANCOVA) analysis will be conducted to account for baseline differences and covariates, ensuring precise comparisons of intervention effects.

A biostatistician will oversee the statistical analysis to ensure accuracy and validity. All analyses will be conducted in SPSS version 19, with R software, for advanced checks if necessary.

Results

This study will include 196 participants, with 49 individuals assigned to each of the four intervention groups. Based on the study design and anticipated outcomes, the *Ziziphus jujuba* group is expected to demonstrate significant improvements in glucose metabolism and insulin resistance, as measured by fasting blood glucose (FBG) levels and the triglyceride-glucose (TyG) index, compared to the other groups.

Additionally, improvements in lipid profiles are anticipated in the *Ziziphus jujuba* group, including reductions in triglycerides (TG) and low-density lipoprotein (LDL) cholesterol, along with increases in high-density lipoprotein (HDL) cholesterol.

Furthermore, markers of inflammation, including C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), are expected to decrease more significantly in the *Ziziphus jujuba* group compared to the other groups. These changes are hypothesized to result from the metabolic benefits associated with *Ziziphus jujuba*.

Discussion

This randomized clinical trial aims to investigate the potential effects of Ziziphus jujuba on anthropometric parameters, biochemical factors, and pregnancy outcomes in infertile women with Polycystic Ovary Syndrome (PCOS) undergoing letrozole-induced ovulation. Additionally, a comparative analysis of metformin and myoinositol is included. Previous studies have demonstrated the effectiveness of metformin and myoinositol in reducing insulin resistance and improving the metabolic profile in women with PCOS. However, conflicting evidence exists regarding their impact on pregnancy rates. This study seeks to evaluate the effects of Ziziphus jujuba in these domains and provide new insights into its potential as a therapeutic option (28, 30).

Polycystic Ovary Syndrome (PCOS) is a complex condition characterized by diverse pathophysiological factors that contribute to its heterogeneous clinical manifestations. Among these factors, hyperandrogenism is recognized for its direct and significant role in the development of insulin resistance. Although PCOS is undoubtedly multifactorial, insulin resistance and elevated androgen levels are considered primary contributors to the syndrome's etiology (31).

Obesity significantly exacerbates the clinical manifestations of PCOS, worsening infertility and reducing the effectiveness of fertility treatments (32). Although the intricate relationship between obesity and PCOS is not fully understood, it represents a critical component of patient management, underscoring the importance of addressing weight loss in this population (33). Furthermore, dyslipidemia is increasingly prevalent in women with PCOS, adding complexity to their clinical profile. As a result, interventions targeting these specific aspects of PCOS—such as obesity and dyslipidemia—offer promising potential for improving outcomes in infertile patients (34).

Lifestyle modifications are the first-line treatment for PCOS, emphasizing their critical role in managing the condition. Metformin and myoinositol are considered secondary therapeutic options. Metformin, a biguanide medication, reduces hepatic glucose production and improves insulin resistance. However, uncertainties persist regarding its efficacy in improving clinical outcomes, alongside concerns about its mild side effects (35). Furthermore, its effects on birth outcomes, fasting glucose levels, serum lipids, and anthropometric parameters remain inconclusive (8).

Inositol, comprising myo-inositol and di-chiro inositol, is an emerging insulin-sensitizing agent with significant efficacy in women with PCOS. Functioning as a second messenger, it is critical in insulin signal transduction. However, previous studies have not demonstrated significant effects on parameters such as BMI, triglycerides, fasting blood glucose, cholesterol levels, or ovulation compared to a placebo (35). Concerns persist regarding its potential risks, including hypoglycemia and suboptimal nutrient absorption. Furthermore, inositol does not appear to have a substantial impact on pregnancy outcomes (8).

Ziziphus jujuba has emerged as a promising natural reservoir of nutraceutical and therapeutic compounds (36). Recent studies on jujube fruit's phytochemical and pharmacological properties have highlighted its diverse biological effects. These include improvements in anthropometric indices, immunomodulation, antioxidant activity, antitumor properties, hepatoprotection, hypoglycemic effects, gastrointestinal protection, anticancer potential, anti-inflammatory action, anti-hyperlipidemic and anti-hyperglycemic activities, neuroprotection, sedative effects, and antiviral functions (20, 21, 37). Importantly, recent evidence suggests that *Ziziphus jujuba* can reduce insulin resistance, decrease triglyceride levels, and lower fasting blood glucose, thereby enhancing insulin sensitivity (21-23).

Previous studies have investigated the effects of *Ziziphus jujuba* on insulin resistance, primarily by measuring blood insulin levels. However, these studies did not incorporate the recently introduced Triglyceride-Glucose (TyG) Index, considered a more sensitive and reliable marker of insulin resistance. Our study seeks to evaluate insulin resistance using the TyG index, offering a more comprehensive and accurate assessment of metabolic dysfunction. Moreover, previous research has not explicitly examined the effects of *Ziziphus jujuba* on Polycystic Ovary Syndrome (PCOS), particularly in infertile patients undergoing ovulation induction.

Several mechanisms have been proposed to explain the effects of *Ziziphus jujuba* on various health-related factors. The phenolic compounds in *Ziziphus jujuba*, such as ferulic acid, catechin, and rutin, have been shown to influence glucose metabolism. These compounds exert hypoglycemic effects by inhibiting intestinal α-glycosidase activity, thereby reducing hepatic glucose production and impacting glucose transporters. Additionally, *Ziziphus jujuba* is hypothesized to play a crucial role in regulating glucose and lipid metabolism by activating the adiponectin signaling pathway. Adiponectin is inversely correlated with glucose, triglyceride, very low-density lipoprotein (VLDL), and cholesterol levels while positively associated with high-density lipoprotein (HDL) cholesterol (22, 38, 39).

An alternative mechanism by which *Ziziphus jujuba* may enhance lipid and glucose metabolism involves the preferential utilization of glucose as an energy source over lipids. This process promotes enhanced acetyl-CoA synthesis derived from pyruvic acid, facilitating its entry into the Krebs cycle rather than triglyceride biosynthesis. As a result, the triglyceride-lowering effect of *Ziziphus* extract leads to a significant reduction in very low-density lipoprotein (VLDL) levels. Since VLDL contributes indirectly to forming low-density lipoprotein (LDL) particles, a significant reduction of VLDL levels is anticipated to correspond with decreased LDL levels. Additionally, given the inverse relationship between plasma high-density lipoprotein (HDL) concentration and plasma triglyceride levels, the triglyceride-reducing effects of *Ziziphus* are expected to result in increased HDL levels. Furthermore, improved glucose metabolism may promote the conversion of proteins into anabolism, enhancing protein synthesis. Apo-A1, a primary structural component of HDL, accounts for approximately 70% of its composition. This suggests that increased HDL levels may be attributed to increased protein anabolism, potentially influenced by *Ziziphus* (40).

Ziziphus jujuba contains substantial amounts of pectin, inulin, and unsaturated fatty acids, possibly contributing to its hypocholesterolemic effects. Additionally, its high saponin content positively influences plasma lipid levels. Furthermore, the phytosterols present in *Ziziphus* may inhibit intestinal cholesterol absorption, leading to reductions in both total cholesterol and low-density lipoprotein (LDL) cholesterol (22).

Furthermore, *Ziziphus jujuba* demonstrates significant potential in mitigating inflammation. This fruit is notably rich in vitamin C, which plays a critical role in preventing the production of free radicals. Additionally, ascorbic acid structurally resembles glucose, enabling it to inhibit the non-enzymatic glycosylation of proteins. Studies suggest that *Ziziphus jujuba* may protect against acute and chronic inflammatory responses by inhibiting nitric oxide synthase (NOS). Its effectiveness in reducing oxidative stress is attributed to its abundant natural antioxidant components, including flavonoids, tannins, carotenes, polysaccharide fractions, and vitamins (41).

Previous studies on the effects of *Ziziphus jujuba* on Polycystic Ovary Syndrome (PCOS) have been limited, with only two investigations reported to date. Of these, one study was conducted in animals, while the other involved a very small sample size (42, 43). Furthermore, neither study assessed pregnancy outcomes, focusing solely on clinical symptoms. In contrast, our study is the first to evaluate *Ziziphus jujuba* as a pre-treatment compared to other widely used therapies for PCOS, such as metformin and myoinositol. These treatments are commonly utilized globally for managing PCOS. No previous study has undertaken such a comparative analysis, positioning this investigation as a unique and valuable contribution to the literature.

Ziziphus jujuba emerges as a promising herbal pre-treatment option for individuals with Polycystic Ovary Syndrome (PCOS) undergoing ovulation induction. This study is unique in employing the hydroalcoholic extract of *Ziziphus jujuba* as a pre-treatment for infertile PCOS patients undergoing induction ovulation. Additionally, this research represents the first comparative investigation evaluating the outcomes of metformin, myoinositol, and *Ziziphus* extract in this demographic, explicitly focusing on pregnancy outcomes. This pioneering approach highlights the potential benefits and distinctive attributes of *Ziziphus jujuba* in PCOS management, particularly in the context of fertility treatments.

Strength and limitations

Our study possesses several strengths that enhance its credibility. First, including PCOS individuals across the BMI spectrum mitigates selection bias, ensuring a more representative sample. Second, the extended duration and larger sample size contribute to the robustness of the findings. Additionally, the involvement of a multidisciplinary team ensures precision and comprehensive oversight. A notable strength is using *Ziziphus jujuba* extract, which offers a unique and potentially impactful intervention. However, some limitations exist, including variability in product usage and dietary regimens, which may introduce confounders. Nonetheless, patient commitment and regular follow-up are designed to minimize these risks, bolstering the study's internal validity.

Trial Status

The commencement of this clinical trial is scheduled for November 2023, with the hypothesis that data collection will extend through September 2024. [Clinical Trial Number:IRCTID: IRCT20230712058752N1, Registration date: 2023-07-18/ (https://irct.behdasht.gov.ir/trial/71240)]

Declarations

Ethics Considarations and Consent to Participate

The study was approved by the Ethics Committee of Mashhad University of Medical Sciences (ID: 4020097; IR.MUMS.REC.1402.191). The Human Research Ethics Committee of Mashhad University of Medical Sciences (MUMS) reviewed and approved the study protocol. Written informed consent will be obtained from all participants, with each component of the agreement explained in detail to ensure participants fully understand and agree to the terms of the study.

Consent for Publication

Not applicable.

Availability of Data and Materials

All research data will be securely archived in the data repository of Mashhad University of Medical Sciences. Access to this data by external investigators or industrial entities will be granted upon formal request, subject to approval by the corresponding author and university administration, ensuring the controlled availability of research materials.

Conflicts of Interest

The authors declare no competing interests.

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Authors' Contributions

M.N. and F.R. conceptualized the study and provided overarching supervision throughout all stages. N.KH. and F.M. was responsible for data collection, while M.KH. designed the methodology. H.R. developed the interventions. All authors actively contributed to the manuscript writing process.

Composition of Data Monitoring Committee (DMC)

Mashhad University of Medical Science constitutes the Data Monitoring Committee (DMC) for this research, ensuring the committee's complete independence from any conflicts of interest.

Data Confidentiality

The personal information of the participants and study data are managed by Mashhad University of Medical Sciences and stored in their secure database to ensure confidentiality. Researchers seeking access to this data must submit a request via email to the research team. The research team will grant access to the requested data upon review and approval.

Protocol Amendments

Significant modifications will be promptly communicated to the corresponding author and, if deemed necessary, will be forwarded to the journal responsible for publishing the article to ensure correction.

References

1. Carson SA, Kallen AN. Diagnosis and management of Infertility: A Review. JAMA. 2021;326(1):65-76.
2. Daar AS, Merali Z. Infertility and social suffering: the case of ART in developing countries. Current Practices and Controversies in Assisted Reproduction. 2002;15:21.
3. Mehri N, Messkoub M, Kunkel S. Trends, Determinants and the Implications of Population Aging in Iran. Ageing International. 2020;45(4):327-43.
4. Mascarenhas MN, Flaxman SR, Boerma T, Vanderpoel S, Stevens GA. National, regional, and global trends in infertility prevalence since 1990: a systematic analysis of 277 health surveys. PLoS medicine. 2012;9(12):e1001356.
5. Mustafa M, Sharifa A, Hadi J, IIIzam E, Aliya S. Male and female infertility: causes, and management. IOSR Journal of Dental and Medical Sciences. 2019;18(9):27-32.
6. Melo AS, Ferriani RA, Navarro PA. Treatment of infertility in women with polycystic ovary syndrome: approach to clinical practice. Clinics. 2015;70:765-9.
7. Zehravi M, Maqbool M, Ara I. Polycystic ovary syndrome and infertility: an update. International journal of adolescent medicine and health. 2021;34(2):1-9.
8. Collée J, Mawet M, Tebache L, Nisolle M, Brichant G. Polycystic ovarian syndrome and infertility: overview and insights of the putative treatments. Gynecological Endocrinology. 2021;37(10):869-74.
9. Morley LC, Tang T, Yasmin E, Norman RJ, Balen AH. Insulin-sensitising drugs (metformin, rosiglitazone, pioglitazone, D-chiro-inositol) for women with polycystic ovary syndrome, oligo amenorrhoea and subfertility. Cochrane Database of Systematic Reviews. 2017(11).
10. Legro RS, Barnhart HX, Schlaff WD, Carr BR, Diamond MP, Carson SA, et al. Clomiphene, metformin, or both for infertility in the polycystic ovary syndrome. New England Journal of Medicine. 2007;356(6):551-66.
11. Johnson N, Stewart A, Falkiner J, Farquhar C, Milsom S, Singh V-P, et al. PCOSMIC: a multi-centre

randomized trial in women with PolyCystic Ovary Syndrome evaluating Metformin for Infertility with Clomiphene. Human reproduction. 2010;25(7):1675-83.
12. Bailey CJ, Day C. Metformin: its botanical background. Practical diabetes international. 2004;21(3):115-7.
13. Tarry-Adkins JL, Grant ID, Ozanne SE, Reynolds RM, Aiken CE. Efficacy and side effect profile of different formulations of metformin: a systematic review and meta-analysis. Diabetes Therapy. 2021;12(7):1901-14.
14. Greff D, Juhász AE, Váncsa S, Váradi A, Sipos Z, Szinte J, et al. Inositol is an effective and safe treatment in polycystic ovary syndrome: a systematic review and meta-analysis of randomized controlled trials. Reproductive Biology and Endocrinology. 2023;21(1):10.
15. Fauser BC, Tarlatzis BC, Rebar RW, Legro RS, Balen AH, Lobo R, et al. Consensus on women's health aspects of polycystic ovary syndrome (PCOS): the Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. Fertility and sterility. 2012;97(1):28-38. e25.
16. Pundir J, Psaroudakis D, Savnur P, Bhide P, Sabatini L, Teede H, et al. Inositol treatment of anovulation in women with polycystic ovary syndrome: a meta-analysis of randomised trials. BJOG: An International Journal of Obstetrics & Gynaecology. 2018;125(3):299-308.
17. Facchinetti F, Unfer V, Dewailly D, Kamenov ZA, Diamanti-Kandarakis E, Laganà AS, et al. Inositols in polycystic ovary syndrome: An overview on the advances. Trends in Endocrinology & Metabolism. 2020;31(6):435-47.
18. Morgante G, Orvieto R, Di Sabatino A, Musacchio MC, De Leo V. The role of inositol supplementation in patients with polycystic ovary syndrome, with insulin resistance, undergoing the low-dose gonadotropin ovulation induction regimen. Fertility and sterility. 2011;95(8):2642-4.
19. Akbaribazm M, Goodarzi N, Rahimi M. Female infertility and herbal medicine: An overview of the new findings. Food science & nutrition. 2021;9(10):5869-82.
20. Lu Y, Bao T, Mo J, Ni J, Chen W. Research advances in bioactive components and health benefits of jujube (Ziziphus jujuba Mill.) fruit. Journal of Zhejiang University-Science B. 2021;22(6):431-49.
21. Farhadnejad H, Asghari G, Hedayati M, Sahranavard S, Teymoori F, Mirmiran P, et al. Effect of Ziziphus jujube on cardiometabolic factors and systemic inflammation in type 2 diabetic patients: A randomized controlled trial. Clinical Nutrition ESPEN. 2022;49:53-60.
22. Yazdanpanah Z, Ghadiri-Anari A, Mehrjardi AV, Dehghani A, Zardini HZ, Nadjarzadeh A. Effect of Ziziphus jujube fruit infusion on lipid profiles, glycaemic index and antioxidant status in type 2 diabetic patients: a randomized controlled clinical trial. Phytotherapy Research. 2017;31(5):755-62.
23. Irannejad niri Z, Shidfar F, Jabbari M, Zarrati M, Hosseini A, Malek M, et al. The effect of dried Ziziphus vulgaris on glycemic control, lipid profile, Apo-proteins and hs-CRP in patients with type 2 diabetes mellitus: A randomized controlled clinical trial. Journal of Food Biochemistry. 2021;45(3):e13193.
24. Tripathi S. Ziziphus jujuba: A phytopharmacological review. 2014.
25. Tahergorabi Z, Abedini MR, Mitra M, Fard MH, Beydokhti H. "Ziziphus jujuba": A red fruit with promising anticancer activities. Pharmacogn Rev. 2015;9(18):99-106.
26. Liu C, Dai M. The effects of Chinese herbal medicine on the pregnancy outcomes of infertile women with polycystic ovary syndrome undergoing in vitro fertilization-embryo transfer: a systematic review and meta-analysis. 2021.
27. Azami Movahed M, Mahboubi Rabbani M, Bayanati M. Research advances in bioactive components and therapeutic benefits of jujube fruit, a mini review. Research Journal of Pharmacognosy. 2024;11(4):93-104.
28. Agrawal A, Mahey R, Kachhawa G, Khadgawat R, Vanamail P, Kriplani A. Comparison of metformin plus myoinositol vs metformin alone in PCOS women undergoing ovulation induction cycles: randomized controlled trial. Gynecological Endocrinology. 2019;35(6):511-4.
29. Mostafa UE-S, Labban L. Effect of Zizyphus jujuba on serum lipid profile and some anthropometric measurements. Advancement in Medicinal Plant Research. 2013;1(3):49-55.
30. Prabhakar P, Mahey R, Gupta M, Khadgawat R, Kachhawa G, Sharma JB, et al. Impact of myoinositol with metformin and myoinositol alone in infertile PCOS women undergoing ovulation induction cycles-randomized controlled trial. Gynecological Endocrinology. 2021;37(4):332-6.
31. Xu Y, Qiao J. Association of insulin resistance and elevated androgen levels with polycystic ovarian syndrome (PCOS): a review of literature. Journal of healthcare engineering. 2022;2022.
32. Hazlehurst JM, Singh P, Bhogal G, Broughton S, Tahrani AA. How to manage weight loss in women with obesity and PCOS seeking fertility? Clinical endocrinology. 2022;97(2):208-16.
33. Hu L, Ma L, Xia X, Ying T, Zhou M, Zou S, et al. Efficacy of bariatric surgery in the treatment of women with obesity and polycystic ovary syndrome. The Journal of Clinical Endocrinology & Metabolism. 2022;107(8):e3217-e29.
34. Guo F, Gong Z, Fernando T, Zhang L, Zhu X, Shi Y. The lipid profiles in different characteristics of women with PCOS and the interaction between dyslipidemia and metabolic disorder states: a retrospective study in Chinese population. Frontiers in Endocrinology. 2022;13:892125.

35. International evidence-based guideline for the assessment and management of polycystic ovary syndrome 2018. 2018.
36. Li YM, Hao Kx, Xie H, Jiang JG. The Hypoglycemic Activities and Underlying Mechanisms of Two Saponins-Rich Components from Fried Ziziphus jujuba Mill. Kernel. Molecular Nutrition & Food Research. 2023:2200364.
37. Ji X, Peng Q, Yuan Y, Shen J, Xie X, Wang M. Isolation, structures and bioactivities of the polysaccharides from jujube fruit (Ziziphus jujuba Mill.): A review. Food chemistry. 2017;227:349-57.
38. Qiao L, Zou C, van der Westhuyzen DR, Shao J. Adiponectin reduces plasma triglyceride by increasing VLDL triglyceride catabolism. Diabetes. 2008;57(7):1824-33.
39. Hemmati M, Asghari S, Zohoori E, Karamian M. Hypoglycemic effects of three Iranian edible plants; jujube, barberry and saffron: Correlation with serum adiponectin level. Pak J Pharm Sci. 2015;28(6):2095-9.
40. Shirdel Z, Maadani H, Mirbadalzadeh R. Investigation into the hypoglycemic effect of hydroalcoholic extract of Ziziphus Jujuba Leaves on blood glucose and lipids in Alloxan-Induced diabetes in rats. Journal of Diabetes and Metabolic Disorders. 2009;8:2.
41. Hemmati M, Zohoori E, Mehrpour O, Karamian M, Asghari S, Zarban A, et al. Anti-atherogenic potential of jujube, saffron and barberry: anti-diabetic and antioxidant actions. EXCLI journal. 2015;14:908.
42. Asadi Nejad K, Arzaei M. Comparison of the effect of Shilan (Jujube) medicinal capsule with metformin on ovarian cysts in patients with polycystic ovary syndrome. Journal of Traditional Medicine of Islam and Iran. 2016;7(1):53-8. [in Persian]
43. Mirazi, Naser, Poursher, Izadi. Study of the effect of hydroalcoholic extract of jujube fruit (Zizyphus jujube L.) in rats with polycystic ovary syndrome. Armaghane Danesh. 2019;24(5):746-62.[in Persian]

JOURNAL OF NUTRITION FASTING AND HEALTH

Factors Affecting Food Waste Management Behavior in Iran: A Systematic Review Based on Behavioral Theories

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ABSTRACT

Introduction: Food waste in Iran is approximately six times higher than the global average, posing significant challenges to food security, the environment, and economic sustainability at regional, national, and international levels. Despite substantial evidence on consumer behavior in waste management, limited knowledge exists regarding the factors influencing food waste management behavior in Iran. This systematic review identifies and analyzes these factors based on behavioral theories.

Methods: A comprehensive search was conducted across five databases—PubMed, Scopus, Web of Science, ScienceDirect, and Magiran—to identify cross-sectional studies examining food waste behavior through the lens of behavioral theories. Studies published in English from inception to October 2022 were included. The Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) framework guided the identification, screening, and inclusion of studies in this review.

Results: Out of 14 screened articles, four met the eligibility criteria and were included in the study. The behavioral theories applied in these studies were the Theory of Planned Behavior (TPB), the Theory of Reasoned Action (TRA), and the Social Cognitive Theory (SCT). The most commonly identified predictors of food waste behavior were attitudes, perceived behavioral control, and subjective norms. Additionally, enhancing food waste reduction skills emerged as a valuable strategy to increase perceived control and individuals' ability to adopt sustainable food waste management practices.

Conclusions: The key constructs of attitude, subjective norms, and perceived behavioral control from the Theory of Planned Behavior (TPB) significantly predict food waste management behavior. However, incorporating an expanded version of TPB may yield a more significant impact on behavior modification.

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Introduction

Food waste (FW) encompasses both edible and inedible portions of food that require disposal or recycling (1). It also refers to food lost at various stages of the food supply chain, including harvesting, production, processing, distribution, and consumption (2, 3). Approximately 1.3 billion tons of edible food are annually wasted globally (4), with nearly 50 million tons lost in the eastern Middle East subregion (5). In Iran, where 85% of the food supply is derived from agriculture, 35% is wasted, amounting to 28 million tons—a figure nearly six times higher than the global average (6). The adverse consequences of food waste pose significant threats to economic development, environmental sustainability, public health, food safety, and food security (7). Since more than 800 million people worldwide suffer from hunger and malnutrition (8, 9), preventing and reducing FW is a critical strategy for fostering a sustainable food system and mitigating environmental impacts (10). Consequently, behavioral changes at both individual and collective levels are essential for reducing FW. Despite numerous studies, the determinants of

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FW generation remain a topic of ongoing debate (11, 12). Moreover, FW is a complex behavioral phenomenon influenced by multiple factors and motivations. Therefore, identifying an appropriate conceptual framework to explain FW behavior is crucial (13). Behavioral theories, such as Social Cognitive Theory (SCT), the Theory of Planned Behavior (TPB), and the Theory of Reasoned Action (TRA), provide valuable insights into FW management behaviors and serve as foundational models for designing and implementing effective intervention strategies (14-17).

Therefore, this study aimed to examine the extent to which behavioral theories have been utilized to investigate food waste (FW) management and identify the key constructs that predict FW-related behaviors. Accordingly, this systematic review was conducted to determine the factors influencing FW management behavior based on behavioral theories in Iran.

Materials & Methods

Study Design

This systematic review was conducted under the PRISMA guidelines (13). Ethical approval was not required since the study relied solely on secondary literature sources.

Search Strategy

A comprehensive systematic search was conducted across five electronic databases: PubMed, Scopus, Web of Science, ScienceDirect, and Magiran. The same search strategy was applied uniformly across all databases, covering records published from inception until October 2022. The search terms included a combination of Medical Subject Headings (MeSH) and non-MeSH terms as follows: (“domestic waste” OR “food loss” OR “food waste*” OR “kitchen waste*” OR “leftovers” OR “lost food” OR “plate waste*” OR “wasted food”) AND ("theory of planned behavior*" OR "theory of reasoned action" OR "TPB" OR "TRA" OR "planned behavior*" OR "social cognitive theory" OR "reasoned action") AND (Iran*). To ensure comprehensive coverage and minimize the risk of missing relevant studies, we manually searched all eligible studies' reference lists and related review articles.

Initially, two researchers independently screened the titles and abstracts of the retrieved references. In cases of disagreement, a chief investigator was consulted to resolve discrepancies. Full-text evaluation and data extraction were performed for studies that met the inclusion criteria. Studies lacking full-text availability in English or not peer-reviewed were excluded.

Inclusion and Exclusion Criteria

Studies were included in this review if they were conducted in Iran, published in English, available in full text, and addressed food waste. The eligibility assessment was performed by screening the retrieved articles' titles, abstracts, and full texts. Review articles and duplicate studies were excluded.

Data Extraction and Quality Assessment

Validated quality assessment tools were used to evaluate the studies (18), incorporating seven criteria to assess selection bias, measurement bias, and analysis bias: (1) clear definition of the target population; (2) representative sampling of potential respondents; (3) adequate response rate; (4) standardized data collection methods; (5) use of reliable survey instruments; (6) use of valid survey instruments; and (7) appropriate data analysis. The total quality score ranged from 0 to 7, based on responses of “Yes” (scored 1) or “No” (scored 0) (Table 2). Two authors (S.F.F and Z.N.) independently assessed all selected studies for this systematic review, with a third author consulted to resolve any discrepancies in the assessment results.

Table 1. Articles included in the review.

	Author, Year Country	Population	Age Mean or range	Sample Size	Design of study	Items measuring	Measurement	Assessment
1	Bijan Abadi/2020 /Kermanshah(17)	All wholesalers of Kermanshah city.	Wholesalers aged 22 and above Male (n=265), Female (n=0)	265	Cross-sectional	TPB variables a Cultural factors: (-Egalitarian personality -Fatalistic personality -Individualistic personality -Hierarchical personality) -Facilitators/impediments	Likert	The waste management of fruit and vegetable in wholesale markets
2	Ava Heidari/2019/ Mashhad(15)	A person from a household	Participants aged 22 and above	382	Cross-sectional (A case study)	TPB variables [a] Moral attitude -Perceived ascription of responsibility	A 5-point Likert scale (from	Household food management

	Author, Year Country	Population	Age Mean or range	Sample Size	Design of study	Items measuring	Measurement	Assessment
			Male (n=132), Female (n=242)			-Marketing addiction -Waste-preventing behavior	1=strongly disagree to 5=strongly agree).	
3	Amir H Pakpour/2013/ city of Qazvin(14)	A person from a household	Age ranged from 20 to 68 years Male (n=660), Female (n=1122)	1782	Cross-sectional	TPB variables [a] Moral obligation Self-identity Action planning Past recycling behaviour	A 5-point Likert scale (from 1=strongly disagree to 5=strongly agree) Action planning: (from 1=totally disagree, to 5=totally agree) Past behaviour (from 1=never, to 5=frequently/at every collection)	Household waste behaviours
4	Fatemeh Soorani/ 2018/ Yasouj(16)	A person from a household	Age ranged from 15 to 64 years	405	Cross-sectional	TPB variables [a] Feeling of guilt	A 5-point Likert scale (from 1=strongly disagree to 5=strongly agree)	Food consumption management behavior (- Shopping routine, - Reusing leftover routine, - Food storage routine)

[a] TPB variable: attitudes, perceived behavioral control, subjective norms, and intention; [b] PBC: perceived behavioral control; [c] SN: subjective or social norms

Table2. Quality assessment of the reviewed article

	Title of Paper	Defining clearly the target population	Sampling is representative of potential respondents	Adequate response rate	Standardized data collection methods	Reliable survey instruments	Valid survey instruments	Analyzing the data appropriately	Result of quality assessment
1	The waste management of fruit and vegetable in wholesale markets: Intention and behavior analysis using path analysis	1	1	1	1	1	1	1	7
2	A theoretical framework for explaining the determinants of food waste reduction in residential households: a case study of Mashhad, Iran	1	1	0	1	1	1	1	6
3	Household waste behaviours among a community sample in Iran: An application of the theory of planned behaviour.	1	0	1	1	1	1	1	6

Results

The primary search identified a total of 14 articles. One article was excluded due to duplication, and seven studies were removed for not meeting the inclusion criteria. Consequently, six studies remained for full-text screening, of which two were excluded for lacking the required information. Ultimately, four studies, comprising data from 2,834 participants, met all inclusion criteria and were included in this systematic review. Figure 1 presents the PRISMA flowchart of the search process.

The selected studies were conducted in four cities: Mashhad, Kermanshah, Qazvin, and Yasuj. Table 1 provides a summary of the included studies.

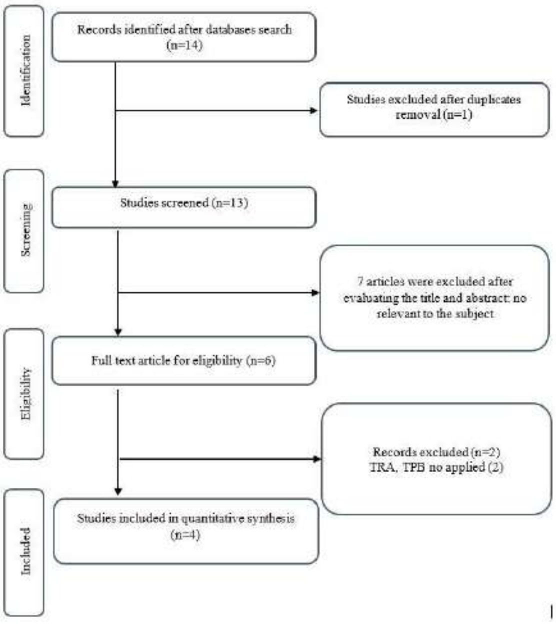

Figure1. Flowchart of study selection for inclusion trials in the systematic review.

Discussion

Based on behavioral theories, this study aimed to identify the factors influencing food waste management behavior in Iran. The findings revealed that, according to the inclusion criteria of this review, the Theory of Planned Behavior (TPB) is the only behavioral theory applied in food waste management studies in Iran. In contrast, other behavior change theories have not been utilized. The TPB constructs of attitude, subjective norms, and perceived behavioral control predicted food waste management behavior. However, when TPB is applied in an expanded form, its predictive power increases significantly. Incorporating additional constructs such as guilt, marketing addiction, the perceived ascription of responsibility, moral attitude, waste-preventing behavior, moral commitment, self-identity, action planning, past recycling behavior, and the cultural theory of risk (individualistic and hierarchical characteristics) enhances the model's explanatory capacity and effectiveness in food waste management behavior.

A review study by Raghu et al. (19) indicated that most research on solid waste recycling has been conducted using the Theory of Planned Behavior (TPB). Similarly, in our study, most of the reviewed research focused on TPB. However, while our review was limited to studies conducted in Iran, Raghu et al. (19) did not impose any geographical restrictions and examined solid waste recycling more broadly.

Additionally, Etim et al. (20) systematically analyzed the factors influencing household food waste behavior based on TPB. Their findings highlighted the importance of targeting attitudes, subjective norms, and perceived behavioral control for effectively reducing household food waste, which aligns with our results. However, unlike our study, which focused solely on Iran, Etim et al. (20) examined food waste behavior across 17 countries, employing a broader search scope.

Srivastava et al. (21) conducted a review study to introduce the concept of a systematic literature review with meta-analysis to examine the application of the Theory of Planned Behavior (TPB) in food waste behavior research. A total of 26 studies were analyzed. The findings revealed that the most substantial relationship between attitude and behavioral intention was observed. In contrast, the relationships between subjective norms and perceived behavioral control with intention emerged later.

All reviewed studies were conducted in Iran, applying either the original, an adapted, or an extended version of the Theory of Planned Behavior (TPB). The research focused on factors influencing household food waste management

(14-16) and waste management in wholesale fruit and vegetable markets (17). Within the TPB framework, attitude (14-17), perceived behavioral control (PBC) (14-17), and subjective norms (14-17) Individuals ' food waste management intentions were identified as the most frequent predictors. Additionally, several extended constructs were recognized as influencing FW management behavior, including feelings of guilt (16), moral attitude, waste-preventing behavior, perceived ascription of responsibility (15), past behavior, moral obligation, self-identity, action planning (14), and personality-related traits such as individualistic, hierarchical, and fatalistic characteristics (17). All studies utilizing the TPB model confirmed that attitude plays a significant role in shaping food waste management behavior (14-17). Attitude toward behavioral intention refers to an individual's favorable or unfavorable evaluation of a behavior (18). Accordingly, attitudes toward food waste reduction were assessed by evaluating individuals' desire and willingness to minimize food waste.

Three studies found that attitude has a significant positive effect on household food waste management in Iran (14-16). The findings indicate that individuals with a positive attitude toward the environment and the importance of food waste reduction are more likely to exhibit stronger intentions to minimize food waste. However, this influence is partially mediated by subjective norms and attitudes (15). Additionally, another study demonstrated that attitudes toward food waste management positively impact behavior (17). Specifically, attitudes play a crucial role in shaping waste management practices in the wholesale fruit and vegetable sector. Attitudes toward waste management directly influence how fruit and vegetables are supplied and consumed. Wholesalers with positive attitudes toward food waste management are more likely to acknowledge its significance, voluntarily adopt more efficient behaviors, and implement waste reduction strategies. Consequently, by actively reducing waste and maintaining sustainable management initiatives, organizations are more likely to achieve long-term waste reduction goals (22).

Perceived behavioral control (PBC) refers to an individual's perception of the ease or difficulty of reducing food waste (16). Studies have shown that PBC significantly influences the intention to reduce household food waste (14-16) as well as fruit and vegetable waste management (17).

Subjective norms (SNs) represent the social support or pressure exerted by influential groups, such as family and friends, in shaping an individual's behavior. In other words, SNs reflect what people perceive as acceptable or unacceptable behavior at a given moment (23). If significant others disapprove of food waste, individuals are likelier to eliminate the practice. All reviewed studies emphasized the importance of understanding the role of subjective norms in shaping food waste reduction intentions (14-17). Positive responses to social influences from significant individuals strengthen intentions to reduce food waste—particularly in fruit and vegetable waste management (17). Conversely, when individuals receive negative feedback from others, some may exhibit rebellious tendencies, resisting behavioral change (17).

The studies assessed in this review identified several additional constructs influencing food waste management behavior. These include perceived responsibility, moral obligation, self-identity, intention, action planning, and past behavior (14); waste-preventing behavior and perceived responsibility (15); guilt (16); and individualism, hierarchical personality, and fatalism (17). These factors serve as significant predictors for enhancing food waste management practices.

Moral obligation refers to an individual's judgment of whether a behavior is morally right or wrong (23). Developing educational materials that emphasize moral obligation and action planning may be beneficial in promoting responsible food waste management. Action planning involves specifying when, where, and how an individual will act based on their intentions, serving as a voluntary, post-intentional process (23). Research suggests that strategies emphasizing individuals' intrinsic and moral motivations for recycling can effectively enhance household waste reduction efforts (24). One of the most significant predictors of the intention to reduce food waste is waste-preventing behavior, which includes activities such as waste reuse, minimization, and recycling (15).

The findings of this review indicate that food waste can induce feelings of guilt, which, in turn,

motivates individuals to reduce food waste and engage in pro-environmental behaviors (27).
Additionally, individualism has been found to influence attitudes toward fruit and vegetable waste management (17). In highly individualistic societies, flexibility and adaptability to environmental changes are valued, and adjusting to these changes is considered essential for disaster prevention and maintaining ecological balance (27, 28). Individualists tend to favor competitive and innovative market-driven solutions over government intervention in achieving equilibrium within markets (28, 29). Conversely, fatalism negatively impacts attitudes toward food waste management (17), as fatalists believe that natural forces operate beyond human control, leading to the perception that human efforts have little influence on waste reduction (28). On the other hand, hierarchical personality traits have a positive impact on attitudes toward fruit and vegetable waste management (17). Individuals with a hierarchical worldview perceive nature as controllable and are more likely to support structured waste management efforts, emphasizing expertise, leadership, regulation, and forecasting as key drivers of effective waste reduction strategies (26, 27).
This is the only study in Iran that has examined the factors influencing food waste management behavior. Despite our extensive efforts to employ multiple search strategies and select eligible studies, it is possible that some relevant studies were unintentionally overlooked. Similarly, as with any research, we acknowledge the possibility that certain studies may have omitted key factors in their findings, potentially affecting validity and precision. Consequently, we cannot draw definitive conclusions about these associations. However, our findings provide valuable insights and may serve as a foundation for future research.

Conclusion

The findings of this study indicate that, based on the inclusion criteria, the Theory of Planned Behavior (TPB) is the only theoretical framework applied to food waste management research in Iran. Within this model, the constructs of attitude, subjective norms, and perceived behavioral control were identified as key predictors of food waste management behavior. However, when TPB is applied in an expanded form, its predictive power is significantly enhanced by incorporating additional constructs such as guilt, marketing addiction, perceived ascription of responsibility, moral attitude, waste-preventing behavior, moral commitment, self-identity, action planning, past recycling behavior, and the cultural theory of risk (individualistic and hierarchical characteristics). Therefore, educational interventions for food waste management can be effectively designed and implemented using an extended version of TPB, incorporating these additional constructs to foster more sustainable waste reduction behaviors.

Declarations

Conflicts of Interest

The authors declared no conflict of interest.

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Ethical Considerations

This study is based on a research project approved by Mashhad University of Medical Sciences (Project Code: IR.MUMS.REC.1400.185).

Authors' Contributions

Conceptualization, N.P., and S.F.F.; methodology, S.F.S., and M.A.A.; writing—original draft preparation, S.F.S., and Z.N.; writing—review and editing, S.F.S., E.Ch.Kh, and Z.N. All authors have read and agreed to the published version of the manuscript.

References

1- Stangherlin I, Pires A. Food Waste: A Review of the Literature. Waste Management.2018; 74:1-12.
2-Gustavsson J, Cederberg C, Sonesson U. The extent and causes of food loss and waste in the food supply chain: A systematic review. Food Security.2021;13(5):1247-65.
3-Pereira LM, Silva LF, Lima EG. Food loss and waste in the food supply chain: A comprehensive review. Journal of Cleaner Production. 2020; 273: 122741.
4- Stenmarck Å, Jensen C, Quested T, Moons E. Food waste in Europe – a review of the extent, causes, and prevention strategies. Waste Management.2020;105: 1-13.
5-Ziegler D, Allen B. Food waste in the Middle East and North Africa: A critical review of the status, causes, and mitigation strategies. Environmental Science & Policy. 2020;113: 44-56.
6-Khosravani F, Rac G, Farhadian H. Investigating consumers' purchasing behavior toward reducing

agricultural wastes (case: fruit and vegetable markets, Tehran). Iranian Journal of Agricultural Economics and Development Research. 2018;49(1):75-92.

7- Wang L, Xue L, Li Y, Liu X, Cheng S, Liu G. Horeca food waste and its ecological footprint in Lhasa, Tibet, China. Resources, Conservation and Recycling. 2018;136:1-8.

8- Khorasani EC, Peyman N, Moghzi M. Application of the Theory of Planned Behavior to predict low-nutrient junk food consumption among male students. Journal of Health Sciences and Technology. 2017;1(2):75-9.

9- Peyman NO, Khorasani EC, Moghzi MO. The Impact of Education on the Basis of the Theory of Planned Behavior on Junk Food Consumption in high school in Chenaran. Razi Journal of Medical Sciences. 2016;23(8):62-72.

10- Priefer C, Jörissen J, Bräutigam K-R. Food waste prevention in Europe–A cause-driven approach to identify the most relevant leverage points for action. Resources, Conservation and Recycling. 2016;109:155-65.

11- Abdelradi F. Food waste behaviour at the household level: A conceptual framework. Waste Management. 2018;71:485-93.

12- Mondéjar-Jiménez J-A, Ferrari G, Secondi L, Principato L. From the table to waste: An exploratory study on behaviour towards food waste of Spanish and Italian youths. Journal of Cleaner Production. 2016;138:8-18.

13- Page MJ, Moher D, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. PRISMA 2020 explanation and elaboration: updated guidance and exemplars for reporting systematic reviews. BMJ. 2021;372.

14- Pakpour AH, Zeidi IM, Emamjomeh MM, Asefzadeh S, Pearson H. Household waste behaviours among a community sample in Iran: An application of the theory of planned behaviour. Waste Management. 2014;34(6):980-6.

15- Heidari A, Mirzaii F, Rahnama M, Alidoost F. A theoretical framework for explaining the determinants of food waste reduction in residential households: a case study of Mashhad, Iran. Environmental Science and Pollution Research.2020;27(7):6774-6784.

16- Soorani F, Ahmadvand M. Determinants of consumers' food management behavior: Applying and extending the theory of planned behavior. Waste Management. 2019;98:151-9.

17- Abadi B, Mahdavian S, Fattahi F. The waste management of fruit and vegetable in wholesale markets: Intention and behavior analysis using path analysis. Journal of Cleaner Production. 2021;279:123802.

18- Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, et al. Cochrane Handbook for Systematic Reviews of Interventions (2nd ed.). Wiley-Blackwell; 2019.

19-Raghu SJ, & Rodrigues LLR. Behavioral aspects of solid waste management: A systematic review. Journal of the Air & Waste Management Association. 2020; 70(12): 1268–302.

20-Etim E, Choedron KT, Ajai O, Duke O, Jijingi HE. Systematic review of factors influencing household food waste behaviour: Applying the theory of planned behaviour. Waste Management & Research. 2024; 734242X241285423.

21-Srivastava SK, Mishra A, Singh S et al. Household food waste and theory of planned behavior: A systematic review and meta-analysis. Environ Sci Pollut Res. 2023; 30, 97645–59.

22- Smith J, Johnson A. Waste management practices in fruit and vegetable wholesale markets: Impact of attitudes and behaviors. Journal of Agricultural Economics. 2020;45(2):112-26.

23- Verplanken B, Wood W. Interventions to break and create consumer habits. Journal of Consumer Psychology.2020;30(4), 747-57.

24- Miafodzyeva S, Brandt N, Andersson M. Recycling behaviour of householders living in multicultural urban area: a case study of Järva, Stockholm, Sweden. Waste Management & Research. 2013;31(5):447-57.

25- Qi D, Roe BE. Household food waste: Multivariate regression and principal components analyses of awareness and attitudes among US consumers. PloS one. 2016; 11(7):e0159250.

26- Yazdanpanah M, Hayati D, Thompson M, Zamani GH, Monfared N. Policy and plural responsiveness: Taking constructive account of the ways in which Iranian farmers think about and behave in relation to water. Journal of Hydrology. 2014;514:347-57.

27- Steg, L., & Vlek, C. Encouraging pro-environmental behaviour: An integrative review of psychological models and their applications. Journal of Environmental Psychology. 2018; 56:42-57.

28- Bennett L, Coudrain V. Cultural attitudes and environmental behavior: The role of fatalism in perceptions of climate change and waste management in the Middle East. Environmental Sociology. 2019; 5(4): 306-18.

JOURNAL OF NUTRITION FASTING AND HEALTH

The Effect of Dietary Glycemic Index on Inflammatory Biomarkers: A Systematic Review with Consideration of Confounders

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ABSTRACT

Introduction: The glycemic index (GI) and inflammation are associated with several diseases; however, the relationship between GI and inflammation remains unclear. In this systematic review, the authors hypothesize that GI influences inflammatory biomarkers but can be significantly affected by unrecognized statistical confounders.

Methods: A comprehensive search was made in ScienceDirect, Web of Science, PubMed, Directory of Open Access Journals (DOAJ), and Google Scholar from 2010 to April 2022 using MESH and un-MESH keywords. Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) were used.

Results: Out of 24,577 studies, 14, including one master's thesis, were included in this review. Seven of these studies were conducted on individuals with a disease, six were on healthy or obese individuals without other illnesses, and one focused on pregnant women. IL-6 was measured in 8 studies, TNF-α in 7, CRP in 6, and hs-CRP in 2. Five well-designed studies confirmed that GI can influence inflammation, while seven found no association. Several unaddressed confounders and limitations were identified across the studies. The primary factors affecting the results were dietary patterns, metabolic factors, and food processing.

Conclusion: Based on the results, evidence supports a slight effect of GI on inflammatory biomarkers. The bias risk in different studies is high. More studies are required, and this review provides essential considerations to lower the bias risks for further studies.

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Introduction

Inflammation is a protective biological response involving the immune system, tissues, and organs to various harmful stimuli, such as pathogens, cellular damage, and surgery (1-3). Overall, inflammation is a key driver of many diseases (1, 2). Several factors can be used to assess the severity of inflammation, with inflammatory blood biomarkers being among the most crucial (1, 2, 4).

Recent studies have demonstrated that dietary intake significantly influences pro-inflammatory processes and the severity of chronic diseases (3, 5-8). Notably, strong associations have been found between carbohydrate and sugar consumption, insulin levels, and the risk of inflammation and chronic diseases (9-11).

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The glycemic index (GI), introduced by Jenkins et al. (12) in the 1980s, is a key measure for assessing the quality of carbohydrates. It is defined as the degree and duration of blood glucose elevation following fasting in response to the consumption of a specific carbohydrate, compared to a standard (typically glucose or white bread). The GI is scaled from 0 to 100 and is categorized into Low GI (<56), Medium GI (56-69), and High GI (>69) (12-14).

Recent studies have highlighted an association between dietary GI and various chronic diseases, particularly diabetes, cardiovascular diseases, and breast cancer (9, 10, 15-21). Obesity is another factor that increases the risk of inflammation in individuals, and some studies have also shown a significant association between GI and weight management (22-25). Furthermore, the inflammatory effects of GI and carbohydrate intake have been discussed in systematic reviews and meta-analyses as potential mediators of breast cancer (10). However, a meta-analysis in 2018 found no significant relationship between GI and inflammatory cytokines, including CRP, leptin, IL-6, and TNF-α (26).

Despite some studies indicating a pro-inflammatory effect of Glycemic Load (GL) (9, 10, 26), the overall impact of GI on inflammation remains unclear. GL is a measure that estimates the increase in blood glucose levels after consuming carbohydrates (9, 10, 26). In other words, it is still uncertain whether the quality of carbohydrates contributes to inflammation or if only the quantity plays a role. While GL estimates the blood glucose increase after carbohydrate consumption, it does not fully account for carbohydrate quality. Previous research suggests that GL is confounded by carbohydrate quantity, making it an inadequate independent measure (13). In contrast, GI is independent of carbohydrate weight, allowing it to more accurately represent carbohydrate quality (12-14).

The conflicting results in the existing literature highlight a significant research gap: while some studies support the role of GI in promoting inflammation, others fail to find a significant correlation between GI and inflammatory cytokines such as CRP, leptin, IL-6, and TNF-α (26). Therefore, this systematic review aims to evaluate the impact of the Glycemic Index on inflammatory biomarkers, specifically IL-6, IL-1, TNF-α, CRP, and hs-CRP, independent of GL. This review seeks to clarify the association between carbohydrate quality and inflammation, identify biases in prior studies, and provide recommendations for future research. The authors hypothesize that GI influences inflammatory biomarkers; however, previous studies have often overlooked significant confounders that must be addressed.

Materials and Methods

Search Strategy

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were followed for this study. Three independent researchers (PM, PZSh, and AV) searched scientific databases, including ScienceDirect, Web of Science, PubMed, and the Directory of Open Access Journals (DOAJ), covering the period from 2010 to April 2022 (Table 1).

Table 1. PICO criteria for inclusion of studies in the systematic review

PICO component	Description
Population	age≥18 years old, in any country, with or without a disease
Intervention	With low GI or high GI diet pattern or report the GI score of diet (GL studies excluded)
Comparators	N/A
Outcomes	Reported any changes in IL-6, IL-1, TNF-a, CRP, and HS-CRP
Study design	All original studies on human subjects include: Case-Control Studies, Intervention Studies, Cross-sectional studies, cohort studies
Language	English, Farsi

GI: Glycemic Index, GL: Glycemic Load, IL-6: Interleukin-6, IL-1: Interleukin-1, TNF-a: Tumor Necrosis Factor Alfa, CRP: C-Reactive Protein, HS-CRP: High Sensitive C-Reactive Protein, N/A: Not applied

The search timeline was limited based on two factors: 1) the update to the GI table in 2008 (27) and 2) a comprehensive discussion by Galland et al. (9) followed by Milajerdi et al.'s (26) study in 2010. Additionally, a thorough search was conducted in Google Scholar from 2010 to April 2022, and relevant articles from this database were included in the study.

Search Keywords

In this systematic review, a comprehensive search strategy was employed, utilizing both Medical Subject Headings (MeSH) and non-MeSH keywords tailored to the search protocols of each database to identify relevant studies on the relationship between the glycemic index and inflammation. The search included keywords such as "Glycemic Index," along with various MeSH terms related to its epidemiology, etiology, immunology, physiology, and more, alongside non-MeSH terms like "glycemic index," "GI," and "glycaemic indices." Inflammation-related terms included both MeSH and non-MeSH keywords such as "inflammation," inflammatory biomarkers, specific interleukins (e.g., IL-1, IL-6, IL-10), tumor necrosis factor (TNF), C-reactive protein (CRP), and other inflammatory indices and mediators. To ensure comprehensiveness, related systematic reviews were consulted, and a secondary search was performed by a fourth researcher using a simplified query of ("glycemic index" OR GI) and "inflammatory biomarkers." The search results from all databases were consolidated, and duplicate articles were removed. Finally, the findings were systematically organized into a single comprehensive file for analysis.

Inclusion and Exclusion Criteria

All clinical trials, case-control studies, cohort studies, and cross-sectional human studies published from 2010 to April 2022 that examined the effect of diet based on GI (Low/High GI) on inflammatory biomarkers or inflammation were considered. Studies such as duplicates, reviews, systematic reviews, meta-analyses, preprints, open-review manuscripts, editorial letters, conference abstracts, and short communications were excluded. Other exclusion criteria included: 1) studies conducted in children or animals due to biological and physiological differences, 2) studies that did not consider GI as a separate factor from GL, 3) studies that did not report inflammatory biomarkers in measurable values, 4) studies involving interventions other than dietary patterns, including medical, physical activity, exercise, or supplementary interventions, 5) studies for which the full text was unavailable, and 6) studies published in languages that the authors could not read. The main reason for excluding GL was its potential confounding effect on inflammation due to the amount of carbohydrate consumed. Ultimately, only studies that directly evaluated the effect of GI on inflammatory biomarkers were included in this review.

Study Selection

During the study selection process, researchers independently reviewed all papers, and the final findings were merged. A total of 24,577 articles were found in databases and Google Scholar. Three researchers (KE, PM, and AV) initially reviewed each article's title and general information to identify animal studies, children's studies, and review articles. Meta-analyses, reviews, letters, systematic reviews, animal studies, and studies conducted on children were excluded. The abstracts of 989 papers were thoroughly reviewed by three reviewers (PM, PZSh, and KS). Nine hundred and twelve articles met the exclusion criteria, and seventy-seven articles were deemed eligible for full-text review, which was conducted by three reviewers (PZSh, MR, and MRSh). The final number of relevant articles suitable for this systematic review was fourteen. Two judges (FK and RR) were involved throughout the review process. The review process was repeated once more by three reviewers (KE, PM, and AV), and no significant differences were found between the two rounds. A full description of this process is provided in Figure 1.

Risk of Bias Assessment

The Risk of Bias in Non-Randomized Studies of Interventions (ROBINS) checklist was used to assess the risk of bias and visualized using the robvis tool. The assessment is structured around seven domains: pre-intervention biases (D1: Confounding), during-intervention biases (D2: Selection of participants, D3: Classification of interventions), and post-intervention biases (D4: Deviation from intended interventions, D5: Missing data, D6: Measurement of outcomes), as well as biases in the selection of reported results (D7: Selection of reported results).

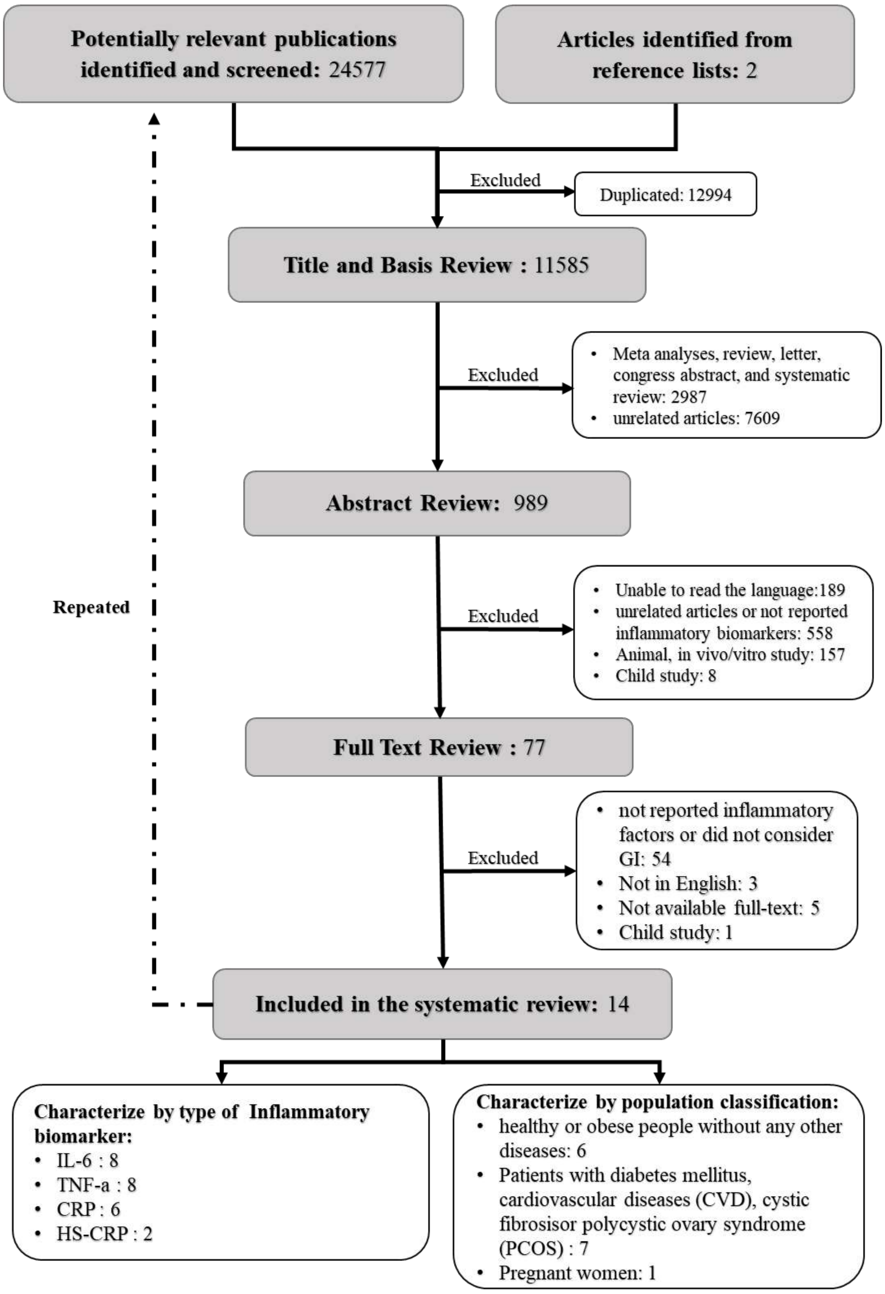

Figure 1. Flow diagram of study selection. GI: Glycemic Index, GL: Glycemic Load, IL-6: Interleukin-6, IL-1: Interleukin-1, TNF-α: Tumor Necrosis Factor Alfa, CRP: C-Reactive Protein, HS-CRP: High Sensitive C-Reactive Protein, N/A: Not applied

Results

Of the 14 studies included in this review (28-40) which also encompassed one MSc thesis (41) seven studies (28, 29, 32, 33, 36, 37, 39) were conducted on individuals with diabetes mellitus, cardiovascular diseases (CVD), and polycystic ovary syndrome (PCOS). Six studies (30, 31, 34, 38, 40, 41) investigated healthy or obese individuals without any underlying diseases, and one study (35) focused on pregnant women.

Of the reviewed studies, nine (29-36, 40, 41) evaluated the impact of GI on inflammatory biomarkers, while five studies (28, 33, 37, 39, 40) assessed both GI and GL. The inflammatory markers studied included IL-6 in eight studies (30, 35-41), TNF-α in eight studies (28, 32, 35-38, 40, 41), CRP in six studies (30, 31, 33, 34, 40, 41) and HS-CRP in two studies (29, 39). The risk of bias for these studies is illustrated in Figure 2, with a comprehensive summary of the findings in Table 2.

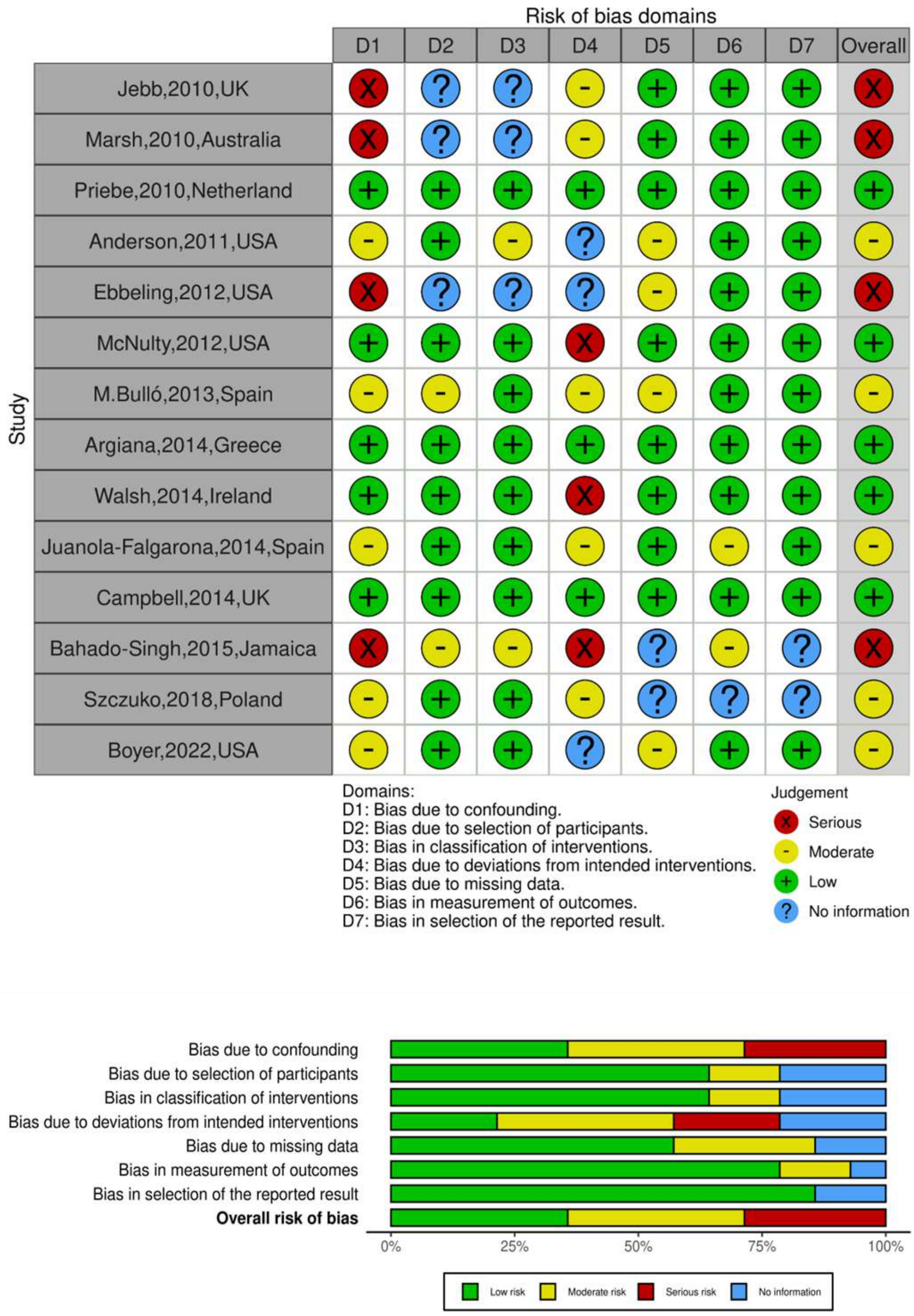

Figure 2. The risk of bias assessment visualized by robvis (visualization tool).

Table 2. Summarize findings by publishing order.

Study (author, year, country)	Study design	Study group	Population (number, gender, and age)	Inflammatory factors analyzed	Duration of follow-up	Outcome Mean ± SD						Outcome P-value	Confounding factors adjustment	Conclusion
Jebb, 2010, UK (35)	dietary intervention	Healthy people	548 (230 men/ 318 women, mean age for men 52 ± 10 and women 51 ± 9)	CRP (mg/L)	24 weeks		HS/HGI (N=85)	HM/HGI (N=111)	HM/LGI (N=116)	LF/HGI (N=116)	LF/LGI (N=121)	0.86	Adjusted for sex, center, ethnicity, baseline waist circumference, (log)HDL cholesterol, and age	No significant relation between GI and CRP in groups
						CRP (mg/L)								
						baseline	0.7 (0.16, 2.30)	0.54 (0.20, 1.90)	0.4 (0.14, 1.10)	0.5 (0.10, 1.95)	0.57 (0.16, 1.90)			
						Follow-up	0.95 (0.30, 1.85)	0.65 (0.20, 2.30)	0.7 (0.20, 2.00)	0.7 (0.20, 2.40)	0.6 (0.20, 1.70)			
						Percentage change	+21.3 (-5.8, 55.2)	+3.8 (-21.4, 35.6)	+36.3 (3.0, 78.2)	+22.4 (-7.6, 60.3)	+8.0 (-13.5, 33.9)			
Marsh, 2010, Australia (34)	dietary intervention	overweight and obese premenopausal women with PCOS	96 (0 men/96 women, mean age: 30.1)	CRP (mg/L)	12 months or until they achieved a 7% weight loss		Low GI (n=50)	CHD (n=46)				0.24	Adjusted for baseline (weight and body fat) or baseline and metformin (other variables) by using the general linear model ANOVA	No significant relation between GI and CRP in groups
						CRP (mg/L) change								
						Baseline	5.3 ± 0.8	4.6 ± 0.5						
						Changes from baseline	-1.2 ± 0.3	-0.6 ± 0.7						
Priebe, 2010, Netherland (39)	randomized Cross over study	Healthy people	10 (10men/ 0 women, age 21 ± 2.0)	IL-6 (pg/mL) TNF-a (pg/mL)	60 minutes before consumption to 240 minutes after consumption		Fasting concentration	0–2 h Postprandial concentration	0–4 h Postprandial concentration				N/M	non-digestible carbohydrate (generally low GI carbohydrate) increases inflammatory biomarkers less than digestible carbohydrate (high GI carbohydrate)
						IL-6 (pg/mL)								
						Low GI (n=10)	7.0 ± 0.8	5.2 ± 0.8	5.1 ± 0.7			**<0.05**		
						High GI (n=10)	13.1 ± 4.2	15.0 ± 4.5	19.7 ± 5.1					
						TNF-a (pg/mL)								
						Low GI (n=10)	5.7 ± 1.9	5.5 6 1.8	5.3 6 1.6			**<0.05**		

Study (author, year, country)	Study design	Study group	Population (number, gender, and age)	Inflammatory factors analyzed	Duration of follow-up	Outcome Mean ± SD							Outcome P-value	Confounding factors adjustment	Conclusion
						High GI (n=10)	6.6 ± 1.8		6.7 6 1.7		7.8 6 2.1				
Anderson, 2011, USA (27)	cohort study	Aged healthy people	1751 (percentage of men is different from 36.7% to 83.9% in groups, aged 70–79)	IL-6 (pg/mL) CRP (mg/ml) TNF-a (pg/mL)	from baseline and year 2 of the study	reported GI of groups	50.2 (n=31)	54.4 (n=319)	55.2 (n=284)	55.8 (n=570)	58.8 (n=284)	59.6 (n=258)		Adjusted for gender, age, and race	no analyses were performed according to the GI
						IL-6 (pg/mL) Geometric mean									
							2.2	1.6	1.9	1.9	1.8	1.8	N/M		
						CRP (mg/ml) Geometric mean									
							1.5	1.6	1.8	1.8	1.8	1.7	N/M		
						TNF-a (pg/mL) Geometric mean									
							2.9	2.9	3.2	3.2	3.2	3.0	N/M		
Ebbeling1, 2012, USA (32)	controlled 3-way crossover study	overweight and obese young adults	21 (13 men/8 women, aged 30.3)	CRP (mg/L)	Not clear but was During Weight-Loss Maintenance	Pre–Weight-Loss Baseline	Low Fat (n=21)		Low Glycemic Index (n=21)		Very Low Carbohydrate (n=21)		Overall= 0.13 **Trend= 0.05**	None but Rank transformed for analysis	No significant relation between GI and CRP in groups but the trend of change was significant
						CRP (mg/L) changes									
						1.75 (0.44 to 4.61)	-0.78 (0.38 to 1.92)		-0.76 (0.50 to 2.20)		-0.87 (0.57 to 2.69)				
McNulty, 2012, USA (41)	dietary intervention	overweight and obese women	20 (0 men/20 women, aged 30.3)	TNF-α (pg/mL) IL-6 (pg/ml) CRP (mg/l)	Less than a week (24 to 48 h)			Low GI (n=10)		High GI (n=10)				N/M	no significant relation was found in both TNF and CRP but IL-6 was significantly decreased in the low GI group
						TNF-α (pg/mL)									
						Pre-exercise		1.13 ± 0.18		1.79 ± 0.43			0.24		
						Post-exercise		1.06 ± 0.11		1.67 ± 0.26					
						24 h		0.98 ± 0.13		1.20 ± 0.13					
						48 h		1.12 ± 0.16		1.32 ± 0.18					
						IL-6 (pg/ml)									
						Pre-exercise		2.28 ± 0.52*		3.05 ± 0.61			0.42		
						Post-exercise		3.0 ± 0.52*		3.96 ± 0.59					
						24 h		2.59 ± 0.49*		2.84 ± 0.50					
						48 h		2.23 ± 0.39*		2.64 ± 0.51					
						CRP (mg/l)									
						Pre-exercise		2.06 ± 0.48		2.46 ± 0.86			0.59		
						Post-exercise		2.00 ± 0.51		2.87 ± 1.06					
						24 h		2.23 ± 0.36		2.81 ± 1.13					
						48 h		2.13 ± 0.38		2.40 ± 0.77					

Study (author, year, country)	Study design	Study group	Population (number, gender, and age)	Inflammatory factors analyzed	Duration of follow-up	Outcome Mean ± SD				Outcome P-value	Confounding factors adjustment	Conclusion
M. Bulló2, 2013, Spain (38)	cohort dietary intervention	no cardiovascular disease and met one or more of the two following criteria: three or more cardiovascular risk factors, or type 2 diabetes mellitus	568, Q1: 126 Q2: 129 Q3: 128 Q4: 128 (Men: 227/Women: 284, men aged 55-80 years and women 60-80 years)	IL-6 (pg/mL) TNF-α (pg/mL)	1 year	Q1 (n=126)	Q2 (n=129)	Q3 (n=128)	Q4 (n=128)		Adjusted for sex, age, changes in waist circumference, changes in body mass index, intervention group, physical activity in leisure time, smoking, insulin use, presence of type 2 diabetes mellitus, w-3 fatty-acid intake, and fib.er	a significant relation between TNF-α and GI at the baseline but changes in GI after 1 year did not have any significant relation between GI and both IL-6 and TNF
						IL-6 (pg/mL) change relative to the change in quartile (Q) 1						
						0	-0.61 (-3.92 to 2.70)	-1.80 (-5.13 to 1.53)	0.33 (-3.11 to 3.78)	0.969		
						TNF-α (pg/mL) change relative to the change in quartile (Q) 1						
						0	-0.04 (-5.45 to 5.36)	-2.49 (-7.93 to 2.94)	1.63 (_3.99 to 7.25)	0.798		
Argiana, 2014, Greece (40)	dietary intervention	type 2 diabetes	61 (Men: 27/women: 31, age: 61.3 and 63 for Low GI/GL and Controls groups respectively)	HS-CRP (µg/mL) IL-6 (pg/mL)	12 weeks	Low GI/GL (n=28)	Controls (n=31)				N/M	GI has a significant relation with HS-CRP but no significant change was found in IL-6
						HS-CRP (µg/mL) change						
						−1.4 ± 0.7*	0.7 ± 0.5			**0.007**		
						IL-6 (pg/mL) change						
						−0.1 ± 0.3	0.7 ± 0.5			0.718		
Walsh, 2014, Ireland (36)	dietary intervention	Pregnant women	621 (0 men/621 women, age not mentioned)	TNF-a (pg/mL) IL-6 (pg/mL)	early pregnancy and to 28 weeks		Intervention Group	Control Group			N/M	no significant relation was found between GI with either IL-6 or TNF-a
						TNF-a (pg/mL)						
						First trimester	4.82 (3.02-7.50)	4.60 (2.91-7.57)		NS		
						28 weeks	5.36 (3.19-7.83)	4.65 (3.09-7.71)				
						Cord	5.62 (0.58-9.2)	5.08 (0.88-8.49)				
						IL-6 (pg/mL)						
						First trimester	9.70 (4.17-21.1)	9.48 (4.38-26.26)				
						28 weeks	9.98 (5.38-20.8)	9.37 (4.21-22.34)		NS		
						Cord	10.95 (3.55-29.0)	9.17 (3.06-23.91)				
Juanola-Falgarona,	Controlled clinical trial	overweight and obese adults	122 (men: 25/women: 97,	IL-6 (pg/mL)	6 month	Low GI (n = 41)	High GI (n = 40)	Low Fat diet (n = 40)		0.457	N/M	No significant relation between
						CRP (mg/mL)						

Study (author, year, country)	Study design	Study group	Population (number, gender, and age)	Inflammatory factors analyzed	Duration of follow-up	Outcome Mean ± SD				Outcome P-value	Confounding factors adjustment	Conclusion
2014, Spain (31)			aged 42.5 to 44.1 in groups)	CRP (mg/mL)		Baseline	2.99 ± 4.34	3.58 ± 6.25	3.70±5.59			groups for both IL-6 and CRP
						6-m change	-20.19 ± 1.78	-20.07 ± 2.74	-20.04 ± 1.72			
						IL-6 (pg/mL)						
						Baseline	1.67 ± 1.18	1.36 ± 0.90	1.66 ± 1.11	0.162		
						6-m change	-20.27 ± 0.86	0.12 ± 0.91	-20.01 ± 0.72			
Campbell3, 2014, UK (37)	dietary intervention	type 1 diabetes	10 (10men/ 0 women, aged 27 ± 5)	IL-6 (pg/mL) TNF-a (pg/mL)	60 minutes before consumption to 180 minutes after consumption	time	Low GI (n=5)	High GI (n=5)			N/M	There is a significant relation between GI with IL-6 and TNF-a
						IL-6 (pg/mL)						
						-60	5.8	6		**<0.05**		
						meal	4.8	5.2				
						60	4.2	5.3				
						120	4.5[a]	8.7[a]				
						180	4.2[b]	8.2[b]				
						TNF-a (pg/mL)						
						-60	5.8	5.6		**<0.05**		
						meal	4.5	4.5				
						60	3.6[c]	6.7[c]				
						120	4.2[d]	6.6[d]				
						180	3.8[e]	6.1[e]				
Bahado4-Singh, 2015, Jamaica (30)	dietary intervention	overweight people with type 2 diabetes	53 (24 men/ 29 women, mean age 42 ± 2.0 years)	HS-CRP (mg/dL)	24 weeks		Low-Intermediate GI	Conventional Diet/ High GI		**<0.05**	Adjusted for age, BMI, smoking, alcohol consumption, history of hypertension, history of hypercholesterolemia, and duration of diabetes.	A diet low in GI can significantly reduce HS-CRP in comparison with a Conventional Diet
						HS-CRP (mg/dL)						
						Baseline	1.36 ± 0.21	1.12 ± 0.30				
						Difference between week 12 and baseline	-0.65 ± 0.19	-0.33 ± 1.09				
						Difference between week 24 and baseline	-0.52 ± 0.17	-0.17 ± 0.31				
						% Difference between week 24 and baseline	-38.24	-15.18				
Szczuko, 2018, Poland (33)	Dietary Intervention	Women with PCOS	22 (0men/22 women, age 26.76 ± 5.08)	TNF-α (pg/mL)	3 months	**TNF-α (pg/mL)**				NS	N/M	No significant relation between before and after intervention
						before		after				
						59.69 (35.79–104.4)		57.626 (43.48–98.83)				
					3 d ‹	**TNF-α (pg/mL)**				NS		

Study (author, year, country)	Study design	Study group	Population (number, gender, and age)	Inflammatory factors analyzed	Duration of follow-up	Outcome Mean ± SD	Outcome P-value	Confounding factors adjustment	Conclusion
Boyer, 2022, USA (29)	Dietary Intervention	Premenopausal women at high genetic risk of breast cancer	137 (0men /137women, (mean age =34.2)	TNF-α (pg/mL)		Mean of the population = 4.6 ± 1.3 β without adjustment = 0.008, p>0.05 β with adjustment = 0.005, p>0.05		BMI and total energy intake	No significant association between GI change before and after the intervention

1. To convert nmol/L to mg/L CRP, it is multiplied by 9.524
2. Population quartile to their glycemic index at baseline.
3. Outcome's mean is extracted from the article chart by JavaTpoint software (Approximate)
4. The sampling of studies is 1:1 grouping but the specific size of each group was not mentioned in the text.

* Was significant within-group after the intervention
outcomes with the same alphabet (abcd) are significant to each other
N/M: Not Mentioned, GI: Glycemic Index, GL: Glycemic Load, IL-6: Interleukin-6, IL-1: Interleukin-1, TNF-α: Tumor Necrosis Factor Alfa, CRP: C-Reactive Protein, HS-CRP: High Sensitive C-Reactive Protein, CHD: conventional healthy diet, PCOS: polycystic ovary syndrome, NS: not significant without P.value

Among the studies that explored the relationship between GI and inflammatory biomarkers, five studies—mainly clinical trials with a total sample size of 155 and a mean sample size of 31—identified a significant association between GI and at least one inflammatory biomarker (35-39). In contrast, seven studies, primarily population-based interventions with a total sample size of 3,300 and a mean sample size of 471, found no significant relationship between GI and inflammation (28-34). One study observed a significant association at baseline, but this was not maintained upon follow-up (37). Additionally, one study (40) did not perform any statistical analyses regarding the relationship between GI and inflammatory biomarkers.

The findings highlight several important considerations, including the influence of dietary patterns on the inflammatory effects of GI, the significance of study design, and the variability in GI's impact. While most studies with larger sample sizes did not find a strong association, a detailed review suggests that GI may have a minor effect on inflammation. The heterogeneity of the studies, methodological differences, and confounding factors complicate the interpretation of these results. Despite the inconclusive evidence, the authors propose a potential link between GI and inflammation while acknowledging the limitations of the studies reviewed. These limitations should be carefully considered in future research exploring the relationship between GI and inflammatory biomarkers.

Discussion

The reviewed studies generally support the authors' hypothesis. However, the studies are heterogeneous, and differences influence their findings in methodology and confounding factors. Despite the majority of studies with larger sample sizes showing no significant association, a detailed review suggests that GI may have a minor effect on inflammation. However, the complexity of GI and the lack of sufficient studies with consistent findings prevent us from providing a definitive answer to this question. In this review, the authors propose a possible link between GI and inflammation and outline the main limitations that should be considered in future studies.

In 2010, findings from a multicenter diet intervention study reported no significant differences in CRP levels between groups, both before and after adjustment (34). However, further analysis within the study revealed two completely different effects of GI on CRP—one positive and one negative—associated with two distinct diet patterns. The findings led to the hypothesis that diet patterns, particularly fat content, can influence the effect of GI (34). This is the first confounder identified in the study that was not adequately addressed. It is worth noting that the potential effect of food components and GL on GI and inflammatory responses has been reported several times before (9, 10, 26).

According to reports from a cohort study, diet patterns with higher GI scores were associated with slightly higher TNF-α and CRP levels than lower GI groups (40). However, no in-group analysis was performed in this study. A key finding from this study is that GI may vary significantly depending on the diet patterns, supporting previous hypotheses (9, 10, 26). For example, in this study, diet patterns involving sweets and desserts had a lower GI than those involving refined grains and breakfast cereals, which were believed to have a higher GI (40). This represents another significant limitation for studies on GI and GL. Based on the current research, it is recommended to consider the population's diet patterns and the consumption of unhealthy foods—characterized by a higher inflammatory index and lower GI—as confounders. However, it must be acknowledged that controlling a population's diet in a real-life environment, which contains multiple confounders that affect both GI (e.g., diet) and inflammation (e.g., stress, physical activity, injuries), is nearly impossible. Therefore, a high risk of bias can be expected in population-based and cohort studies investigating GI.

Designing studies with appropriate methodology that can isolate samples from confounders presents a significant challenge. While such studies can provide suitable laboratory conditions, their main limitations often include small sample sizes and short follow-up periods. Some studies with strong methodologies fall into this category (28, 32, 33). However, three studies with well-controlled, low-bias protocols demonstrated a significant direct relationship between GI and inflammation despite their small sample sizes (31, 36, 38). In all of these studies, participants adhered to a closely monitored diet during the assessment, highlighting the importance of controlling confounders over the sample size (31, 36, 38). Additionally, one study showed that providing linear graphs for small sample-sized studies could offer valuable insights (38). These findings underscore the significant impact of confounders on study results.

In another study with a large population, a 137-item Food Frequency Questionnaire (FFQ) was used to assess dietary intake and GI (37). This study, based on the Brand-Miller GI table (27), found a significant association between TNF-α and GI at baseline (P-ANOVA = 0.046) (37). However, no significant differences were observed after a one-year intervention between GI and IL-6 or TNF-α (37). The main reason for this discrepancy is the study's methodology, which involved low-inflammatory diet patterns in the groups (42-44). This study compared two potential anti-inflammatory diet patterns, which could have influenced the results. Nonetheless, the nature of the survey may also have impacted the findings, similar to previous population-based studies.

One of the notable findings in the Bahado-Singh et al. study (29) showed a 38.24% decrease in HS-CRP levels in the low-intermediate GI group, compared to a 15.18% decrease in the high GI group. Despite the decline in both groups, the reduction in the low-intermediate GI group was significantly smaller than in the high GI group ($p < 0.05$). However, the study did not explain the anti-inflammatory effect observed in both high and low-intermediate GI diets. Although both groups followed the same diet during the assessment, the decrease in HS-CRP could have been influenced by other anti-inflammatory components in the diets. Nevertheless, the low GI diet demonstrated a more potent anti-inflammatory effect. The adherence of the sample population to their diet plan and environmental factors played a key role in these findings.

These confounding effects can influence the current understanding of the topic. Inflammatory biomarkers are more sensitive than outcomes like disease incidence, which may explain the variability in findings. Despite supporting data on the effect of GI on various diseases (7, 9, 10, 15-21, 45, 46), results on inflammatory biomarkers vary widely. A meta-analysis shows a significant difference between low and high GI groups in CRP levels for both models in obese individuals with and without diabetes (47). At the same time, a meta-analysis by Milajerdi et al. found no inflammatory effect of GI, supporting the findings of Buyken et al. (26, 48). Conversely, another study demonstrated an association between GI and oxidative stress (49). These discrepancies highlight the importance of sample size and the methodology used in selecting studies for systematic reviews. In general, the sample size of studies significantly impacts the weight of findings in meta-analyses. Consequently, the results of cross-sectional and population-based studies, which have limited control and a higher risk of bias, tend to outweigh those of controlled interventions. Therefore, it is recommended that future studies in this field focus on interventions in individuals within controlled conditions, with equal carbohydrate intake and similar characteristics.

Nevertheless, the most significant finding supporting the association between GI and inflammation was reported in the study by Yeon-Soo et al. in 2018 (50). In this study, an association was found between GI and the Dietary Inflammatory Index (DII), which was developed by Dr. Shivappa and Dr. Hebert (51-54) to assess dietary inflammatory potential. This study, along with the reported effect of GI on CRP by Schwingshackl et al. (47), suggests a need to reconsider the effect of GI on inflammation, as previously reported by Milajerdi et al. (26).

One of the main weaknesses of the GI is related to its nature, which, if not adequately controlled, increases the risk of bias. Factors such as food processing, sugar content, other nutrients, food pH, speed of eating, blood glucose levels, and insulin levels can all affect the body's GI response, as illustrated in Figure 3 (12-14, 55-57). Another significant weakness of the GI is its food classification pattern (12-14). In this pattern, some pro-inflammatory foods—such as pizza (GI=39), fructose (GI=15), chocolate (GI=40), ice cream (GI=51), soft drinks/soda (GI=59), and potato crisps (GI=56)—are classified as low to moderate GI foods, while some fruits—like pineapple (GI=59), mango (GI=51), and watermelon (GI=76)—have a higher GI (12-14). Considering these issues, it is possible that an unhealthy diet pattern could have a lower GI than a healthier one, but further research is needed to confirm this hypothesis. These factors represent potential confounders that can influence the results of population-based studies, although they can be controlled in isolated conditions.

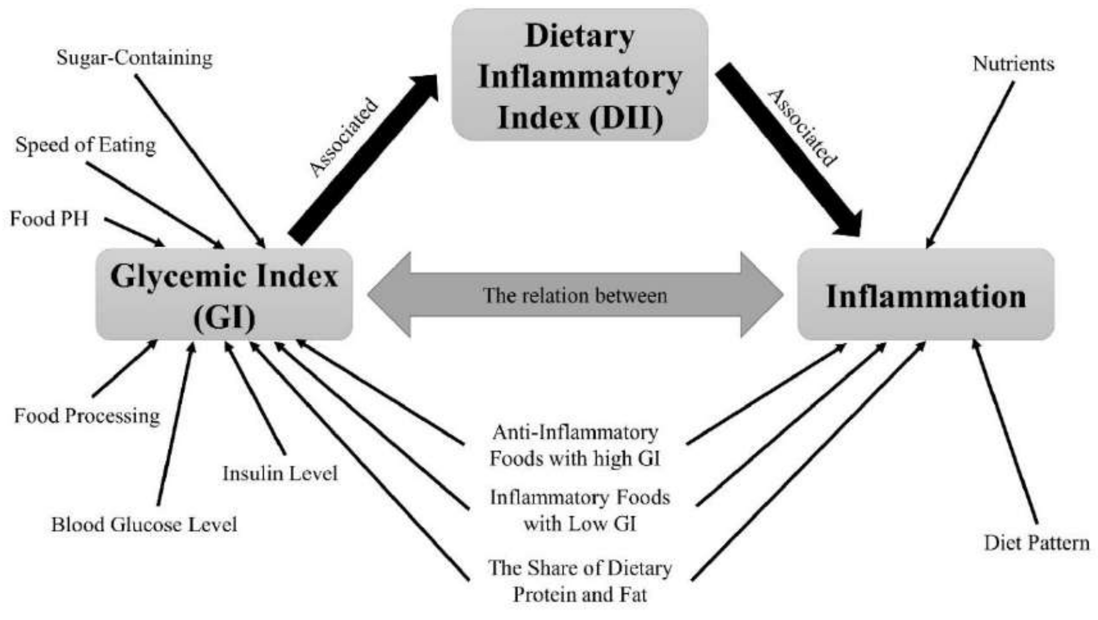

Figure 3. The possible direct and indirect confounders of the effect of GI on inflammatory biomarkers

Based on the findings and considering the limitations, conducting a well-designed GI study presents several complications that must be addressed. Among all food components, it seems that diet patterns have the most confounding effect, though further investigation is still needed

(3, 6, 29, 34). Continued follow-ups in controlled clinical trials, with isolated conditions or ensuring participants' diet adherence, could also be beneficial. Additionally, studies to explore the association between diet patterns and GI are recommended. To better understand the effect of GI on inflammation, using more homogenous populations and controlling for differences in diet patterns— which can introduce biases— would provide considerable benefits. Nevertheless, a dietary pattern high in fruits, vegetables, fish, poultry, legumes, and whole grains, and low in red and processed meats, sweetened beverages, sweets, refined grains, and fried potatoes, has been linked to lower levels of inflammatory biomarkers, regardless of GI and GL (3, 5, 6, 40). Therefore, understanding the association between GI and inflammation may benefit clinical settings, particularly in hospitals and intensive care units. This could inform the design of oral or enteral formulas to control inflammation and glycemic responses in these settings and for sensitive patients who need to follow specific diets at home.

The strength of this study lies in the perspectives of the reviewers. At each step, at least two researchers with differing opinions reviewed the studies, providing a fresh perspective and potential hypotheses for further research. However, the main weaknesses of this study are related to the nature of GI and the lack of sufficient studies. Another limitation was the absence of statistical analysis. Nevertheless, the authors recognized that the current findings on GI are not suitable or homogenized for this purpose. Given the unclear effect of dietary patterns in the reviewed studies, any analysis could introduce bias, though it may still provide a statistically specific answer to this issue.

Conclusion

Despite research in this field, the findings of studies remain inconsistent, and numerous confounders can affect the results. There is evidence supporting a slight effect of GI on inflammatory biomarkers. Based on the available evidence, diet and underlying factors can significantly influence the relationship between GI and inflammation. However, further research is needed to establish a clear link between GI and inflammation. Specifically, studies should focus on homogenized populations with similar diet patterns, and continuous monitoring through follow-up studies is recommended. Given the previous meta-analyses on this subject, it is likely that diet-related biases, which are not statistically recognized, may have influenced the findings.

Declarations

Ethics Approval and Consent to Participate

The protocol is approved by an in-house committee at Varastegan Institute for Medical Sciences

Consent for Publication

The earliest version of this publication has been pre-printed at https://doi.org/10.21203/rs.3.rs-1558724/v1, which was significantly improved after several revisions.

Availability of Data and Materials

Data is available upon reasonable request.

Conflict of Interest

The authors of this paper declare no conflict of interest.

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Authors' Contributions

All authors participate in the search and review of the papers described in the method. KE, PM, MRSH, and AV drafted the paper, RR and FK made the final revision, and RR and MRSH accepted the responsibilities of the corresponding authorship. MRSH submitted.

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Abbreviations

Interleukins-1: IL-1
Interleukins-6: IL-6
Interleukins-10: IL-10
Tumor Necrosis Factor-α: TNF-α
C-Reactive Protein: CRP
High-Sensitive C-Reactive Protein: HS-CRP
Glycemic Index: GI
Glycemic Load: GL
Food Frequency Questionnaire: FFQ
Preferred Reporting Items for Systematic Review and Meta-analysis: PRISMA

References

1. Hotamisligil GS. Inflammation, metaflammation and immunometabolic disorders. Nature. 2017;542(7640):177-85.
2. Netea MG, Balkwill F, Chonchol M, Cominelli F, Donath MY, Giamarellos-Bourboulis EJ, et al. A guiding map for inflammation. Nat Immunol. 2017;18(8):826-31.
3. Ricordi C, Garcia-Contreras M, Farnetti S. Diet and Inflammation: Possible Effects on Immunity, Chronic Diseases, and Life Span. J Am Coll Nutr. 2015;34 Suppl 1(sup1):10-3.
4. Olson TP, Dengel DR, Leon AS, Schmitz KH. Changes in inflammatory biomarkers following one-year of moderate resistance training in overweight women. Int J Obes (Lond). 2007;31(6):996-1003.
5. Sears B, Ricordi C. Anti-inflammatory nutrition as a pharmacological approach to treat obesity. Journal of Obesity. 2011;2011.
6. Sears B, Ricordi C. Role of fatty acids and polyphenols in inflammatory gene transcription and their impact on obesity, metabolic syndrome and diabetes. Eur Rev Med Pharmacol Sci. 2012;16(9):1137-54.
7. Jenkins DJ, Dehghan M, Mente A, Bangdiwala SI, Rangarajan S, Srichaikul K, et al. Glycemic index, glycemic load, and cardiovascular disease and mortality. New England Journal of Medicine. 2021;384(14):1312-22.
8. Bujtor M, Turner AI, Torres SJ, Esteban-Gonzalo L, Pariante CM, Borsini A. Associations of dietary intake on biological markers of inflammation in children and adolescents: a systematic review. Nutrients. 2021;13(2):356.
9. Galland L. Diet and inflammation. Nutrition in Clinical Practice. 2010;25(6):634-40.
10. Schlesinger S, Chan DS, Vingeliene S, Vieira AR, Abar L, Polemiti E, et al. Carbohydrates, glycemic index, glycemic load, and breast cancer risk: a systematic review and dose–response meta-analysis of prospective studies. Nutrition reviews. 2017;75(6):420-41.
11. Davis NJ, Crandall JP, Gajavelli S, Berman JW, Tomuta N, Wylie-Rosett J, et al. Differential effects of low-carbohydrate and low-fat diets on inflammation and endothelial function in diabetes. J Diabetes Complications. 2011;25(6):371-6.
12. Jenkins DJ, Wolever TM, Taylor RH, Barker H, Fielden H, Baldwin JM, et al. Glycemic index of foods: a physiological basis for carbohydrate exchange. Am J Clin Nutr. 1981;34(3):362-6.
13. Augustin LS, Kendall CW, Jenkins DJ, Willett WC, Astrup A, Barclay AW, et al. Glycemic index, glycemic load and glycemic response: an International Scientific Consensus Summit from the International Carbohydrate Quality Consortium (ICQC). Nutrition, Metabolism and Cardiovascular Diseases. 2015;25(9):795-815.
14. Foster-Powell K, Holt SH, Brand-Miller JC. International table of glycemic index and glycemic load values: 2002. Am J Clin Nutr. 2002;76(1):5-56.
15. Pi-Sunyer FX. Glycemic index and disease. Am J Clin Nutr. 2002;76(1):290S-8S.
16. Willett W, Manson J, Liu S. Glycemic index, glycemic load, and risk of type 2 diabetes. Am J Clin Nutr. 2002;76(1):274S-80S.
17. Livesey G, Taylor R, Livesey HF, Buyken AE, Jenkins DJA, Augustin LSA, et al. Dietary Glycemic Index and Load and the Risk of Type 2 Diabetes: A Systematic Review and Updated Meta-Analyses of Prospective Cohort Studies. Nutrients. 2019;11(6):1280.
18. Vega-López S, Venn BJ, Slavin JL. Relevance of the glycemic index and glycemic load for body weight, diabetes, and cardiovascular disease. Nutrients. 2018;10(10):1361.
19. Zazpe I, Santiago S, Gea A, Ruiz-Canela M, Carlos S, Bes-Rastrollo M, et al. Association between a dietary carbohydrate index and cardiovascular disease in the SUN (Seguimiento Universidad de Navarra) Project. Nutrition, Metabolism and Cardiovascular Diseases. 2016;26(11):1048-56.
20. Evans CE, Greenwood DC, Threapleton DE, Gale CP, Cleghorn CL, Burley VJ. Glycemic index, glycemic load, and blood pressure: a systematic review and meta-analysis of randomized controlled trials. The American Journal of Clinical Nutrition. 2017;105(5):1176-90.
21. Gao R, Duff W, Chizen D, Zello GA, Chilibeck PD. The Effect of a Low Glycemic Index Pulse-Based Diet on Insulin Sensitivity, Insulin Resistance, Bone Resorption and Cardiovascular Risk Factors during Bed Rest. Nutrients. 2019;11(9):2012.
22. Brand-Miller JC, Holt SH, Pawlak DB, McMillan J. Glycemic index and obesity. Am J Clin Nutr. 2002;76(1):281S-5S.
23. Larsen TM, Dalskov SM, van Baak M, Jebb SA, Papadaki A, Pfeiffer AF, et al. Diets with high or low protein content and glycemic index for weight-loss maintenance. N Engl J Med. 2010;363(22):2102-13.
24. Lennerz B, Lennerz JK. Food Addiction, High-Glycemic-Index Carbohydrates, and Obesity. Clin Chem. 2018;64(1):64-71.
25. Petrella E, Tamborrino V, Di Cerbo L, Neri I, Facchinetti F. An early, customized low-glycemic-index diet prevents adverse pregnancy outcomes in overweight/obese women. Minerva Ginecol. 2018;70(3):254-60.
26. Milajerdi A, Saneei P, Larijani B, Esmaillzadeh A. The effect of dietary glycemic index and glycemic load on inflammatory biomarkers: a systematic review and meta-analysis of randomized clinical trials. Am J Clin Nutr. 2018;107(4):593-606.
27. Atkinson FS, Foster-Powell K, Brand-Miller JC. International tables of glycemic index and glycemic load values: 2008. Diabetes care. 2008;31(12):2281-3.

28. Boyer AL, Arikawa AY, Schmitz KH, Sturgeon KM. Association of inflammatory diets with inflammatory biomarkers in women at high genetic risk for breast cancer. Nutrition and cancer. 2022;74(3):816-9.
29. Bahado-Singh PS, Riley CK, Wheatley AO, Boyne MS, Morrison EY, Asemota HN. High Fiber Caribbean Diets with Low-Intermediate GI Improve Glycemic Control, Cardiovascular and Inflammatory Indicators in Overweight Persons with Type 2 Diabetes: A Randomized Control Study. Current Research in Nutrition and Food Science. 2015;3(1):36.
30. Juanola-Falgarona M, Salas-Salvado J, Ibarrola-Jurado N, Rabassa-Soler A, Diaz-Lopez A, Guasch-Ferre M, et al. Effect of the glycemic index of the diet on weight loss, modulation of satiety, inflammation, and other metabolic risk factors: a randomized controlled trial. Am J Clin Nutr. 2014;100(1):27-35.
31. Ebbeling CB, Swain JF, Feldman HA, Wong WW, Hachey DL, Garcia-Lago E, et al. Effects of dietary composition on energy expenditure during weight-loss maintenance. JAMA. 2012;307(24):2627-34.
32. Szczuko M, Zapalowska-Chwyc M, Drozd A, Maciejewska D, Starczewski A, Wysokinski P, et al. Changes in the IGF-1 and TNF-alpha synthesis pathways before and after three-month reduction diet with low glicemic index in women with PCOS. Ginekologia polska. 2018;89(6):295-303.
33. Marsh KA, Steinbeck KS, Atkinson FS, Petocz P, Brand-Miller JC. Effect of a low glycemic index compared with a conventional healthy diet on polycystic ovary syndrome. Am J Clin Nutr. 2010;92(1):83-92.
34. AB RSGJSALJAGBAFGSMCSCMDBLJWCMST. Effect of changing the amount and type of fat and carbohydrate on insulin sensitivity and cardiovascular risk: the RISCK (Reading, Imperial, Surrey, Cambridge, and Kings) trial. The American journal of clinical nutrition. 2010;92(4):748-58.
35. Walsh JM, Mahony RM, Culliton M, Foley ME, McAuliffe FM. Impact of a low glycemic index diet in pregnancy on markers of maternal and fetal metabolism and inflammation. Reprod Sci. 2014;21(11):1378-81.
36. Campbell MD, Walker M, Trenell MI, Stevenson EJ, Turner D, Bracken RM, et al. A low–glycemic index meal and bedtime snack prevents postprandial hyperglycemia and associated rises in inflammatory markers, providing protection from early but not late nocturnal hypoglycemia following evening exercise in type 1 diabetes. Diabetes care. 2014;37(7):1845-53.
37. Bulló M, Casas R, Portillo M, Basora J, Estruch R, García-Arellano A, et al. Dietary glycemic index/load and peripheral adipokines and inflammatory markers in elderly subjects at high cardiovascular risk. Nutrition, Metabolism and Cardiovascular Diseases. 2013;23(5):443-50.
38. Priebe MG, Wang H, Weening D, Schepers M, Preston T, Vonk RJ. Factors related to colonic fermentation of nondigestible carbohydrates of a previous evening meal increase tissue glucose uptake and moderate glucose-associated inflammation. Am J Clin Nutr. 2010;91(1):90-7.
39. Argiana V, Kanellos P, Makrilakis K, Eleftheriadou I, Tsitsinakis G, Kokkinos A, et al. The effect of consumption of low-glycemic-index and low-glycemic-load desserts on anthropometric parameters and inflammatory markers in patients with type 2 diabetes mellitus. Eur J Nutr. 2015;54(7):1173-80.
40. Anderson AL, Harris TB, Tylavsky FA, Perry SE, Houston DK, Lee JS, et al. Dietary patterns, insulin sensitivity and inflammation in older adults. European journal of clinical nutrition. 2012;66(1):18-24.
41. McNulty KAM. Inflammation, insulin, and glucose differences between high and low glycemic index diets following downhill running in overweight and obese women: Doctoral dissertation, Montana State University-Bozeman, College of Education, Health & Human Development; 2012.
42. Sears B. Anti-inflammatory Diets. J Am Coll Nutr. 2015;34 Suppl 1(sup1):14-21.
43. Ricker MA, Haas WC. Anti-Inflammatory Diet in Clinical Practice: A Review. Nutr Clin Pract. 2017;32(3):318-25.
44. Tolkien K, Bradburn S, Murgatroyd C. An anti-inflammatory diet as a potential intervention for depressive disorders: A systematic review and meta-analysis. Clinical Nutrition. 2019;38(5):2045-52.
45. Tracy RP. Emerging relationships of inflammation, cardiovascular disease and chronic diseases of aging. Int J Obes Relat Metab Disord. 2003;27 Suppl 3(3):S29-34.
46. Chiavaroli L, Lee D, Ahmed A, Cheung A, Khan T, Mejia SB, et al. Low Glycemic Index/Load Dietary Patterns and Glycemia and Cardiometabolic Risk Factors in Diabetes: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. Current Developments in Nutrition. 2021;5(Supplement_2):1018-.
47. Schwingshackl L, Hoffmann G. Long-term effects of low glycemic index/load vs. high glycemic index/load diets on parameters of obesity and obesity-associated risks: a systematic review and meta-analysis. Nutr Metab Cardiovasc Dis. 2013;23(8):699-706.
48. Buyken AE, Goletzke J, Joslowski G, Felbick A, Cheng G, Herder C, et al. Association between carbohydrate quality and inflammatory markers: systematic review of observational and interventional studies. The American journal of clinical nutrition. 2014;99(4):813-33.
49. Papachristoforou E, Lambadiari V, Maratou E, Makrilakis K. Association of glycemic indices (hyperglycemia, glucose variability, and hypoglycemia) with oxidative stress and diabetic complications. Journal of Diabetes Research. 2020;2020.
50. Kim Y, Chen J, Wirth MD, Shivappa N, Hebert JR. Lower dietary inflammatory index scores are

associated with lower glycemic index scores among college students. Nutrients. 2018;10(2):182.
51. Shivappa N, Steck SE, Hurley TG, Hussey JR, Hebert JR. Designing and developing a literature-derived, population-based dietary inflammatory index. Public Health Nutr. 2014;17(8):1689-96.
52. Shivappa N, Hébert JR, Rietzschel ER, De Buyzere ML, Langlois M, Debruyne E, et al. Associations between dietary inflammatory index and inflammatory markers in the Asklepios Study. British Journal of Nutrition. 2015;113(4):665-71.
53. Wirth M, Burch J, Shivappa N, Violanti JM, Burchfiel CM, Fekedulegn D, et al. Association of a dietary inflammatory index with inflammatory indices and the metabolic syndrome among police officers. Journal of occupational and environmental medicine/American College of Occupational and Environmental Medicine. 2014;56(9):986.
54. van Woudenbergh GJ, Theofylaktopoulou D, Kuijsten A, Ferreira I, van Greevenbroek MM, van der Kallen CJ, et al. Adapted dietary inflammatory index and its association with a summary score for low-grade inflammation and markers of glucose metabolism: the Cohort study on Diabetes and Atherosclerosis Maastricht (CODAM) and the Hoorn study. Am J Clin Nutr. 2013;98(6):1533-42.
55. Brand JC, Nicholson PL, Thorburn AW, Truswell AS. Food processing and the glycemic index. Am J Clin Nutr. 1985;42(6):1192-6.
56. Jenkins DJ, Wolever TM, Jenkins AL. Starchy foods and glycemic index. Diabetes care. 1988;11(2):149-59.
57. Livesey G, Taylor R, Hulshof T, Howlett J. Glycemic response and health—a systematic review and meta-analysis: relations between dietary glycemic properties and health outcomes. The American Journal of Clinical Nutrition. 2008;87(1):258S-68S.

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Association between Systemic Immune-Inflammation Index, Body Composition, and Mortality among Older Adults

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ABSTRACT

Introduction: Chronic inflammation in older adults is associated with various age-related diseases and may contribute to functional decline and reduced quality of life. An imbalance in body composition, characterized by excess fat and inadequate muscle mass levels, has been identified as an underlying cause of inflammation. Numerous prognostic factors, such as indicators, have been employed to measure inflammation. The Systemic Immune-Inflammation Index (SII) is a reliable indicator of inflammation that correlates with mortality in numerous investigations substantially. This paper aims to examine the relationship between body composition, mortality, and SII.

Methods: This cross-sectional study was conducted on 60 years old or older adults using the Neyshabur Longitudinal Study on Ageing (NeLSA) data. SII scores were calculated using data from individual blood bank records. Data analysis involved analytical techniques such as correlation coefficient, logistic regression, and linear regression.

Results: A total of 3,534 individuals participated, of whom 1,858 were male. The median age of participants was 65.71. The study revealed a significant association between the percentage of body fat and SII (p<0.001). The overall mortality rate was 0.93 in 1000. Mortality was linked to SII after adjusting for confounding variables (OR=1.001, 95% CI=1.000 to 1.002, P=0.047).

Conclusion: SII only correlated with blood pressure and body fat. A weak correlation was observed between SII and hs-CRP, which was associated with overall mortality in older adults.

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Introduction

Aging is defined differently by various researchers, and older adults are defined as 50–80 years old. However, the World Health Organization (WHO) classifies people as elderly when they are 65 years or older [1]. The global aging population is increasing, with over 500 million people, or 8% of the world's population, over 65 [2, 3]. The National Institute on Aging and the United States National Institute of Health predict a quadrupling of adults over 80 by 2050 [4]. Around one-fifth of the population will be over 60 by the middle of this century [5]. This demographic transition affects individuals and groups globally, with 30% of individuals who are 80 or older living alone [6]. The aging population and labor unavailability cause economic challenges, with lower national income expansion, impacting elderly socioeconomic status through tax-transfer systems and the dissatisfaction of older workers due to delayed retirement [7]. Older adults often suffer from multifaceted diseases such as back and neck pain, inflammation, dementia, chronic obstructive pulmonary disease, diabetes, and osteoarthritis, which often require multiple drug treatments [8,

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9]. Older adults frequently face challenges in their independence due to physical and mental limitations, which cause feelings of isolation and a lack of social assistance [10]. The decline in the quality of life of older adults due to diseases necessitates public health to address healthcare needs and develop products and services tailored to the specific needs of older adults [11].

Significant changes in body composition, such as an increase in body fat percentage and a decrease in lean mass and bone density, are related to aging. These variations can affect the onset of chronic diseases and unfavorable health outcomes. Several factors, including hormone imbalance, ongoing inflammation, and metabolic changes, can influence the onset and progression of chronic diseases [12]. Inflammation is a defense mechanism against harmful assaults and is vital for healing injured tissue and eliminating harmful stimuli. Acute inflammation is related to innate immunity and the host's initial defense against chemicals and foreign invaders. New research has suggested its functions as a complex molecular system [13]. In contrast, chronic inflammation is related to various age-related diseases like cancer, diabetes, atherosclerosis, and hypertension.

Various techniques are used to measure inflammation. The overall inflammation status of the body can be measured using inflammation markers in addition to erythrocyte sedimentation rate (ESR) and high sensitivity C-reactive protein (hs-CRP). Inflammation indicators include neutrophil-to-lymphocyte ratios (NLR), platelet-to-lymphocyte ratios (PLR), and systemic immune-inflammation indexes (SII). SII is a quantitative, accessible, and affordable measurement of the systemic immune-inflammatory response in the human body. SII has been related to poor prognosis in malignant tumors and an increased risk of all-cause, cardiovascular, and cancer-related mortality [14, 15].

The association between aging and chronic diseases such as diabetes [16], cancer [17], and heart disease [15] has been the subject of several studies. Research has suggested that higher levels of oxidative stress and inflammation may be related to increased mortality in older adults. However, there is a lack of evidence and insufficient data regarding this relationship in older adults, which requires population-based studies.

Chronic inflammation has many causes and mechanisms, and while lifestyle changes can help prevent these disorders, understanding the causes and mechanisms is crucial as healthy aging becomes more prevalent in developed and developing countries. This study was conducted on Iranian older adults because of the correlation between body composition and inflammation, the SII index's importance in predicting mortality, and the limited number of studies examining SII, body composition, and mortality among the general elderly population.

Materials & Methods

Study Population

Neyshabur Longitudinal Study on Ageing (NeLSA) is a large-scale and population-based study conducted in Neyshabour, Iran, among individuals between 50 and 94 years old [18]. NeLSA is intended to include 7460 subjects and assess various aspects of aging. Data collected in the NeLSA include demographic information, including sex, age, income, education level, and all causes of mortality and smoking, collected through interviews, blood tests, and comprehensive questionnaires, including physical activity.

Study design

This cross-sectional study used the NeLSA database. The Mashhad University of Medical Sciences Ethics Committee approved each procedure involving human subjects (IR.UMS.MEDICAL.REC.1401.325).

The inclusion criteria for NeLSA and the current study were as follows: (i) Having Iranian nationality, (ii) being older than sixty years, and (iii) willingness to participate in the cohort by signing the informed consent form. The exclusion criteria were those without fully documented information.

Study Measurements

Laboratory Measurements

The blood biochemistry data were the complete blood count (CBC) and serum high sensitivity-CRP (hs-CRP), which were measured in the registration & enrollment phase of NeLSA [18].

Body Composition Measurements

Body composition, including anthropometric and bioelectric impedance (BIA), were recorded. The BIA data were recorded using the InBody 770, BIOSPACE KOREA connected to a BSM. The BIA variables were total body water (TBW), percent body fat (PBF), fat-free mass (FFM), skeletal muscle mass (SMM), visceral fat level (VFL), and visceral fat area (VFA).

Socioeconomic and Lifestyle

The participants' level of physical activity was estimated using the Physical Activity Scale for older adults (PASE), which has been appropriately confirmed in previous Iranian research [19]. Socioeconomic status indications included educational levels categorized as illiterate, less than a high school diploma, high school diploma, and university degree. The income adequacy of the respondent was assessed based on their financial status. Smoking status was determined using a self-report.

Past Medical History

A physician performed clinical examinations, took histories, and double-checked participants' medical records. The current study included chronic diseases, including diabetes, heart disease, and hypertension (HTN).

Mortality

All-cause mortality was obtained from the cohort records from the initiation of the study till the time the data were collected.

Systematic Inflammation Index (SII)

The total blood count results from tests were used to determine SII. In addition, platelet count (PC), neutrophil count (NC), and lymphocyte count (LC) were measured in 1000 cells/ml. Based on previous research, the SII was computed as PC * (NC/LC) [17, 20].

Statistical Analysis

The Statistical Package for Social Sciences (SPSS) software version 26 was used for data analysis. The Kolmogorov-Smirnov test was used to evaluate the normality of the data. Continuous variables were presented using Cromedian and interquartile range (IQR), while categorical variables were presented as percentages and frequencies. The Spearman correlation coefficient was used to evaluate the correlation between variables and identify the confounders. The relationship between the study variables and SII was examined using linear regression after adjusting for age, sex, educational attainment, smoking, income, and other medical conditions. Univariate models were performed first, and variables with p-values less than 0.1 were included in the final model. The multivariate regression analysis was performed using backward elimination. The logistic regression analysis assessed the relationship between mortality and study variables. A P<0.05 was considered significant in all statistical analyses.

Results

The current study was conducted on 3534 participants, including 1676 females (47.4%) and 1858 males (52.6%). The median age of the included participants was 65.71(62.17-71.83) years. Table 1 summarizes the anthropometric measurements of the participants. The education level of the majority of the participants was less than a high school diploma (46.1%).

The participants' median SII was 284.42 (203.74, 392.76), and their median serum hs-CRP was 0.2 (0.1, 0.5) mg/dl, respectively.

Table 2 presents the relationship between SII and study variables. The relationship was evaluated using five different adjusted models. SII was positively associated with HTN in the crude model (Model 1) (β=28.673, 95% CI=8.516 to 48.830, P=0.005). No significant relationship was found between SII and the body composition variables (PBF, VFL, and VFA), sex, or education. In Model 4 (adjusted for VFA, education, and VFL). SII was significantly related to HTN (β=28.809, 95% CI=8.696 to 48.923, P=0.005) and PBF (β=1.92, 95% CI=0.994 to 2.86, P<0.001). Furthermore, SII continued to be positively related to both PBF (β=1.922, 95% CI=0.987 to 2.857, P=0.00) and HTN (β=28.81, 95% CI=8.690 to 48.933, P=0.005) after adjustment for VFA, education, VFL, and sex.

Table 1. Body composition measurements of the study participants.

Variables	Median (Q_1-Q_3)
PBF	37.6 (30.3, 44.3)
FFM	42.4 (37.5, 49.5)
SMM	23 (20, 27.3)
TBW	31.3 (27.6, 36.6)
VFL	13 (9, 17)
VFA	135.6 (94.65, 174.25)

For non-normal distribution variables, the median and first and third quartiles were used. PBF, Percent Body Fat; FFM, Fat-Free Mass; SMM, Skeletal Muscle Mass; TBW, Total Body Water; VFL, Visceral Fat Level; VFA, Visceral Fat Area.

Table 2. Relationship between SII and study variables.

		Sex	PBF	VFL	VFA	HTN	Education
Model 1	**β (95%CI low-up)**	14.34 (-3.877, 32.56)	1.55 (-1.02, 4.13)	1.41 (-28.81, 31.64)	-0.68 (-3.12, 2.98)	28.67 (8.51, 48.83)	-0.43 (-11.52, 10.66)
	P-value	0.12	0.23	0.92	0.96	0.005*	0.93
Model 2	**β (95%CI low-up)**	14.34 (-3.87, 32.56)	1.54 (-1.01, 4.10)	0.74 (-3.89, 5.38)	-	28.68 (8.53, 48.83)	-0.43 (-11.52, 10.66)
	P-value	0.12	0.23	0.75		0.005*	0.93
Model 3	**β (95%CI low-up)**	14.53 (-2.99,32.97)	1.54 (-1.00,4.10)	0.744 (-3.89,5.38)	-	28.71 (8.58,48.84)	-
	P-value	0.10	0.23	0.75		0.005*	
Model 4	**β (95%CI low-up)**	14.60 (-2.91,32.13)	1.92 (0.994,2.86)	-	-	28.80 (8.69,48.92)	-
	P-value	0.10	<0.001*			0.005*	
Model 5	**β (95%CI low-up)**	-	1.92 (0.987,2.85)	-	-	28.81 (8.69,48.93)	-
	P-value		<0.001*			0.005*	

SII, Dependent variable. A significant difference is shown by P < 0.05
Model 1: Unadjusted model. ***Model 2:*** VFA was adjusted. ***Model 3:*** VFA and education were adjusted. ***Model 4:*** VFA, education, and VFL were adjusted. ***Model 5:*** VFA, education, VFL, and sex were adjusted.
PBF, Percent Body Fat; VFL, Visceral Fat Level; VFA, Visceral Fat Area; HTN, Hypertension; CI, confidence Interval
* Significant relationship at α=0.05.

Figure 1 illustrates the relationship between the SII and hs-CRP. There was a significant weekly positive relationship between SII and hs-CRP among the study participants (r=0.076, p=0.001). The mortality rate was 0.93 in 1000 (328 from 3534 persons). The crude model had a significant relationship between mortality and SII (OR=1.001, 95% CI=1.000 to 1.001, p=0.016). The relationship between SII and mortality remained significant after adjustment for income, education, sex, diabetes, heart disease, HTN, and heart disease (OR=1.001, 95% CI=1.000 to 1.002, P=0.047).

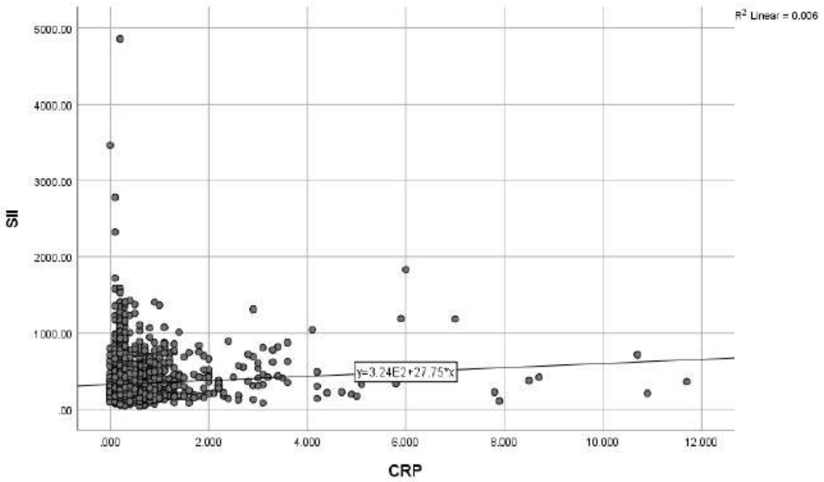

Figure 1. Relationship between high sensitivity C-reactive protein (hs-CRP) and systemic immune inflammation index (SII). A significant difference is shown by P < 0.05.

Discussion

The current study investigated the relationship between the immune systemic inflammation index (SII) and body composition among older adults. The higher body fat percentage and hypertension were associated with increased SII. The study also indicated that increased SII was related to an increased mortality risk, even after adjusting for confounders.

The direct association between SII and PBF was in line with the findings of the previous studies. Funghetto et al. reported that PBF categorization based on dual-energy X-ray absorptiometry (DEXA) and biochemical tests accurately predicted obesity, systemic inflammation, and atherogenic lipid profiles in older women than BMI [21]. According to another cross-sectional study using data from the National Health Survey on 8-18-year-old children and adolescents by

Singer et al. (2023), increased SII was associated with an increased likelihood of losing muscle mass, suggesting a strong association between inflammation and childhood obesity [22]. Eren et al. studied the association between childhood obesity and inflammatory mediators in children aged 6-16 and reported that increased SII was associated with increased body fat, waist circumference, and BMI [23]. In contrast to the current study's findings, Hestiantoro et al. reported that PBF was an accurate measure for assessing inflammation associated with body fat mass among women with polycystic ovarian syndrome (PCOS) [24]. This difference might be attributed to the difference in the study population between Hestiantoro et al. (only women with PCOS) and the present study (elderly men and women).

Different mechanisms have been proposed for the association between fat mass and inflammation among obese individuals. The low-grade chronic inflammation due to the increased adipose tissue might be, to some extent, due to the release of inflammatory mediators from adipose tissue. The buildup of aberrant or excess fat associated with obesity causes adipose tissue to emit inflammatory mediators, including CRP, tumor necrosis factor-alpha (TNF-α), and interleukin 6 (IL-6). The decrease in the synthesis of the anti-inflammatory adiponectin exacerbates the pro-inflammatory state and oxidative stress. The liver produces and secures CRP in response to increased IL-6, indicative of inflammation. Additionally, white adipose tissue (WAT) can cause inflammation in obesity through immune cell-adipocyte interactions, hypoxia, and increased adipocyte death [25]. The relationship between inflammation and body fat is primarily explained by the release of inflammatory mediators and the intricate interactions between immune cells and adipocytes [26]. Research has also been conducted on adipokines, including adiponectin, in the inflammatory processes associated with obesity. Adipose tissue secretes adipokines, and the dysregulation of adipokine synthesis can lead to inflammation and metabolic dysfunction in obesity. For example, reduced production of adiponectin and an anti-inflammatory adipokine might lead to a pro-inflammatory condition in obese individuals [27].

Based on the findings, hypertension was associated with increased SII, similar to the findings of a prospective population-based cohort study conducted in China that indicated a positive correlation between hypertension and increased SII. The cohort study discovered that those with higher baseline SII levels had a higher risk of developing hypertension throughout the follow-up period [28]. As this was a cross-sectional study, the priority of the events could not be determined. However, the significant association between hypertension and increased inflammation in the current study could align with the findings of the mentioned cohort study.

Higher SII index and hypertension may be associated with intricate interactions between inflammation, immunological response, and cardiovascular health. Low-grade chronic inflammation that elevated SII levels can indicate can result in endothelial dysfunction, oxidative stress, and arterial stiffness. This inflammatory milieu can lead to elevated blood pressure by upsetting the balance of vasoactive chemicals, including nitric oxide bioavailability, and generating pro-inflammatory cytokines. Furthermore, inflammatory mediators and immune cells may directly affect myocardial remodeling and contribute to hypertension [29].

The current study found a direct but weak relationship between SII and CRP. Similarly, Ömür et al. showed that SII and CRP were positively correlated, particularly in individuals with persistent atrial fibrillation (AF) [28]. In contrast, Ustundag et al. reported no correlation between the CRP value and SII [30]. Various factors, including variations in study populations, sample sizes, research methodologies, and confounding variables, may have caused these contradictory results.

The current study showed a significant relationship between increased SII and mortality. Studies have demonstrated a nonlinear link between the SII and all-cause mortality and a correlation between the SII and cardiovascular, cardio-cerebrovascular, and all-cause mortality in the general population. Cardiovascular and cardiocerebrovascular mortality is strongly correlated with increased SII, and a twofold increase in SII was related to a 42% increase in mortality while reducing SII to half was associated with a 15% reduction in mortality. Furthermore, a linear relationship has been identified between the SII and insulin resistance and inflection [15].

Several variables, including age, gender, and comorbidities, mediate the relationship between SII and mortality. Given the nonlinear relationship between SII and mortality, SII may have varying effects on different forms of mortality [16]. Cao et al. reported a J- or U-shaped pattern, indicating that high or low SII may be associated with an increased risk of mortality due to cancer, cardiovascular disease, and all-cause mortality [31]. Wang et al. found that the SII was associated with the lowest mortality risk at a specific cut-off, and the association was nonlinear [15]. Similarly, another study reported a cut-off for SII concerning the risk of insulin resistance among individuals with abdominal obesity [32]. Similarly, a substantially strong correlation was reported between SII and cardiovascular mortality, indicating that SII and cardiovascular mortality may be more tightly related [15]. The results suggested that the association between SII and mortality is complex and may be affected by several variables, including demographics, gender, and some medical issues. Consequently, more investigation is required to comprehend the intricate connection between SII and mortality.

Strength and limitations

This study is bolstered by its extensive sample size and the inclusion of multiple confounding factors. However, the study's limitations include its cross-sectional design and the absence of a clear understanding of the causality of the SII and mortality or other conditions.

Conclusion

Based on the results, the Systemic Inflammation Index (SII) was associated with percentage body fat (PBF) and hypertension. Additionally, there was a weak correlation between SII and high-sensitivity C-reactive protein (hs-CRP), which was linked to overall mortality in older individuals. The body fat percentage can be a suitable predictive variable for the SII inflammatory index; therefore, controlling body fat may help improve inflammation in older adults. However, further prospective and large-scale studies are warranted to establish the results.

Declarations

Conflict of Interest

The authors declare no conflicts of interest.

Author Contributions

MM, PK, SD, JJ, SMA, SRM, ST, AGH, and AJ designed the study and were involved in the manuscript's data collection, analysis, and drafting. MS was involved in the study's design and critically reviewed the manuscript. All authors read and approved the final manuscript.

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Ethical Considerations

The Mashhad University of Medical Sciences Ethics Committee authorized the procedures performed under its ethical guidelines. Before the data collection, the participants also signed an informed consent form, and Neyshabur Longitudinal Study on ageing protocols were adhered to for data confidentiality and anonymization.

Code of Ethics

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References

1. Sabharwal S, Wilson H, Reilly P, Gupte CM. Heterogeneity of the definition of elderly age in current orthopaedic research. Springerplus. 2015;4:516.
2. Sabri SM, Annuar N, Rahman NLA, Musairah SK, Mutalib HA, Subagja IK. Major Trends in Ageing Population Research: A Bibliometric Analysis from 2001 to 2021. Proceedings. 2022;82(1):19.
3. Derbie L, Oumer A, Ayele K, Berhane A. Determinants of nutritional status among old age population in eastern Ethiopia: a general linear model approach. J Nutr Sci. 2022;11:e70.
4. Jaul E, Barron J. Age-Related Diseases and Clinical and Public Health Implications for the 85 Years Old and Over Population. Front Public Health. 2017;5:335.
5. Kiani S, Bayanzadeh M, Tavallaee M, Hogg RS. The Iranian population is graying: are we ready? Archives of Iranian medicine. 2010;13(4):333-9.
6. Doshmangir L, Khabiri R, Gordeev VS. Policies to address the impact of an ageing population in Iran. Lancet. 2023;401(10382):1078.
7. Bos D, Von Weizsacker RK. Economic consequences of an aging population. Eur Econ Rev. 1989;33(2-3):345-54.
8. Noto S. Perspectives on Aging and Quality of Life. Healthcare (Basel). 2023;11(15).
9. Viegas R, Alves da Costa F, Mendes R, Deidda M, McIntosh E, Sansano-Nadal O, et al. Relationship of the SITLESS intervention on medication use in community-dwelling older adults: an exploratory study. Front Public Health. 2023;11:1238842.

10. Salimi F, Garmaroudi G, Mustafa Hosseini S, Batebi A. Effect of Self-Care Educational Program to Improving Quality of Life among Elderly Referred to Health Centers in Zanjan. J Educ Community Health. 2015;2(2):28-37.
11. Abdi S, Spann A, Borilovic J, de Witte L, Hawley M. Understanding the care and support needs of older people: a scoping review and categorisation using the WHO international classification of functioning, disability and health framework (ICF). BMC Geriatr. 2019;19(1):195.
12. Ponti F, Santoro A, Mercatelli D, Gasperini C, Conte M, Martucci M, et al. Aging and Imaging Assessment of Body Composition: From Fat to Facts. Frontiers in endocrinology. 2019;10:861.
13. Ahmed AU. An overview of inflammation: mechanism and consequences. Frontiers in Biology. 2011;6(4):274-81.
14. Pettersson-Pablo P, Nilsson TK, Breimer LH, Hurtig-Wennlöf A. Body fat percentage is more strongly associated with biomarkers of low-grade inflammation than traditional cardiometabolic risk factors in healthy young adults – the Lifestyle, Biomarkers, and Atherosclerosis study. Scandinavian Journal of Clinical and Laboratory Investigation. 2019;79(3):182-7.
15. Wang H, Nie H, Bu G, Tong X, Bai X. Systemic immune-inflammation index (SII) and the risk of all-cause, cardiovascular, and cardio-cerebrovascular mortality in the general population. European Journal of Medical Research. 2023;28(1):575.
16. Deng X, Liu D, Li M, He J, Fu Y. Association between systemic immune-inflammation index and insulin resistance and mortality. Scientific Reports. 2024;14(1):2013.
17. Hu B, Yang XR, Xu Y, Sun YF, Sun C, Guo W, et al. Systemic immune-inflammation index predicts prognosis of patients after curative resection for hepatocellular carcinoma. Clin Cancer Res. 2014;20(23):6212-22.
18. Aminisani N, Azimi-Nezhad M, Shamshirgaran SM, Mirhafez SR, Borji A, Poustchi H, et al. Cohort Profile: The IRanian Longitudinal Study on Ageing (IRLSA): the first comprehensive study on ageing in Iran. International Journal of Epidemiology. 2022;51(4):e177-e88.
19. Keikavoosi-Arani L, Salehi L. Cultural adaptation and psychometric adequacy of the Persian version of the physical activity scale for the elderly (P-PASE). BMC Res Notes. 2019;12(1):555.
20. Qin Z, Li H, Wang L, Geng J, Yang Q, Su B, et al. Systemic Immune-Inflammation Index Is Associated With Increased Urinary Albumin Excretion: A Population-Based Study. Front Immunol. 2022;13:863640.
21. Funghetto SS, de Oliveira Silva A, de Sousa NMF, Stival MM, Tibana RA, Pereira LC, et al. Comparison of percentage body fat and body mass index for the prediction of inflammatory and atherogenic lipid risk profiles in elderly women. Clinical Interventions in Aging. 2015;10(null):247-53.
22. Singer K, Eng DS, Lumeng CN, Gebremariam A, J ML. The relationship between body fat mass percentiles and inflammation in children. Obesity (Silver Spring, Md). 2014;22(5):1332-6.
23. Eren C, Cecen S. The relationship between childhood obesity with inflammatory mediators. J Pak Med Assoc. 2020;70(10):1737-41.
24. Hestiantoro A, Kapnosa Hasani RD, Shadrina A, Situmorang H, Ilma N, Muharam R, et al. Body fat percentage is a better marker than body mass index for determining inflammation status in polycystic ovary syndrome. International journal of reproductive biomedicine. 2018;16(10):623-8.
25. Hildebrandt X, Ibrahim M, Peltzer N. Cell death and inflammation during obesity: "Know my methods, WAT(son)". Cell Death & Differentiation. 2023;30(2):279-92.
26. Ellulu MS, Patimah I, Khaza'ai H, Rahmat A, Abed Y. Obesity and inflammation: the linking mechanism and the complications. Archives of Medical Science. 2017;13(4):851-63.
27. Kirichenko TV, Markina YV, Bogatyreva AI, Tolstik TV, Varaeva YR, Starodubova AV. The Role of Adipokines in Inflammatory Mechanisms of Obesity. International Journal of Molecular Sciences. 2022;23(23):14982.
28. Ömür SE, Zorlu Ç, Yılmaz M. Comparison of the Relationship Between Inflammatory Markers and Atrial Fibrillation Burden. Anatolian journal of cardiology. 2023;27(8):486-93.
29. Cao Y, Li P, Zhang Y, Qiu M, Li J, Ma S, et al. Association of systemic immune inflammatory index with all-cause and cause-specific mortality in hypertensive individuals: Results from NHANES. Frontiers in Immunology. 2023;14.
30. Üstündağ Y. Relationship between CRP, systemic immune-inflammation index and routine hemogram related inflammatory markers in low grade inflammation. International Journal of Medical Biochemistry. 2018.
31. Cao Y, Li P, Zhang Y, Qiu M, Li J, Ma S, et al. Association of systemic immune inflammatory index with all-cause and cause-specific mortality in hypertensive individuals: Results from NHANES. Front Immunol. 2023;14:1087345.
32. Yang C, Yang Q, Xie Z, Peng X, Liu H, Xie C. Association of systemic immune-inflammation-index with all-cause and cause-specific mortality among type 2 diabetes: a cohort study base on population. Endocrine. 2023.

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The Effect of Detraining Followed by Endurance Training and *Salvia Officinalis* supplementation on Psychological Indicators and Physiological Performance in Rats

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ABSTRACT

Introduction: This study examined the effects of two and four weeks of detraining, followed by four weeks of endurance training (ET) and Salvia *officinalis* (S) supplementation, on anxiety-like behaviors, aerobic capacity (AC), depression, and pain threshold (PT) in rats.

Methods: In this experimental study, 40 adult female rats were randomly assigned to five groups: (1) Control, (2) Sham, (3) S, (4) ET, and (5) ETS. Rats in groups 4 and 5 underwent treadmill training for four weeks (five sessions per week, 60 minutes per session). Groups 3 and 5 received *Salvia officinalis* extract at 100 mg/kg/day. Data were analyzed using analysis of covariance (ANCOVA) and one-way analysis of variance (ANOVA) with repeated measures.

Results: The percentage of time spent in the open arms (OA) of the elevated plus maze in the S, ET, and ETS groups was significantly higher than in the control group during both two and four weeks of detraining. However, after four weeks of detraining, this percentage was significantly lower compared to two weeks of detraining ($P \le 0.05$). No significant differences were observed in AC, depression levels, or PT between the two- and four-week detraining periods following four weeks of ET+S ($P \le 0.05$).

Conclusion: The findings suggest that four weeks of *Salvia officinalis* supplementation and endurance training can reduce anxiety-like behaviors. However, two and four weeks of detraining may lead to an increase in anxiety-like behaviors.

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Introduction

Activity and movement are fundamental characteristics of most living organisms, including humans. However, with increasing urbanization, the type and amount of physical activity have changed, leading to a decreased necessity for movement in daily life. Consequently, to compensate for this reduced physical activity and maintain overall well-being, individuals should engage in structured exercise programs to reap its benefits (1).

The rise in body weight and obesity is closely linked to an increase in blood lipid levels (2). Data indicate that physical inactivity is the fourth leading risk factor for global mortality, accounting for six percent of deaths worldwide. Furthermore, evidence suggests that inactivity has severe adverse effects on both physical and mental health, contributing to the development of cardiovascular diseases, diabetes, cancer, and mental disorders (3).

Evidence suggests that a decline in physical activity is primarily associated with increased body fat and obesity, reduced physical performance, and decreased overall fitness. Additionally, insufficient physical activity can disrupt the body's homeostasis, potentially leading to both physical and psychological disorders (4). Furthermore, research indicates a significant relationship between reduced physical activity and the occurrence of anxiety disorders, psychological distress, and appetite dysregulation (5).

On the other hand, given the critical role of regular physical activity in public health, evidence suggests that structured exercise enhances physical performance, boosts self-confidence, improves overall fitness, and reduces mental disorders. Research indicates that

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exercise improves lipid metabolism and promotes mitochondrial biogenesis (6), as well as increases neurotrophins, serotonin, and dopamine, thereby enhancing both physical and psychological well-being (7). Furthermore, studies have demonstrated that sports activity is associated with improved myokine levels, increased ephedrine production, enhanced pain tolerance, and better physical fitness in animal models with psychological disorders (8). Additionally, previous research has shown that exercise, even under varying temperature conditions, can improve liver enzyme function (9). Moreover, improvements in memory and reductions in depression have been linked to diabetes in animal models (10). Regular physical activity has also been reported to prevent mental disorders such as depression and anxiety, particularly in the elderly population (11). However, evidence suggests that detraining reverses the benefits of exercise, leading to a return to baseline physiological states. In this context, Dabidi Roshan et al. observed a non-significant increase in C-reactive protein levels after four weeks of detraining in the training group, while a significant increase was reported in the control group (12). Similarly, Coyle et al. (1983) reported a 7% decrease in aerobic capacity following two weeks of detraining (13). Costill et al. (1985) extensively studied detraining and reported an 8–10% reduction in anaerobic threshold after one week of detraining (14), a 25% decline in buffering capacity after three weeks (15), and a 39% decrease in muscle glycogen levels following four weeks of detraining (14).

On the other hand, it is widely believed that a diet rich in anti-aging compounds plays a crucial role in reducing the risk of obesity-related and metabolic disorders (7). In addition to pharmaceutical interventions, medicinal plants have been explored—both traditionally and experimentally—for their potential in treating cognitive disorders. Among these, *Salvia officinalis* is particularly noteworthy (16). *Salvia officinalis* is one of the largest genera in the mint family, comprising over 900 species, many of which grow naturally as weeds in parks and gardens (17). Moreover, various studies have examined the effects of exercise and herbal medicines on obesity and its associated psychological and physiological consequences, including anxiety, aerobic capacity, depression, and pain tolerance. For instance, one study reported that swimming training combined with fenugreek supplementation improved lipid profiles and glycemic indices in diabetic rats (6). Similarly, swimming training alongside cinnamon extract enhanced spatial memory in diabetic rats (10). Additionally, endurance training combined with beetroot extract was found to reduce anxiety and depression while increasing serotonin and dopamine expression in diabetic rats (7). Despite existing research, the long-term effects of exercise and medicinal plants on physiological and psychological health remain insufficiently explored in both applied and fundamental studies. Understanding the sustained impact of these interventions could provide valuable insights for optimizing physical and mental health. Given the limited information on the effects of detraining (two and four weeks) on health indicators, the present study aims to investigate the impact of two and four weeks of detraining following four weeks of endurance training and *Salvia officinalis* supplementation on anxiety, body mass index, aerobic capacity, depression, and pain threshold in rats.

Materials and Methods

In this experimental study, 40 female Wistar rats (average weight: 150–200 grams; average age: 8 weeks) were obtained from the Laboratory Animal Reproduction and Breeding Center at Islamic Azad University, Marvdasht Branch. The animals were housed for seven days in the sports physiology laboratory of the same institution to acclimate to the new environment. Throughout the study, the rats were maintained under standard laboratory conditions, including polycarbonate cages with autoclave capability, an optimal temperature of 20–24°C, relative humidity of 55–65%, and a 12-hour light-dark cycle. All procedures complied with ethical guidelines for laboratory animal research under the Helsinki Convention. Following the adaptation phase, the rats were randomly divided into six experimental groups (n = 8 per group): Control (C), Endurance training (ET), Salvia officinalis consumption (S), Sham (Sh), and Endurance training + Salvia officinalis (ETS).

Endurance Training Protocol

Initially, to familiarize the animals with the endurance training protocol, they were placed on a treadmill and ran 8 meters per minute with a zero-degree incline for 10 minutes. This warm-up intensity was chosen as it did not affect the study variables. A mild electric stimulus was installed at the end of the treadmill to encourage continuous movement. To minimize potential stress or injury from the stimulus, the animals were conditioned beforehand using gentle tapping on the treadmill, soft auditory cues, or light tail touches. The endurance training protocol lasted four weeks, consisting of five weekly sessions of progressively increasing treadmill running, without incline (0% incline), at speeds of 18–22 meters per minute for 60 minutes per session. The intensity was adjusted to correspond to 70% of the rats' maximum oxygen consumption (VO_2 max) in the first week, 75% in the second week, and 80% in the third week. Each training session began with a warm-up phase, where the animals ran for 10 minutes at 8 meters per minute before transitioning into the primary training protocol. Upon completing the training session, a cool-down phase was implemented by gradually reducing the treadmill speed until it reached zero, lasting approximately five to seven minutes (18).

Preparation of Salvia officinalis Extract

The Salvia officinalis plant extract was prepared using the soaking method. To achieve this, 50–100 grams of dried Salvia officinalis was first ground and passed through a sieve with a defined mesh size. Depending on the type of extract, distilled water or 80% ethanol was added to moisten the powdered plant material. After approximately one minute, an additional volume of the same solvent was added until the powder was fully submerged, forming an 8–10 cm liquid layer above the plant material. The mixture was then stored in a dark environment for 30 hours before being placed on a shaker for 30 minutes to enhance extraction. Following this step, the solution was filtered using filter paper, separating the extract from the plant residue. The remaining plant material was further extracted by adding fresh solvent, and this process was repeated two to three times. Finally, the filtered extracts from each step were combined and concentrated using a vacuum distillation method with a rotary evaporator. The rats in Groups 3 and 5 received 100 mg/kg of Salvia officinalis extract via intraperitoneal injection.

Evaluation of Anxiety-Like Behaviors

The elevated plus-maze (EPM) was used to assess anxiety-like behaviors in rats. This test is based on the model first introduced by Pellow et al. The apparatus is constructed of wood and consists of four arms arranged in a plus-shaped configuration. Two opposing arms are open, while the other two are enclosed. The dimensions of both the open and closed arms are 10 × 50 cm, with the enclosed arms featuring 40 cm high walls at the sides and ends. These arms extend from a central platform measuring 10 × 10 cm. The maze was elevated 50 cm above the ground using a supporting base. At the beginning of the test, each rat was placed in the central area, facing an open arm. Over five minutes, the animal was allowed to explore freely, and the following parameters were recorded: the number of entries into the open arms, the number of entries into the closed arms, the total time spent in the open arms, and the total time spent in the closed arms. An arm entry was defined as placing all four paws within the respective arm. The duration spent in each arm was calculated based on the same criterion (5).

Assessment of Depression

The forced swimming test (FST) was used to assess depressive-like behavior in rats. For this purpose, a glass container with a height of 25 cm and a diameter of 12 cm was filled with water to a depth of 8 cm, maintained at a temperature of 25°C. Each rat was gently placed in the water from a height of 20 cm. Conventionally, the cessation of limb movements was considered immobility. The total duration of the test was six minutes. The first two minutes were designated an adaptation period, during which immobility time was not recorded. After this initial phase, the rats' movements were observed, and periods during which the animal exhibited no movement or response and remained floating passively were recorded as immobility time using a stopwatch. Following the test, the animals were dried in a chamber maintained at 30 ± 1°C (5).

Assessment of Aerobic Capacity

A progressive treadmill running protocol was implemented to determine maximum oxygen consumption (VO_2 max) or maximum running speed in rats. The rats ran for five minutes at an 8 m/min speed. In the next stage, they ran for eight minutes at speeds ranging from 10 to 15 m/min. In the third stage, they ran for five minutes at 20 m/min, followed by a fourth stage in which they ran for 10 minutes at 25 m/min and then continued for 20 minutes at 30 m/min. In the final stage, the running speed was increased to 35 m/min and maintained until the rats made contact with the end of the treadmill three times within one minute, considered the point of exhaustion (VO_2 max). Based on the obtained results, the training intensity was subsequently determined at 55–60% of the maximum running speed (2).

Assessment of Pain Tolerance Threshold

The Hot-Plate test was used to assess pain tolerance. This test employs a heated plate, which is warmed using an electric current. In this study, each rat was individually placed on the plate, maintained at 55°C, and the start time (time zero) was recorded. The latency to the first nociceptive response, defined as either paw licking or a distinct alteration in gait, was measured as the pain tolerance threshold. The endurance threshold was recorded when the animal exhibited jumping behavior. To prevent tissue damage, the maximum duration of the test was limited to 60 seconds per rat (8).

Data Analysis Method

or statistical analysis, the Kolmogorov-Smirnov test was used to assess the normality of data distribution. A one-way analysis of variance (ANOVA) with repeated measures and analysis of covariance (ANCOVA) was performed, followed by Bonferroni post hoc tests for pairwise comparisons. Data analysis was conducted using SPSS version 21, with a significance level set at $p < 0.05$.

Results

Table 1 presents the mean and standard deviation of the study variables. The results of the analysis of covariance (ANCOVA) indicated a significant difference in the percentage of time spent in the open arms (OA) among the experimental groups (P = 0.001). Furthermore, Bonferroni's post hoc test revealed no significant difference between the control and sham groups (P = 0.27). However, the percentage of time spent in the OA was significantly higher in the Salvia officinalis (P = 0.001), endurance training (P = 0.001), and endurance training combined with Salvia officinalis (P = 0.001) groups compared to the control group after two weeks of detraining.

A significant difference was observed in the percentage of time spent in the open arms (OA) among the experimental groups (P = 0.001). Additionally, the percentage of time spent in the OA was significantly higher in the Salvia officinalis (P = 0.001) and endurance training combined with Salvia officinalis (P = 0.003) groups compared to the control group. The time factor significantly affected changes in the percentage of time spent in the OA (P = 0.005). However, the interaction effect between group and time was insignificant (P = 0.40). In other words, the percentage of time spent in the OA after four weeks of detraining was significantly lower than after two weeks of detraining (P = 0.005) (Figure 1).

There was no significant difference in aerobic power levels of rats after two weeks (P = 0.32) and four weeks (P = 0.51) of detraining, following four weeks of endurance training and Salvia officinalis consumption. Additionally, the results indicated that the time factor (P = 0.051) and the interaction effect between time and group (P = 0.43) had no significant impact on aerobic power during the two- and four-week detraining period (Figure 2).

Similarly, there was no significant difference in depression levels of rats after two weeks (P = 0.42) and four weeks (P = 0.31) of detraining, following four weeks of endurance training and Salvia officinalis extract consumption. Furthermore, the results showed that the time factor (P = 0.99) and the interaction between time and group (P = 0.15) had no significant effect on depression levels during the two- and four-week detraining period.

There was no significant difference in the depression levels of rats after two weeks (P = 0.44) and four weeks (P = 0.07) of detraining, following four weeks of endurance training and Salvia officinalis extract consumption. Additionally, the time factor (P = 0.47) did not significantly affect the pain tolerance threshold during the two- and four-week detraining period. However, the interaction between time and group was significant (P = 0.008), indicating that changes in pain tolerance threshold varied depending on the experimental conditions.

Table 1. Mean and standard deviation of research variables in five groups after two and four weeks of detraining following four weeks of endurance training and consumption Salvia officinalis

Factor	Group	Four weeks of detraining	Four weeks of detraining	After training
Anxiety-like behaviors (percentage of time spent in the open arm)	C	10.43 ± 6.28	10.43 ± 6.53	12.78 ± 9.84
	Sh	17.29 ± 13.30	19.29 ± 13.43	26.67 ± 13.26
	S	48.66 ± 6.92	53.14 ± 3.14	47.46 ± 24.87
	ET	48.66 ± 6.92	55.22 ± 3.00	37.04 ± 12.04
	ETS	47.42 ± 9.94	54.96 ± 3.57	63.10 ± 21.66
Aerobic power (m/min)	C	21.61 ± 6.23	21.42 ± 3.22	20.63 ± 3.01
	Sh	20.82 ± 1.73	20.61 ± 0.68	10.43 ± 6.28
	S	20.03 ± 4.91	22.70 ± 2.44	23.51 ± 2.04
	ET	25.33 ± 2.02	27.16 ± 2.52	30.65 ± 4.38
	ETS	25.13 ± 0.83	24.92 ± 1.70	28.75 ± 1.48
Depression (immobility in seconds)	C	50.54 ± 42.90	43.19 ± 18.41	47.81 ± 25.43
	Sh	37.51 ± 17.21	36.62 ± 24.91	34.00 ± 26.30
	S	7.18 ± 3.81	6.50 ± 3.49	2.62 ± 4.26
	ET	24.21 ± 21.25	8.63 ± 12.56	17.00 ± 13.40
	ETS	10.10 ± 5.54	17.29 ± 15.79	14.75 ± 8.28
Pain tolerance threshold (seconds)	C	11.65 ± 4.61	7.49 ± 4.62	9.58 ± 3.03
	Sh	9.62 ± 4.46	4.75 ± 1.54	5.42 ± 1.60
	S	5.65 ± 2.92	11.30 ± 4.21	18.02 ± 9.23
	ET	9.77 ± 3.72	9.64 ± 5.84	11.40 ± 10.17
	ETS	10.51 ± 5.28	10.97 ± 4.82	20.42 ± 12.17

*** (P≤0.001) Significant increase compared to control group

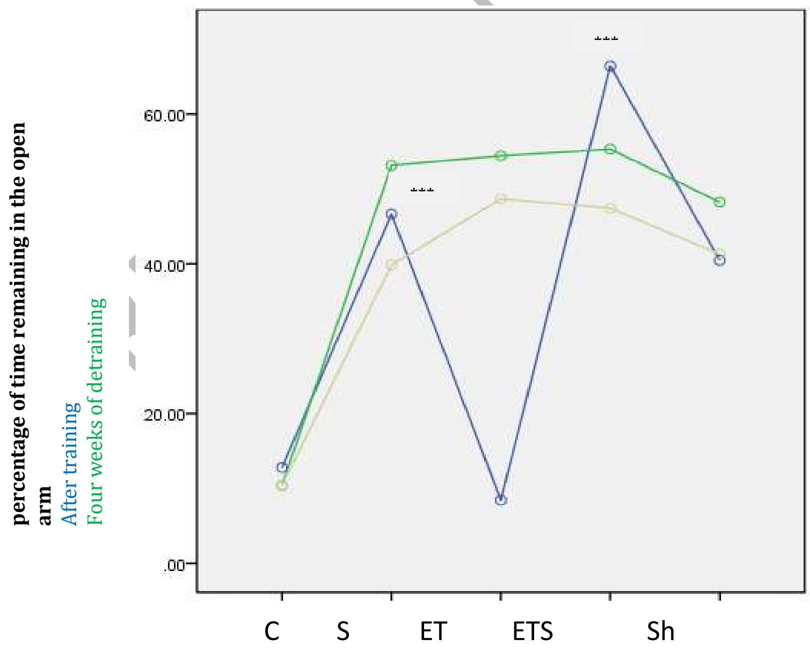

Figure 1. The percentage of time remaining in the open arm in the five research groups after end of training, two and four weeks of detraining followed by four weeks of endurance training and consumption of Salvia officinalis extract

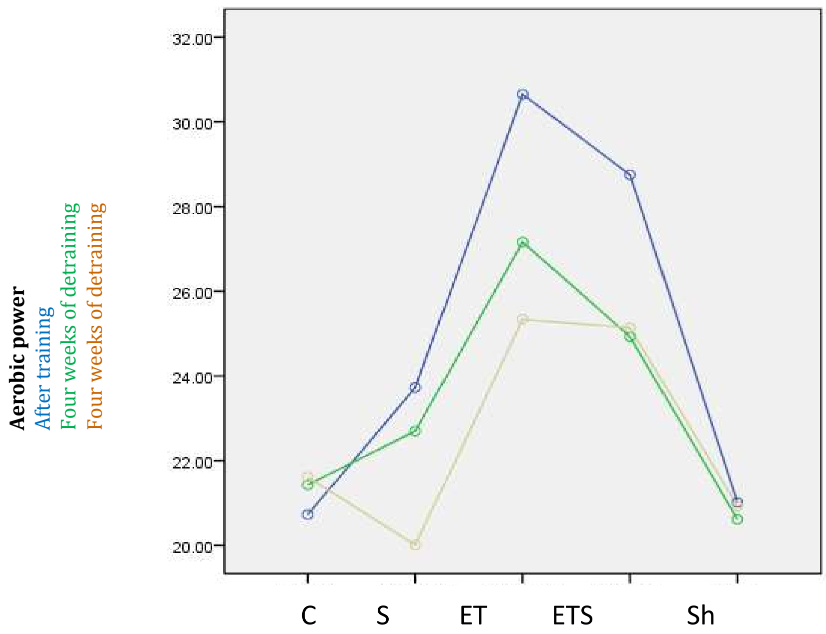

Figure 2. Aerobic power in the five research groups after end of training, two and four weeks of detraining followed by four weeks of endurance training and consumption of Salvia officinalis extract

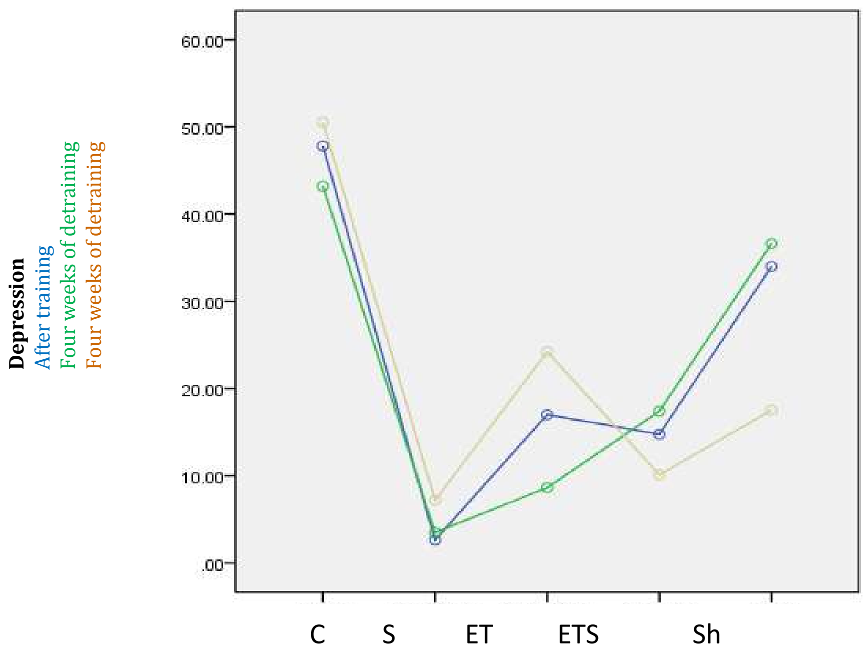

Figure 3. Depression (time of inactivity) in the five research groups after end of training, two and four weeks of detraining followed by four weeks of endurance training and consumption of Salvia officinalis extract

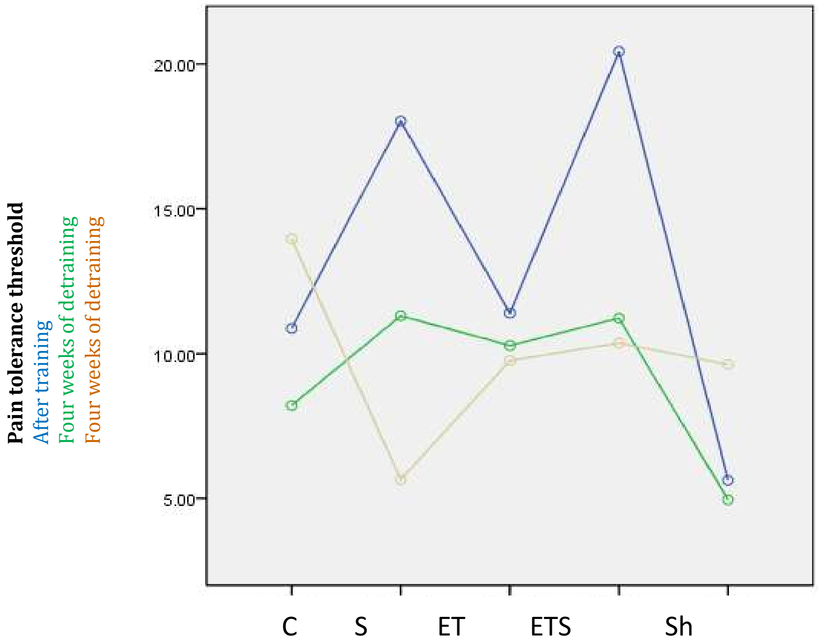

Figure 4. Pain tolerance threshold in the five research groups after end of training, two and four weeks of detraining followed by four weeks of endurance training and consumption of Salvia officinalis extract

Discussion

This study aimed to investigate the effects of two and four weeks of detraining, following four weeks of endurance training and Salvia officinalis consumption, on anxiety-like behaviors, aerobic power, depression, and pain threshold in rats. The results demonstrated that the percentage of time spent in the open arms (OA) was significantly higher in the Salvia officinalis extract, endurance training, and endurance training combined with Salvia officinalis groups after two and four weeks of detraining compared to the control group. Additionally, the percentage of time spent in the OA after four weeks of detraining was significantly lower than after two weeks of detraining.

There were no significant differences in aerobic power, depression levels, and pain tolerance threshold between the two- and four-week detraining periods following four weeks of endurance training and Salvia officinalis consumption. Anxiety is a natural response to psychological stress and encompasses behavioral, physiological, and cognitive components. It is a subjective experience that lacks a tangible external manifestation; however, it plays a crucial role in the interpretation of observable phenomena (19). Prolonged or excessive anxiety is commonly associated with physiological responses, including increased metabolic activity, suppression of immune function, and heightened cardiovascular workload. Moreover, a significant relationship exists between anxiety and mortality (19–22).

Exercise reduces the sympathetic nervous system's activity while enhancing the parasympathetic system's function. As a result of these physiological and neural adaptations, heart rate and blood pressure decrease, leading to improved alertness, enhanced mood, and increased energy levels, collectively contributing to better daily functioning and overall well-being. This physiological regulation plays a crucial role in promoting mental health (7). The observed discrepancies between the present findings and previous studies may be attributed to differences in the study population, exercise duration, and the specific types of physical activity employed. Aerobic exercise, as a non-pharmacological intervention, is efficacious in improving sleep quality and duration (23,24). Therefore, it is recommended that aerobic exercise be incorporated as an intervention to reduce anxiety and enhance sleep quality in rats.

Consistent with the present study's findings, Motaghi et al. (2016) reported the anxiolytic and sleep-inducing effects of Salvia officinalis. Additionally, Sharifipour et al. demonstrated that Salvia officinalis significantly reduced anxiety levels in women during childbirth (25). In the elevated plus-maze (EPM) test, exploratory behavior drives rats to enter the open arms (OA). In contrast, anxiety-related avoidance of open, brightly lit, and elevated spaces encourages them to spend more time in closed arms (CA). Consequently, higher anxiety levels lead to a greater preference for the CA. Previous studies have further confirmed the anxiolytic effects of Salvia officinalis (4). The bioactive compounds in Salvia officinalis interact with γ-aminobutyric acid (GABA) receptors, functioning as negative, positive, or neutral allosteric modulators, and can influence the effects of other allosteric agonists (26).

A study on the flavones present in the methanolic extract of Salvia officinalis identified apigenin, hispidulin, sircimaritin, and the diterpene 7-methoxyrosmanol as compounds that bind to benzodiazepine receptors in the human brain. In the present study, the alcoholic extract of Salvia officinalis exhibited anxiolytic effects, which may be attributed to the biphasic action of flavonoids. Specifically, at low doses, flavonoids enhance GABAergic function, whereas at higher doses, they exhibit inhibitory effects (27,28). Other phytochemicals soluble in water and alcohol may reduce sleep onset latency by acting on GABAA receptors or through alternative regulatory mechanisms. In this regard, research has identified silyol, a flavonoid in Salvia guaranitica, as one of its key active compounds with sleep-inducing properties (29).

Furthermore, the interactive effect of exercise and Salvia officinalis on anxiety reduction after two and four weeks of detraining suggests that endurance training induces physiological adaptations that contribute to improved mental and physical well-being. These adaptations include increased muscle mass and plasma volume, enhanced pulmonary ventilation and blood circulation, greater cardiac reserve, elevated concentrations of muscle oxidative enzymes, and stimulation of hematopoietic factors. Collectively, these changes help mitigate mental and physical fatigue while promoting a greater sense of control, independence, and self-confidence (7).

The methanolic extract of Salvia officinalis leaves has been found to contain three flavones and two types of diterpenes that function as benzodiazepine receptor activators (30). Flavonoids, plant-derived compounds containing a phenyl-benzopyrone nucleus, can interact with ionotropic GABA receptors through various mechanisms. These compounds are believed to influence different modulatory sites on GABA receptors, thereby contributing to their anxiolytic effects (31). Regarding the lack of a significant effect of exercise and Salvia officinalis supplementation, the absence of differences observed after two and four weeks of detraining may be attributed to the principle of training reversibility. Detraining, a potential increase in caloric intake, and an imbalance between energy expenditure and consumption may lead to weight gain. As body weight increases, more significant energy expenditure is required for movement, imposing additional strain on the cardiopulmonary system (5). One of the limitations of the present study was the inability to precisely monitor the rats' food intake and spontaneous physical activity during non-training periods. Future research should aim to quantify daily food consumption and physical activity levels, alongside investigating the effects of exercise and inactivity to better understand their interactions.

Conclusion

Regarding the effects of Salvia officinalis (S) and endurance training (ETS) on depression, aerobic capacity, and pain tolerance threshold, the findings suggest that these two interventions do not interact. Although four weeks of Salvia officinalis supplementation and endurance training can effectively reduce anxiety-like behaviors, the subsequent two- and four-week detraining periods appear to lead to an increase in anxiety-like behaviors.

References

1. Yao CA, Rhodes RE. Parental correlates in child and adolescent physical activity: a meta-analysis. Int J Behav Nutr Phys Act. 2015;12:1–38.

2. Hosseini SA, Norouzi S, Rafiee N, Farzanegi P, Salehi O, Farkhaie F. Interactive Effects of Endurance Training and Crocin on Aerobic Capacity, Dietary Intake and Weight of High-Fat Diet-Induced Type 2 Diabetic Rats. J Nutr Sci Diet. 2018;4(3).

3. Ahmadi DK. The Comparison efficiency of two kind of morning exercise training on Lipid Profile of Teenagers in 2012. 2013; 18-24.
4. Williams CL, Hayman LL, Daniels SR, Robinson TN, Steinberger J, Paridon S, et al. Cardiovascular health in childhood: a statement for health professionals from the Committee on Atherosclerosis, Hypertension, and Obesity in the Young (AHOY) of the Council on Cardiovascular Disease in the Young, American Heart Association. Circulation. 2002;106(1):143–60.
5. Hosseini SA, Salehi O, Keikhosravi F, Hassanpour G, Ardakani HD, Farkhaie F, et al. Mental health benefits of exercise and genistein in elderly rats. Exp Aging Res. 2022;48(1):42–57.
6. Hosseini SA, Hamzavi K, Safarzadeh H, Salehi O. Interactive effect of swimming training and fenugreek (Trigonella foenum graecum L.) extract on glycemic indices and lipid profile in diabetic rats. Arch Physiol Biochem. 2023;129(2):349–53.
7. Ghanbari P, Khajehzadeh S, Sayyed A, Raeisi D, Salehi O. The effect of high intensity interval training with beetroot (Beta vulgaris) juice supplementation on serotonin and dopamine receptors expression, anxiety and depression in middle-aged diabetic rats. Avicenna J Phytomedicine. 2022; 12(6):627-37.
8. Hosseini SA, Salehi OR, Farzanegi P, Farkhaie F, Darvishpour AR, Roozegar S. Interactive Effects of Endurance Training and Royal Jelly Consumption on Motor Balance and Pain Threshold in Animal Model of the Alzheimer Disease. Arch Neurosci. 7(2):e91857.
9. Arammi S, Sahragard M, Seyed A, Salehi O, Hosseini SA, Mosallanezhad Z. The effects of swimming training at different temperatures along with cinnamon supplementation on liver enzymes and thyroid hormones in diabetic rats. Avicenna J Phytomedicine. 2024; 14(1):126-37.
10. Salehi O, Sheikholeslami-Vatani D, Negarandeh Z, Yarahmadi J. The effect of swimming training at different temperatures with cinnamon consumption on avoidance memory, spatial memory and aerobic power in streptozotocin-induced diabetic rats. 2020; 1(1).
11. Schuch FB, Vancampfort D. Physical activity, exercise, and mental disorders: it is time to move on. Trends psychiatry Psychother. 2021;43(3):177–84.
12. Dabidi roshan, V., Gaeeni, A. A., Ravasi, A. A. The Effect of 4 Weeks of Detraining after 12 Weeks of Continuous and Intermittent Aerobic Training on High Sensitive C-Reactive Protein in Rats. 2007; 1-14.
13. Coyle EF, Martin WH, Holloszy JO. Cardiovascular and metabolic rates of detraining. Med Sci Sport Exerc. 1983;15(2):158.
14. Costill DL, Gollnick PD, Jansson ED, Saltin B, Stein EM. Glycogen depletion pattern in human muscle fibres during distance running. Acta Physiol Scand. 1973;89(3):374–83.
15. Costill DL, Fink WJ, Hargreaves M, King DS, Thomas R, Fielding R. Metabolic characteristics of skeletal muscle during detraining from competitive swimming. Med Sci Sports Exerc. 1985;17(3):339–43.
16. Hosseinzadeh H, Ziaei T. Effects of Crocus sativus stigma extract and its constituents, crocin and safranal, on intact memory and scopolamine-induced learning deficits in rats performing the Morris water maze task. J Med Plants. 2006;5(19):40–50.
17. Alijanpour B, Sheidai M. Numerical taxonomy study of Salvia L.(Lamiacae) species in southern slope of Central Alborz, Iran. Nov Biol Reper. 2017;4(2):107–15.
18. Salehi OR, Hosseini SA, Farkhaie F, Farzanegi P, Zar A. The Effect of Moderate Intensity Endurance Training with Genistein on Brain-Derived Neurotrophic Factor and Tumor Necrosis Factor-Î±in Diabetic Rats. J Nutr Fasting Heal. 2019;7(1):44–51.
19. Shahinfar J, Zeraati H, Nasimi F, Shojaei S. Effect of medicinal plants on anxiety, a review article. 2017.
20. Hosseini MA, Shirzad F, Ahmadzad-Asl M, Hadi F. Depression, Anxiety, Stress, and Resiliency in Iranian Families with Autistic Children. J Adv Med Biomed Res. 2022;30(139):123–8.
21. Bakhtiari-Dovvombaygi H, Askari M, Rahimkhani M, Abdollahi M, Baladastian M, Alipour A, et al. Prevalence of death anxiety and its related factors in the population of eastern Iran: a cross-sectional study in the era of COVID-19. Front Public Heal. 2024;12:1427995.
22. Jangi Jahantigh L, Latifi Z, Soltanizadeh M. Effect of self-healing training on death anxiety and sleep quality of older women living in nursing homes. Iran J Ageing. 2022;17(3):380–97.
23. Li L, Wang C, Wang D, Li H, Zhang S, He Y, et al. Optimal exercise dose and type for improving sleep quality: a systematic review and network meta-analysis of RCTs. Front Psychol. 2024;15:1466277.
24. Patel A, Cheung J. The Effect of Exercise on Daytime Sleepiness in Healthy Individuals. Sleep Med. 2024.
25. Motaghi S, Teimouri M. Investigation of anxiolytic and hypnotic effects of aqueous and hydroalcoholic extracts of salvia officinalis in adult mice. Iran J Physiol Pharmacol [Internet]. 2018;2(3). Available from: http://ijpp.phypha.ir/article-1-303-en.html
26. Hanrahan JR, Chebib M, Johnston GAR. Interactions of flavonoids with ionotropic GABA receptors. Adv Pharmacol. 2015;72:189–200.
27. Johnston GAR. Flavonoid nutraceuticals and ionotropic receptors for the inhibitory neurotransmitter GABA. Neurochem Int. 2015;89:120–5.
28. Sadeghi A H, Bakhshi M, Behboodi Z, Goodarzi S, Haghani H. Effect of sage extract on hot flashes in postmenopausal women. Complement Med J. 2013;2(4):324–35.
29. Viola H, Wasowski C, Marder M, Wolfman C, Paladini AC, Medina JH. Sedative and hypnotic properties of Salvia guaranitica St. Hil. and of its active principle, Cirsiliol. Phytomedicine. 1997;4(1):47–52.
30. Kavvadias D, Monschein V, Sand P, Riederer P,

Schreier P. Constituents of sage (Salvia officinalis) with in vitro affinity to human brain benzodiazepine receptor. Planta Med. 2003;69(02):113–7.
31. Karami M, Hossini E, Shahbi Majd N, Ebrahimzadeh MA, Alemy S. Salvia limbata: Botanical, Chemical, Pharmacological and Therapeutic Effecte. Clin Excell [Internet]. 2015;3(2).

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The Effects of Aerobic Exercise and Crocin on Metabolic Indices, Oxidative Stress, and Blood Pressure in Overweight/Obese Women

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ABSTRACT

Introduction: Excess body weight is associated with increased oxidative stress, altered lipid profiles, and elevated blood pressure levels. This study aimed to investigate the effects of aerobic exercise combined with crocin supplementation on various indicators of metabolic syndrome, oxidative stress, and blood pressure in overweight or obese women.

Methods: Forty women, aged 30 to 40 years, with a BMI between 30 and 40 kg/m^2, were randomly assigned to one of four groups (n=10 per group): aerobic exercise, crocin supplementation, a combination of exercise and supplementation, and a placebo control group. Blood pressure measurements were taken in a fasted state 24 hours before the start of the intervention, followed by the collection of 5 mL blood samples from the brachial vein. Baseline assessments included metabolic syndrome markers and oxidative stress indicators. The exercise groups participated in an 8-week aerobic training program consisting of three 40-50 minute weekly sessions, performed at 65-80% of their maximum heart rate. The supplementation groups received 30 mg of crocin daily. Post-intervention measurements were taken 48 hours after the final exercise session. Statistical analyses were conducted using ANCOVA and ANOVA with Bonferroni post-hoc tests. Data were analyzed with SPSS version 26, and statistical significance was set at $p<0.05$.

Results: Significant differences were observed among the four groups for weight, body fat percentage, BMI, waist-to-hip ratio (WHR), glucose, insulin, triglycerides (TG), superoxide dismutase (SOD), malondialdehyde (MDA), glutathione peroxidase (GPX), systolic blood pressure (SBP), and diastolic blood pressure (DBP) (p=0.0001), as well as for high-density lipoprotein (HDL) (p=0.003), low-density lipoprotein (LDL) (p=0.023), and insulin resistance (IR) (p=0.049). Bonferroni's posthoc analysis revealed significant differences in weight, body fat percentage, BMI, WHR, glucose, insulin, MDA, GPX, and DBP between the control and all three intervention groups. Notable differences in SOD and SBP were observed not only between the control and intervention groups but also when comparing the exercise group with the exercise + supplementation group, as well as between the supplementation group and the exercise + supplementation group.

Conclusion: Aerobic exercise and crocin supplementation significantly improved metabolic syndrome indices, oxidative stress markers, and blood pressure in overweight/obese women. The combined approach of exercise and supplementation yielded enhanced benefits.

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Introduction

Obesity and overweight are associated with increased oxidative stress, lipid profile abnormalities, and hypertension. In the modern era, obesity and overweight are major global health concerns, with their prevalence rising significantly (1, 2). This growing trend has negative health implications and is linked to various diseases, including type 2 diabetes, dyslipidemia, cardiovascular diseases, hypertension, and cancer. These conditions reduce life expectancy and premature mortality, leading to substantial healthcare costs (1). Studies have shown that cholesterol reverse transport is impaired, and its clearance is reduced in obese mice, which may help explain some of the mechanisms underlying obesity-induced hypertension (3). Obesity increases systemic vascular resistance and causes triglyceride accumulation in blood vessels, which

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can lead to fibrosis of the vascular walls and an elevated risk of hypertension (4). Furthermore, it has been reported that the endogenous antioxidant system, including glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT), is primarily suppressed in obese individuals, making them more susceptible to disease (5). As a result, a heightened state of oxidative stress and inflammation, coupled with a compromised antioxidant defense system, is commonly observed (6). Although the complications of obesity may vary among individuals, the consequences are broadly similar across the population, particularly the presence of oxidative stress and inflammation in all obese patients (7). The Eighth Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC8) and the American College of Sports Medicine (ACSM) recommend aerobic exercise as a primary treatment for individuals with hypertension. Park et al. (2022) reported that moderate-intensity aerobic exercise may help reduce inflammation and oxidative stress independent of fat reduction, potentially lowering the risk of obesity-related disorders in middle-aged obese women (8). Krause et al. (2014) reported that 16 weeks of low- and moderate-intensity aerobic exercise, although not significantly affecting body composition, aerobic capacity, or inflammatory markers in obese individuals, improved oxidative stress by increasing muscle nNOS expression and tNOx levels in skeletal muscles (9). Another non-pharmaceutical, natural weight management approach is herbal supplements (10). Saffron (Crocus sativus L.), a perennial plant from the iris family, is rich in a compound called picrocrocin, with crocin being recognized as its main bioactive component. The beneficial properties of saffron, especially compared to its other compounds, are primarily attributed to crocin (11). Crocin has demonstrated various biological functions, including antioxidant, anti-inflammatory, and anti-obesity effects (12, 13). Studies have shown that crocin reduces high-fat diet-induced inflammation in brown adipose tissue through SIRT1 activation, which may help improve brown adipose tissue function in obesity (14). Saffron extract appears to reduce inflammation caused by a high-calorie diet (HFD) and the expression of microRNAs that negatively regulate SIRT1. It also prevents the nuclear translocation of NFκB in the brown adipose tissue of HFD-induced obese mice and inhibits the NFκB signaling pathway by modulating SIRT1 activity (15). Several meta-analyses have examined the effects of saffron extract supplementation on lipids, blood pressure, glucose, and insulin, but the results have been conflicting. For example, Sahebkar et al. (2017) reported that quercetin supplementation does not have a significant favorable effect on plasma lipids (16). In contrast, Huang et al. (2020) reported that after 8 weeks of quercetin supplementation, HDL-C and triglyceride (TG) concentrations improved significantly (17). Additionally, it has been reported that saffron extract supplementation significantly reduces blood pressure by modifying the renin-angiotensin and autonomic nervous systems, sensitizing the baroreflex's parasympathetic component, and reducing vascular resistance and compliance (18). However, a meta-analysis showed that quercetin supplementation has a relatively small effect on fasting plasma glucose, HOMA-IR, or hemoglobin A1c (19). Another study indicates that saffron extract increases cytosolic chloride concentration by activating the NKCC1 membrane transport protein, subsequently leading to an anti-hypertensive effect (20). Given that both aerobic exercise and crocin supplementation have independently shown a positive impact on metabolic syndrome indices, oxidative stress, and blood pressure in obesity, the present study aims to investigate whether the combination of crocin supplementation and aerobic exercise, compared to each intervention alone, has a more pronounced effect on overweight/obese individuals.

Methodology

Three experimental groups and one control group were included in this study, which employed a quasi-experimental design with a pretest-posttest format. The statistical population consisted of overweight/obese women (weight: 76.07 ± 7.11 kg, height: 162.4 ± 6.5 cm, age: 33.95 ± 4.75 years) from Ilam City, all of whom were either non-athletes or had not engaged in regular physical activity for at least six months before the study. Initially, a public summons was used to recruit interested participants, who voluntarily completed questionnaires detailing their medical history, personal characteristics, and level of physical activity and provided written informed consent.

Forty participants were randomly selected from eligible candidates and assigned to one of four groups: the control group, supplementation group, exercise group, or exercise plus supplementation group. Participants were instructed to maintain consistent communication with the researcher and refrain from altering their lifestyle or dietary habits during the study period. Following baseline measurements of height, weight, and body composition, participants attended training sessions three times weekly for eight weeks. They were required to fast for at least 10 hours before blood sample collection, performed before and after the 8-week training period. The ethics committee of Ilam University approved the study under the code IR.ILAM.REC.1403.002.

Blood Sampling

To measure blood variables during both the pre-test and post-test phases, 6 mL of blood was drawn from the left antecubital vein. Participants were required to fast for 10 hours to standardize metabolic conditions before blood sampling, which was performed 48 hours before and after the training protocol. To control for nutritional status, which could potentially influence specific measured parameters, a 24-hour food recall questionnaire was administered one day before both the pre-test and post-test (21). Superoxide dismutase (SOD), glutathione peroxidase (GPX), and malondialdehyde (MDA) were measured using research kits from Zellbio (Germany). Insulin levels were assessed using the ELISA method with Monobind kit protocols. Blood glucose was determined using the glucose oxidase method, and lipid profile analysis was conducted using photometric methods with Pars Azmoon kits (Iran). The Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) was calculated using the formula: fasting insulin (μU/mL) × fasting glucose (mmol/L) ÷ 22.5 (22, 23).

Blood Pressure Monitoring

For resting blood pressure measurements, participants were instructed to refrain from physical activity for at least 30 minutes before measurement. A nurse subsequently measured blood pressure using an OMRON blood pressure monitor.

Crocin Supplementation

Based on previous studies, the supplementation groups received two 15 mg crocin tablets daily for eight weeks. The placebo group received an equivalent amount of starch, following the same protocol as the crocin supplementation group (24).

The Aerobic Exercise Protocol

The aerobic exercise protocol consisted of treadmill running three times per week at 65-80% of the maximum heart rate, as outlined in Table 1. Each session began with a 10-15 minute general warm-up, including stretching exercises, followed by the main treadmill exercise, and concluded with a 10-minute cool-down period (25).

Table 1. Protocol of aerobic training (25).

	Weeks							
	1st	**2nd**	**3rd**	**4th**	**5th**	**6th**	**7th**	**8th**
Intensity (HR_{Max})	65%	65%	70%	70%	75%	75%	80%	80%
Duration(Min)	20	20	24	24	28	28	32	32

Statistical Methods

In this study, data are presented as means with standard deviations. The Shapiro-Wilk test was used to assess the distribution of the data. Analysis of covariance (ANCOVA) was employed for factors where the assumption of homogeneity of regression slopes was met, including weight ($p = 0.25$), body fat percentage ($p = 0.67$), body mass index (BMI) ($p = 0.45$), waist-to-hip ratio (WHR) ($p = 0.93$), HDL ($p = 0.13$), LDL ($p = 0.06$), superoxide dismutase (SOD) ($p = 0.74$), malondialdehyde (MDA) ($p = 0.76$), glutathione peroxidase (GPX) ($p = 0.11$), systolic blood pressure (SBP) ($p = 0.40$), and diastolic blood pressure (DBP) ($p = 0.33$). One-way analysis of variance (ANOVA) was used for factors where this assumption was violated, including glucose ($p = 0.0001$), insulin ($p = 0.0001$), insulin resistance (IR) ($p = 0.001$), and triglycerides (TG) ($p = 0.0001$). When significant differences were detected by either ANCOVA or one-way ANOVA, Bonferroni post-hoc tests were performed, given the equal sample sizes across the four groups. Data analysis was conducted using SPSS software version 26 for Windows, with statistical significance set at $\alpha \leq 0.05$.

Results

The analysis revealed significant differences among the four groups for weight (p = 0.0001), body fat percentage (p = 0.0001), BMI (p = 0.0001), WHR (p = 0.0001), glucose (p = 0.0001), insulin (p = 0.0001), MDA (p = 0.0001), GPX (p = 0.0001), and DBP (p = 0.0001). Bonferroni post-hoc tests revealed significant differences between the control and all intervention groups (exercise, supplementation, and exercise plus supplementation groups). Significant differences were observed only between the control and exercise plus supplementation groups for insulin resistance, triglycerides, HDL, and LDL. For SOD and SBP, in addition to differences between the control group and the other three groups, significant differences were also observed between the exercise group and the exercise plus supplementation group, as well as between the supplementation group and the exercise plus supplementation group.

Table 2. Mean and standard deviation of the body composition in the four study groups.

Variables		Control	Training	Supplementation	training plus supplementation
Weight (kg)	Pre test	76.42 ± 3.98	75.83 ± 4.33	77.5 ± 4.3	74.55 ± 3.94
	Post test	76.6 ± 4.55	72.2 ± 4.34	75.01 ± 3.88	70.8 ± 3.98
Body fat percentage (%)	Pre test	38.4 ± 2.45	38.9 ± 1.85	37.5 ± 2.36	37.4 ± 2.17
	Post test	38.4 ± 2.31	36.01 ± 1.69	34.4 ± 1.89	33.60 ± 1.9
BMI (kg/m²)	Pre test	29.98 ± 3.58	28.79 ± 1.83	28.58 ± 2.12	28.46 ± 2.82
	Post test	30.05 ± 3.67	27.42 ± 1.92	27.65 ± 1.93	27.01 ± 2.57
WHR (%)	Pre test	0.903 ± 0.09	0.924 ± 0.1	0.922 ± 0.09	0.938 ± 0.05
	Post test	0.892 ± 0.09	0.891 ± 0.09	0.893 ± 0.088	0.895 ± 0.06

Table 3. Results of ANCOVA analysis in oxidative stress parameters

Variables	Groups	Pre-test	Post-test	Paired sample t-test		ANCOVA	
		M±SD	M±SD	p	t	p	f
SOD U/ml	Control	65.5±16.7	65.3±15.6	0.86	0.17	P<0.001	32.96
	Training	69.7±9.9	75.2±9.5	0.0001	-14.73		
	Supplementation	61.26±12.7	65.7±12.5	0.0001	-11.72		
	T+S	66.6±9.8	75±9.3	0.0001	-23.34		
MDA µM	Control	27.9±9.1	28.4±8.1	0.28	-1.14	P<0.001	31.54
	Training	24.98±8.4	22.6±8	0.0001	7.88		
	Supplementation	23.6±6.5	21±5.9	0.0001	6.97		
	T+S	26.7±6.4	23±6	0.0001	7.82		
GPX U/ml	Control	202 ± 38	203 ± 36	0.85	-0.25	P<0.001	18.05
	Training	232 ±76	242 ± 77	0.0001	-11.54		
	Supplementation	230 ± 61	244 ± 62	0.0001	-13.46		
	T+S	220 ± 62	236 ± 65	0.0001	-0.9.92		

Discussion

This study demonstrated that aerobic exercise, crocin supplementation, and their combined effect (exercise plus supplementation) led to significant improvements in metabolic syndrome indicators among overweight/obese women, with the combined intervention group showing the most pronounced changes. These findings are consistent with previous research on the individual effects of crocin, such as studies by Taherifard et al. (26), Shirali et al. (27), and Javandoost et al. (24), as well as studies on aerobic exercise by Frączek et al. (28) and Davies et al. (29) in overweight and obese populations.

The mechanisms by which crocin and exercise improve metabolic syndrome indicators are multifaceted. Crocin has been shown to inhibit hepatic lipogenesis, enhance lipolysis, and upregulate genes involved in beta-fatty acid oxidation (30). Additionally, crocin's antioxidant properties may help reduce oxidative stress-induced lipid metabolism dysregulation, commonly observed in obesity (26). Conversely, aerobic exercise can increase lipoprotein lipase activity, an enzyme responsible for clearing triglyceride-rich lipoproteins, and enhance gene expression in reverse cholesterol transport, leading to increased high-density lipoprotein cholesterol (HDL-C) levels. Exercise-induced weight loss and improved insulin sensitivity may also contribute to the favorable changes in metabolic syndrome indicators observed in overweight and obese individuals (28). The synergistic effect observed in the combined intervention group appears to stem from crocin's ability to modulate lipid metabolism and the capacity of aerobic exercise to enhance lipid clearance and reverse cholesterol transport (31). The effects of crocin supplementation and aerobic exercise on insulin resistance in overweight/obese individuals have been extensively studied. Research indicates that crocin supplementation can significantly reduce fasting blood glucose, HbA1c, and insulin levels in obese individuals with type 2 diabetes (T2DM). Daily saffron supplementation combined with aerobic exercise over eight weeks has improved insulin levels and overall blood glucose control in middle-aged, overweight women with T2DM (32). Another study found that saffron consumption was associated with reduced insulin resistance, as measured by HOMA-IR, in diabetic mice, demonstrating its potential to enhance insulin sensitivity through various mechanisms, including modulation of oxidative stress and inflammation (33). Regular aerobic exercise has also been reported to significantly reduce insulin levels and improve metabolic markers in obese individuals. A 12-week study demonstrated significant reductions in insulin levels and inflammatory markers among participants (34). The combination of crocin supplementation and aerobic exercise appears to have synergistic effects. Studies have shown that when both interventions are applied together, more significant improvements in insulin sensitivity and reduced insulin levels are observed compared to either intervention alone. Rajabi et al. (2022) reported that participants who simultaneously used saffron extract and engaged in aerobic exercise exhibited more significant reductions in insulin and other metabolic markers compared to the other groups (35). Aerobic exercise has been reported to improve insulin sensitivity, which enhances the clearance of triglyceride (TG)-rich lipoproteins and promotes hepatic low-density lipoprotein cholesterol (LDL-C) uptake. This results in favorable changes in the lipid profile, including decreased TG and LDL-C levels (36, 37). Aerobic exercise can also influence the expression and activity of key enzymes involved in hepatic lipid synthesis and oxidation, such as acetyl-CoA carboxylase and carnitine palmitoyltransferase I. These adaptations reduce TG and low-density lipoprotein (VLDL) production, improving lipid profiles (38, 39). Additionally, weight loss from aerobic exercise can further enhance the lipid profile in overweight and obese individuals (40). This study revealed that both crocin supplementation and aerobic exercise significantly improved oxidative stress markers, yielding even more pronounced positive effects. Both interventions resulted in notable reductions in malondialdehyde (MDA), a marker of lipid peroxidation, while significantly enhancing the activity of antioxidant enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GPX). Given that obesity is associated with increased oxidative stress, which can contribute to a variety of chronic diseases, including cardiovascular diseases and type 2 diabetes, the ability of crocin and aerobic exercise to mitigate oxidative stress in overweight/obese women is a significant finding. Previous research has demonstrated that crocin scavenges free radicals, inhibits lipid peroxidation, and regulates the expression of antioxidant enzymes. (41, 42). These mechanisms can help reduce the excessive production of reactive oxygen species (ROS) and restore the body's balance between oxidants and antioxidants (26). Additionally, aerobic exercise can stimulate endogenous antioxidant systems, such as superoxide dismutase (SOD) and glutathione peroxidase (GPX), through the activation of transcription factors like Nrf2 (43). Moreover, exercise-induced weight loss and improved insulin sensitivity can help reduce obesity-related oxidative stress (8). The combination of crocin supplementation and aerobic exercise appears to exert a synergistic effect on oxidative stress markers in overweight/obese women. In conjunction with the exercise-induced enhancement of endogenous antioxidant defenses, Crocin's antioxidant properties may significantly reduce lipid peroxidation and maximal increases in antioxidant enzyme activity. Crocin also enhances the activity of hormone-sensitive lipase and carnitine palmitoyltransferase I—enzymes involved in fatty acid mobilization and oxidation, respectively—contributing to reduced triglyceride (TG) levels and an improved overall fat profile (30). Studies have shown that aerobic exercise stimulates endogenous antioxidant enzymes, including SOD, catalase, and GPX. This exercise-induced enhancement of antioxidant defense mechanisms can help neutralize excessive reactive oxygen species (ROS) production associated with obesity (38, 44). Furthermore, aerobic exercise can stimulate mitochondrial biogenesis, leading to more efficient energy utilization and reduced electron leakage, decreasing ROS production (45). Additionally, since obesity is associated with low-grade chronic inflammation that contributes to oxidative stress, aerobic exercise has been shown to reduce the production of pro-inflammatory cytokines, such as TNF-α and IL-6, thereby mitigating inflammation-induced oxidative stress (46).

The results showed that aerobic exercise, crocin supplementation, and their combined effect led to significant changes in blood pressure among overweight/obese women, with the combination group showing the most substantial improvements. These findings aligned with studies by Chen et al. (47), Yang et al. (48), Wang et al. (49), and Razavi et al. (30). The beneficial effects of crocin on blood pressure can be attributed to its potent antioxidant and anti-inflammatory properties. Crocin has been reported to inhibit the renin-angiotensin-aldosterone system (RAAS), which plays a central role in blood pressure regulation (30). As a carotenoid compound derived from saffron (Crocus sativus), crocin has garnered increasing attention for its potential therapeutic applications in managing metabolic and cardiac disorders, including obesity. Crocin has been shown to improve endothelial function and enhance nitric oxide bioavailability, leading to vasodilation and reduced peripheral resistance (47). In addition, crocin's role in blood pressure reduction is partly due to its inhibition of key enzymes involved in RAAS, such as the angiotensin-converting enzyme (ACE) and the angiotensin II type 1 receptor (AT1R). This inhibition leads to reduced production and activity of angiotensin II, consequently decreasing peripheral resistance and lowering blood pressure. Aerobic exercise has also been shown to have favorable effects on blood pressure in overweight/obese individuals (50). Exercise can enhance baroreflex responsiveness, improve autonomic nervous system balance, and promote structural and functional adaptations in blood vessels, all contributing to blood pressure reduction (51) (52). The synergistic effect of crocin supplementation and aerobic exercise on blood pressure reduction observed in our study is supported by previous research. The combination of exercise and antioxidant supplements has been reported to be more effective in reducing blood pressure than either intervention alone in hypertensive individuals (44, 53). The underlying synergistic mechanisms may include improved endothelial function, reduced oxidative stress, and enhanced nitric oxide signaling (52). Obesity is associated with excessive sympathetic nervous system activation, which can contribute to hypertension. Aerobic exercise has been shown to improve autonomic nervous system balance by increasing parasympathetic activity and reducing sympathetic tone (54). This can reduce heart rate and peripheral vascular resistance, resulting in lower blood pressure (44). Regular aerobic exercise can also induce structural changes in blood vessels, such as increased arterial compliance and reduced arterial stiffness. These adaptations improve blood flow efficiency and decrease peripheral resistance, ultimately lowering blood pressure (25). Furthermore, aerobic exercise has been shown to regulate antioxidant defense systems and reduce the production of reactive oxygen species (ROS), thereby decreasing obesity-related oxidative stress. Exercise also reduces the production of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), which have detrimental effects on vascular function (44). Additionally, aerobic exercise can promote weight loss and enhance insulin sensitivity in overweight and obese individuals. These metabolic adaptations contribute to blood pressure reduction, as excess body weight and insulin resistance are strongly associated with the development of hypertension (55).

Conclusion

In conclusion, overweight and obese women experienced significant improvements in metabolic syndrome indicators, oxidative stress, and blood pressure as a result of aerobic exercise and crocin supplementation. The combined use of these interventions may offer additional benefits, particularly for individuals with metabolic disorders such as type 2 diabetes (T2DM). To optimize the dosage and duration of these interventions for maximal efficacy, further research is needed to explore the underlying mechanisms.

Declaration

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Declaration of Interest

The authors declare no conflict of interest.

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Ethics Considerations

The study protocol adhered to the principles outlined in the Helsinki Declaration, which provides guidelines for ethical research involving human participants. The ethics committee of Ilam University approved the study using the code IR.ILAM.REC.1403.002

References

1. Marinou K, Tousoulis D, Antonopoulos AS, Stefanadi E, Stefanadis C. Obesity and cardiovascular disease: from pathophysiology to risk stratification. International Journal of Cardiology. 2010;138(1):3-8.
2. Ghorbani S, Alizadeh R, Moradi L. The effect of high intensity interval training along with consumption of caraway seeds (Carum carvi L.) on liver enzymes, lipid profile, and blood glucose in obese and overweight women. Ebnesina. 2017;19(2):12-20.
3. Duong M, Uno K, Nankivell V, Bursill C, Nicholls SJ. Induction of obesity impairs reverse cholesterol transport in ob/ob mice. PLoS One. 2018;13(9):e0202102.
4. Bogaert YE, Linas S. The role of obesity in the pathogenesis of hypertension. Nature clinical practice Nephrology. 2009;5(2):101-11.
5. Martínez-Martínez E, Cachofeiro V. Oxidative stress in obesity. MDPI; 2022. p. 639.
6. Jiang S, Liu H, Li C. Dietary regulation of oxidative stress in chronic metabolic diseases. Foods. 2021;10(8):1854.
7. Nguyen JC, Killcross AS, Jenkins TA. Obesity and cognitive decline: role of inflammation and vascular changes. Frontiers in Neuroscience. 2014;8:375.
8. Park K-S, Nickerson BS. Aerobic exercise is an independent determinant of levels of inflammation and oxidative stress in middle-aged obese females. Journal of Exercise Rehabilitation. 2022;18(1):43.
9. Krause M, Rodrigues-Krause J, O'Hagan C, Medlow P, Davison G, Susta D, et al. The effects of aerobic exercise training at two different intensities in obesity and type 2 diabetes: implications for oxidative stress, low-grade inflammation and nitric oxide production. European Journal of Applied Physiology. 2014;114:251-60.
10. Alizadeh R, Salehi O, Rezaeinezhad N, Hosseini SA. The effect of high intensity interval training with genistein supplementation on mitochondrial function in the heart tissue of elderly rats. Experimental Gerontology. 2023;171:112039.
11. Pei Y, Otieno D, Gu I, Lee S-O, Parks JS, Schimmel K, et al. Effect of quercetin on nonshivering thermogenesis of brown adipose tissue in high-fat diet-induced obese mice. The Journal of Nutritional Biochemistry. 2021;88:108532.
12. Moon J, Do H-J, Kim OY, Shin M-J. Antiobesity effects of quercetin-rich onion peel extract on the differentiation of 3T3-L1 preadipocytes and the adipogenesis in high fat-fed rats. Food and Chemical Toxicology. 2013;58:347-54.
13. Ahn J, Lee H, Kim S, Park J, Ha T. The anti-obesity effect of quercetin is mediated by the AMPK and MAPK signaling pathways. Biochemical and Biophysical Research Communications. 2008;373(4):545-9.
14. Lee SG, Parks JS, Kang HW. Quercetin, a functional compound of onion peel, remodels white adipocytes to brown-like adipocytes. The Journal of Nutritional Biochemistry. 2017;42:62-71.
15. Pei Y, Parks JS, Kang HW. Quercetin alleviates high-fat diet-induced inflammation in brown adipose tissue. Journal of Functional Foods. 2021;85:104614.
16. Sahebkar A. Effects of quercetin supplementation on lipid profile: A systematic review and meta-analysis of randomized controlled trials. Critical Reviews in Food Science and Nutrition. 2017;57(4):666-76.
17. Huang H, Liao D, Dong Y, Pu R. Effect of quercetin supplementation on plasma lipid profiles, blood pressure, and glucose levels: a systematic review and meta-analysis. Nutrition Reviews. 2020;78(8):615-26.
18. Serban MC, Sahebkar A, Zanchetti A, Mikhailidis DP, Howard G, Antal D, et al. Effects of quercetin on blood pressure: a systematic review and meta-analysis of randomized controlled trials. Journal of the American Heart Association. 2016;5(7):e002713.
19. Ostadmohammadi V, Milajerdi A, Ayati E, Kolahdooz F, Asemi Z. Effects of quercetin supplementation on glycemic control among patients with metabolic syndrome and related disorders: A systematic review and meta-analysis of randomized controlled trials. Phytotherapy Research. 2019;33(5):1330-40.
20. Marunaka Y. Actions of quercetin, a flavonoid, on ion transporters: its physiological roles. Annals of the New York Academy of Sciences. 2017;1398(1):142-51.
21. Rasooli SA, Fathi R, Golzar FA-K, Baghersalimi M. The effect of circuit resistance training on plasma levels of amino acids, alpha-hydroxybutyrate, mannose, and urinary levels of glycine conjugated adducts in obese adolescent boys. Applied Physiology, Nutrition, and Metabolism. 2021;46(6):561-70.
22. Zhang H, Fealy CE, Kirwan JP. Exercise training promotes a GDF15-associated reduction in fat mass in older adults with obesity. American Journal of

Physiology-Endocrinology and Metabolism. 2019;316(5):E829-E36.
23. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. Diabetes Care. 2004;27(6):1487-95.
24. Javandoost A, Afshari A, Nikbakht-Jam I, Khademi M, Eslami S, Nosrati M, et al. Effect of crocin, a carotenoid from saffron, on plasma cholesteryl ester transfer protein and lipid profile in subjects with metabolic syndrome: A double blind randomized clinical trial. ARYA Atherosclerosis. 2017;13(5):245.
25. Sadeghian Shahi M, Bagherpour Z, Aria B, Ayati Zadeh F. The effect of eight weeks of aerobic training and Quercetin supplementation on blood pressure, C-reactive protein, and interleukin-6 in inactive overweight women. Journal of Sports and Biomotor Sciences. 2021;13(25):20-8.
26. Taherifard MH, Shekari M, Mesrkanlou HA, Asbaghi O, Nazarian B, Khosroshahi MZ, et al. The effect of crocin supplementation on lipid concentrations and fasting blood glucose: A systematic review and meta-analysis and meta-regression of randomized controlled trials. Complementary Therapies in Medicine. 2020;52:102500.
27. Shirali S, Zahra Bathaie S, Nakhjavani M. Effect of crocin on the insulin resistance and lipid profile of streptozotocin-induced diabetic rats. Phytotherapy Research. 2013;27(7):1042-7.
28. Franczyk B, Gluba-Brzózka A, Ciałkowska-Rysz A, Ławiński J, Rysz J. The impact of aerobic exercise on HDL quantity and quality: a narrative review. International Journal of Molecular Sciences. 2023;24(5):4653.
29. Doewes RI, Gharibian G, Zaman BA, Akhavan-Sigari R. An updated systematic review on the effects of aerobic exercise on human blood lipid profile. Current Problems in Cardiology. 2023;48(5):101108.
30. Razavi BM, Hosseinzadeh H, Movassaghi AR, Imenshahidi M, Abnous K. Protective effect of crocin on diazinon induced cardiotoxicity in rats in subchronic exposure. Chemico-Biological Interactions. 2013;203(3):547-55.
31. Hosseini SA, Norouzi S, Rafiee N, Farzanegi P, Salehi OR, Farkhaie F. Interactive effects of endurance training and crocin on aerobic capacity, dietary intake and weight of high-fat diet-induced type 2 diabetic rats. Journal of Nutritional Sciences and Dietetics. 2018:65-74.
32. Rajabi A, Akbar Nezhad Gharehlo A, Madadizadeh E, Basereh A, Khoramipoor K, Pirani H, et al. The effect of 12 weeks of aerobic exercise training with or without saffron supplementation on diabetes-specific markers and inflammation in women with type 2 diabetes: A randomized double-blind placebo-controlled trial. European Journal of Sport Science. 2024.
33. Liu J, Yang Y, Qi Y. Effect of saffron supplementation on the glycemic outcomes in diabetes: a systematic review and meta-analysis. Frontiers in Nutrition. 2024;11:1349006.
34. Ho SS, Dhaliwal SS, Hills AP, Pal S. The effect of 12 weeks of aerobic, resistance or combination exercise training on cardiovascular risk factors in the overweight and obese in a randomized trial. BMC Public Health. 2012;12:1-10.
35. Rajabi A, Khajehlandi M, Siahkuhian M, Akbarnejad A, Khoramipour K, Suzuki K. Effect of 8 weeks aerobic training and saffron supplementation on inflammation and metabolism in middle-aged obese women with type 2 diabetes mellitus. Sports. 2022;10(11):167.
36. Yazdandoost-Baygi H, Talebi-Garakani E, Nasiri K, Safarzade A. The effect of two different intensities of aerobic training and crocin consumption on visceral adipose cell size and insulin resistance in ovariectomized rats fed with high-fat Diet. Journal of Applied Exercise Physiology. 2022;18(35):15-30.
37. Rezaeinezhad N, Alizadeh R, Ghanbari-Niaki A. Short-term circuit resistance training improves insulin resistance probably via increasing circulating Adropin. Journal of Diabetes & Metabolic Disorders. 2022;21(1):583-8.
38. Wang Y, Xu D. Effects of aerobic exercise on lipids and lipoproteins. Lipids in Health and Disease. 2017;16:1-8.
39. Akhavan Rasoolzadeh E, Nazarali P, Alizadeh R. Effect of endurance and resistance training on adropin and insulin resistance among overweight men: a randomized clinical trial. Physiology and Pharmacology. 2022;26(3):239-47.
40. Slentz CA, Houmard JA, Kraus WE. Modest exercise prevents the progressive disease associated with physical inactivity. Exercise and Sport Sciences Reviews. 2007;35(1):18-23.
41. Ronsisvalle S, Panico A, Santonocito D, Siciliano EA, Sipala F, Montenegro L, et al. Evaluation of Crocin Content and In Vitro Antioxidant and Anti-Glycation Activity of Different Saffron Extracts. Plants. 2023;12(20):3606.
42. Nassar R, Eid S, Chahine R, Chabi B, Bonnieu A, El Sabban M, et al. Antioxidant effects of lebanese Crocus sativus L. and its main components, crocin and safranal, on human skeletal muscle cells. European Journal of Integrative Medicine. 2020;40:101250.
43. Radak Z, Zhao Z, Koltai E, Ohno H, Atalay M. Oxygen consumption and usage during physical exercise: the balance between oxidative stress and ROS-dependent adaptive signaling. Antioxidants & Redox Signaling. 2013;18(10):1208-46.
44. Ruangthai R, Phoemsapthawee J. Combined exercise training improves blood pressure and antioxidant capacity in elderly individuals with hypertension. Journal of Exercise Science & Fitness. 2019;17(2):67-76.
45. Wang CH, Wang CC, Wei YH. Mitochondrial dysfunction in insulin insensitivity: implication of

mitochondrial role in type 2 diabetes. Annals of the New York Academy of Sciences. 2010;1201(1):157-65.
46. Pedersen BK. The anti-inflammatory effect of exercise: its role in diabetes and cardiovascular disease control. Essays in Biochemistry. 2006;42:105-17.
47. Chen X, Huang J, Lv Y, Chen Y, Rao J. Crocin exhibits an antihypertensive effect in a rat model of gestational hypertension and activates the Nrf-2/HO-1 signaling pathway. Hypertension Research. 2021;44(6):642-50.
48. Yang H, Li X, Liu Y, Li X, Li X, Wu M, et al. Crocin improves the endothelial function regulated by Kca3. 1 through ERK and Akt signaling pathways. Cellular Physiology and Biochemistry. 2018;46(2):765-80.
49. Wang Y, Wang Q, Yu W, Du H. Crocin attenuates oxidative stress and myocardial infarction injury in rats. International Heart Journal. 2018;59(2):387-93.
50. Börjesson M, Onerup A, Lundqvist S, Dahlöf B. Physical activity and exercise lower blood pressure in individuals with hypertension: narrative review of 27 RCTs. British Journal of Sports Medicine. 2016;50(6):356-61.
51. Ayatipour O, Nazarali P, Karimi H, Rezaeinezhad N, Alizadeh R. Acute effect of resistance exercise with and without blood-flow restriction on blood pressure in pre-hypertensive and hypertensive middle-aged women. Journal of Applied Health Studies in Sport Physiology. 2021;8(2):119-26.
52. Pescatello LS, MacDonald HV, Lamberti L, Johnson BT. Exercise for hypertension: a prescription update integrating existing recommendations with emerging research. Current Hypertension Reports. 2015;17:1-10.
53. Wei K, Wei Y, Xu W, Lu F, Ma H. Corn peptides improved obesity-induced non-alcoholic fatty liver disease through relieving lipid metabolism, insulin resistance and oxidative stress. Food & Function. 2022;13(10):5782-93.
54. Lee CK, Lee J-H, Ha M-S. Comparison of the effects of aerobic versus resistance exercise on the autonomic nervous system in middle-aged women: A randomized controlled study. International Journal of Environmental Research and Public Health. 2022;19(15):9156.
55. Shaw KA, Gennat HC, O'Rourke P, Del Mar C. Exercise for overweight or obesity. Cochrane Database of Systematic Reviews. 2006(4).

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Pomegranate Peel Extract Powder: Mitigating Cadmium Accumulation and Oxidative Damage in Common Carp Fillets

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ABSTRACT

Introduction: Cadmium (Cd) contamination in aquatic environments threatens fish health by inducing oxidative stress and bioaccumulation. Natural antioxidants like pomegranate peel extract powder (PPEP) may help mitigate these effects through metal chelation and oxidative defense. This study evaluates PPEP's potential to reduce Cd accumulation and oxidative damage in common carp.

Methods: The protective effects of PPEP, as a natural dietary supplement, against Cd accumulation was evaluated by an inductively coupled plasma mass spectrometer, and the effect of PPEP on the oxidative effects of Cd in common carp was assessed via the determination of 2-thiobarbituric acid reactive substances (TBARs) and carbonyl content. Moreover, the bioaccumulation of some minerals (copper (Cu), magnesium (Mg), and zinc (Zn)) was also evaluated in the studied carps.

Results: The feeding with different concentrations of PPEP (1, 2 and 4% wt) could significantly reduce the level of Cd in the fillet samples ($P<0.05$). Moreover, this supplementation also resulted in lower concentrations of Cu and Mg, while Zn was unaffected. Based on TBARs analysis, the levels of oxidation in Cd-supplemented samples were reduced (27-38%) by the PPEP treatments ($P<0.05$). However, no correlation was detected between protein oxidation and lipid peroxidation markers in the fish samples ($P>0.05$).

Conclusion: This study demonstrates that PPEP effectively reduces cadmium accumulation and oxidative stress in common carp fillets. While PPEP mitigates lipid peroxidation, its impact on essential minerals like Cu and Mg requires further investigation. These findings support PPEP as a natural dietary strategy to improve fish health in aquaculture.

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Introduction

Food security, both quantitatively and qualitatively, is a top priority for sustainable global development. In recent decades, the unexpected adverse effects of contaminants on food quality have threatened food security and human health. Among these contaminants, heavy metals (HMs) are chemicals that can disrupt metabolism and enter food chains via the environment, contributing to disease and even death (1). Heavy metals are generally classified into two categories: essential heavy metals, like copper and zinc, which are necessary for vital processes such as metabolism and the growth and development of various organs, and non-essential heavy metals, like cadmium and Lead, which are not required by organisms for any metabolic processes (2). Due to human activities, such as mining, improper waste disposal, and fuel combustion, levels of heavy metals in the environment have increased, particularly in aquatic ecosystems adjacent to industrial areas (3). Among the most important of these are fish, aquatic organisms that live in these polluted ecosystems. As a widely consumed nutritious food group, fish can contain significant amounts of various types of heavy metals, such as cadmium (Cd) and lead (4). This pollution is a serious problem that may outweigh the benefits of consuming seafood. Of all heavy metals, cadmium is one of the most biologically and kinetically toxic, and its toxicity against all life

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forms, including mammals, fish, and plants, has been sufficiently demonstrated. High intake of cadmium in the human diet can lead to various chronic toxicities, such as impaired kidney function, and the International Agency for Research on Cancer has classified it as a Group 1 human carcinogen (5). Furthermore, cadmium causes various systemic toxic effects in fish, including changes in behavior, gill morphology, energy balance, endogenous antioxidant status, increased micronuclei, and death (6). It is known that tissue damage caused by cadmium is mainly attributed to oxidative stress caused by toxicants (7). Cadmium stimulates the formation of reactive oxygen species (ROS) and increases lipid peroxidation, causing oxidative damage to various tissues and leading to loss of membrane function (8).

Researchers are currently working on several strategies to mitigate the harmful oxidative effects of HMs in animal tissues. In this regard, chelating HMs and enhancing endogenous antioxidant defense mechanisms through herbal supplements containing antioxidant compounds is one innovative approach that has been introduced and applied in various animal models to combat oxidative stress induced by HM toxicity. These compounds can destroy free radicals and are responsible for initiating or propagating the reaction. This is done through a chain-breaking mechanism by donating free electrons to active oxygen radicals and lipids present in biological systems, converting them into stable molecules. This prevents or delays the oxidation process. Key components include flavonoids and ascorbic acid (9–11).

The most important of these herbal supplements is pomegranate (Punica granatum), which belongs to the Punicaceae family and is widely cultivated in Central Asian countries such as Iran and Afghanistan (12). This fruit is a valuable source of natural phenolic compounds such as catechins, anthocyanins, tannins, gallic acid, and ellagic acid, known for their beneficial health-promoting properties (9). The protective effects of pomegranate extracts, especially its juice, against oxidative stress resulting from chronic exposure to various high-impact chemicals such as aluminum and lead have previously been reported in animal models such as mice (13,14). Indeed, these effects have been linked to the presence of certain bioactive compounds, particularly polyphenols, in pomegranate. Although almost all parts of the pomegranate, including fruit juice, peel, and leaf extracts, have been shown to possess potent antioxidant activity (15), the majority of studies have focused on fruit juice or evaluated in vivo animal models (16), while information on the use of the peel in various meat products is scarce. Pomegranate peel (PP, approximately 40–50% of the total fruit weight), the main by-product of industrial processing of pomegranate fruit, is usually discarded as waste. However, numerous nutritious and health-promoting compounds, such as phenolics, proteins, bioactive peptides, and polysaccharides, can be found in PP (17). Therefore, it has great potential for use as a natural, antioxidant-rich, and low-cost substance for various purposes.

This study aimed to evaluate the protective effects of polyphenol extract powder (PPEP), as a natural food supplement, against cadmium accumulation and the oxidative effects of this toxicant in farmed carp. Furthermore, the effect of PPEP supplementation on the bioaccumulation of some metals, including copper (Cu), magnesium (Mg), and zinc (Zn), was evaluated. Common carp was chosen due to its high consumption rate among various seafood species in Iran and its susceptibility to HM-induced tissue damage (18). The use of herbal supplements from food industry waste, such as pomegranate peels, to eliminate the bioaccumulation of heavy metals such as cadmium in common carp meat, which ranks first in global consumption, and to improve its quality in terms of food safety and health, as well as the elimination of the most important elements that cause cancer in humans, represents a new approach to preserving human health, food safety, and improving the environment.

Materials and Methods

Pomegranate Peel Extraction

PPEP was prepared following the method of Abdel Moneim et al. (2012) with some modifications (1). In brief, 3 kg of sweet-type whole Iranian pomegranates grown in Kashmar, Iran, was purchased from a local store in Mashhad and transported to the laboratory. Then, they were peeled, cut into small pieces, washed with distilled water and dried in an oven at 40 °C for 48 hours. After drying, the peels were ground to obtain a fine powder. The pomegranate peel powder was mixed with ethanol (96% v/v) in a 1:10 ratio with continuous stirring for 2 min, and after 48 hours it was filtered with a vacuum pump. The obtained extract was concentrated using a rotary evaporator (Model N-100, Eyela, Tokyo, Japan) at 40 °C. Finally, the concentrated extract was poured into a Petri dish and held in an incubator at 40 °C for 24 h to completely dry.

Experimental Design and Sampling

A total of 100 common carp (*Cyprinus carpio*) at the age of or fingerlings (20.3 ± 0.8 g) were obtained from a local fish farm or hatchery (Saft Khalid, Khorasan Province, Iran), transported to the lab, and acclimated to the new environment for 10 days, during which the subjects were fed on a basal commercial diet (EX-TG2, Beyza Feed Mill, Iran) containing 43-46% crude protein and 11-15% crude fat. The fish were then randomly divided into five groups, each containing 20 fish, and stocked in five 120-liter aquariums equipped with air pumps for continuous aeration. The level of Cd in the water was measured before fish stocking and reported as 1.1 ± 0.82 μg/L. The chemical standards for drinking water issued by the Environmental Protection Agency (EPA) and the World Health Organization (WHO). The optimal intake for cadmium set by the WHO is 0.002 and 0.005 mg per liter. The amount of cadmium found in fish flesh is 15.3 parts per billion. Cadmium concentrations in environmental water can be significant, ranging from 0.01 to 1.16 mg per liter. Therefore, the cadmium concentration (0.5 mg/L) was adopted. The five treatments were subjected to the following conditions: the first group (control) stocked in plain water; the second group (Cd) exposed to $CdCl_2$ (0.5 mg/L); third (Cd/1%PPEP), fourth (Cd/2%PPEP) and fifth (Cd/4%PPEP) groups were exposed to $CdCl_2$ (0.5 mg/L) and fed PPEP at levels of 1, 2, and 4% (w/w) of the daily diet, respectively, according to a previous study (2).

Different concentrations of PPEP were dispersed in 2 mL of distilled water (at 30 °C) and then sprayed on the applied diets. The fish were fed three times per day (7, 1, 17) at a rate of 2.5% body weight day^{-1}. The length of the experiment was 30 days and the health status of the fish was evaluated by visual examination during this period. Throughout the experiment, to maintain clear healthy water, the water of the aquaria was replaced with fresh well-aerated water twice a week to remove uneaten food and feces. The temperature, pH and oxygen concentration of the water were maintained at 20±1°C, ~7.5 and 5.6-6 ppm respectively during the acclimation time and afterward. These values are acceptable for fish farming (3).

At the end of the experiment, eight fish from each group were randomly selected and anesthetized with clove powder (0.5 g/L). Studies have shown that cadmium accumulates in fish tissues within days of exposure, following the order: gills > liver > muscle > brain. Fish primarily eliminate metals through bile, urine, gill excretion, and mucus. Among these, the gills exhibited the highest rate of demineralization, while the liver showed a significant but lower degree of metal removal. In contrast, the brain and muscles displayed minimal demineralization even after 30 days. Consequently, muscles were selected for measuring cadmium accumulation. The fish were then headed and cleaned, and appropriate muscle samples were manually excised using a sterile scalp. Subsequently, the fillets were washed with physiological serum and stored at −70 °C until analysis.

Heavy Metals Analysis

Each sample (0.5 g) was digested in concentrated nitric acid (98%, 5 mL) and hydrogen peroxide (3 mL) in a beaker. Then, the samples were diluted to 50 ml with ultra-pure water. Afterward, the prepared samples were determined for Cu, Mg, Zn and Cd using an inductively coupled plasma mass spectrometer (ICP-MS, model 7700 series, Agilent Technologies, Tokyo, Japan). Metals assayed in the present study were: copper (Cu), magnesium (Mg), zinc (Zn) and cadmium (Cd). Standard solution of the element (Perkin Elmer) was prepared by diluting stock solutions of 100 mg/mL of each HM based on Taweel et al. (2013) (4). The concentrations of HMs in the fish samples were reported in mg/kg tissue.

Oxidative Status Tests

In order to use the frozen samples for oxidative status tests, they were quickly thawed and homogenized (Heidolph homogenizer, Germany) for five min in 10 volumes (w/v) of ice-cold 0.05 M phosphate buffer (pH 7.4). Finally, the resultant mixture was centrifuge (Eppendorf 5417c centrifuge, Germany) at 5000 rpm for 10 min, and the clear supernatant fraction was collected and used for oxidative assays.

TBARs (2-thiobarbituric acid reactive substances) method was carried out to measure the lipid oxidation in the samples according to the spectrophotometric (Optizen 2120 UV Plus spectrophotometer, Korea) procedure described by Maraschiello et al. (1999) (5). The TBARs value was expressed as mg malonaldehyde (MDA) equivalents/kg tissue.

Protein oxidation in the tissue samples as measured by the protein carbonyl content was assessed using the 2,4-dinitrophenylhydrazine (DNPH) method as described by Levine et al. (1990) (6). The carbonyl content was estimated by a spectrophotometric assay at 370 nm and expressed as nmol/mg tissue.

Statistical Analysis

The data were expressed as mean values with their standard deviation indicated (mean ± SD). All data were statistically analyzed using SPSS 16 software by one-way analysis of variance (ANOVA), and multiple comparisons were done by Tukey's tests. A level of probability $P<0.05$ were considered statistically significant.

Results

Heavy Metal Trace in Fish Samples

Cadmium

The concentration of Cd in the muscle tissue of common carp was drastically increased in the fish groups exposed to 0.5 mg/L of Cd, compared to the control ($P<0.05$) (Table 1). Among those groups, maximum and minimum Cd contents were recorded for the Cd group (0.02 mg/kg) and Cd/1%PPEP group (0.011 mg/kg) respectively. Feeding with different concentrations of PPEP could efficiently reduce the level of Cd in the carp fillets ($P<0.05$). Based on the result, the changes in Cd content of cd/PPEP-exposed samples were not dependent on the concentration of PPEP ($P<0.05$).

Table 1. Metal accumulation (mg/kg tissue) in carp fillets exposed to cadmium (Cd: 0.5 mg/L) and pomegranate peel extract powder[1].

	Treatment				
Heavy metals	Control	Cd	Cd/1%PPEP	Cd/2%PPEP	Cd/4%PPEP
Cd	0.0012 ± 0.0005[a]	0.02 ± 0.0023[b]	0.011 ± 0.0015[c]	0.013 ± 0.0021[c]	0.013 ± 0.0028[c]
Cu	1.27 ± 0.2[a]	0.84 ± 0.38[ab]	0.66 ± 0.21[b]	0.68 ± 0.28[b]	0.91 ± 0.51[ab]
Mg	256 ± 30[a]	247.5 ± 33[a]	216.7 ± 11[ab]	222.2 ± 17[ab]	196.4 ± 17[b]
Zn	45 ± 14.2[a]	32.6 ± 16.2[a]	32.1 ± 17.3[a]	36.3 ± 5.9[a]	25.7 ± 3.4[a]

[1]The mean ± standard deviations are presented.

[a-c] Different lowercase letters within a row indicate significant differences ($P<0.05$)

Copper, Magnesium and Zinc

Table 1 shows the level of metal accumulation in the fish fillets induced by dietary cadmium and PPEP. The results of the analysis of copper concentration in muscle tissue of common carp showed that although all of the Cd-exposed samples exhibited lower Cu concentrations (0.66-0.91 mg/kg) compared to the control group (1.27 mg/kg), only the Cu content of Cd/1%PPEP and Cd/2%PPEP carp fillets were significantly lower than the relevant value in control ($P<0.05$) (Table 1).

The concentrations of Mg ranged between 196.4 and 256 mg/kg (Table 1). The supplementation of PPEP resulted in lower content of Mg in the fillet samples, particularly in the Cd/4%PPEP group ($P<0.05$), compared to the control.

The analysis of Zn concentration revealed that the control group and the fifth group of carps contained maximum (45 mg/kg) and minimum (25.7 mg/kg) concentrations of Zn respectively (Table 1). However, the differences recorded between the treated samples were not significant, mainly due to the variations among the Zn values in each group of fish fillets. Moreover, it seems that the addition of Cd to the experimental diets reduced the concentrations of the metal in the fillets.

Oxidative Markers in Tissue Samples

Lipid Oxidation

The TBARs values of the fish muscle samples from different experimental groups are presented in Fig. 1A The minimum and maximum lipid oxidation were recorded for the control group and group 2 with the TBARs values of 0.13 and 0.22 mg MDA equivalents/kg muscle tissue respectively. With the addition of Cd, the level of lipid oxidation was significantly increased in the group 2 samples, while the differences in TBARs values among the actual treatments and also the control group were not significant ($P<0.05$). However, some slight differences can be observed between the fish fed with different levels of PPEP, and they exhibited slightly higher lipid oxidation compared to the control.

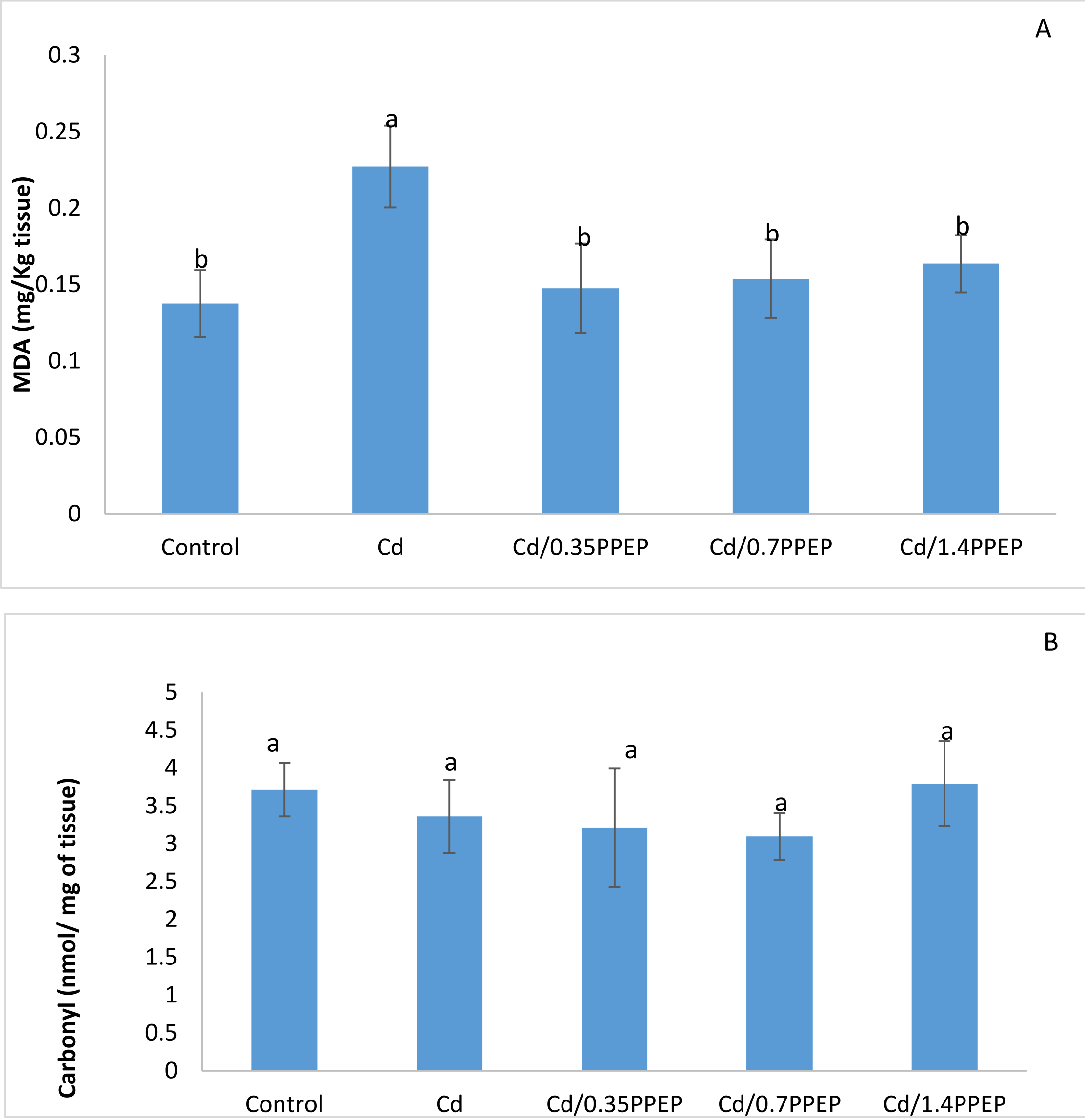

Figure 1. Effect of cadmium and different concentrations of pomegranate peel extract powder (PPEP) on TBARS (A) and carbonyl content (B) of carp fillets. Data are presented as means of three replicates and the error bars show the standard deviation. Different letters represent a significant difference ($P<0.05$).

Protein Oxidation

The measurement of protein carbonyl content is the prevalent method used for the determination of the extent of protein oxidation in food systems (7). As shown in figure 1B, carbonyl content in the fish samples ranged between 3.1 and 3.8 (nmol/mg tissue), and minimum and maximum values were recorded for Cd/2%PPEP and Cd/4%PPEP respectively. These values are in accordance with the values reported in the literature for carp muscles (8). Neither the addition of Cd to the aquarium water nor the feeding with PPEP significantly changed the carbonyl content of muscle proteins compared to the control ($P>0.05$). However, among the tested groups, the fish fed with 1% or 2% PPEP showed lower values. No correlation was detected between protein oxidation and lipid peroxidation markers in the fish samples.

Discussion

The elevation of Cd content in the fish samples exposed to Cd was predictable as it has been frequently stated that higher levels of heavy metals, like cadmium and lead, in the aquatic ecosystems finally lead to higher levels of the elements in the tissues of the resident aquatic organisms (9,10). In general, muscles accumulate fewer HMs than the metabolically active tissues, like liver, kidneys, or gills, and this bioaccumulation occurs in a species-specific manner (9). The previous study by Vinodhini et al. (2008) showed that HMs, particularly Cd, could significantly accumulate in the tissues of common carp exposed to sublethal concentrations of the metals for periods of 32 days (10).

Based on the result, PPEP had a decreasing effect on the Cd content of the carp fillets. As it has been widely demonstrated in the literature, some synthetic and natural compounds can chelate metals and limit their reactivity (11). Moreover, metal chelators can be used for the detoxification of HMs-contaminated foods or feeds. Among natural metal chelators, flavonoids and vitamin C have shown promising chelating properties (11). The chemical composition of PPE was evaluated in several studies, which indicated that PPE is a good source of flavonoids (12). Furthermore, vitamin C was also detected in PPEs obtained from different sources (13,14). Consequently, the lower Cd concentrations in the carp groups fed with different levels of PPEP might be related to the presence of active agents like flavonoids and vitamin C that probably chelate the metal and decreased its level in the flesh of tested fish. The levels of cadmium reduction in the fourth and fifth groups were lower than in the third group. This may be due to the fact that pomegranate peels also contain some important minerals like manganese, copper, iron, zinc, lead, and cadmium at different concentrations (15). It has been demonstrated that due to the competition and interaction between the ions, the rate of heavy metals removal using pomegranate peel is relatively low when applied to mixed ions (16).

Similar effects were observed by Aksu et al. (2017) in the rats exposed to lead and fed with pomegranate juice (17). They reported that the levels of lead in all tested rats' tissues including kidney, liver, heart and testis, significantly declined with the addition of pomegranate juice to their diets ($P<0.05$).

Regarding the impacts of dietary bioactive herbal materials on mineral bioaccumulation in animal tissues various studies have been published. Some reports mentioned that tissue metal uptake was not affected by herbal supplementation, while others demonstrated different impacts of dietary herbs on the accumulation of minerals in animal tissues (18). Stef and Gergen (2012) reported a poor or moderate correlation between total phenols and the bioaccumulation of some minerals, like Zn and Cu in the muscles of chickens supplemented with medicinal herbs rich in polyphenols (19). On the other hand, the four-week inclusion of polyphenol-rich herbal products in the diet of piglets had a marginal effect on the levels of Zn, Cu and Fe (20). Kalay and Canli (2000) indicated that Cu and Zn are both non-chelatable, but due to the metabolism of copper and the lack of zinc metabolism in the body, the level of Cu and Zn in the tissues decreased and remained almost unchanged during the study, respectively (21). In another study, similar to the results of the current study, antioxidant agents caused the negative removal of some beneficial mineral elements such as calcium or magnesium (4). Čobanová et al. (2020) mentioned that the uptake of Zn and Cu by lamb tissues was not affected by herbal supplementation (18). Therefore, great variability in the effects of the supplementation of plant materials on minerals uptake is evident, but it seems that the extent of chelation is depend on the chemical constituents of the herbs particularly their polyphenols (19). In the present study, the concentrations of Cu and Mg in the fillets were mainly affected by supplementation of some levels of PPEP, probably due to the chelating ability of PPEP constituents, while Zn contents were less influenced. The results of analysing the concentration of magnesium in the muscle tissue of common carp showed a significant decrease in the concentration of magnesium in the fifth group compared to the control group. In another study similar to our findings, antioxidant agents, in addition to the removal of heavy metals, caused the loss of some beneficial minerals such as calcium or magnesium from the body in an inappropriate manner through the chelation of those elements (22).

It is noted that toxic levels of cadmium can impede zinc absorption (23). Then, the lower levels of zinc in the cadmium-induced samples may be justified by this fact.

MDA is a highly reactive dialdehyde that is produced through the oxidation of unsaturated fatty acids with toxic nature. This substance is a common marker widely used for tracing the progress of lipid oxidation in different matrices like foodstuffs and animal tissues (24). Reactive oxygen species (ROS) like superoxide radicals, hydroxyl radicals, and hydrogen peroxide are active compounds that can trigger the peroxidation of lipids, resulting in the formation of MDA. Among different agents that can stimulate the formation of ROS, HMs, like Cd, have shown strong potential to develop those active species (25). Therefore, as the level of Cd was higher in the Cd-exposed fish samples, then the increase of lipid peroxidation in those samples was probably attributed to the stimulating effects of the deposited Cd on the formation of ROS. In accordance with our findings, the induction of oxidative stress by several HMs like Cd and Pb were similarly reported in animal models like rat, common carp and several aquatic organisms (25–27).

The lower MDA values in the PPEP-fed groups compared to group 2 indicate the protective effects of the extract against the oxidative effects of Cd. Regarding the effects of feeding with herbal substances on the MDA content of different animal tissues, several reports have been published. The analysis of TBARs values in different animal tissues including liver, kidney, heart and testis showed that pomegranate juice and PPE both provided a protection against the oxidative stresses induced by HMs (28,29). The effect of piper betle leaf extract (PBE) against Cd-induced oxidative hepatic dysfunction in rats was examined by Milton Prabu et al. (2012) (30). Besides, the elevation of TBARs values in the liver samples exposed to Cd, the authors observed that the supplementation of PBE (200 mg/kg BW) significantly reduced the level of TBARs in the Cd-induced samples ($P<0.05$). This antioxidant effect has similarly attributed to the presence of polyphenols in the PBE. The supplementation of grape pomace in the diets of sheep was also effective in bringing down the level of lipid oxidation (31).

The antioxidant activity of dietary PPEP in fish samples implies that the active antioxidant agents of PPEP, like polyphenols, can be absorbed through the gastrointestinal tract and transferred to the muscle tissue. This phenomenon was previously demonstrated by several authors for other natural dietary compounds (31). In this regard, Nardoia et al. (2018) showed that polyphenols present in wine by-products can be absorbed, distributed, and remain their active antioxidant activity in chicken breast meat (32).

Although the aforementioned studies have suggested that the inclusion of dietary phenolic compounds favors the antioxidant stability of flesh food products during storage, the exact mechanisms of action have not been fully established. For a better understanding of the mechanism that PPEP confront the lipid oxidation progress, the chemical composition of the extract must be considered. Pomegranate peel contains various active compounds with strong antioxidant activity like polyphenols, flavonoids, and anthocyanins that can destroy ROS (33). In fact, high levels of phenols were previously reported for pomegranate peel obtained from different geographic areas (12). It has been demonstrated that phenolic compounds possess a cell membrane stabilizing activity by inhibiting the generation of ROS induced by Cd (30). However, it seems that the Cd chelating activity of PPEP was the leading factor that inhibited lipid peroxidation and limited the production of MDA in the carp samples as the Cd analysis showed lower content of the metal in the PPEP-supplemented samples.

To explain the lack of correlation between protein oxidation and lipid peroxidation markers, it should be mentioned that protein carbonyl groups are formed by the oxidation of certain amino acids like lysine, threonine, arginine, proline, and histidine, while other amino acids might be oxidized without any alteration in carbonyl content (34). Moreover, it has been stated that the onset of muscle protein oxidation takes place slower than the oxidative degradation of lipids in meat systems (35). So, although TBARs analysis showed significant differences between different groups of carps, the inclusion of Cd or PPEP in the diets did not significantly change the carbonyl content of the samples.

The effects of dietary herbal materials and heavy metals on the carbonyl content of different animal tissues have been assessed in various studies. Milton Prabu et al. (2012) stated that the oxidizing effects of Cd in the liver of rats were significantly hampered by the pre-oral supplementation of piper betle leaf extract (200 mg/kg BW) (30). Moreover, a positive correlation between lipid oxidation and carbonyl content of the liver tissue was also reported.

Ortuño et al. (2016) observed a similar phenomenon in chilled meat obtained from lambs fed on a diet supplemented with rosemary extract (35). In fact, both TBRAs values and carbonyl content of the lamb meat supplemented with rosemary declined compared with the control (fed only with basal diet). However, it is worth mentioning that the differences between the carbonyl content of the reinforced lamb samples and the control were significant since day 11 of storage, while the effects of the inclusion of rosemary extract on the lipid oxidative degradation were observable from day 7 ($P<0.05$). So, as the fish samples in the present study were immediately frozen and used for antioxidant assays, further storage of the samples might be needed for detecting the differences in protein oxidation among the treatments

Mechanism of Action of PPEP against Cadmium Toxicity

The protective effects of PPEP against cadmium toxicity are primarily attributed to its metal-chelating properties, antioxidant activity, and regulation of metal metabolism. The polyphenols and flavonoids in PPEP can bind to cadmium ions, reducing their bioavailability and limiting their accumulation in fish tissues. Additionally, PPEP acts as a potent antioxidant by scavenging reactive oxygen species (ROS), thereby mitigating oxidative stress-induced lipid and protein oxidation (36). This study demonstrated a significant reduction in thiobarbituric acid reactive substances (TBARs) in PPEP-fed groups, supporting its protective role against lipid peroxidation. Furthermore, PPEP appears to influence the uptake of essential minerals, such as copper and magnesium, which may be due to competitive interactions between cadmium and these elements. While these findings suggest PPEP as a promising dietary strategy to reduce heavy metal toxicity in aquaculture, further research is needed to clarify its long-term effects and optimize its application.

Conclusion

This study demonstrated that PPEP supplementation effectively reduced cadmium accumulation and oxidative stress in common carp fillets, likely due to its known antioxidant and metal-chelating properties. These findings suggest that PPEP could be a valuable dietary strategy for mitigating heavy metal toxicity in aquaculture. However, this study has some limitations. The exact molecular mechanisms underlying PPEP's protective effects remain unclear and require further investigation. Additionally, the potential impact of PPEP on the sensory properties and overall quality of fish fillets was not assessed. Future studies should explore the long-term effects of PPEP supplementation, optimize its dosage for different exposure levels of heavy metals, and evaluate its influence on fish health and product quality in real aquaculture conditions. Investigating the synergistic effects of PPEP with other natural antioxidants could also provide valuable insights into enhancing its efficacy.

Declarations

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Hayder Al-Iessa, Davar Shahsavani, Hasan Baghishani and Mohammadreza Rezaeigolestani. The manuscript was written by Hayder Al-Iessa and Mohammadreza Rezaeigolestani, and revised by Hasan Baghishani. All authors read and approved the final manuscript.

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References

1. Ahmed E. Moneim A. Antioxidant activities of Punica granatum (pomegranate) peel extract on brain of rats. J Med Plants Res. 2012;6(2):195–9.
2. Rakhnareh A, Safari O, Armament Y. Alteration of bacteria and yeast populations of Carassius auratus in response to diets containing Punica granatum extract. In: 7th National Conference of Iranian Fisheries. 2019.
3. Abdel-Latif HMR, Abdel-Tawwab M, Khafaga AF, Dawood MAO. Dietary oregano essential oil improved the growth performance via enhancing the intestinal morphometry and hepato-renal functions of common carp (Cyprinus carpio L.) fingerlings. Aquaculture. 2020;526:735432.
4. Taweel A, Shuhaimi-Othman M, Ahmad AK. Assessment of heavy metals in tilapia fish (Oreochromis niloticus) from the Langat River and Engineering Lake in Bangi, Malaysia, and evaluation of

the health risk from tilapia consumption. Ecotoxicol Environ Saf. 2013;93:45–51.
5. Maraschiello C, Sárraga C, García Regueiro JA. Glutathione Peroxidase Activity, TBARS, and α-Tocopherol in Meat from Chickens Fed Different Diets. J Agric Food Chem. 1999;47(3):867–72.
6. Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz A-G, et al. Determination of carbonyl content in oxidatively modified proteins. In: Oxygen Radicals in Biological Systems Part B: Oxygen Radicals and Antioxidants. Academic Press; 1990: 464–78.
7. Badr HM, Mahmoud KA. Antioxidant activity of carrot juice in gamma irradiated beef sausage during refrigerated and frozen storage. Food Chem. 2011;127(3):1119–30.
8. Zhang L, Gui P, Zhang Y, Lin J, Li Q, Hong H, et al. Assessment of structural, textural, and gelation properties of myofibrillar protein of silver carp (Hypophthalmichthys molitrix) modified by stunning and oxidative stress. LWT. 2019;102:142–9.
9. Zaynab M, Al-Yahyai R, Ameen A, Sharif Y, Ali L, Fatima M, et al. Health and environmental effects of heavy metals. J King Saud Univ - Sci. 2022;34(1):101653.
10. Vinodhini R, Narayanan M. Bioaccumulation of heavy metals in organs of fresh water fish Cyprinus carpio (Common carp). Int J Environ Sci Technol. 2008;5(2):179–82.
11. Zhai Q, Narbad A, Chen W. Dietary strategies for the treatment of cadmium and lead toxicity. Nutrients. 2014;7(1):552-71.
12. Gullón P, Astray G, Gullón B, Tomasevic I, Lorenzo JM. Pomegranate Peel as Suitable Source of High-Added Value Bioactives: Tailored Functionalized Meat Products. Molecules. 2020;25(12):2859.
13. Mphahlele RR, Fawole OA, Makunga NP, Opara UL. Effect of drying on the bioactive compounds, antioxidant, antibacterial and antityrosinase activities of pomegranate peel. BMC Complement Altern Med. 2016;16(1):143.
14. Barros Z, Salgado J, Melo P, Biazotto F. Enrichment of Commercially-Prepared Juice With Pomegranate (Punica granatum L.) Peel Extract as a Source of Antioxidants. J Food Res. 2014;3.
15. Ozcan M, Dursun N, Sağlam C. Heavy Metals Bounding Ability of Pomegranate (Punica granatum) Peel in Model System. Int J Food Prop. 2011;14:550–6.
16. Rasheed M, Tayh W. Removal of Heavy Metals from Wastewater Using Pomegranate Peel. IOP Conf Ser Mater Sci Eng. 2020;881:12187.
17. Aksu DS, Sağlam YS, Yildirim S, Aksu T. Effect of pomegranate (Punica granatum L.) juice on kidney, liver, heart and testis histopathological changes, and the tissues lipid peroxidation and antioxidant status in lead acetate-treated rats. Cell Mol Biol. 2017;63(10 SE-Original Research Articles):33–42.
18. Čobanová K, Váradyová Z, Grešáková Ľ, Kucková K, Mravčáková D, Várady M. Does herbal and/or zinc dietary supplementation improve the antioxidant and mineral status of lambs with parasite infection?. Antioxidants. 2020;9(12):1172.
19. Stef DS, Gergen I. Effect of mineral-enriched diet and medicinal herbs on Fe, Mn, Zn, and Cu uptake in chicken. Chem Cent J. 2012;6(1):19.
20. Fiesel A, Ehrmann M, Geßner DK, Most E, Eder K. Effects of polyphenol-rich plant products from grape or hop as feed supplements on iron, zinc and copper status in piglets. Arch Anim Nutr. 2015;69(4):276–84.
21. Kalay M, Canli M. Elimination of essential (Cu, Zn) and non-essential (Cd, Pb) metals from tissues of a freshwater fish Tilapia zilli. Turkish J Zool. 2000;24:429–36.
22. Jomova K, Valko M. Advances in metal-induced oxidative stress and human disease. Toxicology. 2011;283(2):65–87.
23. Lönnerdal B. Dietary Factors Influencing Zinc Absorption. J Nutr. 2000;130(5):1378S-1383S.
24. Okolie N, Okugbo O. A comparative study of malondialdehyde contents of some meat and fish samples processed by different methods. J Pharm Sci Innov. 2013;2:26–9.
25. Rajeshkumar S, Liu Y, Ma J, Duan HY, Li X. Effects of exposure to multiple heavy metals on biochemical and histopathological alterations in common carp, Cyprinus carpio L. Fish Shellfish Immunol. 2017;70:461–72.
26. Orji OU, Awoke JN, Harbor C, Igwenyi IO, Obasi OD, Ezeani NN, et al. Ethanol leaf extract of Psychotria microphylla rich in quercetin restores heavy metal induced redox imbalance in rats. Heliyon. 2020;6(9):e04999.
27. Jafari SS, Rahbarian R, Noghreie M. Protective effect of pomegranate peel (Punica granatum) ethanolic extract on common carp (Cyprinus carpio) exposed to cadmium. Iran Sci Fish J. 2023;31(6):95–105.
28. Kumar D, Singh S, Singh AK, Rizvi SI. Pomegranate (Punica granatum) peel extract provides protection against mercuric chloride-induced oxidative stress in Wistar strain rats. Pharm Biol. 2013;51(4):441–6.
29. Aksu DS, Saglam YS, Yildirim S, Aksu T. Effect of pomegranate (Punica granatum L.) juice on kidney, liver, heart and testis histopathological changes, and the tissues lipid peroxidation and antioxidant status in lead acetate-treated rats. Cell Mol Biol. 2017;6(10):33–43.
30. Milton Prabu S, Muthumani M, Shagirtha K. Protective effect of Piper betle leaf extract against cadmium-induced oxidative stress and hepatic

dysfunction in rats. Saudi J Biol Sci. 2012;19(2):229–39.

31. Vieira C, Guerra-Rivas C, Martínez B, Rubio B, Manso T. Effects of grape pomace supplementation on the diet of lactating ewes as compared to vitamin E on the meat shelf life of suckling lambs. Meat Sci. 2022;184:108666.

32. Nardoia M, Ruiz-Capillas C, Casamassima D, Herrero AM, Pintado T, Jiménez-Colmenero F, et al. Effect of polyphenols dietary grape by-products on chicken patties. Eur Food Res Technol. 2018;244(2):367–77.

33. Zhao C, Sakaguchi T, Fujita K, Ito H, Nishida N, Nagatomo A, et al. Pomegranate-derived polyphenols reduce reactive oxygen species production via SIRT3-Mediated SOD2 activation. Saretzki G, editor. Oxid Med Cell Longev. 2016;2016:2927131.

34. Kolgiri V, Patil VW. Protein carbonyl content: a novel biomarker for aging in HIV/AIDS patients. Brazilian J Infect Dis. 2017;21(1):35–41.

35. Ortuño J, Serrano R, Jordán MJ, Bañón S. Relationship between antioxidant status and oxidative stability in lamb meat reinforced with dietary rosemary diterpenes. Food Chem. 2016;190:1056–63.

36. Adhami VM, Mukhtar H. Anti-Oxidants from Green Tea and Pomegranate for Chemoprevention of Prostate Cancer. Mol Biotechnol. 2007;37(1):52–7.