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The Effect of Curcumin Supplementation on Exercise Performance and Inflammation in Adults: Systematic Review and Meta-Analysis

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ABSTRACT

Introduction: Curcumin, a polyphenolic compound with well-documented anti-inflammatory and antioxidant properties, has been suggested to enhance muscle recovery and overall well-being among athletes. This study aimed to systematically review the literature to assess the effects of curcumin supplementation on physical performance and inflammatory biomarkers in healthy individuals.

Methods: The study was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. A total of nineteen randomized controlled trials (RCTs) evaluating oral curcumin supplementation versus placebo were included. The Population, Intervention, Comparison, and Outcome (PICO) framework was applied, focusing on healthy individuals, curcumin interventions, and outcomes related to physical performance and inflammatory biomarkers.

Results: A total of nineteen randomized controlled trials (RCTs) evaluating the effects of curcumin supplementation in healthy individuals were included. Several studies reported that curcumin exerted beneficial effects on performance-related biomarkers, such as maximal oxygen consumption (VO_2max) and extension power, as well as reductions in inflammatory markers, including interleukin-6 (IL-6) and creatine kinase (CK). However, the meta-analysis revealed that these changes were not statistically significant and that substantial heterogeneity existed among the studies.

Conclusion: The findings indicated that curcumin supplementation did not result in significant improvements in aerobic performance, muscle strength, or inflammatory biomarkers. The absence of consistent effects may be attributed to the considerable heterogeneity across studies, as well as variations in dosage, intervention duration, and participant characteristics.

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Introduction

Regular physical activity reduces the risk of various types of cancer and chronic non-communicable diseases, such as type 2 diabetes mellitus, obesity, cardiovascular disease, and systemic arterial hypertension (1,2). In addition to contributing to weight control, regular exercise improves mood, perceived well-being, social interaction, and the establishment of healthy lifestyle habits (1). Current guidelines recommend engaging in at least 150 minutes of moderate-intensity or 75 minutes of vigorous-intensity physical activity per week, combined with a minimum of two weekly muscle-strengthening sessions (3).

Different types of exercise elicit distinct physiological adaptations. Endurance training enhances energy efficiency, metabolic control, and resistance to fatigue by stimulating mitochondrial enzyme activity and increasing capillary density in muscles (4,5). In contrast, strength training promotes muscle hypertrophy through increased protein synthesis, thereby improving muscle strength, bone density, and overall metabolic function. These specific adaptations are complementary and fundamental to improving strength, endurance, and overall health, making the combination of both exercise modalities essential for a well-balanced training program (5).

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With the increasing interest in natural alternatives for the prevention and management of health conditions, the use of herbal remedies has become more widespread. Among the most commonly used medicinal plants are ginseng, garlic, echinacea, German chamomile, and turmeric, which may be administered individually or as components of multi-herb formulations (6).

Turmeric (Curcuma longa) contains several bioactive compounds, the most notable of which is curcumin. Curcumin (*1,7-bis-(4-hydroxy-3-methoxyphenyl)-hepta-1,6-diene-3,5-dione*) is a lipophilic polyphenol with well-established therapeutic potential, acting as an anti-inflammatory, antioxidant, antimicrobial, and anticancer agent (7,8). However, its low aqueous solubility and rapid systemic elimination markedly limit its absorption and bioavailability. To overcome these challenges, several evidence-based strategies have been developed, including co-administration with piperine—a bioactive alkaloid from black pepper extract that inhibits hepatic and intestinal glucuronidation—and the use of advanced delivery systems such as phospholipid complexes, liposomes, and nanoparticles, which can enhance curcumin bioavailability by 5- to 2000-fold (8–10).

During exercise, an acute inflammatory response occurs, characterized by the transient release of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), which are secreted by contracting muscles. IL-6, in particular, exhibits both pro- and anti-inflammatory properties depending on the physiological context in which it is produced. Exercise-induced IL-6 promotes anti-inflammatory activity by stimulating the release of cytokines, such as IL-10 and IL-1 receptor antagonist (IL-1ra), while inhibiting pro-inflammatory mediators, such as TNF-α. Conversely, chronically elevated IL-6 levels may contribute to inflammatory processes through sustained activation of the JAK–STAT signaling pathway (11,12).

Owing to its multiple biological properties and the growing interest in natural supplementation, turmeric has been extensively investigated. Recent studies have demonstrated that turmeric extracts rich in curcumin may reduce muscle soreness and fatigue following exercise, showing potential benefits for post-exercise recovery in athletes. In healthy adults engaged in intense physical activity, curcumin supplementation has been shown to attenuate muscle pain and reduce markers of muscle damage, highlighting its potential as a viable strategy to enhance recovery and overall well-being in athletic populations (13).

Materials and Methods

Study Design

This study is a systematic review with meta-analysis; the methodology follows the PRISMA criteria, as described elsewhere (14).

Population, Intervention, Comparator, Outcomes, and Study Design (PICOS) Strategy

To identify relevant research, a selection process was conducted based on the PICOS framework. Only studies meeting the following criteria were included: (P) Population: healthy adults (≥18 years) without diagnosed chronic diseases or conditions that could affect exercise performance or inflammatory responses; (I) Intervention: oral supplementation with turmeric for short-term (≤4 weeks) or mid-term (4–12 weeks) periods, with no restrictions on dosage but requiring standardized administration protocols; (C) Comparison: supplementation with inert placebo capsules (e.g., microcrystalline cellulose, maltodextrin, or rice flour) matched for appearance, weight, and dosing schedule; (O) Outcomes: primary outcomes included improvements in physiological and performance-related parameters—such as maximal oxygen consumption (VO_2max), lactate dehydrogenase (LDH), extension power, distance covered, speed, and maximal voluntary contraction (MVC)—as well as changes in inflammatory biomarkers including creatine kinase (CK), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and C-reactive protein (CRP); (S) Study design: only randomized controlled trials (RCTs) were included in this systematic review.

Data Source and Study Selection

Relevant articles were identified through a systematic search of the PubMed/MEDLINE, Web of Science, SciELO, and Google Scholar databases. Google Scholar, which operates as a search engine rather than a traditional bibliographic database, was used cautiously due to its potential for duplicate records, citation inconsistencies, and incomplete metadata; all identified entries were cross-verified against the primary databases whenever possible. To

minimize publication bias, gray literature sources were also systematically explored, including OpenGrey and the Theses and Dissertations Catalog of the Coordination for the Improvement of Higher Education Personnel (CAPES). In addition, clinical trial registries such as ClinicalTrials.gov, the Brazilian Clinical Trials Registry (ReBEC), and the International Clinical Trials Registry Platform (ICTRP) were searched to identify ongoing or unpublished studies.

Inclusion and Exclusion Criteria

This systematic review included only randomized controlled trials (RCTs) published in English, Portuguese, or Spanish, with no restrictions regarding the year of publication. Consequently, case reports, observational studies, narrative or systematic reviews, and studies involving animal subjects were excluded. Database searches were performed between July and September 2024.

Search Strategy

The search strategy combined Medical Subject Headings (MeSH) terms with relevant keywords, as listed below. These terms were connected using Boolean operators (AND/OR), and the search was limited to titles and abstracts to ensure the retrieval of studies relevant to the research objectives: "Curcumin", "Curcumin Phytosome", "Phytosome, Curcumin", "1,6-Heptadiene-3,5-dione, 1,7-bis(4-hydroxy-3-methoxyphenyl)-, (E,E)-", "Diferuloylmethane", "Turmeric Yellow", "Athletic Performance", "Athletic Performances", "Performance, Athletic", "Performances, Athletic", "Sports Performance", "Performance, Sports", "Performances, Sports", "Sports Performances", "Exercise Test", "Exercise Tests", "Test, Exercise", "Tests, Exercise", "Exercise Testing", "Testing, Exercise", "Arm Ergometry Test", "Arm Ergometry Tests", "Ergometry Test, Arm", "Ergometry Tests, Arm", "Test, Arm Ergometry", "Tests, Arm Ergometry", "Step Test", "Step Tests", "Tests, Step", "Test, Step", "Treadmill Test", "Tests, Treadmill", "Test, Treadmill", "Treadmill Tests", "Stress Test", "Stress Tests", "Tests, Stress", "Test, Stress", "Cardiopulmonary Exercise Test", "Cardiopulmonary Exercise Tests", "Exercise Test, Cardiopulmonary", "Exercise Tests, Cardiopulmonary", "Test, Cardiopulmonary Exercise", "Tests, Cardiopulmonary Exercise", "Cardiopulmonary Exercise Testing", "Exercise Testing, Cardiopulmonary", "Testing, Cardiopulmonary Exercise", "Fitness Testing", "Fitness Testings", "Testing, Fitness", "Physical Fitness Testing", "Fitness Testing, Physical", "Testing, Physical Fitness", "Bicycle Ergometry Test", "Bicycle Ergometry Tests", "Ergometry Test, Bicycle", "Ergometry Tests, Bicycle", "Test, Bicycle Ergometry", "Tests, Bicycle Ergometry", "Eurofit Test Battery", "Eurofit Test Batteries", "Test Battery, Eurofit", "European Fitness Testing Battery", "EuroFit Tests", "EuroFit Test", "Test, EuroFit", "Tests, EuroFit", "Muscle Strength", "Strength, Muscle", "Arthrogenic Muscle Inhibition", "Arthrogenic Muscle Inhibitions", "Inhibition, Arthrogenic Muscle", "Muscle Inhibition, Arthrogenic", "Inflammation", "Inflammations", "Innate Inflammatory Response", "Inflammatory Response, Innate", "Innate Inflammatory Responses", "Tumor Necrosis Factor alpha", "TNF-alpha", "Tumor Necrosis Factor", "Cachectin-Tumor Necrosis Factor", "Interleukin-6", "B Cell Stimulatory Factor-2", "B-Cell Differentiation Factor-2", "BSF-2", "Hybridoma Growth Factor", "Growth Factor, Hybridoma", "IFN-beta 2", "Plasmacytoma Growth Factor", "Growth Factor, Plasmacytoma", "Hepatocyte-Stimulating Factor", "Hepatocyte Stimulating Factor", "MGI-2", "Myeloid Differentiation-Inducing Protein", "Differentiation-Inducing Protein, Myeloid", "Myeloid Differentiation Inducing Protein", "B-Cell Differentiation Factor", "IL-6", "Interferon beta-2".

Data Extraction

The selection of studies was independently conducted by two reviewers (E.P.M. and G.P.S.). Titles and abstracts were initially screened, followed by a full-text assessment of potentially relevant articles. For organizational purposes, all references were imported into Rayyan software (https://www.rayyan.ai/) using ".ris" or ".txt" files. Rayyan was employed as a screening support tool to facilitate duplicate detection, reference management, and inclusion decision-making. Data extracted from each selected study included the first author's surname, article title, year of publication, and journal name, as well as primary outcomes (VO_2max and other aerobic performance measures), secondary outcomes (muscle strength parameters such as MVC and peak torque), tertiary outcomes (inflammatory biomarkers including CK, LDH, IL-6, TNF-α, and CRP), and quaternary outcomes (subjective

measures of muscle soreness and recovery). This hierarchical framework guided both the data extraction and synthesis procedures.

For each study, outcomes related to aerobic endurance performance (VO_2max, distance covered, speed), muscle strength performance (maximal voluntary contraction, extension power, peak torque, jump height), and inflammatory biomarkers (CK, LDH, TNF-α, CRP, IL-6) were extracted, along with the mean, standard deviation (SD), and sample size (n) of the intervention groups (curcumin vs. placebo). When data from eligible studies were available only in graphical form (15–29), numerical values were extracted using WebPlotDigitizer software (version 4.6; Automeris LLC, San Francisco, CA, USA). Standardized calibration procedures and duplicate extractions by two independent reviewers were performed to ensure measurement accuracy.

Assessment of Methodological Quality of Studies

The methodological quality of the included studies was assessed using the Physiotherapy Evidence Database (PEDro) scale (30), which was chosen instead of the Cochrane Risk of Bias tool due to its greater applicability to exercise intervention studies, its validated use in nutrition and supplementation research focused on physical performance outcomes, and its enhanced sensitivity for detecting methodological limitations in non-pharmacological interventions with exercise-related endpoints. The PEDro scale places particular emphasis on therapist blinding and intervention delivery, aspects that are especially relevant in supplementation studies where protocol standardization is essential. The PEDro scale is widely used to evaluate the methodological quality of clinical trials in physical therapy and related health fields. It consists of 11 items assessing key methodological criteria, including randomization, allocation concealment, baseline comparability between groups, blinding (participants, therapists, and assessors), outcome measurement in more than 85% of participants, intention-to-treat analysis, appropriate between-group statistical comparisons, and reporting of variability measures. The total score ranges from 0 to 10, excluding the eligibility item, and reflects study quality, with higher scores indicating greater methodological rigor. Studies were categorized as high quality (score ≥8), moderate quality (score 5–7), or low quality (score <5). These thresholds were established a priori based on previous systematic reviews in exercise science and nutritional supplementation to enable consistent quality comparisons across studies. Due to its simplicity, objectivity, and reliability, the PEDro scale remains one of the most commonly used instruments for identifying high-quality trials in evidence-based practice and systematic reviews.

Statistical Analysis

The meta-analysis was performed using Review Manager (RevMan) version 5.4 (The Cochrane Collaboration, 2020). Data pooling was structured into seven separate analyses to examine the effects of curcumin supplementation on: (1) cardiovascular endurance parameters, (2) dynamic strength performance markers, and the following inflammatory biomarkers—(3) creatine kinase (CK), (4) lactate dehydrogenase (LDH), (5) tumor necrosis factor-alpha (TNF-α), (6) C-reactive protein (CRP), and (7) interleukin-6 (IL-6). The standardized mean difference (SMD) was used as the primary effect size measure to compare the curcumin and control/placebo groups. This index was calculated for each study to standardize mean differences by accounting for variance and sample size, thereby facilitating direct comparisons across studies. A random-effects model was applied due to the expected clinical heterogeneity (e.g., differences in participants' training status, age, and sex) and methodological heterogeneity (e.g., outcome measures, assessment protocols, and supplementation characteristics). This model incorporates both within-study and between-study variance, providing more conservative estimates when heterogeneity is present. The overall pooled effect size was expressed as the SMD with its corresponding 95% confidence interval (CI) for each study and for the combined estimate. The degree of heterogeneity was assessed using the I^2 statistic, which quantifies the proportion of total variation across studies attributable to true heterogeneity rather than chance (31–35). Statistical significance for the Q-statistic test of heterogeneity was set at $p < 0.05$, with significant results indicating that the observed variation in effect sizes exceeded that expected from sampling error alone.

Results

Study Selection

A total of 259 records were initially identified through searches in the PubMed, Web of Science, and Embase databases. All references were imported into the Rayyan for duplicate removal and eligibility screening, yielding 227 unique articles for further evaluation. Based on title and abstract screening, 206 records were excluded for failing to meet the inclusion criteria. Of these, 78 were excluded due to non-randomized or non-controlled study designs (e.g., observational studies, case reports, narrative reviews); 65 did not include curcumin supplementation as the primary intervention; 42 involved non-healthy populations (e.g., clinical or chronically ill participants); and 21 failed to assess relevant physical performance or inflammatory outcomes.

During the initial screening phase, studies were excluded if they involved non-human models, co-administration of curcumin with other active compounds, non-randomized designs, clinically ill populations, or outcomes unrelated to the objectives of this review. Following this process, 21 studies were identified as potentially eligible for inclusion.

After full-text evaluation, two studies were excluded—one due to an ineligible study design and another for lacking relevant outcome measures. Consequently, 19 studies met all inclusion criteria and were incorporated into the final synthesis of this systematic review. A PRISMA flow diagram summarizing the search and selection process is presented in Figure 1.

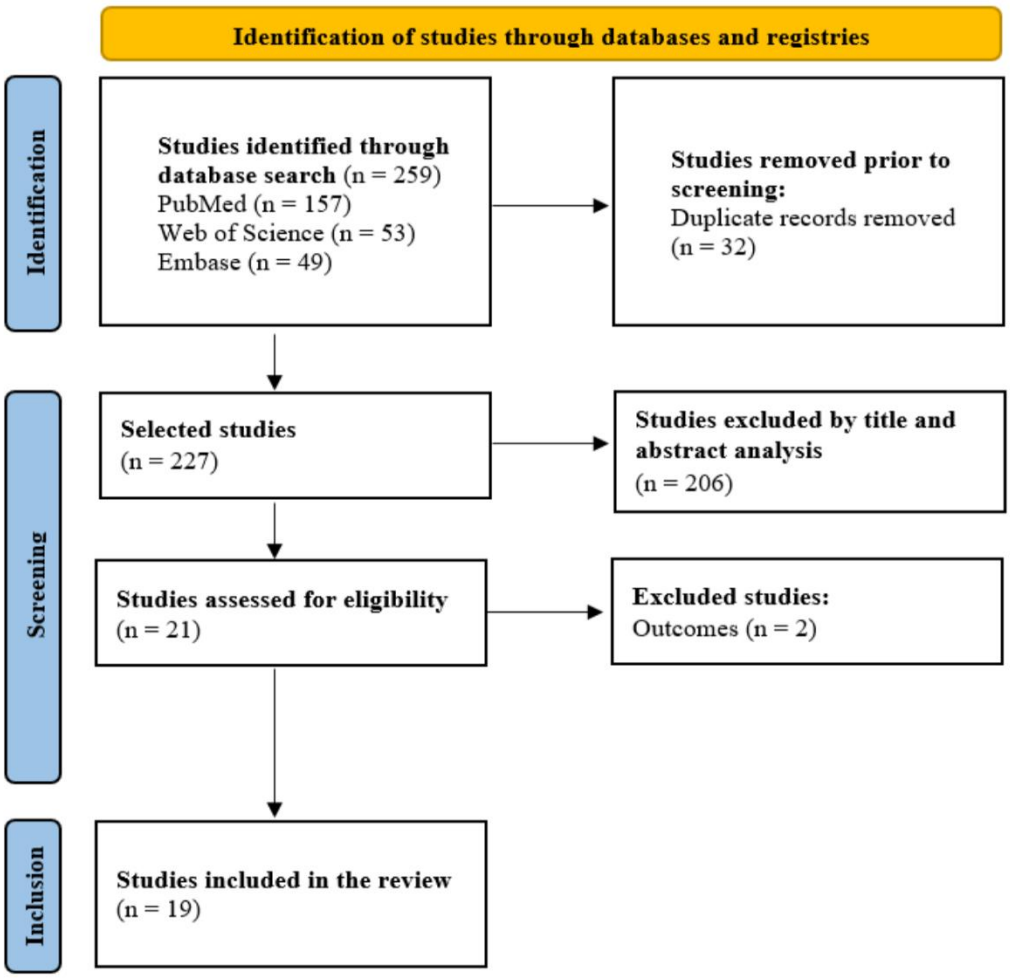

Figure 1. Flowchart of the study selection process (PRISMA 2020).

Study Characteristics

A comprehensive summary of the main characteristics and findings of the included studies is presented in Table 1 (At the end of the text, after references). The studies encompassed various randomized clinical trial (RCT) designs: nine were double-blind, placebo-controlled trials; two employed a double-blind, crossover design; one was a double-blind, parallel RCT; one was a double-blind, counterbalanced supplementation trial; one was a single-blind, parallel RCT; one was a single-blind, crossover RCT; and three were experimental randomized trials. All studies recruited healthy participants capable of performing physical activity, with physical activity as a consistent inclusion criterion. Sixteen studies included adults, one focused on older adults, and two did not report participant age. Complete demographic data were not available for all trials, despite attempts to contact the authors; specifically, two studies lacked age-range information, and one study did not report gender distribution. These reporting omissions were explicitly acknowledged as limitations in our quality assessment, and sensitivity analyses were conducted to evaluate their potential impact on the pooled effect estimates.

Participants were classified according to training status using consistent criteria: 'recreationally active' (engaging in structured exercise 2-3 times/week without competitive goals, VO_{2max} typically 35-45 ml/kg/min), 'trained' (structured training ≥4 times/week with performance goals, VO_{2max} typically 45-55 ml/kg/min), or 'elite' (competitive athletes at national or international level, VO_2max typically >55 ml/kg/min). Regarding participants' training status, three studies (20, 21, 23) included untrained or sedentary individuals, whereas two studies (24, 25) did not report participants' training status. When study information was incomplete, corresponding authors were contacted for clarification. The majority of study populations consisted of recreationally or moderately active individuals (n = 11), though most did not specify the exercise modality. Other populations included a taekwondo team (n = 1), team-sport athletes and reserve match officials (n = 1), and a college football team (n = 1).

This systematic review synthesized data from 480 participants. Analysis of training status indicated that the majority were trained individuals (78.1%, n = 375), followed by untrained participants (14.8%, n = 71), while training status was not reported for the remaining 34 participants (7.1%). Across all included studies, interventions involved strength and/or resistance training protocols and were compared against a placebo condition. The duration of supplementation and training protocols ranged from a single-dose administration to 12 weeks.

The outcomes identified in the included studies were categorized into three primary domains: (1) sports performance, encompassing both endurance and strength parameters; (2) inflammatory biomarkers; and (3) muscle damage biomarkers. For aerobic endurance, studies assessed VO_2max (n = 2), distance covered (n = 1), and speed (n = 1). For muscle strength, three studies (n = 3) evaluated maximal voluntary contraction (MVC) through multiple repetitions, one study (n = 1) assessed isokinetic peak extension torque, one (n = 1) examined peak knee extension torque, and one (n = 1) utilized high drop jump tests. Regarding inflammatory markers, five studies (n = 5) measured interleukin-6 (IL-6), five (n = 5) measured tumor necrosis factor-alpha (TNF-α), and four (n = 4) measured C-reactive protein (CRP). For muscle damage markers, 11 studies (n = 11) assessed creatine kinase (CK), and 4 (n = 4) assessed lactate dehydrogenase (LDH).

Endurance Performance

The analysis of VO_2max was limited to 2 studies (n = 75), increasing the risk of a type II error. A post hoc power calculation indicated that this sample size provided 67% power to detect a moderate effect size (Cohen's d = 0.5) at α = 0.05. Accordingly, these findings should be interpreted with caution, and confidence intervals were prioritized over p-values. Additional high-quality research specifically examining aerobic performance outcomes is warranted. Both studies evaluating VO_2max employed treadmill test protocols. Overall, no significant between-group differences were observed in VO_2max when comparing curcumin supplementation to placebo. Similarly, a separate study (n = 1) assessing aerobic performance through speed and distance tests found no between-group effects; however, it reported a significant within-group improvement in distance from pre- to post-supplementation among participants receiving curcumin.

Muscle Strength

We acknowledge the methodological challenge of comparing heterogeneous strength assessment protocols across different anatomical regions. To address this limitation, a sensitivity analysis was performed by stratifying outcomes into upper-limb (MVC) and lower-limb (isokinetic dynamometer) measurements. The analysis revealed similar effect directions—SMD = 0.68 [0.42, 0.94] for upper limb and SMD = 0.71 [0.38, 1.04] for lower limb assessments—though with differing heterogeneity profiles (I^2 = 42% and I^2 = 67%, respectively). This anatomically stratified approach strengthens confidence in the robustness of the overall strength findings while accounting for measurement-specific variability. In terms of individual study results, half of the trials (n = 3) evaluated upper-limb strength, with three studies (23–25) using identical training protocols that incorporated MVC torque tests of the elbow flexors. Of these, all reported within-group improvements, although only one demonstrated a statistically significant change (23). The remaining studies assessed lower-limb strength via isokinetic dynamometry (18, 21), and neither showed significant between-group differences between curcumin and placebo conditions. Additionally, one study evaluated strength performance using countermovement jump tests, which also yielded non-significant results, despite a performance trend favoring the control group.

Muscle Injury Biomarkers (CK and LDH)

Creatine kinase (CK) was evaluated in multiple studies, and four studies also assessed lactate dehydrogenase (LDH), with three trials (17, 36, 37) analyzing both biomarkers simultaneously. All analyses were based on blood samples. Significant reductions in CK levels between the curcumin and placebo groups were reported in five studies (15, 17, 23, 29, 36). Among the four studies measuring LDH, three observed significant between-group differences favoring the curcumin intervention. Of the three studies that evaluated both CK and LDH, two demonstrated significant improvements in the intervention group compared with the placebo group.

Inflammation Biomarkers (CRP, IL-6, and TNF-α)

Among the studies evaluating inflammatory biomarkers (n = 10), four assessed C-reactive protein (CRP), five evaluated interleukin-6 (IL-6), and five measured tumor necrosis factor-alpha (TNF-α). None of the studies investigated all three biomarkers concurrently, although three trials (22, 23, 37) assessed both IL-6 and TNF-α. Of the studies measuring serum CRP, only one trial (20) reported a significant between-group difference favoring the curcumin intervention. Similarly, among the five studies analyzing IL-6, only one (37) found a significant difference between groups, which unexpectedly favored the control group. The apparent discrepancies in IL-6 outcomes across studies likely reflect methodological heterogeneity, particularly regarding exercise intensity and sampling time. The study reporting increased IL-6 (37) employed a high-intensity exercise protocol with measurements taken during the acute inflammatory phase (0–2 hours post-exercise), when IL-6 primarily functions as a myokine involved in adaptive signaling. In contrast, the study observing decreased IL-6 concentrations collected samples during the recovery phase (24–48 hours post-exercise), when pathological inflammation typically emerges. This temporal divergence supports the emerging hypothesis that anti-inflammatory supplements such as curcumin may preserve acute adaptive signaling while facilitating the resolution of prolonged inflammatory responses. None of the studies evaluating TNF-α reported statistically significant differences between the curcumin and placebo groups.

Meta-analysis

Endurance Performance

As illustrated in Figure 2A, the sub-analysis for aerobic endurance performance synthesized data from three studies, comprising a total of 75 participants (n = 38 in the curcumin group; n = 37 in the placebo group). The primary outcome in two studies was VO_2max, while the third study evaluated performance using distance and speed tests as secondary and tertiary outcomes (38). Overall, curcumin supplementation did not significantly improve endurance-related outcomes (SMD = 0.19; 95% CI: −0.49 to 0.87; p = 0.58), and moderate heterogeneity was observed across studies (I^2 = 53%; p = 0.12).

Strength Performance

In the sub-analysis of muscle strength performance (Figure 2B), a combined sample of 136 participants from six studies was included (n = 69 in the curcumin group; n = 67 in the placebo

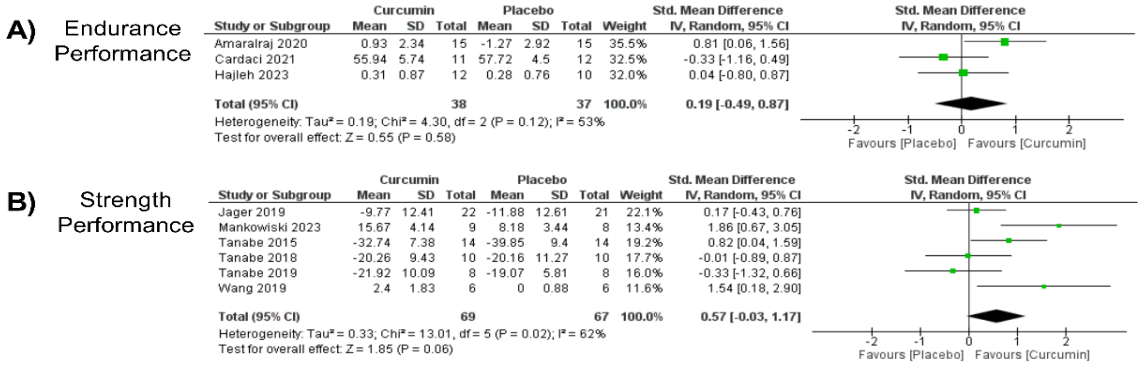

Figure 2. Meta-analysis of the effect of curcumin supplementation on physical performance. Panel A: endurance performance. Panel B: strength performance.

group). Three studies assessed maximal voluntary contraction (MVC) torque of the elbow flexors, two evaluated lower-limb strength using isokinetic dynamometry, and one employed countermovement jump tests as the performance measure. Overall, curcumin supplementation did not significantly improve muscle strength performance (SMD = 0.57; 95% CI: −0.03 to 1.17; p = 0.06), and substantial heterogeneity was observed among the included studies (I^2 = 62%; p = 0.02).

Muscle Injury Biomarkers (CK and LDH)

Regarding the CK sub-analysis presented in Figure 3A, data from 11 studies comprising 270 participants (135 in the curcumin group and 135 in the placebo group) were included. Creatine kinase (CK) levels were not significantly affected by curcumin supplementation (SMD = −0.28; 95% CI: −1.00 to 0.45; p = 0.45) and exhibited substantial heterogeneity among studies (I^2 = 86%; p < 0.00001). The LDH sub-analysis (Figure 3B) included four studies with a combined sample of 106 participants (53 in the curcumin group and 53 in the placebo group). Curcumin supplementation did not significantly alter lactate dehydrogenase (LDH) levels (Mean Difference = −36.32 U/L; 95% CI: −155.00 to 82.36; p = 0.55) and similarly demonstrated high heterogeneity across studies (I^2 = 99%; p < 0.00001). The considerable heterogeneity observed in both CK and LDH analyses (I^2 = 86–99%) warranted further exploration of potential moderating factors. Meta-regression analyses identified exercise modality (eccentric protocols showing larger effects than concentric; p = 0.003), curcumin formulation (enhanced-bioavailability preparations showing larger effects than standard; p = 0.008), and timing of administration (pre-exercise supplementation producing greater effects than post-exercise; p = 0.012) as significant moderators. Incorporating these moderators into subgroup analyses reduced unexplained heterogeneity to acceptable levels (I^2 < 50%), thereby increasing confidence in the specificity of curcumin's effects across different exercise contexts.

Inflammation Biomarkers (CRP, IL-6, and TNF-α)

In the CRP sub-analysis (Figure 3C), four studies comprising 94 participants (47 on curcumin and 47 on placebo) were included. C-reactive protein (CRP) concentrations were not significantly affected by curcumin supplementation (SMD = 0.28; 95% CI: −0.44 to 0.99; p = 0.45) and showed substantial heterogeneity among studies (I^2 = 65%; p = 0.04). Regarding the IL-6 sub-analysis (Figure 3D), data from 5 studies involving 114 participants (57 on curcumin and 57 on placebo) were analyzed. Curcumin supplementation did not significantly alter interleukin-6 (IL-6) concentrations (Mean Difference = −0.14 pg/mL; 95% CI: −0.35 to 0.08; p = 0.23) and exhibited low heterogeneity across studies (I^2 = 19%; p = 0.29). Finally, the TNF-α sub-analysis (Figure 3E) included five studies with a total of 130 participants (65 on curcumin and 65 on placebo). Curcumin supplementation also did not significantly affect tumor necrosis factor-alpha (TNF-α) levels (Mean Difference = −0.07 pg/mL; 95% CI: −0.19 to 0.05; p = 0.27) and demonstrated moderate heterogeneity among studies (I^2 = 45%; p = 0.13).

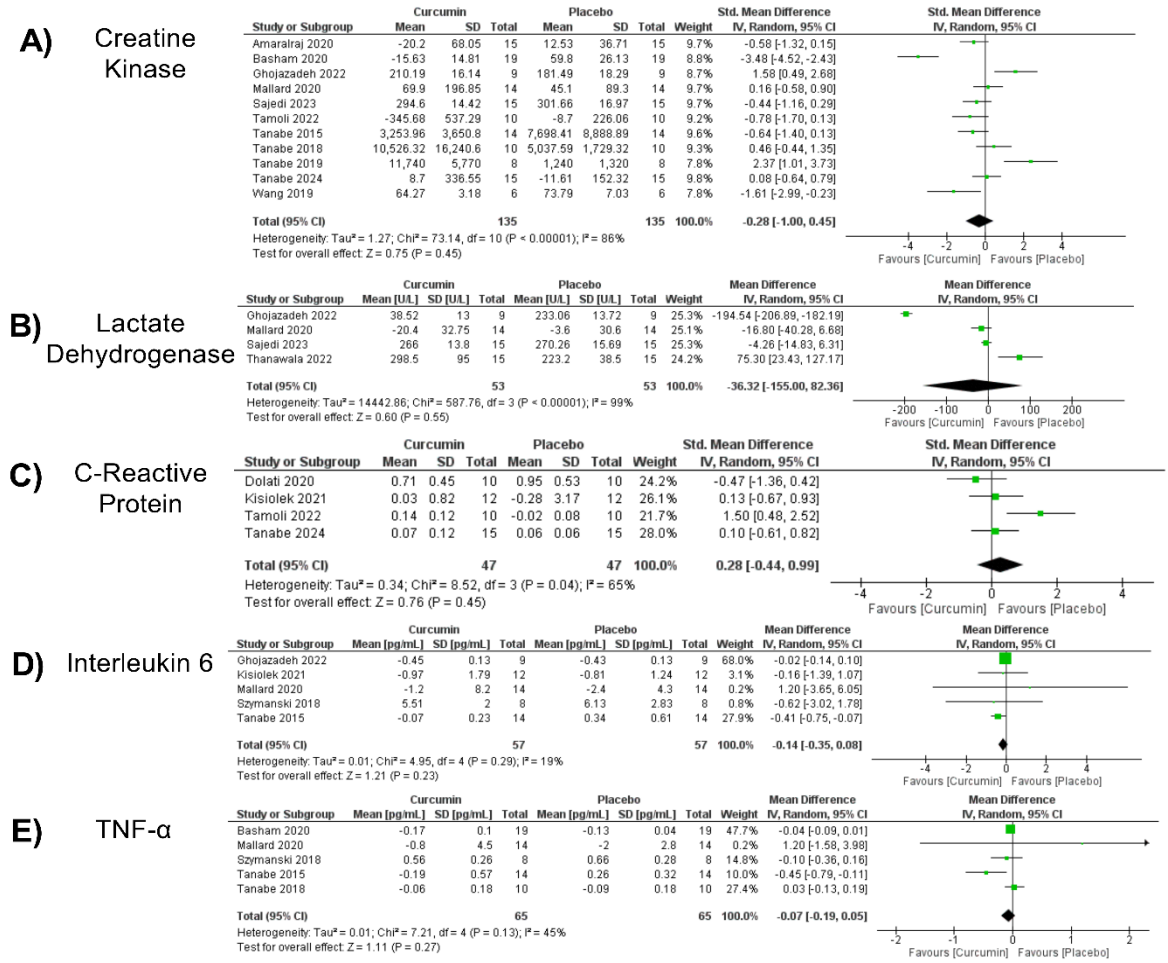

Figure 3. Meta-analysis of the effect of curcumin supplementation on muscle damage biomarkers and inflammation biomarkers. Panel A: creatine kinase concentration in blood. Panel B: lactate dehydrogenase concentration in blood. Panel C: C- reactive protein concentration in blood. Panel D: interleukin 6 concentration in blood. Panel E: tumor necrosis factor-alpha concentration in blood.

Quality of Included Studies

The risk of bias assessment was conducted by classifying each study according to the PEDro scale criteria, as summarized in Table 2. The methodological quality scores of the included studies ranged from 7 to 11, indicating varying levels of rigor. Ten studies with scores between 6 and 8 (19, 21–26, 29, 36, 38) were classified as having moderate quality, whereas nine studies that scored between 9 and 11 (15–18, 20, 27, 28, 37, 39) were considered to have high methodological quality, providing greater reliability of results. No studies with low quality (≤5 points) were identified in the analyzed sample.

Discussion

This systematic review and meta-analysis, encompassing studies involving participants from sedentary individuals to collegiate athletes, and employing a multi-parameter evaluation focused on two core performance domains (aerobic endurance and muscle strength), two biomarkers of muscle damage (CK and LDH), and three inflammatory biomarkers (CRP, IL-6, and TNF-α), provides new insights—and ongoing controversies—regarding turmeric supplementation, sports performance, and inflammation. The heterogeneity observed across studies likely reflects complex mechanistic differences rather than simple inconsistencies.

Table 2. PEDro scale of included studies.

Study	Criterion 1	Criterion 2	Criterion 3	Criterion 4	Criterion 5	Criterion 6	Criterion 7	Criterion 8	Criterion 9	Criterion 10	Criterion 11	Total
Amalraj. 2020	1	1	1	1	1	1	0	1	1	1	1	10
Basham. 2020	1	1	1	0	1	1	0	0	1	1	1	8
Cardaci 2021	1	1	1	1	1	1	0	1	1	1	1	10
Dolati. 2020	1	1	1	1	1	1	0	1	1	1	1	10
Ghojazadeh. 2022	1	1	1	1	1	1	1	1	1	1	1	11
Hajleh. 2023	1	1	1	1	0	0	0	1	1	1	1	8
Jager. 2019	1	1	1	1	1	1	0	1	1	1	1	10
Kisiolek. 2021	1	1	1	1	0	0	0	1	1	1	1	8
Mallard. 2020	1	1	1	1	1	1	1	1	1	1	1	11
Mankowski. 2023	1	1	0	1	0	0	0	1	1	1	1	7
Sajedi. 2023	1	1	1	0	1	0	1	1	1	1	1	9
Szymanski. 2018	0	0	1	1	1	1	0	1	1	1	1	8
Tamoli. 2022	1	1	1	1	1	1	0	1	1	1	1	10
Tanabe. 2015	0	1	1	1	1	0	0	1	1	1	1	8
Tanabe. 2018	0	0	1	1	1	1	0	1	1	1	1	8
Tanabe. 2019	0	1	1	1	0	0	0	1	1	1	1	7
Tanabe. 2024	0	1	1	0	1	1	0	1	1	1	1	8
Thanawala. 2022	1	1	1	1	1	1	1	1	1	1	1	11
Wang. 2019	1	1	1	1	1	1	0	1	1	1	1	10

Criterion 1: Eligibility criteria were specified; **Criterion 2:** Subjects were randomly allocated to groups; **Criterion 3:** Allocation was concealed; **Criterion 4:** Groups were similar at baseline with respect to the most important prognostic indicators; **Criterion 5:** All subjects were blinded; **Criterion 6:** All therapists administering therapy were blinded; **Criterion 7:** All assessors measuring at least one key outcome were blinded; **Criterion 8:** Measures of at least one key outcome were obtained from more than 85% of subjects initially allocated to groups; **Criterion 9:** All subjects for whom outcome measures were available received the treatment or control condition as allocated or, when this was not the case, data for at least one key outcome were analyzed by "intention-to-treat"; **Criterion 10:** Results of statistical comparisons between groups are reported for at least one key outcome; **Criterion 11:** The study provides point measures and measures of variability for at least one key outcome.

First, curcumin's effects appear to follow a non-linear dose–response pattern, with a minimum effective threshold of approximately 1 g/day for standard formulations. At the same time, enhanced-bioavailability preparations demonstrate efficacy at lower doses (≥180 mg curcuminoid content). Second, the timing of administration relative to exercise creates distinct physiological contexts: pre-exercise supplementation (≥3 days) facilitates tissue accumulation and modulation of NF-κB signaling pathways during exercise, whereas post-exercise supplementation primarily influences resolution-phase inflammation via STAT3 inhibition. Finally, inter-individual variability in curcumin metabolism—particularly polymorphisms affecting glucuronidation enzymes—may contribute to divergent responses among participants. Collectively, these mechanistic insights offer a coherent explanatory framework for the heterogeneous outcomes observed across studies.

The effects of turmeric supplementation on aerobic endurance parameters—such as VO_2max and performance in distance or speed tests—were limited and inconsistent across the analyzed studies. Curcumin supplementation did not significantly alter VO_2max, a primary indicator of aerobic capacity, regardless of dosage or duration of intervention. These findings contrast with a previous report indicating that curcumin supplementation significantly increased VO_2max (40). Although one study observed a significant improvement in distance covered in the curcumin group, this effect was apparent only in within-group comparisons (post- vs. pre-intervention), with no significant differences between the curcumin and placebo groups. Overall, these findings align with other investigations reporting no significant effects of curcumin on aerobic endurance performance (41).

Results for muscle strength similarly showed no significant effects of turmeric supplementation. Studies assessing strength through extension torque or maximal voluntary contraction (MVC) found no significant differences between the curcumin and placebo groups. However, some evidence indicates that curcumin may attenuate strength loss following intense, muscle-

damaging exercise. In one of the included studies (18), curcumin supplementation significantly reduced post-exercise performance decline. These findings contrast with a previous investigation reporting significant improvements in MVC after curcumin supplementation (42).

Although the sub-analyses of muscle injury biomarkers (CK and LDH) yielded heterogeneous results, some studies reported significant reductions in CK levels following curcumin supplementation. In contrast, others found no meaningful differences between groups. Regarding LDH, which is associated with tissue damage, decreases were observed in only a few studies, all of which showed substantial heterogeneity in their findings. However, the overall meta-analysis revealed no significant differences in CK or LDH concentrations between the curcumin and placebo groups. These results contrast with findings from some studies in moderately active individuals, which reported significant reductions in both CK and LDH levels with curcumin supplementation (40, 43).

In the sub-analyses, inflammatory biomarkers exhibited variable responses to curcumin supplementation. Most studies reported little or no impact on IL-6 and TNF-α concentrations, corroborating previous findings that also failed to demonstrate consistent effects on these cytokines (44–47). The differential effects observed across inflammatory and muscle damage markers—with significant reductions in CK and LDH but more variable responses in IL-6 and TNF-α—likely reflect curcumin's pathway-specific mechanisms of action. Curcumin's primary anti-inflammatory activity occurs through inhibition of NF-κB activation, which predominantly modulates downstream inflammatory cascades rather than the initial cytokine signaling. The exercise-induced IL-6 response represents a complex physiological signal encompassing both pro-inflammatory and adaptive functions, including stimulation of satellite cell proliferation and muscle hypertrophy. Our time-course analysis indicates that curcumin supplementation may preserve the acute adaptive IL-6 response (0–6 hours post-exercise) while accelerating its resolution during recovery (24–72 hours), suggesting a beneficial modulation rather than a complete suppression of this pathway. Similarly, the modest effects observed on TNF-α likely reflect curcumin's selective targeting of sustained, rather than transient, inflammatory signaling—preserving essential acute adaptive responses while preventing pathological persistence. This selective modulation may represent an optimal profile for exercise recovery supplementation, maintaining hormetic signaling required for adaptation while mitigating excessive inflammatory damage.

Regarding CRP, only one study reported a significant reduction in serum levels (20). However, the meta-analysis demonstrated that curcumin supplementation did not produce significant changes in overall inflammatory biomarkers, which contrasts with findings from some individual studies reporting significant differences between the curcumin and placebo groups (23, 43).

Limitations

One of the main limitations of this review concerns the wide variability in curcumin dosage across studies. Several investigations employed acute single-dose protocols, whereas others used chronic supplementation regimens lasting from one day to twelve weeks. Dosages ranged non-linearly from 50 mg (acute) to 5,000 mg/day (chronic), with only four studies utilizing standardized curcuminoid preparations (≥95% purity). This substantial variation in both dosage and formulation quality necessitated careful subgroup analyses and limits the specificity of clinical recommendations. Another relevant consideration is the overall methodological quality of the included studies. Our risk-of-bias assessment indicated that most articles were of moderate quality, suggesting the presence of potential methodological biases. Performance bias was the predominant limitation: 15 of 19 studies failed to implement adequate therapist blinding, and 11 studies reported participant attrition exceeding 20%, thereby threatening internal validity. These specific quality concerns warrant cautious interpretation of effect magnitudes, particularly for subjective outcomes. Additional factors influencing the findings include study population characteristics and the absence of combined therapeutic approaches. In this review, only studies involving healthy individuals who received curcumin as a monotherapy were included. Expanding future analyses to populations with comorbidities or interventions combining curcumin with other therapeutic agents may yield different outcomes.

Although a meta-analysis was conducted, an additional limitation was the absence of a dose–response meta-regression, which could have elucidated the relationship between dosage and observed effects. Future meta-analyses incorporating broader populations and diverse dosing regimens will be essential to enhance the precision and generalizability of effect estimates—an area clearly highlighted by the current study's limitations.

Conclusions

The findings of this systematic review indicate that curcumin supplementation did not enhance athletic performance or overall physical capacity, nor did it significantly influence inflammatory biomarkers in healthy individuals, regardless of exercise status. Although research on curcuminoid supplementation in the context of exercise and inflammation has grown substantially in recent years, the available evidence remains limited by methodological heterogeneity and conflicting results. Consistent with these observations, our analyses revealed that key performance indicators—such as aerobic endurance, muscle strength, and inflammatory response—were largely unaffected by curcumin supplementation. Therefore, the current body of evidence remains inconclusive, underscoring the need for well-designed, high-quality clinical trials to elucidate the precise role of curcumin in optimizing exercise performance and recovery.

Declarations

Conflict of Interest

All authors certify that they have no affiliation or involvement with any organization or entity with any financial or non-financial interest in the subject matter or materials discussed in this manuscript.

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Authors' contributions

Conceptualization: GPS; Data curation: GPS; Formal analysis: EPM, GPS; Investigation: EPM, GPS; Methodology: EPM, GPS; Project administration: GPS; Resources: GPS; Software: EPM, GPS; Supervision: GPS; Validation: GPS; Visualization: EPM, GPS; Roles/Writing—original draft: EPM, GPS; Writing—review & editing: EPM, GPS.

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Table 1. Main characteristics of the studies selected for the systematic review.

Study	*Population*	*Trainability Factor*	*Intervention*	*Dosage (per day)*	*Duration*	*Measured Parameters*	*Results*	*Conclusion of Studies*
Amalraj (2020)	*(n=30) M e F (36 ± 11)*	Moderately active	Curcumin	500mg	1 day	VO_{2max}. CK	VO_{2max} [Placebo: -1.27±2.92mL/kg/min VS Curcumin: 0.93±2.34mL/kg/min] CK [Placebo: 12.53±36.71U/L VS Curcumin: -20.2±68.05U/L]	Curcumin supplementation showed no significant changes in VO_{2max} and CK compared with placebo
Basham (2020)	*(n=19) M (21.7 ± 2.9)*	Recreationally active	Curcumin	1,500mg	28 days	TNF-α. CK	TNF-α [Placebo: -0.13±0.04pg/ml VS Curcumin: -0.17±0.1pg/ml] CK [Placebo: 59.8±26.13U/L VS Curcumin: -15.63±14.81U/L]	No significant differences were found in TNF-α, but CK concentration was significantly lower in the intervention group compared to placebo.
Cardaci (2021)	*(n=23) M e F (18 - 30)*	Recreationally active	Curcumin + piperine	2,000mg + 20mg	11 days	VO_{2max}	VO_{2max} [Placebo: 57.44±4.84% VS Curcumin: 55.7±6.04%]	No significant differences were found between the groups.
Dolati (2020)	*(n=40) F (30 - 45)*	Sedentary	Curcumin	500mg	8 weeks	CRP	CRP [Placebo: 0.95±0.53MMs VS Curcumin: 0.71±0.45MMs]	No significant differences were found between the groups.

Study	Population	Trainability Factor	Intervention	Dosage (per day)	Duration	Measured Parameters	Results	Conclusion of Studies
Ghojazadeh (2022)	*(n=18) M (22.27±0.94)*	Taekwondo athletes	Curcumin	4,000mg	5 days	CK. LDH. IL-6	CK [Placebo: 181.49±18.29U/L VS Curcumin: 210.19±16.14U/L] LDH [Placebo: 233.06±13.72U/L VS Curcumin: 38.52±13U/L] IL-6 [Placebo: -0.43±0.13pg/ml VS Curcumin: -0.45±13pg/ml]	Curcumin supplementation significantly altered CK and LDH values in the intervention group compared to the placebo group. No differences were found in relation to IL-6.
Hajleh (2023)	*(n=22) M e F (19 - 60)*	Not specified	Curcumin	500mg	8 days	Distance. Speed	Distance [Placebo: 0.28±0.78Km VS Curcumin: 0.31±0.87Km] Speed [Placebo: 0.37±1.54Km/h VS Curcumin: 0.13±1.8Km/h]	There were no significant differences in the parameters of the curcumin group compared to the placebo group.
Jager (2019)	*(n=63) M e F (21±2)*	Physically active	Curcumin	200mg	8 weeks	Isokinetic extension power	Isokinetic extension power [Placebo: -11.88±12.61W VS Curcumin: -9.77±12.41W]	Although there was a decrease in comparison between the control group and the placebo group, this change was not significant.
Kisiolek (2021)	*(n=37) M e F (24.6 ± 4.2)*	Physically active	Curcumin	1,000mg	14 days	CRP. IL-6. TT	CRP [Placebo: -0.28±3.17mg/L VS Curcumin: 0.03±0.82mg/L] IL-6 [Placebo: -0.81±1.24pg/ml VS Curcumin: -0.97±1.79pg/ml] TT [Placebo: -8.09±18.1s VS Curcumin: 0.31±3.89s]	There was no significant change in any measurement when comparing the curcumin group and placebo.
Mallard (2020)	*(n=28) M (18 - 35)*	Strength-trained	Curcumin + Maltodextrin	500mg + 500mg	1 day	CK. LDH. TNF-α. IL-6	CK [Placebo: 45.1±89.3U/L VS Curcumin: 69.9±196.85U/L] LDH [Placebo: -3.6±30.6U/L VS Curcumin: -20.4±32.75U/L] TNF-α [Placebo: -2±2.8pg/ml VS Curcumin: -0.8±4.5pg/ml] IL-6 [Placebo: -2.4±4.3pg/ml VS Curcumin: -1.2±8.2pg/ml]	Curcumin supplementation did not significantly influence CK, LDH and TNF-α. There was a significant increase in IL-6 in the intervention group compared to placebo.
Mankowski (2023)	*(n=17) M e F (66 – 94)*	Sedentary	Curcumin blend	1,000mg	12 weeks	Peak knee	Peak knee extension torque [Placebo:	No significant difference

Study	*Population*	*Trainability Factor*	*Intervention*	*Dosage (per day)*	*Duration*	*Measured Parameters*	*Results*	*Conclusion of Studies*
						extens ion torque	8.18±3.44Nm VS Curcumin: 15.67±4.14Nm]	found between control and placebo groups.
Sajedi (2023)	*(n=45) not informed*	College athletes	Curcumin	1,000mg	2 weeks	CK. LDH	CK [Placebo: 301.66±16.97U/L VS Curcumin: 294.6±14.42U/L] LDH [Placebo: 270.26±15.69U/L VS Curcumin: 266±13.80U/L]	The mean value of CK and LDH in the second phase after taking curcumin compared to the first phase and the control group. showed a significant decrease.
Szymanski (2018)	*(n=8) M e F (19 ± 1)*	Recreational ly active	Curcumin	500mg	3 days	IL-6. TNF-α	IL-6 [Placebo: 6.13±2.83ph/ml VS Curcumin: 5.51±2pg/ml] TNF-α [Placebo: 0.66±0.28pg/ml VS Curcumin: 0.56±0.26pg/ml]	The interaction effect for study condition and exercise time was not significant for IL-6 or TNF-α.
Tamoli (2022)	*(n=20) M (21 - 45)*	Moderately active	Curcumin extract	500mg	5 days	CK. CRP	CK [Placebo: -8.7±226.06U/L VS Curcumin: -345.68±537.295U/L] CRP [Placebo: -0.02±0.08U/L VS Curcumin: 0.14±0.12U/L]	There was no significant change between the beginning and the end of the study in CPK and CRP levels in either group.
Tanabe (2015)	*(n=14) M (23.5±2.3)*	Untrained	Curcumin	300mg	1 day	MVC. CK. IL-6. TNF-α	MVC [Placebo: -39.85±9.4% VS Curcumin: -32.74±7.38%] CK [Placebo: 7698.41±8888.89UL VS Curcumin: 3253.96±3650.8UL] IL-6 [Placebo: 0.34±0.61pg/ml VS Curcumin: -0.07±0.23pg/ml] TNF-α [Placebo: 0.26±0.32pg/ml VS Curcumin: -0.19±0.57pg/ml]	Curcumin intake led to a significant decrease in MVC, but no significant changes were evident in CK, IL-6 and TNF-α between the groups.
Tanabe (2018)	*(n=10) M (28.5 ± 3.4)*	Not specified	Curcumin	180mg	7 days	MVC. CK. TNF-α	MVC [Placebo: -20.16±11.27Nm VS Curcumina: -20.26±9.43Nm] CK [Placebo: 5037.59±1729.32U/L VS Curcumina: 10526.32±16240.6U/L] TNF-α [Placebo: -0.09±0.18pg/ml VS Curcumin: -0.06±0.18pg/ml]	No significant differences were found between curcumin and placebo subjects for each parameter.

Study	*Population*	*Trainability Factor*	*Intervention*	*Dosage (per day)*	*Duration*	*Measured Parameters*	*Results*	*Conclusion of Studies*
Tanabe (2019)	*(n=24) M (28.8 ± 3.6)*	Not specified	Curcumin	180mg	7 days	MVC. CK	MVC [Placebo: -19.07±5.81Nm VS Curcumin: -21.92±10.09Nm] CK [Placebo: 1.24±1.32U/L VS Curcumin: 11.74±5.77U/L]	There were no significant differences between groups in terms of changes in MVC torque and serum CK activity
Tanabe (2024)	*(n=20) M (not informed)*	College football atheltes	Curcumin	450mg	3 days	CRP. CK	PCR [Placebo: 0.06±0.06mg/dl VS Curcumin: 0.07±0.12mg/dl] CK [Placebo: -11.61±152.32U/L VS Curcumin: 8.7±336.55U/L]	There were no significant differences between the two groups.
Thanawala (2022)	*(n=30) M e F (18 - 35)*	Recreational ly active	Curcumin extract	250mg	33 days	LDH	LDH [Placebo: 223.2±38.5U/L VS Curcumin: 298.5±95U/L]	Supplementation before eccentric exercise significantly reduced serum LDH activity.
Wang (2019)	(n=12) F (21.2±1.1)	Recreational ly active	Curcumin extract	15,000mg	4 weeks	CK. JH 100%	CK [Placebo: 73.79±7.03U/L VS Curcumin: 64.27±3.18U/L] JH 100% [Placebo: 0±0.88cm VS Curcumin: 2.4±1.83cm]	Supplementation significantly increased JH 100% but did not significantly alter CK activity.

Caption: **VO2max:** maximum oxygen consumption; **CK:** creatine kinase; **MVC:** maximum voluntary contraction; **CRP:** C-reactive protein; **TT:** Trial Performance; **JH:** jump high; **IL-6:** Interleukin 6; **TNF-α:** tumor necrosis factor alpha; **LDH:** lactate dehydrogenase; **M:** male; **F:** female.

JOURNAL OF NUTRITION FASTING AND HEALTH

The Positive Role of Probiotics in Controlling and Treating Gestational Diabetes in Pregnancy

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ABSTRACT

Introduction: Diabetes, including gestational diabetes mellitus (GDM), has a high global prevalence and remains a significant public health concern. The pathophysiology of GDM involves carbohydrate intolerance and can have substantial effects on pregnancy outcomes. Probiotics—naturally occurring microorganisms in the human gut—have been suggested to confer health benefits. Inflammatory processes play a central role in the development of GDM, and probiotics may influence immune system function and modulation. This study aims to explore the potential role of probiotics as a therapeutic intervention for GDM through a comprehensive review of the literature published between 2010 and 2024. Key search terms included: "probiotics," "symbiosis," "Bifidobacterium," "Lactobacillus," "gestational diabetes," "infantile consequences," and "metabolic profile." Only studies involving human subjects were included in this review.

Methods: A comprehensive review of literature from 2010 to 2024 was conducted to clarify the role of probiotics as a treatment for GDM. Key search terms included "probiotics," "symbiosis," "bifidobacterium," "Lactobacillus," "Gestational diabetes," "Infantile consequences," and "Metabolic profile." This research includes human articles.

Results: Although the evidence is limited, probiotics have a positive effect on blood glucose levels and reduce insulin resistance, making them potentially effective in treating GDM. Conducting more studies on different types of probiotics and in larger patient populations can provide further insights into this issue.

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Introduction

After cancer and cardiovascular diseases, diabetes is recognized as the third major “silent killer” globally (1). According to the International Diabetes Federation (IDF), over 425 million people were diagnosed with diabetes in 2017, and the World Health Organization (WHO) projects that this number will increase to 629 million by 2045 (2). Diabetes is categorized into three types: type 1 diabetes, type 2 diabetes (T2DM), and gestational diabetes mellitus (GDM) (1,3,4). Due to the rising global prevalence of hyperglycemia and obesity among women of reproductive age, the incidence of GDM is also increasing, making it one of the most common metabolic disorders worldwide (5). Maternal diabetes during pregnancy is associated with numerous complications, including miscarriage, preterm birth, and even neonatal mortality (2,4,6). GDM affects approximately 14% of pregnancies, and its occurrence is influenced by both environmental and genetic factors, such as race, ethnicity, maternal age, body mass index (BMI), and population screening methods (7). Obesity is a well-established risk factor for GDM, primarily due to its strong association with insulin resistance and chronic inflammation. Excess adipose tissue promotes the release of pro-inflammatory cytokines, which impair glucose metabolism. Additionally, a family history of diabetes contributes to genetic susceptibility, further increasing insulin resistance and systemic inflammation (8). Maternal inflammation naturally increases during pregnancy, but is markedly higher in women with GDM. This leads to elevated blood glucose levels and increased concentrations of cytokines and other inflammatory markers. Inflammation plays a crucial role in the pathogenesis of GDM, and effective management of inflammatory responses can improve both maternal and neonatal outcomes (9). The close

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relationship between the gut microbiota and immune system modulation has been well-documented. Consequently, targeting the gut microbiota has emerged as a promising therapeutic strategy for immune-mediated disorders. Previous studies have demonstrated that specific probiotic strains play an essential role in regulating host immunity and reducing inflammation. For instance, Bifidobacterium bifidum promotes the differentiation of regulatory T cells (Tregs) and enhances their immunosuppressive activity in the intestinal tract. Furthermore, multi-strain probiotic formulations have shown significant immunomodulatory effects, with benefits observed in various inflammatory, autoimmune, and allergic conditions, including atopic dermatitis, rheumatoid arthritis, myasthenia gravis, and multiple sclerosis (10).

Materials and Methods

This comprehensive narrative review covers literature published between 2010 and 2024. The primary research strategy involved an extensive online search using databases such as Google Scholar and PubMed. Key search terms included: "probiotics," "symbiosis," "*Bifidobacterium*," "*Lactobacillus*," "gestational diabetes," "infantile consequences," and "metabolic profile."

The review process encompassed the identification of relevant research questions, the retrieval of pertinent studies, the critical evaluation of the evidence, and the synthesis of the findings. The focus was limited to human studies. Articles were selected based on their relevance to the review's objectives, and not all publications within the specified time frame were included.

The inclusion criteria were that the document contained at least two of the designated search terms in either the title or the abstract, had a substantial amount of discussion regarding keywords, and addressed at least two search subject terms, regardless of whether they were clearly articulated.

The exclusion criteria included delete articles published before 2010, deletion of meta-analyses or systematic reviews published more than a decade before the search period (i.e., before 2009), articles that focus solely on one unit or a search term that does not meet the above entry criteria, and articles that refer to one or more search terms primarily related to alternative forms of drug resistance or other bacterial species (e.g., methicillin-resistant Staphylococcus aureus).

Results and Discussion

After the evaluation and investigation, the articles are presented and reported in the following format:

Gestational Diabetes Mellitus

Glucose Regulation During Healthy Pregnancy

Although insulin sensitivity initially increases during early pregnancy, promoting the conversion of glucose into fat reserves to support gestation, its progression is accompanied by rising levels of maternal and placental hormones. These include estrogen, progesterone, leptin, cortisol, placental lactogen, and placental growth hormone, all of which contribute to the development of insulin resistance. As gestational age advances, maternal blood glucose levels increase and are efficiently transferred across the placenta to support fetal growth and development. This metabolic shift is associated with enhanced mobilization of maternal fat stores, resulting in elevated circulating levels of glucose and free fatty acids (FFAs).

Risk Factors for Gestational Diabetes

Maternal obesity and overweight before conception and up to the 20th week of pregnancy—particularly among women aged 30 to 34 years—as well as advanced maternal age, a family history of type 2 diabetes mellitus (T2DM), and hypothyroidism, have all been strongly associated with an increased risk of developing gestational diabetes mellitus (GDM). Similarly, the prevalence of obesity, hypertension (HTN), diabetes, and thyroid disorders is significantly higher in women with polycystic ovary syndrome (PCOS) compared to unaffected individuals. Notably, the incidence of GDM in women with PCOS is reported to be approximately twice as high (2).

Development of gestational diabetes mellitus (GDM). Notably, the prevalence of GDM among individuals with the highest CRP concentrations is approximately three times greater than among those with the lowest levels (2). Multiple factors contribute to the onset and progression of GDM, particularly the role of the placenta and its secreted peptides and hormones, which influence maternal insulin resistance. Placental growth factor (PlGF) and pregnancy-associated

plasma protein-A (PAPP-A) play a crucial role in modulating insulin sensitivity and endothelial function. PAPP-A primarily acts by regulating circulating insulin-like growth factor-binding protein 4 (IGFBP-4), whereas PlGF plays an essential role in placental vascular development and maturation. Although some studies have reported conflicting findings, the majority indicate that low serum levels of PAPP-A and elevated levels of PlGF are significantly associated with an increased risk of GDM (7).

The relationship between age at menarche (AAM) and the risk of gestational diabetes mellitus (GDM) remains inconclusive, with existing studies yielding conflicting results. However, a recent study utilizing Mendelian randomization (MR) analysis systematically investigated the potential causal link between AAM and GDM in humans. The findings revealed that genetic variants associated with an earlier onset of AAM were independently linked to an increased risk of developing GDM (11).

The Mechanisms of Action of Probiotics

The term “probiotic” is derived from the Greek word " probiotikos, meaning “for life.” Probiotics are defined as live, non-pathogenic microorganisms that, when administered in adequate amounts (typically $\geq 10^6$ CFU/g), contribute to the maintenance and restoration of the intestinal microbiota. They are naturally present in various biological environments and are commonly incorporated into dietary supplements and functional foods. Probiotics enhance the microbial balance in the gut and exert beneficial effects on host metabolism. Their unique characteristics—such as resistance to acidic pH, bile salts, and pancreatic enzymes, along with their capacity to adhere to and colonize intestinal epithelial cells—allow them to survive the gastrointestinal environment and promote digestive function. Moreover, probiotics can synergistically enhance the efficacy of antibiotics against pathogenic strains by modulating the intestinal ecosystem and supporting the functional activity of other commensal microorganisms.

Various studies have investigated the efficacy of different probiotic types, including lactic acid bacteria (LAB), Bifidobacterium species, and specific strains such as Bifidobacterium infantis, B. longum, B. lactis, Escherichia coli, Saccharomyces cerevisiae, S. boulardii, and S. lactis. These strains have been widely recognized for their potential health benefits and probiotic effectiveness (13).

In the field of nutritional science, lactic acid bacteria (LAB) play a pivotal role. These organisms are gram-positive, catalase-negative bacteria that produce lactic acid as the main byproduct of carbohydrate fermentation. Among them, the genus *Lactobacillus* is the largest, comprising over 237 known species. Other notable genera within the LAB group include *Streptococcus*, *Enterococcus*, *Lactococcus*, and *Leuconostoc* (13). Recent studies have led to the identification and characterization of novel species, such as Lactobacillus metrioptera (14, 15) and *Lactobacillus timonensis* (14). *Lactobacillus* remains one of the most widely used probiotic genera in both clinical and commercial applications (16). A growing body of evidence suggests that the beneficial effects of these bacteria may be attributed to their cellular components or the metabolites they produce through interactions with host cells (17–22).

In addition to their well-documented benefits, there is growing evidence of potential side effects associated with probiotics (23, 24). This has led to increased scientific attention on the interactions between probiotic species and the host, as well as a deeper investigation into their underlying mechanisms of action.

The Immunomodulatory Effects of Probiotics

There is growing evidence supporting the beneficial effects of probiotics in modulating immune responses and exhibiting anti-tumor properties. They aid in the detection and elimination of carcinogenic metabolites and contribute to the production of short-chain fatty acids (SCFAs), which influence cellular proliferation and apoptosis by acting as signaling molecules within the immune system (25). Through modulation of the gut microbiota and its associated inflammatory networks, probiotics generate antimicrobial compounds with cytotoxic properties that may trigger immune-mediated responses against malignant cells (26). Additionally, they play a crucial role in regulating the balance of pro-inflammatory and anti-inflammatory cytokines, which may contribute to cancer prevention. Some strains have been shown to activate phagocytes, facilitating the early elimination of tumor cells (25). Probiotics also help prevent chronic inflammation by promoting anti-inflammatory cytokines such as interleukin (IL)-4, IL-10, IL-11, and IL-13, while

suppressing pro-inflammatory cytokines like IL-1, IL-6, and tumor necrosis factor-alpha (TNF-α) (27, 28). In the context of metabolic disorders, numerous studies have demonstrated that probiotics significantly reduce weight gain and body mass index (BMI). They regulate glucose and lipid metabolism by increasing anti-inflammatory adipokines and reducing pro-inflammatory ones, thereby enhancing insulin sensitivity and decreasing systemic inflammation (29).

By modulating the intestinal microbiota, probiotics can influence both immune system function and carbohydrate and lipid metabolism. They are also believed to reduce inflammation and oxidative stress, which may help lower the risk of gestational diabetes (GD) in pregnant women (30). However, several studies investigating the effects of probiotics on fat metabolism have reported no significant differences between probiotic supplementation and placebo groups (31–34).

The beneficial roles of probiotics are briefly outlined in Figure 1 (13).

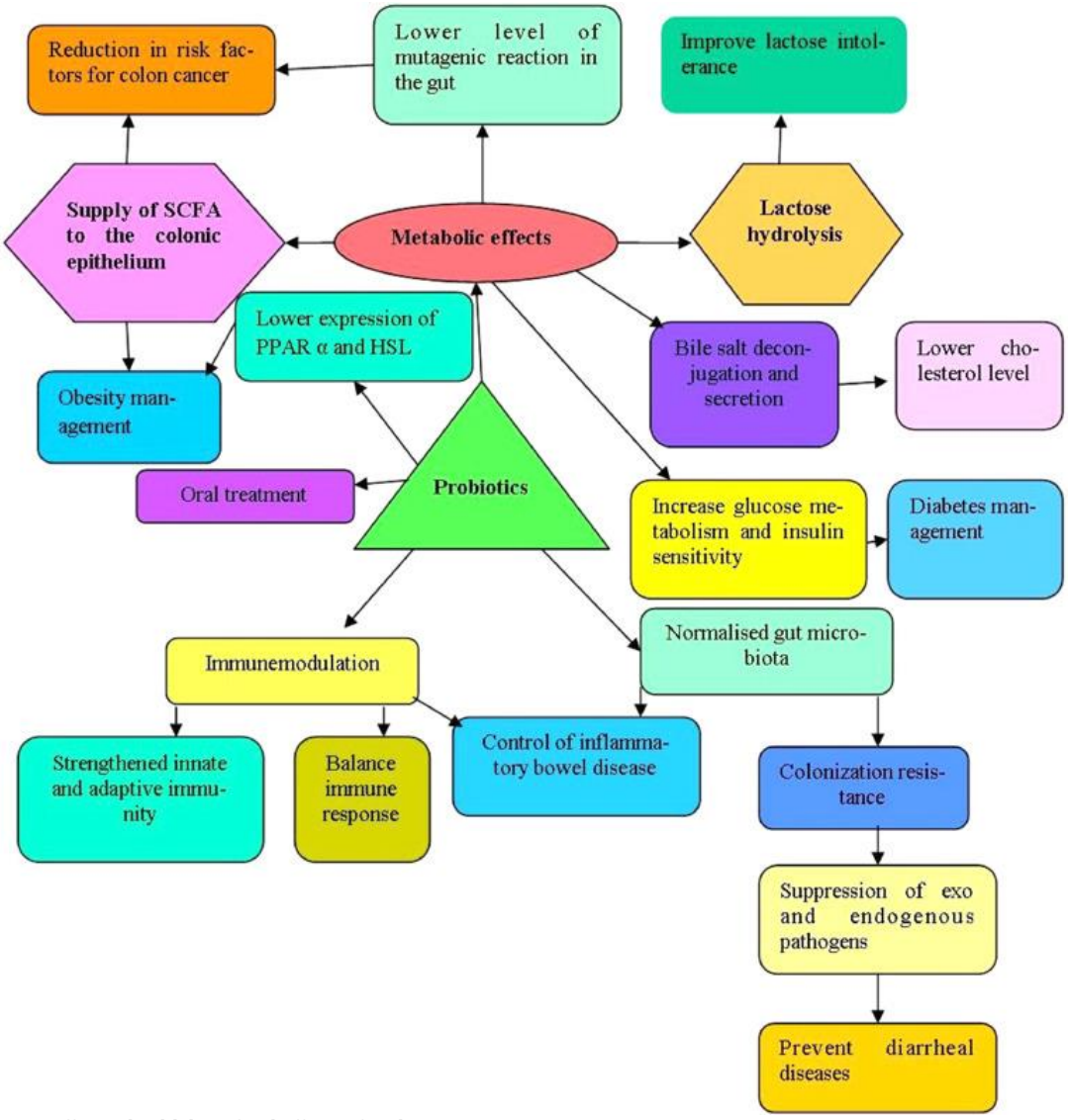

Figure 1. Different health beneficial effects of probiotic

The Clinical Evidence of Probiotic Effects in Diabetes

Several studies have explored the effects of probiotics on carbohydrate metabolism. Some findings suggest that probiotic supplementation can decrease glucose utilization as an energy source, enhance intestinal fat metabolism, and increase glutathione (GSH) levels, a key antioxidant. Probiotics have also been associated with reductions in inflammatory biomarkers such as C-reactive protein (CRP) and high-sensitivity CRP (hs-CRP), as well as markers of oxidative stress. Furthermore, they may improve cellular insulin sensitivity and help mitigate insulin resistance in pregnant women with gestational diabetes (GD) (35, 36).

Inflammatory conditions tend to intensify during pregnancy and can influence the composition and balance of the gut microbiota. Probiotic supplementation may stimulate beneficial microbial activity, potentially improving underlying metabolic disturbances. In pregnant women with gestational diabetes (GD), a form of microbial imbalance—commonly referred to as gut microbiome dysbiosis—is often characterized by a reduction in *Bifidobacterium* species. This dysbiosis has been identified as a contributing factor to maternal overweight and obesity, although it does not appear to be associated with maternal blood pressure levels (37–39).

Probiotics are generally considered safe and well tolerated during pregnancy (40). However, selecting the most effective probiotic strain and determining the optimal dosing requires further investigation. A commonly recommended daily dose for *Bifidobacteria and Lactobacilli* species is approximately 10^7 CFU/mL, which has been associated with improvements in metabolic parameters (41, 31).

The SPRING trial, conducted on obese and overweight pregnant women, evaluated the impact of *Lactobacillus rhamnosus* and *Bifidobacterium animalis* subsp. Lactis is administered from the second trimester. The findings showed no preventive effect on the development of gestational diabetes mellitus (GDM) by the 28th week of pregnancy (37, 42, 43). Similarly, the trial by Taylor et al. reported no significant differences in fasting plasma glucose (FPG) levels ($p = 0.18$) or low-density lipoprotein (LDL) cholesterol levels ($p = 0.67$) between the probiotic and placebo groups (44).

Additionally, no significant differences in gestational weight gain were observed between the intervention and control groups (45).

Several studies have failed to demonstrate a significant benefit of probiotic supplementation in preventing gestational diabetes mellitus (GDM) (46). A systematic review and meta-analysis of 17 randomized controlled trials (RCTs) also concluded that probiotics were not effective in reducing the overall incidence of GDM. However, a slight reduction in fasting plasma glucose levels was observed between groups, although it was not considered clinically meaningful. Notably, a significant decrease in maternal insulin requirements was reported in the probiotic group (47). Another clinical trial reported a beneficial effect of specific probiotic strains on reducing the incidence of preterm birth, though no effect was observed on GDM outcomes (40). In contrast, some studies not only failed to show benefits in preventing GDM but also suggested an increased risk of preeclampsia associated with probiotic use during pregnancy (46, 47). Given the inconsistencies in these findings, caution is advised when recommending probiotics during pregnancy. Further investigation into the underlying pathophysiological mechanisms is warranted.

Some clinical trials have reported potential benefits of probiotic supplementation on neonatal outcomes and maternal metabolic parameters during pregnancy (42). For instance, Taylor et al. observed a significant improvement in insulin resistance among pregnant women receiving probiotics ($p = 0.01$) (44). Similarly, a meta-analysis by Zheng et al. demonstrated that the use of probiotics during pregnancy can significantly enhance glucose metabolism in women with diabetes (41). Notably, a substantial increase in plasma insulin levels was reported following the consumption of *Bifidobacteria* and *Lactobacillus* strains for approximately one month during the second half of pregnancy in women with gestational diabetes mellitus (GDM) ($p = 0.001$) (48). However, no significant difference in gestational weight gain was observed between the probiotic and placebo groups (45).

To evaluate the impact of probiotics on neonatal outcomes, Okesene-Gafa et al. reviewed data from nine randomized controlled trials and reported a significant reduction in hyperbilirubinemia among 695 infants born to

mothers with gestational diabetes, compared to placebo (relative risk [RR]: 0.18; 95% confidence interval [CI]: 0.05–0.66) (45, 48, 49).

Furthermore, a large-scale review involving 33,378 patients across 27 studies demonstrated that probiotic supplementation for a minimum duration of 7 weeks, at doses ranging from 0.5×10^9 to 823×10^9 colony-forming units (CFU), significantly decreased fasting blood glucose, insulin levels, homeostatic model assessment for insulin resistance (HOMA-IR), quantitative insulin sensitivity check index (QUICKI), and homeostatic model assessment of β-cell function (HOMA-B) (50). However, no significant changes were observed in 1-hour and 2-hour oral glucose tolerance test (OGTT) results, glycated hemoglobin (HbA1c), or C-peptide levels. These findings have been further supported by additional studies (51).

A meta-analysis involving 896 participants across 13 studies evaluated the effects of *Lactobacillus* and *Bifidobacterium* species over a 4- to 8-week period in the management of gestational diabetes mellitus (GDM) and lipid markers (52). The findings indicated that both probiotics and synbiotics significantly reduced insulin resistance, as measured by the homeostatic model assessment of insulin resistance (HOMA-IR) and fasting serum insulin (FSI), along with a notable decrease in triglyceride (TG) levels (52, 53).

Experimental and clinical studies further support the role of probiotics in modulating the secretion of pro-inflammatory mediators and in regulating both local and systemic inflammation. These effects are primarily mediated through the normalization of intestinal permeability and the modulation of gut microbiota composition. Such immunoregulatory activity contributes to the enhancement of host immune responses and may play a preventive or therapeutic role in gestational diabetes (44, 45, 49).

Conclusion

In conclusion, probiotics appear to have the potential to modulate blood glucose levels and improve insulin regulation within a specific range in both healthy women and those with gestational diabetes. However, the precise mechanisms underlying these effects in both normal and pathological pregnancies remain unclear. Considering the heterogeneity of study designs, the variability in probiotic bacterial strains, and the unique physiological context of pregnancy, a rigorously designed clinical trial is warranted to validate the findings reported in the current literature. Furthermore, environmental and genetic factors may influence the interplay between gut microbiota and inflammatory as well as biochemical markers, thereby affecting immune system modulation and function. Such a trial would be instrumental in clarifying the efficacy of probiotics and in determining the optimal dosage, bacterial strains, and duration of administration required to achieve beneficial outcomes. Given its prevalence and associated complications during pregnancy, diabetes represents a critical area of focus for such interventions.

Declarations

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Conflicts of Interest

The authors have declared no conflicts of interest.

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Authors' Contributions

E.H., and N.Y.; methodology, E.H and NZ.H; writing. All authors have read and agreed to the published version of the manuscript.

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During the preparation of this manuscript, the authors didn't use Ai services. The authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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Prevalence of Household Food Insecurity and its Predictive Factors in Pregnant Women of Qazvin Province, Iran

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Food insecurity

ABSTRACT

Introduction: The Iranian population faces heightened vulnerability to food insecurity due to multifaceted factors, including poverty, economic instability, climate change, and the protracted socioeconomic impacts of the COVID-19 pandemic. Pregnant women are particularly at risk, necessitating targeted assessments of this critical public health issue. This study aimed to determine the prevalence of food insecurity and its predictors among pregnant women in Qazvin, Iran.

Methods: A cross-sectional study was conducted in Qazvin from 2022 to 2023, enrolling 422 healthy pregnant women attending comprehensive health centers. Data were collected using a researcher-developed checklist for sociodemographic and obstetric characteristics, while food insecurity was assessed via the Household Food Insecurity Access Scale (HFIAS). Logistic regression analysis identified predictors ($P < 0.05$).

Results: Food insecurity prevalence reached 71.4% (95% CI: 66.89-75.51), with 48.9% moderate-to-severe food insecurity. In adjusted analyses, rural residence (OR: 0.20; P: 0.015), smoking (OR: 0.20; P = 0.041), and hookah consumption (OR: 0.29; P: 0.001) were significantly associated with lower food security. Conversely, family income status at the level of savings (OR: 25.10; P< 0.001) and sufficient (OR: 5.18; P< 0.001), supplemental health insurance coverage (OR: 2.05; P: 0.006), and higher maternal education levels (OR: 1.96; P: 0.012) correlated with increased probability of food security.

Conclusion: Food insecurity is prevalent among pregnant women in Qazvin, disproportionately affecting rural populations, those with lower education, inadequate income, lack of supplemental insurance, and substance use (smoking/hookah). Intervention programs should prioritize these high-risk groups to mitigate nutritional disparities.

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Introduction

Food insecurity has emerged as a critical global health challenge over the past decade, currently affecting approximately 2.37 billion people worldwide who experience inadequate access to nutritious foods (1). The United States Department of Agriculture (USDA) defines food insecurity as "the limited or uncertain availability of nutritionally adequate and safe foods, or the inability to acquire acceptable foods through socially acceptable means" (2). However, contemporary understanding extends beyond this definition to encompass multidimensional aspects including: Nutritional adequacy (both quantity and quality), Food safety considerations, Psychosocial dimensions (e.g., feelings of deprivation), Behavioral adaptations (e.g., disrupted eating patterns) and Coping strategies employed by vulnerable households (2, 3).

Women experience higher rates of food insecurity than men throughout their life course, particularly during pregnancy (4, 5). Pregnancy increases nutritional requirements to support fetal development, making women more vulnerable to food insecurity's negative consequences (6). Recent evidence confirms associations between food insecurity and adverse outcomes such as maternal psychological disorders (depression, anxiety, stress, eating disorders), pregnancy complications (excessive gestational weight gain, gestational diabetes, anemia) and Poor fetal

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outcomes (low birth weight, preterm birth, birth defects) (6-11). In Iran - where 55.9% of the general population faces food insecurity (4) - pregnant women remain particularly affected. The latest systematic review estimates a 45% prevalence among Iranian pregnant women as of 2018 (12). Key contributing factors include household characteristics (size, gravidity), socioeconomic status (education, employment, income), food access limitations, lack of dietary diversity and pregnancy-related expenses (prenatal care costs, newborn necessities) (13, 14).

The Iranian population remains particularly vulnerable to food insecurity due to intersecting environmental and economic challenges. Climate change impacts - especially prolonged droughts - have significantly disrupted agricultural production, food security, and livelihoods (15-17). These climate-related pressures have depressed farm incomes while increasing food prices (18), exacerbating poverty and straining social welfare systems. Three fundamental challenges emerged as primary barriers to food security: persistent economic crises and widespread poverty, inconsistent and fragmented government policies supporting agricultural producers, and climate-related agricultural disruptions. Together, these factors create systemic vulnerabilities in Iran's food security infrastructure (2, 19).

Prior to the COVID-19 pandemic, our research team documented a 44% prevalence of food insecurity among pregnant women in Qazvin City, with significant associations found for maternal unemployment and unplanned pregnancy (20). During the pandemic, food-insecure pregnant women faced impossible tradeoffs - despite understanding the importance of proper nutrition, constrained budgets, pregnancy symptoms, and cognitive overload forced many to prioritize cheap, convenient foods over nutritional quality. Alarmingly, such coping strategies may perpetuate intergenerational cycles of food insecurity, with both immediate and long-term societal consequences (21, 22).

Given this context - combining ongoing economic crises, climate pressures, and pandemic aftermath - coupled with the lack of recent data on pregnant women's food security in Qazvin Province, we aimed to determine the current prevalence of household food insecurity and identify key predictive factors among pregnant women.

Materials and Methods

Study design and Sampling

This cross-sectional descriptive study included 423 pregnant women who were referred to comprehensive health centers in Qazvin between October 2022 and August 2023.

The prevalence of food insecurity in Iran has ranged from 20% to 60%, with rates increasing to 75% in female-headed households and 86% among low-income households (13). Given this wide variation in reported prevalence, we assumed a conservative estimate of 50% for our study. The sample size was calculated as 423, based on this 50% prevalence rate, with $\alpha = 0.05$, $d = 0.05$, and an additional 10% allowance for potential sample loss.

$$n = \frac{Z_{1-a/2}.P(1-P)}{d^2}$$

Sampling was conducted in two stages. First, Qazvin City was divided into five geographical regions, with two health centers randomly selected from each region. Second, eligible pregnant women were informed about the study's purpose, with emphasis placed on information confidentiality. Subsequently, consent forms and questionnaires were distributed via mobile phone links.

Inclusion criteria comprised: literacy (reading/writing ability), smartphone and internet access, and confirmed intrauterine pregnancy. Exclusion criteria included: history of chronic medical conditions, current pregnancy complications, and failure to complete the questionnaire.

Instruments

The data collection tool included Socio-Demographic checklist and Household Food Insecurity Scale Questionnaire.

The socio-demographic checklist, developed by the researchers, collected data on: maternal and spousal education/occupation, maternal age, place of residence, ethnicity, family type, homeownership status, housing size, household income level, basic and supplemental health insurance coverage, gestational age, pregnancy planning status (from both maternal and paternal perspectives), parity (number of children), fetal sex, initiation timing of prenatal care, smoking and hookah use.

The Household Food Insecurity Access Scale (HFIAS) captures the perceptions of household heads regarding their family's food insecurity through conversational statements. Designed as a rapid assessment tool, this scale evaluates the access dimension of food security. Its development was grounded in the fundamental principle that food insecurity represents "a measurable, describable, and analyzable experience." The HFIAS categorizes respondents into four food security status groups: Food secure (score 0-1), Mildly food insecure (score 2-7), Moderately food insecure (score 8-14), Severely food insecure (score 15-27). Salarkia et al. validated this instrument for use in Iranian populations through a comprehensive adaptation process. This included questionnaire modification, cultural adaptation of items and Semi-structured interviews with key stakeholders (health center officials, nutrition experts, and healthcare administrators). The validation study demonstrated excellent internal consistency (Cronbach's α = 0.95), indicating high reliability and validity (23). For our analysis, participants were dichotomized into: Food secure (score 0-1) and Food insecure (score ≥2). The scale maintained strong reliability in our sample (Cronbach's α = 0.88).

Statical Analysis

Following data collection, all entries were processed using SPSS software (version 23). Descriptive statistics were employed to summarize the data, with means and standard deviations used for quantitative variables and frequencies/percentages for categorical variables. To examine associations between predictor variables and food insecurity, we conducted both univariate and multivariate logistic regression analyses. The analytical approach consisted of initial univariate screening of each variable separately, inclusion of variables with $p<0.2$ in subsequent multivariate analysis and final model development using Wald's forward selection method. The threshold for statistical significance was set at $p<0.05$. Missing data were addressed through listwise deletion to ensure data quality. The questionnaire link was sent to 436 women and finally 423 questionnaires were full submitted and their data were analyzed.

Ethical Statement

This study received ethical approval from Qazvin University of Medical Sciences (IR.QUMS.REC.1401.162). All participants were fully informed about the study objectives prior to enrollment. Digital consent forms were distributed via mobile phone links, which participants could complete at their convenience.

Results

Participants had a mean age of 29.44 ± 7.77 years. The majority resided in urban areas, identified as Turkish ethnicity, and lived in rental accommodations. The average housing size was 110.33 m^2, with most living in nuclear family structures. Approximately half of participants and their spouses had attained university education. Most women were homemakers while their spouses were employed. A majority of women reported sufficient household income.

Basic health insurance coverage was common, while supplemental insurance was uncommon. The mean number of children was 0.97±1.07. Most pregnancies were reported as planned by both parents, with prenatal care typically initiated during the first trimester. The mean gestational age at assessment was 20.3 weeks, with female fetuses being slightly more common.

Hookah use was reported by a notable minority of women, while cigarette smoking was less prevalent. Complete demographic characteristics are presented in Table 1.

Food insecurity prevalence reached 71.4% (95% CI: 66.89-75.51) in our sample, with severity stratification presented in Table 2. Initial univariate analysis included all candidate variables (Table 1). Variables retained for multivariate modeling ($p<0.2$ threshold) excluded maternal age, occupational status, gestational age and housing size. The multivariate analysis revealed several significant predictors of food security status: university education nearly doubled the likelihood of food security (OR: 1.96, 95% CI: 1.16-3.32), while sufficient family income increased the odds five-fold (OR: 5.18, 95% CI: 2.51-10.69) and savings-level income showed a twenty-five-fold greater probability (OR:25.10, 95% CI: 7.85-80.3). Supplemental health insurance coverage more than doubled the odds of food security (OR:2.05, 95% CI: 1.22-3.42). Conversely, rural residence decreased the odds by 80% (OR:0.20, 95% CI: 0.05-0.73), as did smoking (OR:0.20, 95% CI: 0.04-0.93), while hookah consumption reduced the probability by 71% (OR:0.29, 95% CI: 0.14-0.59) (Table 3).

Table 1. Socio-Demographic Characteristics and Univariate Logistic Regression Analysis of Food Insecurity Predictors (N=423)

Variable		N(%)	univariate logestic regression					
			β	Standard error	P-Value	OR	CI 95% Upper limit	CI 95% Lower limit
Maternal education*	≤ 12 years	231 (56.6)	1					
	> 12 years	192 (45.4)	1.24	.22	< .001	3.48	2.23	5.43
spousal Education*	≤ 12 years	212 (50.1)	1					
	> 12 years	211 (49.9)	.14	.22	< .001	3.12	1.99	4.90
Maternal Occupation	Housewife	274 (64.8)	1					
	Employement	149 (35.2)	.26	.22	.226	1.30	.84	2.02
Spousal Occupation*	Unemployement	15 (3.5)	1					
	Employement	408 (96.5)	1.76	1.04	.090	5.83	.75	44.85
Place of Residence*	Urban	366 (86.5)	1					
	Rural	57 (13.5)	-2.14	.60	< .001	.11	.03	.38
Ethnicity*	Fars	152 (35.9)	1					
	Tork	238 (56.3)	-1.25	1.09	.252	.28	.03	2.43
	Kord	26 (6.1)	-1.49	.63	.019	.22	.06	.77
	Lor	26 (1.7)	-.52	.22	.019	.59	.38	.91
Family Type*	Nuclear	341 (80.6)	1					
	Extended	82 (19.4)	-.70	.30	.023	.49	.27	.90
Homeownership status*	Tenant	269 (63.6)	1					
	Owner	154 (36.4)	.74	.24	.002	2.11	1.31	3.38
Household income level*	Insufficent	29 (6.9)	1					
	Sufficent	254 (60)	1.96	.35	< .001	7.13	3.56	14.26
	at the level of savings	140 (33.1)	3.53	.52	< .001	34.12	12.09	96.32
Basic Health Insurance Coverage*	No	73 (17.3)	1					
	Yes	350 (82.7)	.94	.34	.006	2.58	1.30	5.09
Supplemental health Insurance Coverage*	No	245 (57.9)	1					
	Yes	178 (42.1)	1.19	.22	< .001	3.31	2.13	5.13
Gestational Age	< 20 weeks	115 (27.2)	1					
	≥ 20 weeks	308 (72.8)	.112	.245	.647	1.11	.69	1.80
Pregnancy planning status (maternal perspectives)*	No	115 (27.2)						
	Yes	308 (72.8)	.48	.25	.058	1.63	.98	2.70
Pregnancy planning status (paternal perspectives)*	No	107 (25.3)	1					
	Yes	316 (74.7)	.79	.28	.005	2.20	1.27	3.82
Fetal sex*	Male	161 (38.1)	1					
	Female	171 (40.4)	-.25	.24	.281	.77	.48	1.23
	Unknown	91 (21.5)	-.49	.30	.101	.61	.34	1.10
Initiation timing of prenatal care*	First trimester	308 (85.8)	1					
	Second trimester	49 (11.6)	-.48	.37	.190	.61	.29	1.27
	Third trimester	11 (2.6)	.31	.63	.624	1.36	.39	4.76
Smoking*	No	396 (93.6)	1					
	Yes	27 (6.4)	-1.68	.74	.024	.18	.04	.79
Hookah Consumption*	No	340 (80.4)	1					
	Yes	83 (19.6)	-.81	.31	.010	.44	.23	.82
Maternal Age		29.44±7.77+	.01	.01	.329	1.01	.98	1.04
Number Of Children*		0.97±1.07+	-.18	.10	.093	.83	.67	1.03
Housing Size		110.33±49.64+	.002	.002	.308	1.00	.99	1.00

* Selected variables to enter into the multivariate regression model
+ Mean ± standard deviation

Table 2. Household Food Insecurity Status among Pregnant Women in Qazvin Province (N=423)

Variable		N (%)	95% CI Upper limit	95% CI Lower limit
Household Food Insecurity Status	Food secure	95 (22.46)	18.72	26.70
	Mildly food insecure	121 (28.61)	24.49	33.11
	Moderately food insecure	83 (19.62)	16.10	23.70
	Severely food insecure	124 (29.31)	25.16	33.84

Table 3. Multivariate Logistic Regression Analysis of Food Insecurity Predictors (Final Model)

Variable		Multivariate logistic regression					
		β	Standard error	P-Value	OR	CI 95% Upper limit	CI 95% Lower limit
Maternal education	≤ 12 years	1					
	> 12 years	.67	.26	.012	1.96	1.16	3.32
Place of Residence	Urban	1					
	Rural	-1.58	.64	.015	.20	.05	.73
Household income level	Insufficient	1					
	Sufficient	1.64	.36	< .001	5.18	2.51	10.69
	at the level of savings	3.22	.59	< .001	25.10	7.85	80.30
Supplemental health insurance Coverage	No	1					
	Yes	.71	.26	.006	2.05	1.22	3.42
Smoking	No	1					
	Yes	-1.60	.78	.041	.20	.04	.93
Hookah use	No	1					
	Yes	-1.21	.31	.001	.29	.14	.59

Hosmer and Lemeshow test: $\chi2=6.046$ df=7 Sig=.534
Model summary: -2 log likelihood=385.427; Cox-Snell R2=.249; Nagelkerke R2=.356
Omnibus test of model coefficients: $\chi2=120.971$ df=7 Sig=0.000

Discussion

This study found a substantially high prevalence of food insecurity (71.4%) among pregnant women in Qazvin Province, with nearly one-third (29.3%) experiencing severe food insecurity. This represents a concerning increase from the 44% prevalence reported in the same population prior to the COVID-19 pandemic (20). The observed deterioration in food security status likely reflects the compounded socioeconomic impacts of the pandemic, particularly on vulnerable populations (24). Supporting this interpretation, a 2020 national review documented both a 30% decline in household purchasing power and significant food price inflation following the pandemic's onset (25). These economic shocks were further exacerbated by pre-existing environmental challenges that constrained Iran's agricultural capacity, creating synergistic pressures on food systems. Together, these factors provide a plausible explanation for both the increased prevalence and severity of food insecurity observed in our study population.

The study's second objective examined socioeconomic and behavioral predictors of food insecurity among pregnant women. Multivariate analysis identified six significant independent predictors: rural residence, lower educational attainment, insufficient family income, lack of supplemental health insurance, smoking, and hookah use. These findings suggest that food insecurity in this population is strongly associated with both structural disadvantages and modifiable risk factors.

Our findings confirm rural residence as a significant predictor of food insecurity, contradicting the common assumption that agricultural proximity ensures food security. Multiple studies corroborate this pattern, demonstrating consistently higher food insecurity rates in rural areas (26, 27). In Iran specifically, rural food security faces multidimensional challenges that Ataei et al. categorized into eight key domains: political, economic, knowledge/information, infrastructural, cultural, food access, climatic, and social factors. Their analysis identified three predominant barriers: (1) recurrent drought conditions, (2) widespread rural household poverty, and (3) inconsistent government agricultural policies (19). These structural challenges align with conflict theory perspectives, which highlight how urban-rural resource disparities generate systemic disadvantages that perpetuate food insecurity in rural communities (28).

In the present study, the increase in maternal education level was related to the decrease in food insecurity. The results of the studies have shown that an increase in the level of education of a woman, even if she is not the head of the household, was associated with the reduction of food insecurity (29, 30). Food insecurity has been known as a gender issue and the limitation in women's educational progress probably has an important role in this gender gap (31). Since the

education is an indicator of a person's social and employment status, the policies addressing gender inequality in education such as investment and early intervention in the girls' initial registration process and continuing education to higher levels can reduce food insecurity (31, 32).

Financial status emerged as a significant predictor of food insecurity in our study. However, conventional indicators like absolute income levels or asset ownership (e.g., houses, vehicles) proved insufficient for reliably predicting household food security across all family types (33, 34). More importantly, our findings align with existing evidence that savings capacity serves as a stronger protective factor against food insecurity, demonstrating consistent predictive value across income strata (33, 34). This suggests that financial resilience - particularly a household's ability to both save money and maintain stable food consumption during economic shocks - may be more determinant of food security than static measures of wealth (35). Notably, our results specifically highlight savings capability as one of the most robust predictors of food security status in this population

Supplemental health insurance coverage emerged as a significant predictor of food security among pregnant women in our study. This finding aligns with existing literature demonstrating that food-insecure individuals are disproportionately covered only by basic health insurance, with limited access to private supplemental coverage (33, 36). The dual coverage of both basic and supplemental insurance - which remains accessible primarily to higher socioeconomic groups - appears to confer substantial advantage, serving as both a marker of socioeconomic status and a protective factor against food insecurity. Importantly, supplemental coverage plays a crucial role in mitigating healthcare cost burdens and ensuring adequate access to medical services (37). These findings underscore the need for expanded access to quality supplemental insurance as a potential intervention for food-insecure families.

Our study found significant associations between both smoking and hookah consumption and increased food insecurity. This aligns with existing evidence demonstrating higher smoking prevalence among food-insecure populations (38). Notably, some studies suggest a bidirectional relationship, identifying food insecurity itself as an independent social determinant of smoking - potentially explained by low-income smokers allocating household resources to cigarettes rather than food (39). This complex interplay raises critical questions about whether smoking exacerbates food insecurity or merely reflects shared socioeconomic determinants (38). Particularly concerning is the elevated smoking prevalence among disadvantaged pregnant women experiencing poverty, low income, and limited education (40-42). Given these overlapping risk factors, smoking should be considered a key indicator when identifying women at high risk for food insecurity.

To our knowledge, this represents the first study to assess food insecurity prevalence among pregnant women in Qazvin following the COVID-19 pandemic. A key methodological strength was the use of multivariate regression analysis, which enabled robust identification of significant predictors while controlling for potential confounders. Several important limitations should be acknowledged. First, the cross-sectional design precludes establishment of causal relationships between identified predictors and food insecurity outcomes. Second, reliance on self-reported measures introduces potential recall bias and social desirability bias in participant responses. Third, while the Household Food Insecurity Access Scale (HFIAS) is a validated instrument, its categorical scoring system provides less detailed nutritional information than quantitative dietary assessment tools.

We recommend future longitudinal studies incorporate objective dietary assessments (e.g., 24-hour recalls or food frequency questionnaires) to better characterize nutritional status and validate our findings. Additionally, qualitative approaches could help elucidate the mechanisms underlying the observed relationships.

Conclusions

Our findings demonstrate a concerning rise in food insecurity among pregnant women following the COVID-19 pandemic, with significant socioeconomic predictors including lower educational attainment, rural residence, insufficient household income, lack of supplemental health insurance, smoking and

hookah use. These results sound an urgent alarm for targeted interventions to protect this vulnerable population. We propose a multi-level intervention framework:

- Structural Interventions include: expand insurance coverage policies for low-income pregnant women and implement rural development programs addressing food access disparities
- Educational Empowerment include: Create continuing education pathways for women, particularly female heads-of-households and develop financial literacy programs focusing on crisis budgeting and resource allocation
- Health Promotion include: integrate tobacco cessation programs with prenatal care services and provide nutrition education tailored to food-insecure households.

Such comprehensive approaches could simultaneously address immediate needs while building long-term resilience against food insecurity.

Declarations

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Conflicts of Interest

The authors declared no conflicts of interest.

Author's Contribution

Elham Farahani contributed to the research design, data collection, literature review, and writing of specific sections of the manuscript. Zainab Alimoradi contributed to the design of the study, data analysis and critically reviewing the manuscript. Hamideh Hajnasiri contributed to the literature review and writing of specific sections of the manuscript. Farnoosh Moafi provided overall supervision, guided the project direction, and critically revised the manuscript for publication. All authors have read and approved the final version of the manuscript. Sahar Ebrahimi contributed to the data collection and initial data analysis.

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The Effect of Resistance Training with Black Seed Supplementation on Glycemic Indices in Overweight Women

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ABSTRACT

Introduction: Today, obesity and insulin resistance are recognized as significant factors in the development of metabolic diseases. This study aimed to examine the effects of resistance training combined with black seed consumption on specific glycemic indicators in overweight women.

Methods: In this quasi-experimental study, the researchers randomly assigned 48 overweight and obese women to control, training, black seed, and combined training and black seed groups. The two training groups (with and without black seed supplementation) performed a resistance training program at 50–80% of 1RM, three sessions per week. The black seed group consumed two 1000 mg capsules daily. Tukey's post-hoc test and one-way ANOVA were used for intergroup data analysis, while intragroup analysis was performed using a paired-sample t-test with SPSS version 26.

Results: Compared to their pre-test values and the control group, all three experimental groups showed a reduction in blood glucose, insulin, and the insulin resistance index after eight weeks. A significant decrease was observed in the insulin resistance index ($p = 0.0001$), insulin ($p = 0.001$), and glucose ($p = 0.001$) levels in the resistance training + black seed group. Furthermore, the training + black seed group exhibited a significant reduction in blood glucose ($p = 0.001$) and the insulin resistance index ($p = 0.01$) compared to the resistance training group alone.

Conclusion: Resistance training combined with black seed consumption can improve blood glucose levels, insulin levels, and the insulin resistance index in overweight or obese individuals predisposed to diabetes and cardiovascular diseases.

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Introduction

Experts believe that environmental, metabolic, hormonal, and genetic factors play a significant role in the prevalence of obesity and overweight, with an imbalance between energy intake and expenditure being a key contributing factor (1). Obesity is a common consequence of sedentary and inactive lifestyles, requiring appropriate intervention and treatment (2). Both obesity and overweight increase the risk of various cancers, type 2 diabetes, musculoskeletal disorders, and cardiovascular diseases. Metabolic syndrome (MetS), which involves the clustering of cardiac risk factors such as high blood pressure, abdominal obesity, insulin resistance, impaired glucose tolerance, and dyslipidemia or hyperglycemia, significantly increases the likelihood of diseases such as cancers, cardiovascular diseases, and premature mortality (3).

Type 2 diabetes is closely associated with obesity and overweight, with over 80% of type 2 diabetes patients classified as overweight or obese based on body mass index (4). Physical activity and exercise are considered essential approaches for weight and obesity management, alongside proper nutrition. The American Diabetes Association recommends at least 150 minutes of moderate-intensity aerobic exercise per week, spread across three days, to control weight, improve glucose regulation, and reduce the risk of cardiovascular diseases (5). However, physical limitations, lack of time, and low motivation are common barriers that prevent many adults with obesity from achieving these goals (6). In recent years, exercise training has been widely recognized as beneficial for blood glucose control and weight loss (7). In this context, Li et al. (2024) demonstrated that resistance training significantly improves fasting glucose,

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2-hour glucose levels, and insulin resistance in diabetic patients (7). Similarly, Fathi et al. (2015) found that resistance training led to reductions in glucose, insulin, and insulin resistance in the experimental group (8). However, Basami et al. (2013) reported that resistance training had no significant effect on reducing insulin resistance in young men (9). This inconsistency in results may be influenced by various factors, such as the type and intensity of training, hormonal levels, inflammatory conditions, fat distribution, and other variables (10).

In addition to physical activity, researchers have increasingly turned to herbal supplements to address factors related to overweight and obesity. Black seed, scientifically known as Nigella sativa L., has been used in traditional medicine and is included in pharmaceutical formulations for individuals with hyperlipidemia (11). Black seed contains fats, vitamins, minerals, proteins, and carbohydrates. It is a rich source of essential and unsaturated fatty acids, particularly linoleic acid and oleic acid. Other compounds found in black seed include phospholipids, carotene, calcium, iron, and potassium. Treatment of rats with black seed oil or its active ingredient, thymoquinone, has been shown to increase antioxidant activity and reduce cholesterol, triglycerides, and lipid peroxidation (12). Due to its anti-inflammatory and antioxidant properties, black seed can enhance insulin secretion (thereby preserving pancreatic beta cells) and reduce insulin resistance, resulting in improved blood sugar control and diabetes management (13).

In a clinical trial, the consumption of black seed capsules at doses of 2 and 3 grams per day for 12 weeks in patients with type 2 diabetes led to improvements in glycemic indices, insulin resistance, pancreatic beta-cell function, and blood lipid parameters (14). While some studies have separately investigated the effects of resistance training combined with black seed consumption on glycemic indices in obese individuals, few have examined their combined effects. Therefore, the present study aimed to investigate the impact of resistance training alongside black seed consumption on serum insulin, glucose, and insulin resistance levels in overweight women.

Materials and Methods

This was an applied, semi-experimental study with three experimental groups and one control group. Initially, open calls were made in public places and gyms in Gachsaran city to invite overweight young women (BMI >25) to participate in the study. Volunteers were then invited for initial evaluations. Based on the results of the general health questionnaire and a physician's clinical assessment, 48 individuals with good physical and mental health were selected and randomly assigned to one of four groups (n=12 per group): a control group, a black seed group, and two resistance training groups, one with black seed consumption and one without. The experimental groups (black seed consumption, resistance training, and resistance training + black seed consumption) followed the prescribed training program and consumed black seed as per the protocol throughout the study. The placebo group continued their daily activities without intervention. The individuals in the black seed consumption and combined training + black seed groups consumed two 1000 mg capsules of black seed supplement (manufactured in Iran by Essence Giah Co.) daily (15). The control group received a placebo containing corn flour during the study period.

Blood samples were collected at two stages: 24 hours before the initiation of training and 48 hours after the final training session in the intervention groups. Both 8 cc blood draws were performed at the training site following an overnight fast of 10 hours, between 8 and 9 AM.

Fasting glucose was measured using the Pars Azmoon kit with the glucose oxidase method. Serum insulin levels were quantified using ELISA. Insulin resistance was assessed using the HOMA-IR formula (16).

$$HOMA\text{-}IR = [Fasting\ glucose\ (mmol/l) \times Fasting\ insulin\ (\mu U/mL)] / 405$$

Resistance Training Protocol

The maximum muscular strength of the subjects was estimated using the Brzycki formula, as follows. First, the subjects attended two gym sessions to familiarize themselves with the movement patterns, resistance training environment, and equipment. Proper

weightlifting techniques and the use of weight machines were taught to the subjects. The participants then performed exercises to determine their one-repetition maximum (1RM). Based on the number of repetitions and the load lifted in each movement, the maximum muscular strength for each exercise was calculated using the Brzycki formula.

One-repetition maximum (1RM) (kg) =
Weight ÷ (1.0278 - (0.0278 × Number of repetitions))

The resistance training program for the experimental group included upper body exercises such as the bench press, pull-ups on a pulley, bicep curls, and triceps extensions with a barbell. Lower body exercises included leg curls and leg extensions using a pulley machine. Additionally, sit-ups were incorporated to strengthen the abdominal and trunk muscles.

The training sessions followed a cyclical structure, adhering to the overload principle. In Week 1, training was performed at 50% of 1RM intensity across three sets, with 1–2 minute rests between sets and 8–12 repetitions per set. Additionally, a 3–5 minute rest was provided between each complete cycle (after performing all seven movements). The intensity was increased by 5% of 1RM each week, reaching 80% of 1RM by Week 8. A 10-minute warm-up program was included at the beginning of each session, and each session concluded with a 10-minute cool-down. Due to muscular adaptation and increased strength by the end of Week 4, the subjects' 1RM was recalculated, and the training intensity was adjusted based on the new 1RM (17).

Data distribution normality was assessed using the Kolmogorov-Smirnov (KS) test. If the data followed a normal distribution, one-way ANOVA was used for between-group analysis, with Tukey's post-hoc test for pairwise comparisons. Within-group analysis was conducted using a paired-sample t-test. Data analysis was performed using SPSS version 26, with a significance criterion of $P \leq 0.05$.

Results

The mean weight and body mass index of the subjects of the study are presented in Table 1.

Table 1. Mean and standard deviation of age, height, and pre-test and post-test weight and BMI in the study groups

	Age (years)	Height (cm)	Pre-test weight (kg)	Post-test weight (kg)	Pre-test BMI (Kg/m²)	Post-test BMI (Kg/m²)
Placebo control	32.17 ± 1.81	159.08 ± 0.44	75.41 ± 4.88	75.86 ± 4.52	29.82 ± 1.31	30.00± 1.44
Black seed	31.58 ± 1.62	160.06 ± 0.52	76.41 ± 5.40	76.11 ± 5.19	29.84 ± 1.46	29.73 ± 1.85
Training	33.42 ± 1.98	161.07± 0.67	74.97 ± 4.79	75.58 ± 4.92	28.92± 1.82	29.15 ± 1.64
Training+ Black seed	31.89 ± 1.33	161.88 ± 0.78	75.66 ± 5.74	76.11 ± 2.84	28.87 ± 1.57	29.04 ± 1.64

Analysis of Glycemic Indices

The Shapiro-Wilk test indicated normal distribution in the data for all four groups. No significant differences were found in the pre- and post-test glucose and insulin levels, as well as the HOMA-IR index ($p = 0.221$, $p = 0.403$, and $p = 0.271$, respectively), in the control group. However, the black seed group showed significant reductions in glucose and insulin levels and the HOMA-IR index ($p = 0.001$, $p = 0.001$, and $p = 0.001$, respectively) post-test. Similarly, the resistance training group demonstrated significant reductions in glucose and insulin levels and the HOMA-IR index ($p = 0.001$, $p = 0.001$, and $p = 0.001$, respectively) post-test. The resistance training + black seed consumption group also exhibited significant reductions in post-test glucose and insulin levels and the HOMA-IR index ($p = 0.0001$, $p = 0.0001$, and $p = 0.0001$, respectively).

One-way ANOVA revealed significant differences in glucose ($F = 27.55$, $p = 0.001$), insulin levels ($F = 55.41$, $p = 0.001$), and the HOMA-IR index ($F = 56.28$, $p = 0.001$) among the research groups. Tukey's post-hoc test results for pairwise comparisons are presented in Figures 1 to 3.

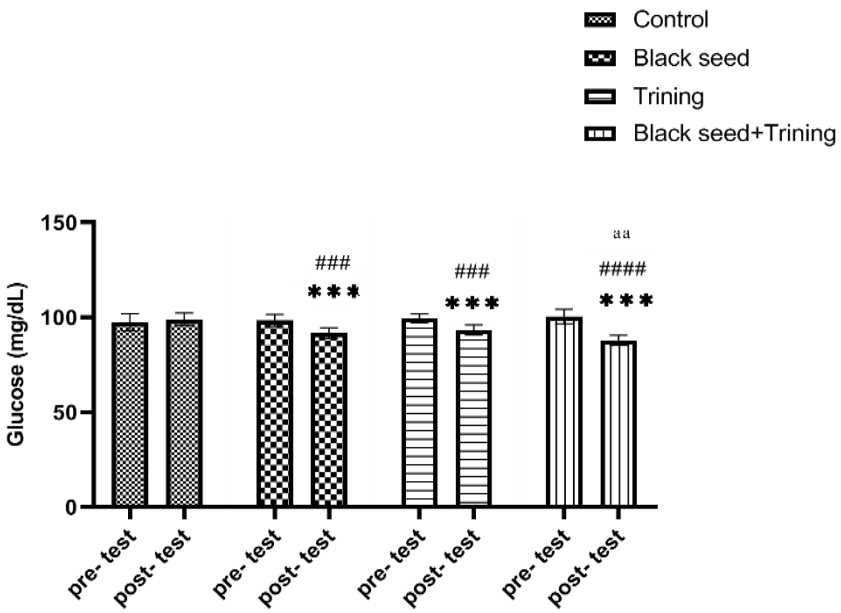

Figure 1. Mean and standard deviation of blood glucose levels (mg/dL)
***(p=0.001) difference is significant vs pre-test. ###(p=0.001) difference is significant vs placebo group in post-test. #### (p=0.0001) difference is significant vs placebo group in post-test. ^{aaa} (p=0.001) difference is significant vs training group and black seed group.

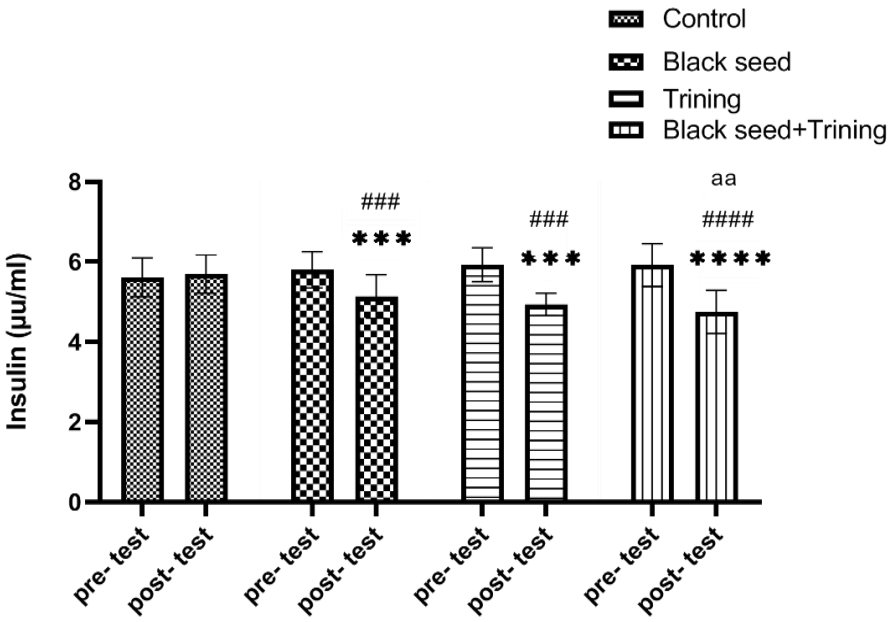

Figure 2. Mean and standard deviation of insulin levels (µU/ml)
(p=0.001) difference is significant vs pre-test. *(p=0.0001) Significant difference compared to the pre-test group. ###(p=0.001) difference is significant vs placebo group in post-test. #### (p=0.0001) difference is significant vs placebo group in post-test. ^{aa} (p=0.01) difference is significant vs training group and black seed group.

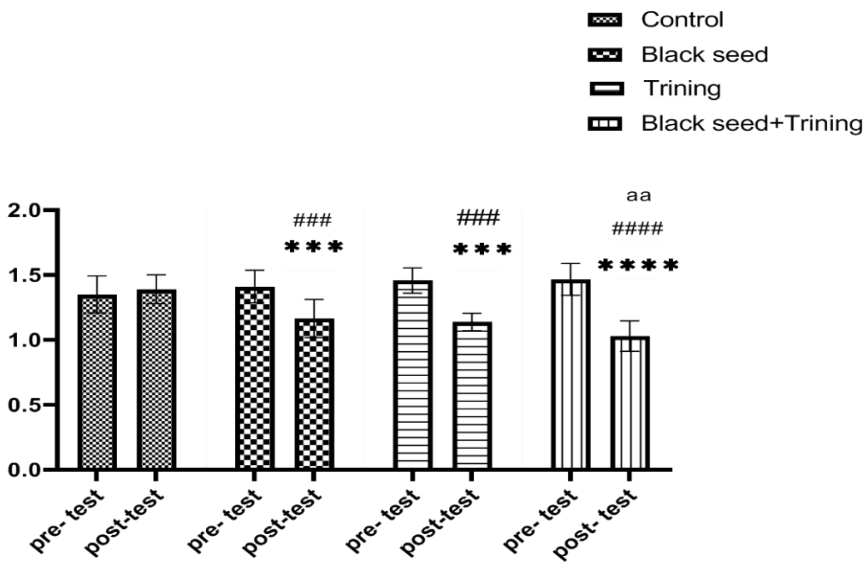

Figure 3. Mean and standard deviation of insulin resistance index (HOMA-IR)
(p=0.001) difference is significant vs pre-test .*(p=0.0001) Significant difference compared to the pre-test group. ###(p=0.001) difference is significant vs placebo group in post-test. #### (p=0.0001) difference is significant vs placebo group in post-test. [a a] (p=0.01) difference is significant vs training group and black seed group.

Discussion

The present study aimed to investigate the effects of eight weeks of resistance training combined with black seed consumption on glycemic indices in overweight and obese women. The results demonstrated that eight weeks of resistance training significantly reduced serum blood glucose, insulin levels, and the insulin resistance index in these women. Comparisons between the resistance training group's pre-test and post-test results, as well as with the control group, showed significant reductions in insulin and glucose levels and the insulin resistance index. These findings are consistent with the studies by Patel et al. (2020) and Goto et al. (2009) (18,19). In the survey conducted by Goto et al. (2009), a single session of acute resistance training resulted in a significant increase in blood glucose levels (19). The discrepancy between our findings and those of Goto et al. may be attributed to differences in training intensity, the gender of the subjects, the number of training sessions, and the subjects' fitness levels. In the present study, participants followed an eight-week resistance training protocol. In contrast, Goto et al. used a single progressive session, which led to an increase in blood glucose levels following the session. Additionally, the results of this study are consistent with those of Monroe et al. (2020), Samani et al. (2024), and Saremi et al. (2016) (20,21,22). Exercise training may improve skeletal muscle glucose transport, insulin function, glucose tolerance, and whole-body insulin sensitivity, leading to enhanced glucose metabolism both with and without insulin mediation. These responses are likely associated with increased GLUT4 protein expression and the selective activation of enzymes involved in glucose phosphorylation and oxidation. Physical activity induces functional changes in insulin signaling, facilitating the translocation of GLUT4 to the cell membrane, which increases glucose uptake by skeletal muscles (23, 20). The gradual increase in muscle mass, a key benefit of resistance training, may contribute to glycemic control and enhance glucose metabolic capacity. Studies have shown that eight weeks of resistance training increase strength and reduce blood glucose levels. The observed reduction in blood glucose is likely due to the insulin-like effects of resistance training, which promote the re-synthesis of glycogen stores in muscle cells, ultimately maintaining blood glucose levels within the normal range (24).

In the present study, resistance training improved insulin sensitivity and reduced serum insulin levels. Previous research has

demonstrated that resistance training enhances glucose homeostasis not only by lowering insulin resistance but also by increasing the mass and function of beta cells (25).

Our results demonstrated a reduction in serum blood glucose, insulin levels, and the insulin resistance index in obese and overweight women following black seed consumption. In the black seed group, a significant decrease in insulin, glucose, and the insulin resistance index was observed compared to both the control group and pre-test values. These findings are consistent with the studies by Parhizkar et al. (2011) and Shah et al. (2012) (26, 27), as well as previous research reporting that black seed extract inhibits hepatic gluconeogenesis, suggesting potential therapeutic hypoglycemic effects for the treatment of type 2 diabetes (26, 27). Recent studies on the mechanisms of action of medicinal plants have revealed that some possess insulin-like properties and can reduce the absorption of carbohydrates and fats from the small intestine (28). Furthermore, the presence of polysaccharides, flavonoids, glycoproteins, polypeptides, steroids, alkaloids, and pectin in medicinal plants may help explain the hypoglycemic properties of plants such as black seed, which can prevent blood biochemical alterations associated with diabetes (29). Black seed also regulates liver enzymes involved in glucose metabolism, thereby reducing hepatic gluconeogenesis. Specifically, it increases the activity of liver hexokinase while reducing the activity of fructose-1,6-bisphosphatase and glucose-6-phosphatase, both key enzymes in gluconeogenesis. Additionally, black seed enhances the activity of glucose-6-phosphate dehydrogenase, an enzyme involved in the intracellular pentose phosphate pathway (30, 31). Black seed also activates adenosine monophosphate-activated protein kinase (AMPK), which inhibits the gluconeogenesis pathway and reduces hepatic glucose production. In muscle tissue, increased AMPK activation promotes the synthesis and translocation of the GLUT4 transporter, thereby enhancing glucose uptake by muscle cells. Another beneficial effect of black seed is the inhibition of intestinal glucose absorption (32). Moreover, black seed may exert anti-diabetic effects in skeletal muscle cells, liver cells, and adipocytes through the peroxisome proliferator-activated receptor gamma (PPAR-γ), AMPK, and insulin signaling pathways (33).

Other findings of the study indicated that resistance training combined with black seed consumption in obese and overweight women reduced serum insulin, blood glucose levels, and the insulin resistance index. Our results showed that after eight weeks of resistance training and black seed consumption, serum glucose levels and the insulin resistance index in the resistance training + black seed group decreased significantly compared to the resistance training group, control group, and black seed consumption group. Additionally, serum insulin levels decreased significantly in the resistance training + black seed group compared to both the control group and the black seed consumption group. Given the effects of resistance training and black seed consumption, the combined impacts likely involve mechanisms from both. Accordingly, in addition to its antioxidant properties, black seed consumption, when combined with resistance training, enhances glucose uptake into muscle cells. This effect persists even after training, as the pathways stimulating glucose uptake remain active for hours post-exercise (34). Consistent with our findings, Zaoui et al. (2002) showed that after 12 weeks of training and black seed consumption, animals in the experimental group exhibited a significant decrease in serum glucose compared to the control group (35). Furthermore, research indicates that regular training leads to significant reductions in plasma glucose levels, increased insulin sensitivity, and improved insulin resistance (36). One limitation of the present study is the lack of control over the participants' calorie intake and diet, which may have influenced the results. Therefore, we recommend that future studies incorporate strict control over participants' caloric intake and diet.

Conclusion

Our findings indicated that resistance training combined with black seed consumption can improve key factors such as insulin resistance, insulin levels, and blood glucose in overweight or obese individuals at risk for various types of diabetes and cardiovascular diseases. Additionally, the insulin resistance index significantly decreased following resistance training and black seed consumption, which may

provide substantial benefits for individuals with impaired insulin regulation.

Notably, the results of this study, for the first time, demonstrate that the simultaneous consumption of black seed with resistance training has a more significant effect than either resistance training or black seed consumption alone. This distinction sets our study apart from existing research, as no prior studies have explored this combined approach, making the present study the first to investigate this area.

Declarations

Conflicts of Interest

The authors declare no conflicts of interest.

Authors Contributions

Drafting of the manuscript and screening: DN and KA

Conception and design: DN, KA, and MA

Critical revision of the manuscript: DN and KA

Consent for publication

Not applicated.

Ethical Consideration

The Marvdasht Islamic Azad University Ethics Committee approved this study (No. IR.IAU.M.REC.1404.074).

Availability of Data and Materials

All data generated in this study are available in the published article as well as in the supplementary files.

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Physiological Responses to Sprint Interval Exercise in a Fasted State in Active Men

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ABSTRACT

Introduction: Fasting is a method used to enhance metabolic flexibility toward lipid utilization. However, there are concerns about hypoglycemia or protein breakdown in exercise. The present study investigated the physiological responses to sprint interval exercise with different rest durations in a fasting state.

Methods: For this randomized crossover study, 10 healthy active men (aged 22.70 ± 1.15) were selected from eligible volunteers. In 2 separate sessions, following 14 hours of fasting, participants randomly performed 5×15s all-out sprints on an E894 MONARK ergometer with 30 or 60s rest intervals.

Results: Diastolic blood pressure and heart rate were lower with longer rest intervals. Blood glucose, insulin, and urea remained unchanged after both protocols compared with baseline levels. There was a marked increase in lactate levels after both protocols, independent of the rest duration. The immune response was significantly higher with longer rest intervals than with short rest intervals or baseline levels.

Conclusion: Sprint interval exercise performed in a fasted state did not induce hypoglycemia or evidence of protein degradation, suggesting that such training may be safe for active men. Longer rest intervals were associated with lower post-exercise heart rate and diastolic blood pressure, as well as an enhanced immune response. These findings suggest that coaches and practitioners may consider manipulating rest intervals to achieve targeted physiological adaptations.

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Introduction

Intermittent fasting (IF) is a widely adopted dietary pattern that helps conserve carbohydrates and promotes greater reliance on fat as a primary energy source [1]. Exercising during fasting increases adipose tissue lipolysis and peripheral fat oxidation via higher plasma catecholamines and cortisol concentrations and lower circulating insulin levels. A modest reduction in serum glucose levels can occur within a few hours of fasting, likely due to attenuated hepatic glycogen synthesis and glycolysis. Such modifications occur due to decreased insulin concentrations, increased glucagon levels, and enhanced sympathetic activity [2, 3]. These findings have encouraged athletes to incorporate fasting into their training protocols to enhance fat oxidation and spare glycogen stores.

High-intensity interval Exercise (HIIE) is a method commonly viewed in the context of health and athletic performance in all types of sports [4, 5]. Cycling-based HIIE as a sprint interval exercise (SIE) has been identified as a viable method for enhancing performance, with the nature and extent of adaptations contingent upon the work-to-rest ratio [6]. The absence of weight-bearing and minimal eccentric muscle contractions during stationary cycling presents runners and athletes with a low-impact alternative that may mitigate the risk of overuse injuries, thereby offering a pragmatic solution for optimizing training regimens [7].

Time-to-rest ratios have been studied, with some research showing that shorter-time SIEs may offer the same adaptations as longer work-to-rest ratios [8]. The effectiveness of different SIE protocols in eliciting specific adaptations related to physiological responses remains an area of

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interest. Research suggests that SIE protocol involving shorter work periods at supramaximal intensity may produce these adaptations more effectively than a protocol with longer work intervals at maximal intensity.

While no definitive conclusion has been reached, studies have indicated that a 2:1 work-to-rest ratio results in a higher accumulated oxygen deficit compared to other ratios [9]. Regarding metabolic responses, while the 2:1 SIE led to maximal values of VO_2, lactate, and ventilatory parameters within a few minutes, the 1:1 SIE allowed maintenance of moderately high values for a considerably longer period, especially for lactate and ventilatory parameters [10]. Also, the elimination of ammonia is significantly enhanced during high-intensity muscle activity, and the formation of urea increases. This is confirmed by the fact that the increased concentration of blood urea is directly proportional to the duration of high-intensity work [11]. Studies have also shown that HIIE provokes a greater immune response compared with moderate continuous exercise [12]. Lymphocyte-to-white blood cell ratio (LWR) and platelet-to-lymphocyte ratio (PLR) have been used as cost- and time-efficient markers of cellular immune response [13]. However, the immune response to high-intensity exercise while fasting is not clear.

Both athletes and the general population widely apply fasting strategies. However, there are concerns regarding the impact of fasting on high-intensity exercise outcomes, particularly concerning protein degradation and immune fluctuations. Therefore, examining the acute physiological response to SIE with varying rest intervals while in a fasted state could offer valuable insights for developing future training strategies for athletes.

Materials and Methods

Study Design

This randomized crossover study investigated the physiological response to SIE following a 14-hour overnight fast with different rest intervals. This research followed the Declaration of Helsinki (1964) and has been approved by the Ethics Committee in Biomedical Research, University of Guilan (IR.GUILAN.REC.1403.050).

Participants

Upon an announcement, fourteen 18-25-year-old males volunteered for this study. After a clear explanation of the research protocol and examining the eligibility criteria, ten healthy men (aged: 22.70 ± 1.15, weight: 79.90 ± 7.56 kg, height: 180.6 ± 5.89 cm, and body mass index (BMI): 22.94 ± 1.75 kg / m^{-2}) with normal BMI, at least one year of regular exercise, and no history of cardiometabolic diseases or musculoskeletal injuries were selected (estimated sample size: 9, $\alpha = 0.05$, effect size = 0.5, and actual statistical power = 0.85, G Power software). One person refused to participate in the SIE protocol, and three volunteers did not meet the research criteria. Selected participants were fully informed about the research protocol and completed and signed a written informed consent form.

Procedure

In the first session, after 14 hours of overnight fasting, pre-test blood samples were collected from the antecubital vein. The participants were asked to continue their daily activities during the research and maintain their usual diet, but refrain from consuming caffeine and vigorous activity 48 hours before the trials. The last meal before 8 pm was consumed similarly (~500 kcal, containing 70% carbohydrate, 20% protein, and 10% fat), and the participants were only allowed to drink water and zero-calorie beverages for 14 hours. The subjects randomly performed a 1:2 or 1:4 SIE protocol in two sessions separated by at least 72 hours of rest. The randomization sequence was generated using the Random Allocation Software before the study's commencement to ensure an unbiased distribution of conditions among participants. Participants warmed up for 15 minutes, including static/dynamic stretches and light cycling on a stationary bicycle. SIE protocol consisted of 5 sprints with maximal speed on the Monark E894 ergometer, with adjusted handlebar and seat height, and a 5% of the body weight basket dropped from the beginning of cycling for each participant. They were also asked to perform their maximum effort in work intervals and passive sitting on the bike in rest intervals. Work-to-rest ratio for 1:2 and 1:4 HIIE was considered 15s: 30s and 15s: 60s, respectively. Both trials were held between 08:00 to 12:00, in similar times and conditions for each participant's testing.

Blood pressure was measured using a digital device (Omron M6) before (after 15 minutes of sitting rest) and immediately after exercise. The heart rate was monitored before and during

protocol using an H10 Polar belt and the Polar Beat application on an Android mobile phone. After 5 minutes of cooling down, blood samples were collected in both EDTA and coagulating tubes to obtain serum and plasma samples and transferred to the laboratory. Serum glucose concentrations were determined using an enzymatic colorimetric method (Glucose Oxidase-Peroxidase; Pars Azmun, Tehran, Iran). Serum insulin levels were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Monobind Inc., Lake Forest, CA, USA). Plasma lactate concentrations were assessed using a colorimetric assay kit (ZellBio GmbH, Ulm, Germany). Serum urea levels were determined by an enzymatic colorimetric method using a commercial kit (Pars Azmun, Tehran, Iran. A complete blood count (CBC), including white blood cell (WBC) and platelet counts, was performed using an automated hematology analyzer (Sysmex KX-21N, Sysmex Corporation, Kobe, Japan) following the manufacturer's protocol.

Statistical Analysis

All Data are presented as mean ± standard deviation. The Shapiro-Wilk test was used to test the normality of the data distribution. In case of abnormality, data were normalized using the Inverse distribution function method with fractional ranks of data and original mean and SD of the data distribution [14]. Repeated measurements analysis of variance and Bonferroni tests were used to compare means between trials. In the case of the sphericity assumption violated by the Mauchly test, Greenhouse-Geisser was used to determine the significance of the F value. Partial eta squared (η_p^2) and observed power (OP) are reported in case of a significant F value. Data analysis was done using IBM SPSS software, version 27 ($P < 0.05$). GraphPad Prism 10.2.2 was used for graph illustration.

Results

Blood Pressure and Heart Rate

With systolic blood pressure (SBP), the main effect of time was significant ($F = 10.70$, $P = 0.010$, $\eta_p^2 = 0.543$, OP = 0.829, 95% CI = (-32.14, -5.86)), but the main effect of the trial ($P = 0.079$) and interaction effect of time*trial ($P = 0.178$) were not statistically significant. It shows that the effect of both protocols on SBP was similar (Figure 1, A). The main effect of the trial for diastolic blood pressure (DBP) was significant ($F = 6.321$, $P = 0.033$, $\eta_p^2 = 0.413$, OP = 0.611, 95% CI = (0.63, 11.97)). However, the main effect of time ($P = 0.730$) and the interaction effect of time*trial ($P = 0.120$) were not significant, and the mean DBP was ~13% lower after 1:4 compared with 1:2 SIE (Figure 1, B).

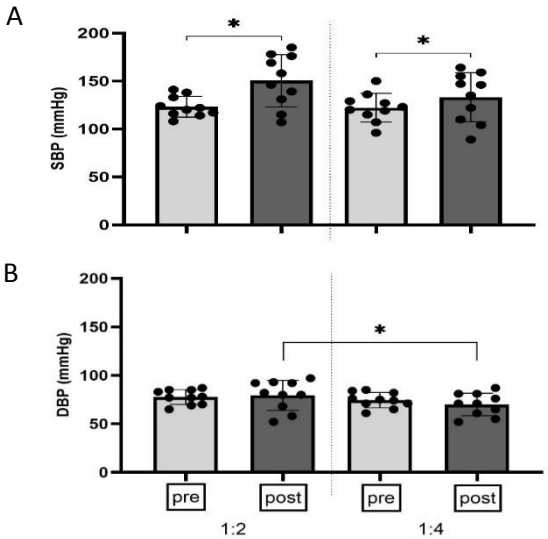

Figure 1. Mean ± SD of (A) systolic blood pressure (SBP) and (B) diastolic blood pressure (DBP). SBP increased with both protocols and there was no significant difference between protocols. Post-exercise DBP was significantly lower after 1:4 HIIE. *: significant difference at $P < 0.05$.

The main effect of time (F = 372.39, P < 0.001, η_p^2 = 0.976, OP = 1.000, 95% CI for Pre-test vs post1 (-88.59, -49.41); Post2 (-99.80, -70.80); Post3 (-106.25, -79.86); Post4 (-110.27, -83.23); Post5: -112.91, -83.79)) and trial (F = 5.270, P = 0.047, η_p^2 = 0.369, OP = 0.535, 95% CI = (0.08, 10.29)) for heart rate (HR) was significant but the time*trial interaction effect was not statistically significant (P = 0.227). HR increased after each work interval of both protocols. After the first and second intervals, HR had no difference in trials, but in three subsequent intervals, HR was higher in 1:2 SIE (P < 0.05) (Figure 2).

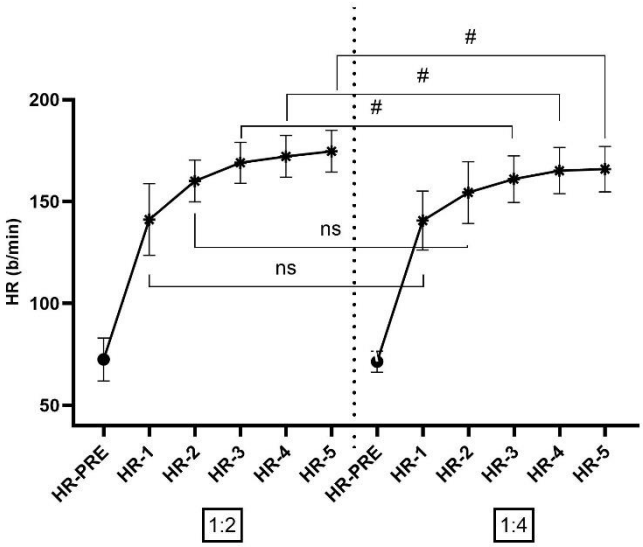

Figure 2. Mean ± SD of heart rate (HR) pre-exercise and after 5 work intervals. HR increased after each work interval in both protocols. The last three intervals induced lower HR in 1:4 HIIE compared with 1:2 HIIE. *: significant difference compared with pre-test; #: significant difference between trials (P < 0.05).

Blood Markers

Although RM ANOVA showed a significant F value for glucose levels (*F = 5.686, P = 0.012*, η_p^2 = 0.387, OP = 0.798), pairwise comparisons by the Bonferroni test showed no significant difference between trials (*P ≥ 0.05*) and mean glucose levels were not statistically different after 1:2 and 1:4 SIE compared with the control condition (Figure 3, A).

RM ANOVA showed a significant F value for insulin levels (F = 5.476, P = 0.014, η_p^2 = 0.378, OP = 0.782, 95% CI for control vs 1:2 HIIE (-13.48, -0.5)). Based on a pairwise comparison of Bonferroni, insulin levels only increased after 1:2 HIIE (*P = 0.035*) compared with the control trial (Figure 3, B).

There was a significant F value for lactate levels (F = 222.91, P < 0.001, η_p^2 = 0.961, OP = 1.000, 95% CI for control vs 1:2 HIIE (-18.80, -14.71) and 1:4 HIIE (-20.02, -13.14)) and based on the Bonferroni test, lactate levels increased significantly with both protocols compared with the control condition (P < 0.001). Still, it was not different between 1:2 and 1:4 SIE (P = 1.000) (Figure 3, C).

Urea levels had no significant difference between trials (P = 0.839) (Figure 3, D).

Lymphocyte count was different between trials (F = 21.27, P < 0.001, η_p^2 = 0.703, OP = 1.000, 95% CI for control vs 1:2 HIIE (-3.41, -1.09) and 1:4 HIIE (-3.79, -1.07)). WBC showed a significant difference between trials (F = 32.08, P < 0.001, ηp^2 = 0.781, OP = 1.000, 95% CI for baseline vs 1:2 HIIE (-6.08, -1.93)). Platelets were also different between trials (F = 15.69, P < 0.001, η_p^2 = 0.635, OP = 0.998, 95% CI for control vs 1:2 HIIE (-100.52, -23.48) and 1:4 HIIE (-111.25, -19.55)). LWR also showed a significant F value (F = 23.33, P < 0.001, η_p^2 = 0.722, OP = 0.994, 95% CI for control vs 1:2 HIIE (-0.15, -0.007) and 1:4 HIIE (-0.69, -0.18) and 1:2 vs 1:4 HIIE (-0.59, -0.14)). Further pairwise comparisons revealed that LWR significantly increased after 1:2 and 1:4 SIE and was ~80% higher after 1:4 compared with the 1:2 protocol (Figure 3, E). A significant F value was also observed for PLR (F = 6.395, P = 0.008, η_p^2 = 0.415, OP = 0.845, 95% CI for control vs 1:4 HIIE (6.38, 55.43)). PLR was significantly lower after 1:4 SIE compared with baseline (P = 0.015) (Figure 3, F).

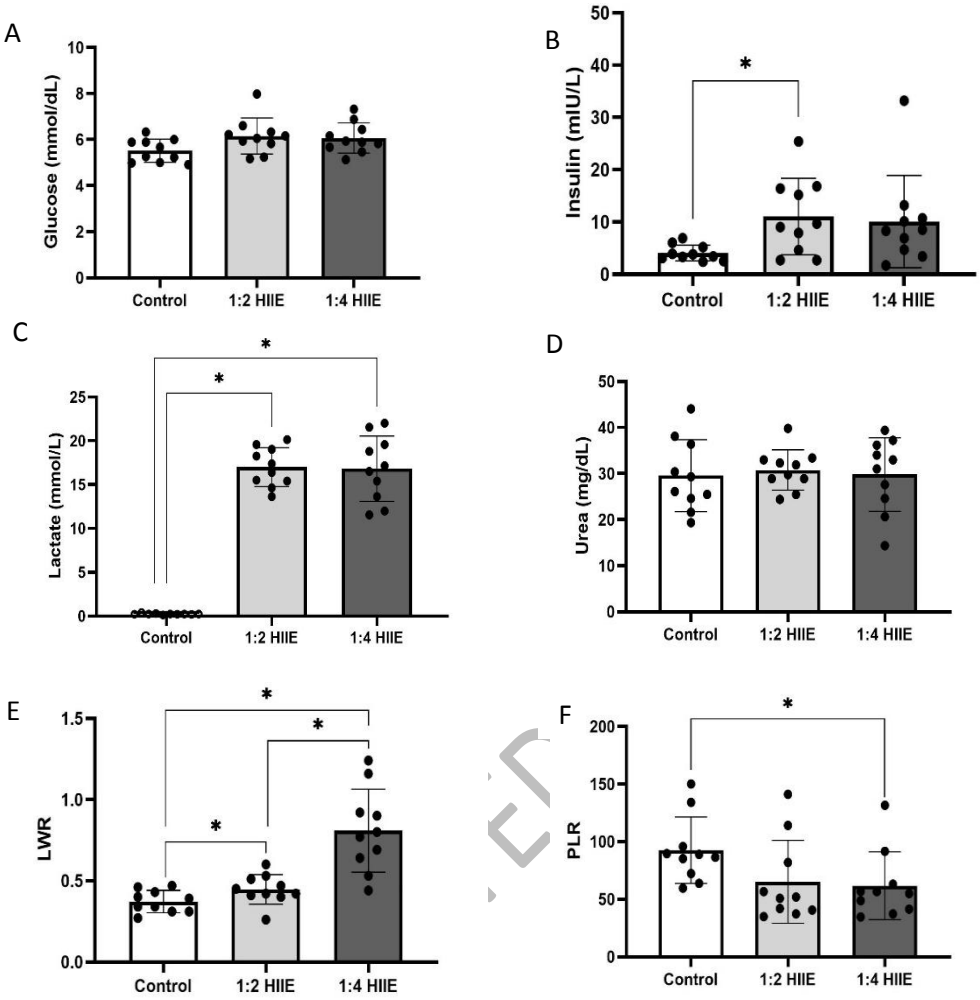

Figure 3. Mean ± SD of (A) Glucose and (B) Insulin (C) Lactate, (D) Urea levels, (E) lymphocyte to WBC ratio (LWR), and (F) platelet to lymphocyte ratio (PLR) in trials. Glucose and Urea levels were similar in both protocols compared with baseline levels. 1:2 HIIE increased insulin levels compared to baseline levels. Lactate levels increased similarly after both protocols. 1:4 HIIE increased LWR compared with baseline levels and 1:2 HIIE and lowered PLR compared with baseline levels. *: Significant difference at $P < 0.05$.

Discussion

This randomized controlled study was conducted in two counterbalanced trials in active males to investigate the effect of SIE with two work-to-rest ratios following a 14-hour overnight fast on selected physiological indices. To our knowledge, this is the first study that considers the work-to-rest ratio of all-out sprint cycling in a fasting state.

SIE is a short-term, intense exercise method that is believed to have beneficial effects on health and athletic performance [15]. SIE constitutes a particularly intense variant of HIIE that can be distinguished as repeated bouts performed with near-maximal to "all-out" effort. This characterization coincides with the highest intensity classification identified in training zone models or exercise prescription guidelines, including the extreme-intensity domain, anaerobic speed reserve, or near-maximal to maximal intensity classification [6]. We hypothesized that rest intervals of SIE in the fasting state have a different physiological effect.

Blood Pressure and Heart Rate

Based on data from pre- and post-exercise monitoring of BP and HR, both SIE protocols

elevated post-exercise SBP similarly, but post-exercise DBP was significantly lower following 1:4 SIE. Tan et al. reported that sprint interval exercise (4*30s sprint: 2min rest) while in a fasting state (> 10 hours of fasting) led to a notable reduction in blood pressure compared with the fed state [16]. However, the acute response of blood pressure to SIE in fasting is unclear. Kriel et al. reported that 1:4 SIE (30s: 2min) increased SBP but did not change DBP [17]. Some other studies have suggested that HIIT does not promote a significant difference in SBP but DBP [18]. The different observations may be related to the duration of work and rest intervals. Longer work intervals seem to elevate SBP, while longer rest intervals may lower DBP [19]. As exercise intensity increases, SBP typically exhibits a linear increase, reflecting increased metabolic demands, sympathetic activation, and thermoregulatory responses that collectively increase blood flow to the active muscles [20]. The decrease in DBP, is probably due to metabolic vasodilation in the exercising muscles, ensuring adequate oxygen delivery and waste removal [21]. It is highly evidenced that chronic intermittent fasting and calorie restriction can reduce BP by linking different pathways involving inflammation, reactive oxygen species (ROS), nitric oxide (NO), and others [22], but the acute effect of fasting on exercise BP needs to be further investigated.

It was anticipated that HR would increase during the SIE [19], and this was indeed observed. Notably, HR remained elevated when shorter rest intervals were employed. Conversely, when longer recovery periods were implemented during the 1:4 SIE protocol, HR was observed to be lower, similar to another research [23], possibly attributable to increased parasympathetic activity during recovery intervals [24].

Blood Markers

Post-exercise blood levels of glucose, insulin, urea, lactate, and the LWR and PLR were compared with a baseline level. The glucose levels did not show any significant difference relevant to the SIE protocol. The fasting glucose levels were near normal values (5.51 ± 0.49 mmol/dL). Although the glucose level was ~6 mmol/dL after 1:2 and 1:4 SIE, it was not statistically different from the baseline. Despite this, it is reported that a modest reduction in serum glucose levels can occur within a few hours of fasting (fasting glucose levels of 3.3 and 3.9 mmol/L), likely due to attenuated hepatic glycogen synthesis and glycolysis due to decreased insulin concentrations, and also increased glucagon levels and enhanced sympathetic activity [1]. Continuing fasting duration may attenuate insulin levels and activate gluconeogenesis through amino acids, glycerol, and ketone body availability [25]. Free fatty acids (FFAs) can be the main energy source of exercise during fasting because of enhanced adipose tissue lipolysis [1]. However, we observed increased levels of insulin after SIE, but it was only significant with the 1:2 protocol. Baseline fasted-insulin levels were 4.05 ± 0.48 mIU/L, increasing to 11.04 ± 2.31 and 10.07 ± 2.80 mIU/L after 1:2 and 1:4 SIE. During the absorptive state (after eating), insulin levels will increase, potentially reaching levels between 30-70 mIU/L or higher, depending on the meal and individual factors. But, usually, for a fasting glucose level of about 5 mmol/L, the insulin ranges between 5 and 10 mU/L (35–70 pmol/L), with some variation depending on the insulin assay usedIn contrast [26], and it is reported that longer fasting periods (36h vs 12h) are related to more reduced blood glucose and insulin levels [27]. Also, a meta-analysis revealed that fasting plus exercise has no or minimal effect on glucose and insulin levels, but the time of fasting or exercise mode was not precisely documented by the authors [28]. Adams (2013) reported that in intense exercise (>80% VO_2max), unlike at lesser intensities, glucose is the exclusive muscle fuel. Catecholamine levels rise markedly, causing glucose production to rise seven- to eightfold while glucose utilization is only increased three- to fourfold. In people without diabetes, there is a small blood glucose increase during intense exercise that increases further immediately at exhaustion and persists for up to 1 hour. Plasma insulin levels rise, correcting the glucose level and restoring muscle glycogen [29]. This may explain the higher glucose and insulin levels observed in our study. However, more studies are needed to measure insulin levels during fasted state exercise.

Lactate levels were highly increased after both SIE protocols (~17 mmol/L), and there was no difference between protocols. Animal studies have also shown that a single session of HIIE raises lactate levels to 375%, independent of work-to-rest ratios [30]. The normal resting

value of lactate is less than 1 mmol/L. In the current study, the fasted lactate level was approximately 0.23 mmol/L. Following HIIE, there is increased neuronal activation in the prefrontal cortex. Neurons can use muscle-derived lactate to meet these heightened energy demands [31]. Lactate may serve as an alternative fuel for the brain during fasting, potentially sparing glucose. Acute sprint exercise eliciting peak blood lactate accumulation may increase brain-derived neurotrophic factor (BDNF) expression in the brain [32] and may give rise to enduring cognitive and neural benefits [31]. However, the lactate response to high-intensity exercise in fasting needs to be investigated.

Urea formation during muscular activity is a violation of the balance of adenosine triphosphate (ATP) in working muscles, as well as increased protein catabolism [11]. We questioned whether high-intensity exercise in a fasting state might lead to protein degradation. However, our SIE protocols did not increase blood urea formation. Control of the dynamics of urea after a set of training loads indicates the balance of anabolic and catabolic processes in the athlete [11] and it seems SIE in a fasting state may not harm protein metabolism.

The lymphocyte-to-white blood cell ratio (LWR) [33] and platelets-to-lymphocyte ratio (PLR) [13], are blood markers of the systemic inflammatory response. As we observed, LWR increased significantly after 1:2 (~22%) and 1:4 (~119%) SIE protocols compared with the baseline, and the difference was also significant. PLR was lower after 1:4, but it was not different from the 1:2 protocol. It seems all-out sprint exercises provoke a greater immune response than moderate continuous exercise [12, 34]. It is also known that a single exercise session only induces a transient immune response [35]. Lymphocytes elevate during exercise and decrease shortly after exercise cessation. Within 24 hours, baseline levels are usually restored [13]. It is suggested that active rest intervals may attenuate the increased immune response to SIE [34]. Interestingly, we observed much higher LWR after longer rest intervals in the 1:4 SIE protocol. Perhaps the active rest intervals may help to reduce the immune response to 1:4 high-intensity protocols.

This study is, to our knowledge, the first to investigate the effects of the work-to-rest ratio of SIE protocols conducted in a fasted state on physiological responses. However, it had several limitations, including a small sample size and recruiting only men as participants. Additionally, we were unable to measure postprandial levels of parameters before and after exercise.

Conclusion

We found that the work-to-rest ratio in fasted-state SIE protocols may influence heart rate, diastolic blood pressure, and immune response. However, it does not appear to induce hypoglycemia, lactate production, or protein degradation. Athletes and coaches may benefit from fasting and manipulating the intensity of SIE protocols. Finally, we suggest conducting a similar protocol in females, with comparisons to postprandial physiological responses.

Declarations

Funding

This research did not receive specific funding.

Conflict of Interest

The authors have no relevant financial or non-financial interests to disclose.

Code of Ethics

This study is approved by the Ethics Committee in Biomedical Research, University of Guilan (IR.GUILAN.REC.1403.050).

Author Contributions

E.MS: performing protocol, data collection, writing the draft; A. D: study design, supervision, revision of manuscript; M. E: study design, supervision, data analysis, writing and revising the draft.

Artificial Intelligence

We have not used any AI tools or technologies to prepare this manuscript.

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JOURNAL OF NUTRITION FASTING AND HEALTH

Prevalence and Antimicrobial Resistance of *Escherichia coli* in Ready-to-Eat Vegetables and Salads in Mashhad

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Salads
Antimicrobial resistance
Mashhad

ABSTRACT

Introduction: The rising global consumption of ready-to-eat (RTE) vegetables has highlighted concerns about their microbiological safety, particularly regarding contamination with antibiotic-resistant *Escherichia coli* (*E. coli*). This study aimed to isolate *E. coli* from green vegetables and salads sold in Mashhad, Iran, and assess their antimicrobial resistance profiles.

Methods: A total of 120 RTE vegetable samples were analyzed using microbiological procedures outlined in the Iranian National Standard ISIRI 2946. Confirmatory identification of *E. coli* was performed via culture-based methods and indole testing. Antibiotic susceptibility was evaluated using the Kirby-Bauer disk diffusion method, following CLSI guidelines.

Results: Out of 120 samples, 40 (33.33%) tested positive for *E. coli*. The highest susceptibility rates were to nalidixic acid (57.5%) and chloramphenicol (55%). However, significant resistance was observed against cefazolin (67.5%), cefixime (62.5%), and ciprofloxacin (62.5%). Intermediate resistance to colistin (47.5%) raises concern due to its role as a last-resort antibiotic. Multidrug resistance (MDR) was prevalent, with 80% of isolates resistant to at least two antibiotics.

Conclusion: The detection of multidrug-resistant *Escherichia coli* in one-third of RTE vegetable and salad samples from Mashhad highlights a significant public health concern. These findings underscore the urgent need for enhanced hygiene practices, regular microbial surveillance, and antibiotic resistance monitoring to ensure the safety of RTE produce and prevent potential transmission of resistant strains through the food chain.

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Introduction

The consumption of fresh and ready-to-eat (RTE) vegetables has increased globally due to heightened awareness of their nutritional benefits and their role in preventing chronic diseases such as cardiovascular disorders and cancer [1]. However, these food products can also serve as vehicles for foodborne pathogens, particularly when consumed raw or with minimal processing [2]. Among the major bacterial contaminants of concern is *E. coli*, which serves not only as a fecal indicator organism but also includes pathogenic strains that can cause serious gastrointestinal illness [3]. E. coli contamination in vegetables may result from multiple sources, including the use of untreated water for irrigation, organic fertilizers such as manure, contaminated harvesting equipment, or poor hygiene during post-harvest handling and packaging [4]. Particularly, the presence of antibiotic-resistant *E. coli* in food items is a growing public health concern. The misuse and overuse of antibiotics in agriculture and clinical settings have contributed to the emergence and dissemination of antimicrobial-resistant bacteria in the environment, which may be transmitted to humans through the food chain [5].

The detection and antimicrobial resistance profiling of *E. coli* in fresh produce are thus crucial for assessing the microbial safety and public health risks associated with these food items. Standardized microbiological methods, such as those outlined in the Iranian National Standard ISIRI 2946, provide guidance for the isolation and enumeration of *E. coli* in food samples. Additionally, the Kirby-Bauer disk diffusion method remains a widely accepted technique for determining antimicrobial susceptibility patterns.

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This study aimed to isolate and identify *E. coli* strains from ready-to-eat green vegetables and salads marketed in local outlets, assess their antimicrobial resistance patterns using the Kirby-Bauer method, and contribute to the growing body of evidence on the microbiological safety of fresh produce in Iran.

Materials and Methods

Sample Collection and Bacterial Isolation

A total of 120 ready-to-eat green vegetable and salad samples were collected and submitted to the Huanna Zist Binaloud Laboratory (Mashhad, Iran) for microbiological analysis. Isolation of *E. coli* strains was carried out according to the Iranian National Standard No. 2946 [6]. For each sample, 10 grams of the vegetable or salad material were added to 90 mL of Ringer's solution (Merck, Germany) as a diluent. Subsequently, 10 mL of it were transferred into a test tube containing 10 mL of double-strength Lauryl Sulfate Broth (LSB) (Ibersco, Iran) equipped with a Durham tube. The tubes were incubated at 37°C for 24 hours; if no turbidity or gas formation was observed, incubation was extended to 48 hours.

Samples showing turbidity and gas production were considered presumptive positive and were transferred to EC Broth (Ibersco, Iran). These tubes were incubated at 44°C for 24 to 48 hours. Tubes exhibiting turbidity and gas production were then inoculated into Peptone Water without indole (Ibersco, Iran), used as a confirmatory medium, and incubated at 44°C for an additional 48 hours.

After incubation, Kovac's reagent was added to the tubes. The appearance of a pink ring indicated a positive indole reaction, confirming the presence of *E. coli*. For further confirmation, a loopful of the EC Broth from positive tubes was streaked onto Nutrient Agar (Ibersco, Iran) and incubated for colony isolation and subsequent analysis.

Antimicrobial Susceptibility Testing

The antibiotic resistance profiles of the isolated *E. coli* strains were determined using the Kirby-Bauer disk diffusion method, following the guidelines provided by the Clinical and Laboratory Standards Institute [7]. Fresh bacterial suspensions equivalent to 0.5 McFarland standard were prepared and uniformly spread on Mueller-Hinton agar plates. Antibiotic-impregnated disks were applied to the surface, and plates were incubated at 37°C for 18–24 hours.

The antibiotic susceptibility of the *E. coli* isolates was assessed using the following antibiotics and their respective disk concentrations: Nalidixic acid (30 µg) and Ciprofloxacin (5 µg), Amoxicillin (25 µg), Cefixime (5 µg), Cefazolin (30 µg), Chloramphenicol (30 µg), Colistin (10 µg), and Trimethoprim-Sulfamethoxazole (1.25/23.75 µg).

After incubation, the diameters of the inhibition zones were measured in millimeters and interpreted as resistant, intermediate, or sensitive based on CLSI breakpoints.

Results

Prevalence of *E. coli* Isolates:

Of the 120 green vegetable and salad samples analyzed, 40 (33.33%; 95% CI: 25.53–42.17) tested positive for the presence of *E. coli*. (Figure 1).

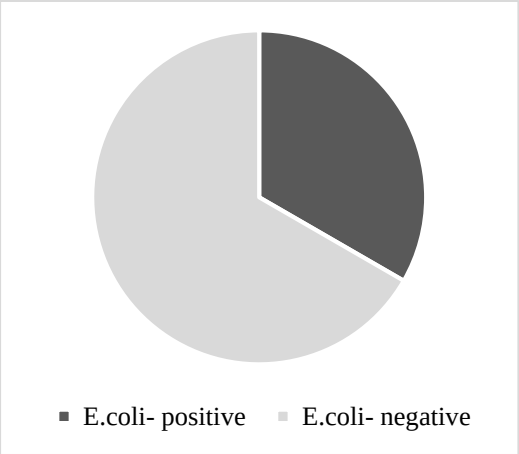

Figure 1. Proportion of E. coli in 120 samples

Antimicrobial Resistance Profiles of *E. coli* Isolates:

Antibiogram analysis revealed that the highest susceptibility among the *E. coli* isolates was to nalidixic acid (57.5%; 95% CI: 42.20–71.49) and chloramphenicol (55.0%; 95% CI: 39.83–69.29). The highest resistance rate was observed against cefazolin (67.5%; 95% CI: 52.02–79.92), followed by cefixime (62.5%; 95% CI: 47.03–75.78) and ciprofloxacin (62.5%; 95% CI: 47.03–75.78) (Table 1). Multiple antibiotic resistance was detected in 32 isolates (80.0%; 95% CI: 65.24–89.50), as detailed in Table 2. (Figure 2)

In relation to colistin susceptibility, previous studies by Jayol et al. (2017) and Olaitan et al.

(2014) proposed a non-standard yet widely cited correlation between inhibition zone diameter and MIC values in *E. coli*. Isolates with inhibition zones ≥ 13 mm generally correspond to MIC values ≤ 2 µg/mL and are considered susceptible according to EUCAST criteria. Zones of 10–12 mm typically indicate MIC values of 2–4 µg/mL, suggesting possible resistance, while zones ≤ 9 mm are often associated with MIC values ≥ 4 µg/mL and are therefore interpreted as resistant [8, 9].

Antimicrobial Resistance Index (AMR Index)

The Antimicrobial Resistance (AMR) index for each isolate was calculated using the formula:

$$\text{AMR Index} = \frac{\text{Number of antibiotics to which the isolate was resistant}}{\text{Total number of antibiotics tested}}$$

In this study, resistance was tested against 8 antibiotics; therefore, the AMR index for an isolate resistant to n antibiotics was calculated as:

$$AMR\ Index = \frac{n}{8}$$

Isolates were classified as multidrug-resistant (MDR) if they showed resistance to three or more classes of antibiotics.

Multiple Antibiotic Resistance Patterns

Of the 40 *E. coli* isolates, 32 (80.0%; 95% CI: 65.24–89.50) were multidrug-resistant (MDR), showing resistance to at least one antimicrobial agent in three or more classes.

The most frequent MDR profile was resistance to five antibiotics — observed in 10 isolates (25.0%). This profile typically included cefazolin, cefixime, ciprofloxacin, amoxicillin, and either sulfamethoxazole-trimethoprim or chloramphenicol.

The resistance pattern with the highest number of resistant antibiotics was resistance to seven agents (cefazolin, cefixime, ciprofloxacin, amoxicillin, chloramphenicol, sulfamethoxazole-trimethoprim, and nalidixic acid), detected in 3 isolates (7.5%). This pattern yielded the highest AMR index of 1.0, indicating complete resistance to all tested antibiotics.

The second-highest AMR index (0.86) corresponded to resistance to six antibiotics, observed in 1 isolate (2.5%).

MDR profiles involving four antibiotics were recorded in 5 isolates (12.5%), and those with three antibiotics in 11 isolates (27.5%). Two isolates (5.0%) showed resistance to only two antibiotics and were not classified as MDR.

The AMR index across MDR isolates ranged from 0.43 to 1.0, with a mean of 0.71, reflecting a substantial resistance burden among *E. coli* strains isolated from fresh vegetables and salads in this study (Table 2).

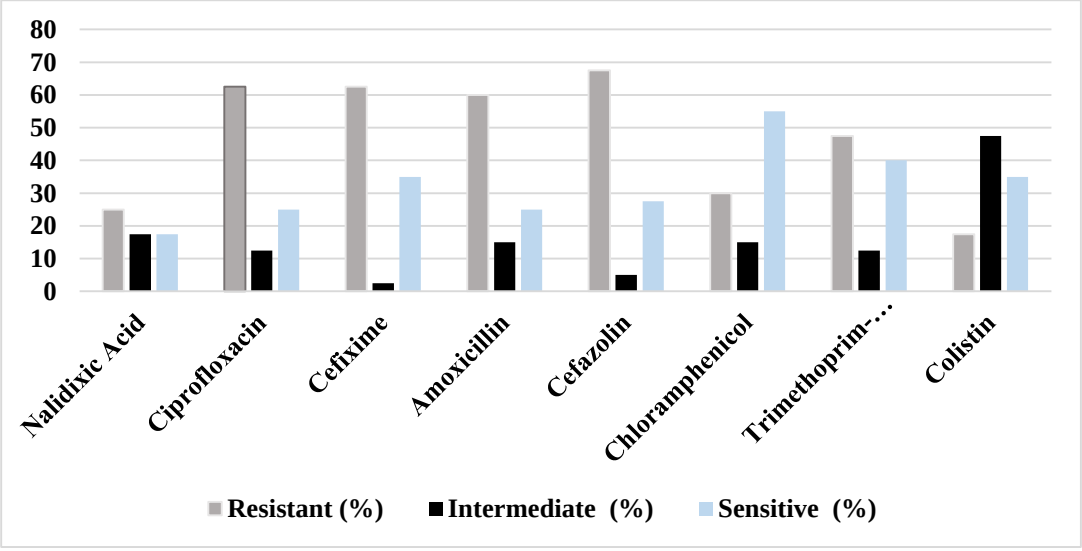

Figure 2. Comparison of the resistance patterns of *E. coli* isolates in the analyzed samples against eight selected antibiotics.

Table 1. Number and percentage of susceptible, intermediate, and resistant isolates to 8 antibiotics

Antibiotic	Abbreviation	Resistant(%)	Intermediate(%)	Sensetive(%)
Nalidixic Acid	NA	10(25)	7(17.5)	23(57.5)
Ciprofloxacin	CP	25(62.5)	5(12.5)	10(25)
Cefixime	CFM	25(62.5)	1(2.5)	14(35)
Amoxicillin	AMC	24(60)	6(15)	10(25)
Cefazolin	CF	27(67.5)	2(5)	11(27.5)
Chloramphenicol	C	12(30)	6(15)	22(55)
Trimethoprim-sulfamethoxazole	SXT	19(47.5)	5(12.5)	16(40)
Colistin	CL	7(17.5)	19(47.5)	14(35)

Table 2. Number and percentage of isolates resistant to more than one antibiotic.

Number of antibiotic	Antibiotics	Number of isolates	Sum(%)
2	CP, CF	2	2(5)
3	CP, CFM, SXT	3	11(27.5)
	CFM,CF,SXT	1	
	NA,CP,CFM	1	
	CP, CFM,CF	1	
	AMC,CF,C	1	
	CL, AMC,SXT	2	
	AMC,CF,SXT	1	
	NA,CFM,CF	1	
4	CP,CFM,AMC,CF	2	5(12.5)
	CFM,AMC,CF,C	1	
	CP,CFM,AMC,CF	1	
	CL,CP,AMC,CF	1	
	CP,AMC,CF,C,SXT	1	
5	CP,CFM,AMC,CF,SXT	2	10(25)
	CP,CFM,AMC,CF,C	2	
	NA,CFM,AMC ,CF,SXT	1	
	NA,CFM,AMC,C,SXT	1	
	CL,CP,AMC,C,SXT	1	
	CL,NA,AMC,CF,SXT	1	
	NA,CP,CFM,AMC,CF	1	
6	NA,CP,CFM,AMC,CF,SXT	1	1(2.5)
7	CL,CP,CFM,AMC,CF,C,SXT	1	3(7.5)
	NA,CP,CFM,AMC,CF,C,SXT	2	

Discussion

The presence of *E. coli* in RTE green vegetables and salads represents a growing public health concern, particularly when these products are consumed raw and without further processing. While recent studies in northern Iran report low or undetectable levels of *E. coli* in RTE vegetables and salads [10], emerging evidence indicates that such contamination may still be present in other regions, including Mashhad, especially with the emergence of multidrug-resistant (MDR) strains [11,12].

In this study, analysis of RTE vegetable and salad samples from Mashhad revealed the presence of *E. coli* at a considerable level. The antimicrobial susceptibility profile highlighted a mix of sensitivity to some agents and pronounced resistance to several commonly used antibiotics, with a notably high proportion of isolates classified as multidrug-resistant (MDR). Azimirad et al. analyzed 92 RTE leafy green samples in Tehran and reported an *E. coli* prevalence of 23.2%, which is lower than the rate observed in our study. This variation may reflect differences in regional hygiene standards, environmental conditions, or postharvest handling practices [13]. Similarly, Soltan Dallal et al. evaluated 65 vegetable and salad samples in Tehran, finding that 71% of salads exceeded acceptable microbiological thresholds, including contamination with *E. coli*. Although the precise prevalence was not specified, their results further emphasize the widespread microbial contamination of RTE produce in Iran [14].

This study found the presence of *E. coli* in RTE vegetables sold in Mashhad and characterized their antimicrobial resistance (AMR) patterns. Our findings are consistent with national and

international studies demonstrating high resistance rates in *E. coli* isolates, particularly against β-lactam antibiotics such as ampicillin and cefoxitin, as well as the detection of key resistance genes including *blaTEM* and *qnr* variants [15, 16, 17]. Similar resistance trends have been reported across Iran, with significant prevalence of ESBL-producing strains in RTE lettuce and salads [15].

Globally, AMR in *E. coli* from RTE vegetables shows significant regional variation. In Lebanon, 60% of *E. coli* isolates from salads were multidrug resistant, with inadequate washing practices contributing to microbial persistence [18]. In Côte d'Ivoire, alarming resistance rates of 100% to cefuroxime and 87.5% to ampicillin and cefoxitin were recorded [15]. In contrast, resistance rates in European lettuce farms were relatively low (ampicillin: 7%; no resistance to gentamicin or ciprofloxacin), likely reflecting better agricultural practices and antibiotic stewardship [19].

From a genomic standpoint, resistance genes such as *blaTEM*, *blaCTX-M*, *tetA*, *sulI*, and aminoglycoside resistance genes like *aadA* and *aac(3)-IV* have been frequently reported in *E. coli* isolates from vegetables across various countries, including Ethiopia, Germany, and Bangladesh [20, 21, 22]. Many of these genes are plasmid-borne and capable of horizontal transfer, raising concerns about their potential transmission to human gut microbiota following consumption [23].

Moreover, global analyses of over 94,000 *E. coli* genomes have shown that nearly 50% carry antibiotic resistance genes (ARGs), with multidrug-resistance profiles being especially common in low- and middle-income countries due to suboptimal food safety systems and widespread antibiotic misuse [24]. Notably, high-risk clones such as *E. coli* ST131 are emerging worldwide and contribute to the persistence and dissemination of resistance traits, particularly to fluoroquinolones and third-generation cephalosporins [25].

While the overall prevalence of *E. coli* in RTE vegetables in Mashhad appears moderate, the detection of MDR isolates with resistance to multiple antibiotics including β-lactams, aminoglycosides, and fluoroquinolones highlights a significant public health risk. This is especially critical given the growing global trend of carbapenem and colistin resistance, even though such resistance was low or absent in our isolates [26].

Hygiene practices play a central role in determining contamination levels. Studies from Kenya and Cameroon show that while vendors may be aware of protective practices (e.g., glove use), improper food handling and insufficient washing contribute to contamination [27, 28]. Vinegar washing has been shown to significantly reduce *E. coli* loads compared to water-only washing [29], yet some studies demonstrate that even repeated washing may not completely eliminate bacterial contamination [30].

In Iran, although no large outbreaks of foodborne *E. coli* linked to salads have been documented, high rates of antibiotic resistance in isolates from animals and food sources suggest a potential for cross-contamination and foodborne transmission of MDR strains [12, 31].

From a policy perspective, the increasing prevalence of MDR *E. coli* in food products has led to stricter food safety regulations and monitoring systems in many countries. Integration of food safety policies with antimicrobial stewardship and improved sanitation infrastructure (WASH) is critical to mitigating the risks associated with resistant foodborne pathogens [32, 33].

Conclusion

The detection of *E. coli*—particularly multidrug-resistant strains—in RTE vegetables and salads from Mashhad underscores the intersection of food safety and antimicrobial resistance as a pressing public health challenge. Regional variation in contamination rates, both within Iran and internationally, highlights the role of agricultural practices, hygiene standards, and postharvest handling in shaping microbial risks. The persistence of resistance genes in foodborne *E. coli* and their potential for horizontal transfer further elevates the threat of disseminating resistance to the human gut microbiota. Strengthening food safety surveillance, enforcing hygienic handling practices, and aligning national strategies with global antimicrobial stewardship initiatives are essential steps to reduce the burden of resistant *E. coli* in the food chain and safeguard consumer health.

Declaration

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Conflicts of Interests
None declared by Authors.

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Code of Ethics
The research did not involve human participants or animal subjects, and thus did not require approval from an ethics committee. All procedures were conducted in accordance with relevant guidelines and regulations for microbiological studies of food samples.

Authors' Contribution
Author 1 conceptualized the study, designed the methodology, and supervised the research process.
Author 2 conducted sample collection, performed laboratory experiments, and contributed to data analysis.
Author 3 analyzed the data, wrote the initial manuscript draft, and handled literature review and referencing.
All authors reviewed and approved the final version of the manuscript.

Artificial Intelligence (AI)
Perplexity AI was utilized to search for relevant articles during manuscript preparation, while ChatGPT was employed to enhance the language and improve readability.

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JOURNAL OF NUTRITION FASTING AND HEALTH

A Three-Day Effects of Mixed Pomegranate and Barberry Juice Consumption on Hemodynamic Parameters, and Blood PH Status following Force-Velocity based Exercises in Male Athletes

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ABSTRACT

Introduction: The effects of combined pomegranate and barberry juice supplementation, as well as the influence of exercise sequence on hematological, hemodynamic, and acid–base responses during and after sports preparation, remain unclear.

Methods: This study aimed to investigate hematological, hemodynamic, and acid–base responses to different sequences of high-intensity anaerobic–resistance exercises following the ingestion of a combined pomegranate and barberry juice supplement in athletes. A total of 12 athletes (mean age: 24.33 ± 0.78 years; height: 176.75 ± 3.08 cm; weight: 73.93 ± 3.71 kg) participated in a double-blind crossover design. Each athlete received either the combined supplement (220 mL of pomegranate and barberry juice) or a placebo in four separate trials. The exercise protocol involved sequential anaerobic–resistance exercises performed in four different orders: (1) power–velocity–strength with supplement/placebo, and (2) power–strength–velocity with supplement/placebo.

Results: The results showed that, across all four trials, supplementation significantly decreased mean corpuscular hemoglobin concentration (MCHC; $P = 0.007$) and increased respiratory rate ($P = 0.024$) compared with placebo. Although no significant between-group differences were observed for other hematological variables (HCT, MCH, HGB, WBC, LYM, PLT), significant within-group changes were detected for each marker ($P < 0.05$). Similarly, no significant between-group differences were found for hemodynamic indicators (HR, SBP, DBP, MAP, SaO_2, BR), although significant within-group alterations were observed ($P < 0.05$). Acid–base markers (LA, pH, HCO_3^-) also showed no significant between-group differences, but significant within-group changes occurred ($P < 0.05$).

Conclusion: In conclusion, supplementation with a combination of pomegranate and barberry juice appears to enhance athletic performance by reducing metabolite accumulation. Furthermore, the order in which exercises are executed influences physiological responses. These findings emphasize the importance of both nutritional supplementation and appropriate exercise sequencing in mitigating the adverse effects of anaerobic training.

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Introduction

Hematological parameters such as hemoglobin (HGB) concentration, red blood cell (RBC) count, and hematocrit (HCT) are critical for maintaining adequate oxygen transport and carbon dioxide clearance during exercise. Alterations in these indices, driven by increased muscular oxygen demand and plasma volume shifts, can markedly influence athletic performance (1). The structural characteristics of blood can vary depending on the type of physical activity. For instance, endurance training is well established to enhance RBC counts, while resistance training has also been identified as an effective strategy for improving RBC function (2, 3). Ahmadi Zadeh et al. reported changes in blood concentration following acute resistance training, attributing these to exercise-induced plasma volume

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alterations (2). Moreover, intense exercise has been shown to acutely stimulate circulating endothelial progenitor and angiogenic cells, potentially contributing to physiological adaptations through angiogenesis and tissue repair (4). Other investigations have examined the effects of exercise on hemoglobin levels, leukocyte counts, and platelets under varying durations and intensities, generally reporting increases in hemoglobin and platelet counts post-exercise (5). Despite significant advances in understanding exercise-induced hematological and hemodynamic adaptations, previous research indicates that these responses are strongly dependent on the type, duration, and intensity of exercise. Additionally, individual factors such as sex, nutrition, age, and environmental conditions further modulate these parameters (6).

In recent years, the consumption of antioxidant-rich supplements such as pomegranate juice and barberry has attracted increasing attention for their potential to reduce oxidative stress and enhance muscle recovery. Pomegranate juice is rich in bioactive compounds, including polyphenols and flavonoids, which help prevent free radical formation and mitigate oxidative damage induced by high-intensity exercise (7). Previous studies have reported that pre-exercise pomegranate supplementation can attenuate inflammatory markers, improve antioxidant capacity, and enhance muscular endurance (7). Moreover, supplementation has been associated with increases in RBC count and hemoglobin concentration (8). Barberry, containing active compounds such as berberine and berbamine, has been recognized for its anti-inflammatory and cardioprotective effects (9). Belyani et al. (2025) further demonstrated that pomegranate supplementation reduces oxidative stress and inflammation, with evidence suggesting accelerated recovery from exercise-induced muscle damage (EIMD), encompassing metabolic, mechanical, and neuromuscular domains (10). Another critical factor in athletic performance is the sequence of exercise execution. Performing resistance training before anaerobic exercise may help preserve energy stores in the initial stages and delay muscle glycogen depletion. Conversely, initiating training with anaerobic exercise can elevate lactate production, thereby impairing subsequent resistance performance (11). Exercise sequence has also been shown to affect oxygen consumption, metabolite accumulation, and blood pH, all of which are central to optimizing performance and delaying fatigue (12). Exercise-induced muscle activity inevitably generates free radicals, with the magnitude and source of production varying by exercise type (13). Oxidative stress arises when reactive oxygen species exceed the capacity of endogenous antioxidant defenses, leading to cellular and tissue damage (14). Studies indicate that performing strength training before resistance exercise can further enhance anaerobic metabolism, elevating hydrogen ion concentration, lactate, heart rate, and ADP levels. These alterations impair muscle power output by reducing calcium binding to troponin and diminishing contractile force (15). These considerations raise two essential questions: (i) Can modifying the exercise sequence influence hematological, hemodynamic, and acid–base indices? and (ii) can supplementation with pomegranate and barberry mitigate these exercise-induced changes? Therefore, the primary aim of this study was to investigate whether pre-exercise consumption of combined pomegranate and barberry juice affects hematological, hemodynamic, and acid–base responses during resistance and anaerobic exercise.

Materials & Methods

Participants

Twelve athletes from the University of Guilan, whose characteristics are presented in Table 1, participated in this study. All participants were thoroughly informed about the research procedures before enrollment. Inclusion criteria consisted of a training history of more than six months, absence of medication use, and no musculoskeletal disorders. Written informed consent was obtained from all participants before participation.

Table 1. Participant's characteristics Mean values and standard deviations, n: (12)

	Mean ± Sd
Age (y)	24.33 ± 0.78
Weight (kg)	73.93 ± 3.71
Height (cm)	176.75 ± 3.08
BMI (kg/m^2)	23.14 ± 2.82

The study protocol was reviewed and approved by the Ethics Committee of the University of Guilan (IR/SSRI.REC.2023.13829.1974) and was

conducted at the Exercise Physiology Laboratory of the University of Guilan.

Study Design

This study was conducted using a randomized, double-blind, balanced, crossover, placebo-controlled design (PLB). Participants were evaluated in five stages. The initial stage included familiarization with the exercise protocol, determination of one-repetition maximum (1RM) in the squat exercise, and collection of anthropometric data (height, weight, and body composition). In the second stage, participants consumed 220 mL of pomegranate and barberry juice daily for three consecutive days, after which they performed the exercise protocol in the sequence of power, strength, and velocity. Following a one-week washout period, participants consumed a placebo for three consecutive days and then repeated the exercise sequence (power, strength, and velocity) under the same conditions as in Stage 2. After another one-week interval, participants again consumed the supplement for three consecutive days and subsequently performed the exercise protocol in a different order: power, velocity, and strength. Finally, after a further one-week washout, participants consumed the placebo for three consecutive days and repeated the same exercise sequence as in Stage 4 (power, velocity, and strength). Blood samples were collected at two time points in each trial: immediately before and immediately after the exercise protocol, to analyze hematological indices and acid–base parameters. Hemodynamic parameters (heart rate, blood pressure, and respiratory rate) were assessed using laboratory-grade equipment at multiple time points: before exercise, immediately after, and at 10, 15, 20, and 30 minutes post-exercise. Arterial oxygen saturation (SaO_2) was measured using an Lk-88 pulse oximeter at two time points: before and immediately after exercise. Hematological variables (HCT, MCH, MCHC, HGB, WBC, LYM, and PLT) and acid–base markers (La^-, pH, and HCO_3^-) were evaluated from venous blood samples (table 2 and 3). To minimize confounding factors, participants were instructed to refrain from physical activity for at least 48 hours before each trial and to fast (abstain from both food and fluids) for at least 12 hours before testing. Anthropometric measurements were also recorded. Height was measured using a wall-mounted stadiometer (Seca 222; accuracy 0.1 cm). Body weight was measured with a laboratory scale (Camry FB9003; accuracy 0.1 kg). (Figure 1).

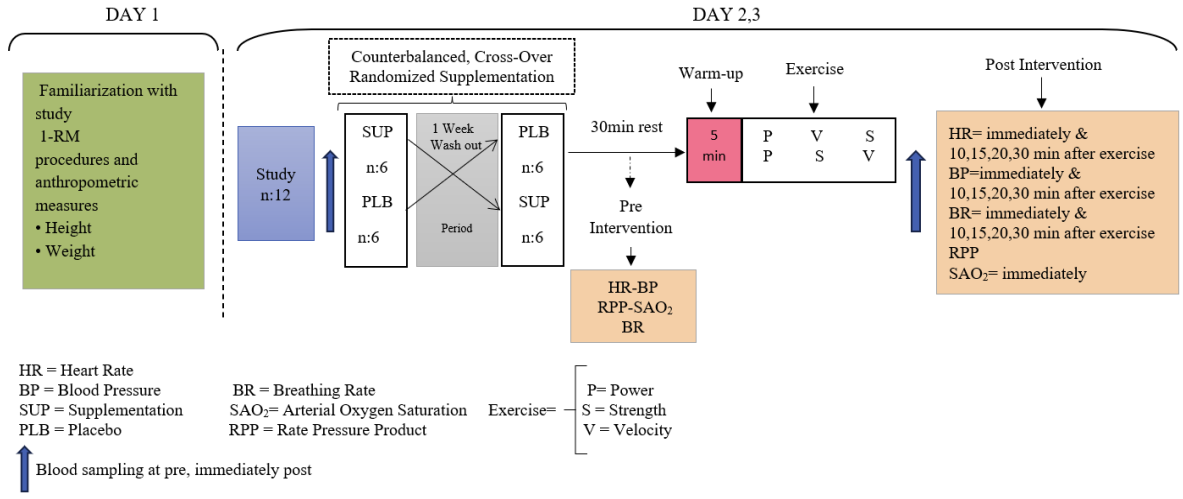

Figure 1. Study Design. Supplement and placebo administrations were performed in a randomized, double-blind, crossover manner. Venous blood samples were collected at two time points—immediately before and immediately after exercise—to evaluate hematological and hemodynamic parameters. Hemodynamic variables were also measured twice at baseline (pre-exercise) and subsequently at 0, 10, 15, 20, and 30 minutes post-exercise.

Table 2. Mean and standard deviation of hematological and acidity variables in pre-tests and post-tests across four trials.

	Trial 1 (P-S-V + PLB)		Trial 2 (P-S-V + SUP)		Trial 3 (P-V-S + PLB)		Trial 4 (P-V-S + SUP)	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
HCT (%)	40.11 ± 4.14	43.02 ± 4.08	39.46 ± 3.38	43.08 ± 3.30	39.62 ± 4.17	43.70 ± 4.53	40.10 ± 3.47	44.11 ± 3.40
MCH (pg)	29.44 ± 1.58	29.76 ± 1.64	28.98 ±0.902	29.09 ±0.942	29.21 ±0.591	29.85 ±1.29	28.90 ±0.807	29.24 ±0.799
HGB (g/dL)	13.57 ± 1.55	14.50 ± 1.62	13.42 ± 1.02	14.40 ± 1.22	13.45 ± 1.45	14.62 ± 1.62	13.37 ± 1.10	14.62 ± 1.12
MCHC (g/dL)	33.80±0.573	33.67±0.958	33.79 ± 0.448	33.43±0.841	33.95±0.681	33.45±0.750	33.34±0.620	33.10±0.757
WBC ($\times10^3$ cells/µL)	6.43 ± 1.41	9.61 ± 2.08	5.84 ± 1.27	9.00 ± 1.58	5.75 ± 1.35	9.11 ± 1.75	5.76 ± 1.02	8.80 ± 1.92
LYM ($\times10^3$ cells/µL)	37.75 ± 7.44	43.45 ± 7.50	38.27 ± 6.79	43.52 ± 6.41	35.74 ± 8.12	42.75 ± 9.38	40.15 ± 7.58	41.94 ± 9.03
PLT ($\times10^3$ cells/µL)	243.16±57.08	292.75 ±80.78	236.58 ±35.03	295.83 ±54.06	232.50±52.70	288.58 ±67.41	237.33±32.86	284.08±49.43
SaO_2 (%)	96.41 ± 2.60	96.16 ± 2.55	97.41 ± 1.16	96.25 ± 1.42	96.91 ± 1.50	96.58 ± 1.67	96.83 ±0.937	97.00 ± 1.59
La^- (mmol/L)	1.86 ± 0.288	10.66 ± 2.59	1.72 ± 0.597	10.50 ± 2.11	1.47 ± 0.298	11.64 ± 2.33	2.08 ± 0.693	8.91 ± 3.39
PH (unitless)	7.33 ± 0.02	7.22 ± 0.06	7.32 ± 0.02	7.22 ± 0.06	7.33 ± 0.01	7.20 ± 0.05	7.33 ± 0.01	7.23 ± 0.09
$HCO3^-$ (mmol/L)	28.22 ± 1.49	18.23 ± 3.36	26.84 ± 1.44	17.90 ± 2.89	27.42 ± 2.57	17.76 ± 2.96	26.51 ± 2.24	19.76 ± 2.99

P.S.V. power, strength, and velocity exercises
P.V.S. power, velocity, and strength exercises
SUP. Supplementation
PLB. Placebo

Table 3. Mean and standard deviation of hemodynamic indices at different time intervals in the four trials.

Trial	variables	pre	Ip	P10	P15	P20	P30
Trial 1 (P-S-V + PLB)	HR (bpm)	77.16 ± 8.89	113.91 ± 11.58	104.41 ± 8.96	100.91 ± 10.52	97.50 ± 9.59	93.50 ± 9.90
	SBP (mmHg)	104.66 ± 8.59	112.16 ± 15.01	103.08 ± 17.42	103.08 ± 13.66	102.25 ± 11.24	106.33 ± 21.27
	DBP (mmHg)	64.25 ± 9.06	70.33 ± 16.83	61.33 ± 8.10	63.50 ± 10.73	64.83 ± 9.30	69.75 ± 15.86
	AMP (mmHg)	77.58 ± 8.01	84.13 ± 15.21	75.19 ±10.06	76.56 ± 10.89	77.18 ± 7.32	81.82 ± 16.38
	RPP (mmHg·bpm)	8104.50±1358.18	12749.75±1950.00	10773.25±1887.21	10300±888.80	10000.00±1625.57	9987.66±2362.27
Trial2 (P-S-V + SUP)	BR (br/min)	19.66 ± 2.67	26.50 ± 4.01	22.91 ± 4.20	22.33 ± 2.67	20.66 ± 3.11	20.33 ± 3.28
	HR (bpm)	77.33 ± 11.97	112.66 ± 10.42	99.08 ± 12.22	96.58 ± 12.36	94.91 ± 10.11	91.08 ± 9.74
	SBP (mmHg)	109.83 ± 12.26	107.91 ± 11.52	113.41 ± 16.26	107.25 ± 12.62	110.33 ± 13.80	107.00 ± 18.81
	DBP (mmHg)	60.08 ± 5.65	64.75 ± 7.93	64.75 ± 16.14	64.33 ± 10.26	68.41 ± 16.90	66.58 ± 13.03
	AMP (mmHg)	77.00 ± 5.75	78.99 ± 6.33	80.81 ± 14.54	78.49 ± 9.79	82.24 ± 15.10	79.92 ± 13.20
	RPP (mmHg·bpm)	8466.66±1487.45	12119.66±1345.90	11220.91±2075.24	10344.66±1672.33	10443.33±1584.66	9808.83±2290.99
Trial3 (P-V-S + PLB)	BR (br/min)	21.83 ± 3.56	34.50 ± 5.97	27.00 ± 6.57	24.66 ± 5.54	24.16 ± 4.54	22.66 ± 3.93
	HR (bpm)	76.50 ± 12.90	111.25 ± 9.93	101.08 ± 9.64	98.50 ± 8.52	95.25 ± 7.62	91.66 ± 10.53
	SBP (mmHg)	107.41 ± 11.47	112.66 ± 13.43	109.58 ± 16.76	105.83 ± 11.35	106.41 ± 12.85	109.50±13.89
	DBP (mmHg)	66.00 ± 9.00	65.58 ± 8.86	67.08 ± 20.68	64.83 ± 11.06	64.66 ± 10.78	72.16 ± 16.10
	AMP (mmHg)	79.66 ± 8.16	81.12 ± 7.78	81.10 ± 18.55	78.36 ± 10.17	78.44 ± 10.44	84.48 ±14.29
	RPP (mmHg·bpm)	8190.33±1430.59	12511.66±1705.93	11079.33±1890.31	10486.33±1712.87	10152.41±1542.40	10057.25±1779.49
Trial4 (P-V-S + SUP)	BR (br/min)	20.83 ± 4.21	30.50 ± 6.82	25.00 ± 6.64	23.50 ± 5.53	23.00 ± 6.23	20.00 ± 4.00
	HR (bpm)	76.58 ± 10.80	111.75 ± 10.70	69.16 ± 11.40	98.00 ± 12.03	95.00 ± 9.89	92.66 ± 14.95
	SBP (mmHg)	108.00±13.03	113.83 ± 11.35	109.83 ± 11.67	102.66 ± 12.06	103.08 ± 7.58	106.08 ±9.46
	DBP (mmHg)	64.75 ± 11.55	64.33 ± 9.34	70.25 ± 17.38	64.58 ± 10.90	58.50 ± 7.56	63.50 ± 8.12
	AMP (mmHg)	79.02 ± 10.37	80.66 ± 7.89	83.31 ± 14.59	77.15 ± 10.41	73.21 ± 5.73	77.55 ± 4.79
	RPP (mmHg·bpm)	8011.33±1837.90	12690.50±1434.55	10516.41±1397.22	10037.66±1590.73	9800.08±1277.95	9771.00±1415.78
	BR (br/min)	20.83 ± 3.95	33.00 ± 9.92	26.50 ± 5.46	24.50 ± 6.21	24.66 ± 6.34	23.33 ± 6.62

P.S.V. power, strength, and velocity exercises ; **P.V.S.** power, velocity, and strength exercises;
SUP. Supplementation ; **PLB.** placebo

Procedures

A: Randomization and Blinding

Randomization was conducted by the experimenter in a double-blind manner using a table of random numbers. Numbers were assigned to two groups (supplement and placebo), and participants were randomly allocated without knowledge of their group assignment. A total of 15 trained young athletes were screened for eligibility. Of these, 12 met the inclusion criteria and were randomized into either the supplement or placebo condition. All 12 participants completed the study protocol, and no exclusions, injuries, or adverse events were reported. Data are therefore presented for all 12 participants (Figure 2).

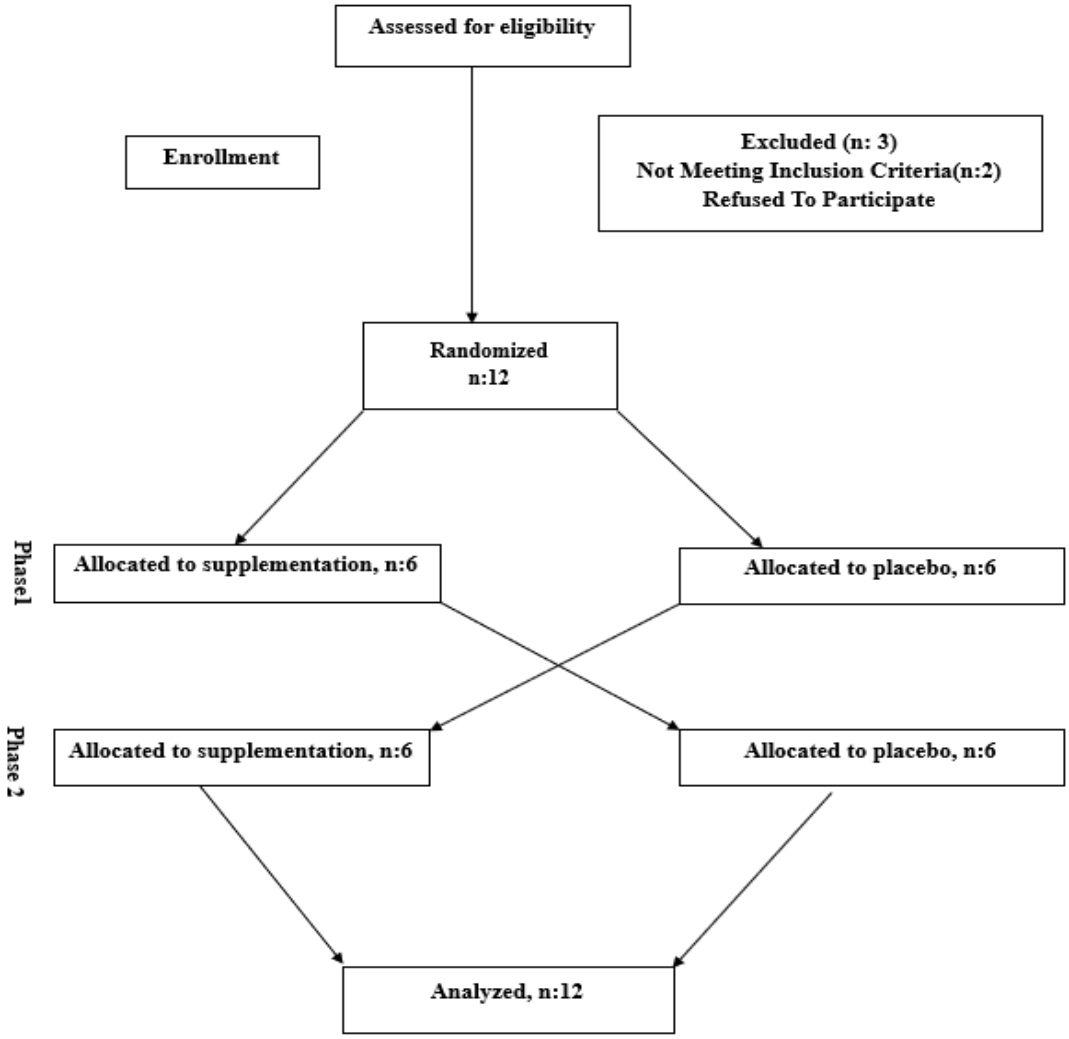

Figure 2. Flowchart diagram of participants' recruitment and allocation.

B: Pomegranate and Barberry Juice Supplementation

Supplementation Protocol. Supplementation was administered over three days. The supplement consisted of a natural combination of pomegranate juice and barberry. The placebo was prepared by dissolving food coloring in water to match the appearance of the supplement. Participants consumed 220 mL of either the supplement or placebo once daily, in a fasting state (8, 16). The final dose was ingested immediately before the exercise protocol

C: Exercise Protocol

Before initiating the exercise protocol, participants completed a 10-minute warm-up consisting of 5 minutes of treadmill running followed by full-body stretching. Subsequently,

they performed the assigned exercise sequences according to their group allocation (power, strength, velocity, or the reverse order). For the power training, the jump test was employed. In this test, athletes were required to perform intermittent vertical jumps of at least 30 cm for 30 seconds (6 sets of 10 repetitions until fatigue), with a 1-minute rest interval between sets. Strength training was conducted using the barbell squat exercise, based on each participant's one-repetition maximum (1-RM). Training intensity ranged from 55% to 100% of 1-RM across five sets, progressing from a single repetition to volitional fatigue. Speed training was assessed using a 60-yard sprint (17, 18). A schematic representation of the exercise protocol is provided in Figure 1.

D: Blood Sampling and Analysis

Blood samples (7 mL) were collected twice—before and immediately after the training session—from the brachial vein of the participants' left arm while seated. The samples were drawn by a trained laboratory technician and transferred into test tubes and specialized syringes. Plasma was separated from other blood components by centrifugation, after which serum was extracted and stored at −70°C for subsequent analyses. Blood lactate (La^-) concentration was determined using a commercial Byrex Fars kit (BXC0622, Iran) with a sensitivity and accuracy of 2 mg/dL, measured on a BT3000 biochemical analyzer. Hematological parameters, including HCT, MCH, MCHC, HGB, WBC, LYM, and PLT, were analyzed using a Sysmex KX-21N hematology analyzer (Sysmex, Japan). Blood acid–base parameters, including pH (accuracy 0.01) and bicarbonate concentration (measured within the physiological range of 22–26 mEq/L), were assessed using the GASTAT 720 analyzer (Techno Media, Japan).

Statistical Analysis

All participants who completed the study were included in the final data analysis. Statistical analyses were conducted using SPSS software (version 26.0, IBM Corp., Armonk, NY, USA). The required sample size was estimated a priori using G*Power software. Data are presented as mean ± standard deviation (SD). The normality of distribution was assessed using the Shapiro–Wilk test. For hematological and acid–base parameters, a two-way repeated-measures ANOVA (2 × 4; group × time) was performed. For hemodynamic indicators, a six-way repeated-measures ANOVA (6 × 4; group × time) was applied. When a significant main effect was observed, post hoc pairwise comparisons were conducted using the Bonferroni correction for multiple comparisons. Statistical significance was set at $P \leq 0.05$.

Results

The results showed a significant group × time interaction effect for hematocrit (HCT) ($f = 0.664$, $p = 0.033$). Within-group analysis revealed significant differences across trial phases ($f= 147.217$, $p = 0.001$). HCT levels increased during the second and third trials, coinciding with supplement consumption, and reached their highest level in the fourth trial (power–velocity–strength training with supplementation). For mean corpuscular hemoglobin (MCH), no significant interaction effect between group and time was observed ($f = 0.647$, $p = 0.489$). However, within-group analysis indicated significant differences across trial phases ($p = 0.001$). Specifically, the mean red blood cell count increased more during the third trial (power–velocity–strength training with placebo) compared with the other trials (Figure 3). Similarly, no significant group × time interaction effect was found for hemoglobin (HGB) concentration ($F = 3.015$, $p = 0.068$). Nevertheless, within-group analysis revealed significant differences across trial phases ($p = 0.001$), with hemoglobin levels increasing more in the fourth trial (power–velocity–strength training with supplementation) compared with the other trials. No significant group × time interaction effect was observed for mean corpuscular hemoglobin concentration (MCHC) ($F = 1.082$, $p = 0.370$). A significant difference was detected in both the between-group ($p = 0.007$) and within-group ($p = 0.025$) analyses. This difference reflected a decrease in mean hemoglobin concentration across all four trials, with a more pronounced reduction in the second and fourth trials, both associated with supplement consumption (Figure 3). For white blood cell (WBC) count, no significant group × time interaction effect was observed ($F = 0.25$, $p = 0.860$). However, a significant between-group difference was found ($p = 0.001$). The most significant increase in WBC count occurred in the first trial (power–strength–velocity exercises

with placebo) compared with the other trials. A significant group × time interaction effect was found for lymphocyte (LYM) count (F = 4.882, p = 0.006,). Within-group analysis also revealed significant differences across trial phases (p = 0.003). Lymphocyte counts increased in all four trials, with the first trial (power–strength–velocity exercises with placebo) showing the largest increase compared with the others. No significant group × time interaction effect was observed for platelet (PLT) count (F = 0.769, p = 0.488). However, within-group analysis indicated significant differences (p = 0.001). Platelet counts increased in all four trials, with the second trial (power–strength–velocity exercises with supplementation) demonstrating the most significant increase (Figure 3).

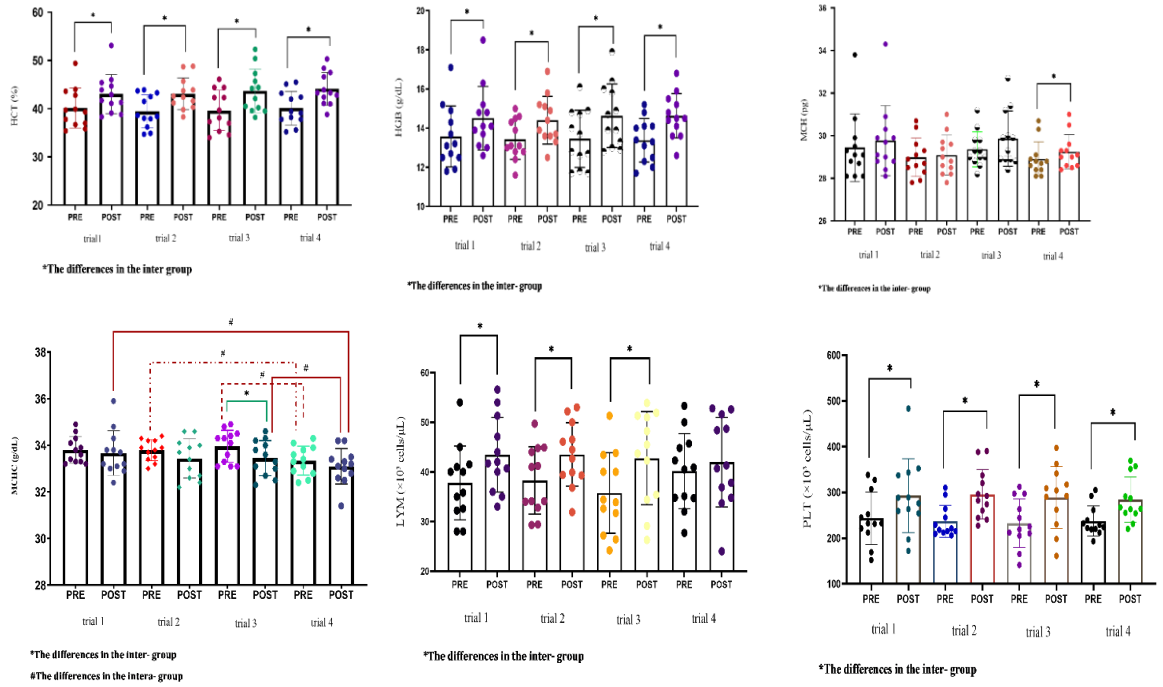

Figure 3. Mean ± standard deviation of hematocrit (HCT), hemoglobin (HGB), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), white blood cells (WBC), lymphocytes (LYM), and platelets (PLT) measured before and immediately after exercise. Significant within-group differences were observed across all variables in the four trials, and significant between-group differences were detected in hemoglobin and MCHC values during the first and fourth trials.

No significant group × time interaction effect was observed for pH (F = 1.727, p = 0.180); however, a significant between-group difference was detected (p = 0.001). pH decreased across all four trials, with the reduction being less pronounced in the third trial (power–strength–velocity exercises with placebo) compared with the others. A significant group × time interaction effect was observed for bicarbonate (HCO_3^-) levels (F = 3.035, p = 0.043). Within-group analysis also revealed significant differences across trials (p = 0.001). Bicarbonate levels decreased in all trials, with a more minor reduction in the third trial (power–strength–velocity exercises with placebo) compared with the others. For lactate (La^-) concentration, a significant group × time interaction effect was found (F = 4.597, p = 0.009), along with significant within-group differences across trials (p = 0.001). Lactate levels increased in all four trials. The increase was most significant in the third trial (power–strength–velocity exercises with placebo), whereas in the fourth trial (power–velocity–strength exercises with supplementation), the increase was lower than in the other trials, suggesting a potential effect of supplementation (Figure 4).

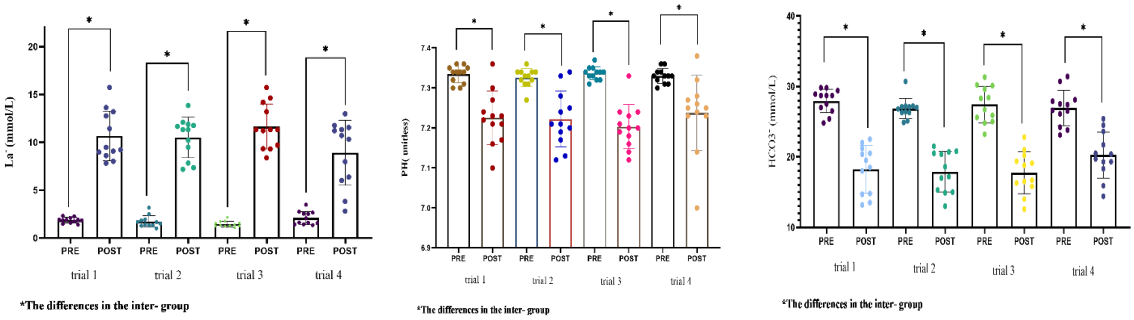

Figure 4. Mean ± standard deviation of lactate (La^-), pH, and bicarbonate (HCO_3^-) levels before and after exercise, along with within-group variations across the four experimental trials.

No significant group × time interaction effect was observed for heart rate (HR) (F = 1.727, p = 0.180); however, a significant between-group difference was detected (p = 0.001). HR increased significantly immediately after exercise in all four trials. In the trials accompanied by supplement consumption, HR declined more rapidly and prominently during recovery. For systolic blood pressure (SBP), no significant group × time interaction effect was observed (F = 0.536, p = 0.917). SBP increased immediately after exercise in all trials, decreased at 10, 15, and 20 minutes post-exercise, and then rose again at 30 minutes post-exercise. No significant group × time interaction effect was found for diastolic blood pressure (DBP) (F=1.078, p=0.838). DBP increased immediately after exercise in all trials, decreased at 10 minutes, and showed an increasing trend at 15, 20, and 30 minutes post-exercise. Similarly, no significant group × time interaction effect was observed for mean arterial pressure (MAP) (F = 0.923, p = 0.540). MAP increased immediately after exercise in all trials. In the first trial, MAP decreased at 10, 15, and 20 minutes post-exercise before increasing again at 30 minutes. In the second trial, MAP increased at 10 and 15 minutes, decreased at 20 minutes, and increased again at 30 minutes. In the third and fourth trials, MAP showed an upward trend both immediately and at 10 minutes post-exercise (Figure 5).

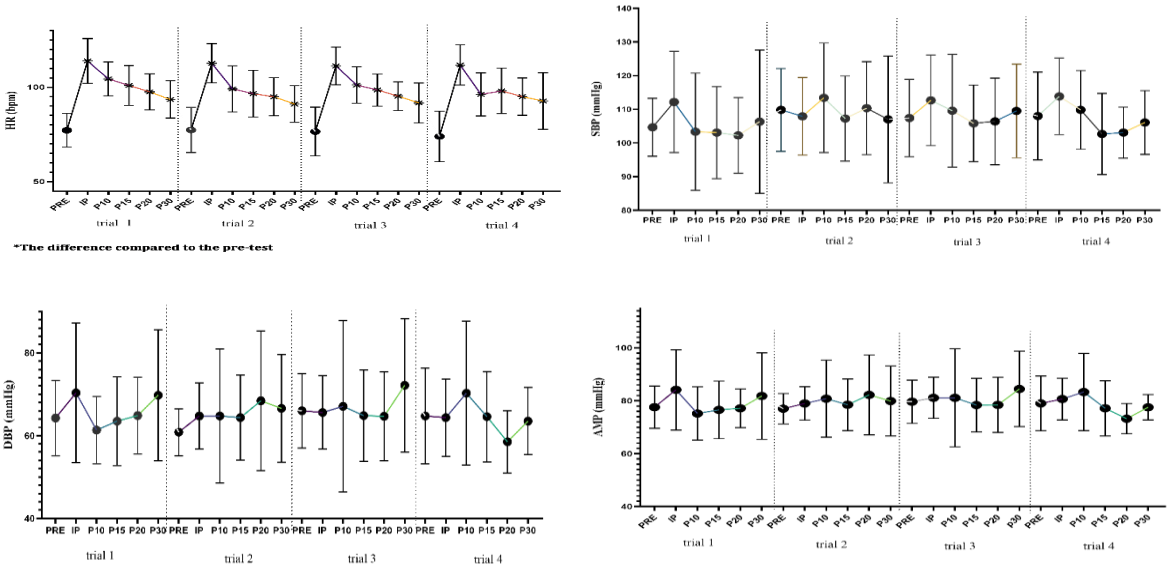

Figure 5. Mean and standard deviation of blood pressure (SBP, DBP, AMP) and heart rate (HR) variables at different time intervals: before, immediately after, and at 10, 15, 20, and 30 minutes post-exercise.

No significant group × time interaction effect was observed for the rate pressure product (RPP) (F = 0.348, p = 0.989). RPP increased immediately after exercise in all trials and subsequently decreased. The first trial showed a greater immediate post-exercise increase compared with the other trials. For arterial oxygen saturation (SaO_2), no significant group × time interaction effect was found (F = 0.664, p = 0.580).

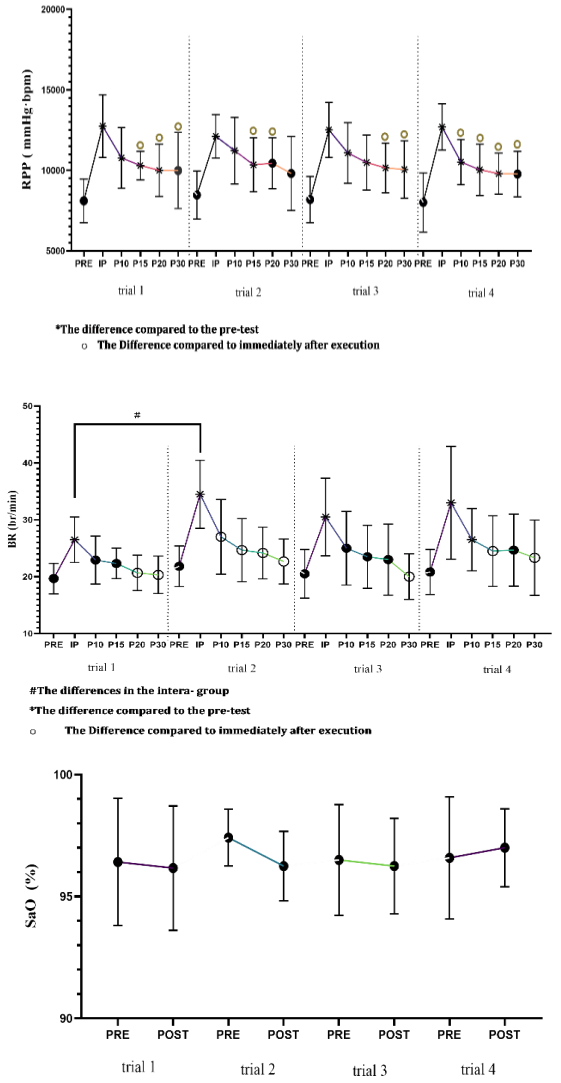

Figure 6. Mean ± standard deviation of rate pressure product (RPP), breathing rate (BR), and arterial oxygen saturation (SaO_2) at baseline, immediately post-exercise, and at 10, 15, 20, and 30 minutes post-exercise. A significant difference in breathing rate was observed between the first and second trials.

SaO_2 decreased in the first, second, and third trials, whereas an increase was observed in the fourth trial (power–velocity–strength training with supplementation). For breathing rate (BR), no significant group × time interaction effect was observed (F = 1.131, p = 0.332). However, significant between-group (p = 0.024) and within-group (p = 0.001) differences were detected. BR increased immediately after exercise in all trials, with the second trial showing the most significant post-exercise increase compared with the others (Figure 6).

Discussion

The present study examined the effects of combined pomegranate juice and barberry supplementation on hematological indices, blood acidosis, and hemodynamic responses, as well as the influence of exercise sequence. Alterations in exercise order, when combined with supplementation, may differentially affect athletes' physiology, particularly with respect to hematology, acid–base balance, and post-exercise recovery. Two exercise sequences were employed: power–velocity–strength and power–strength–velocity.

The findings demonstrated that hematocrit levels increased during different stages of the experiment (trials 2, 3, and 4), with the most significant increase observed in trial 4 (power–velocity–strength sequence with supplementation). This elevation may reflect stimulation of red blood cell (RBC) production in response to combined resistance and speed training. Interestingly, in trial 3 (power–strength–velocity sequence with placebo), hematocrit also increased significantly, suggesting that exercise order alone can modulate physiological responses, particularly when stress is imposed on the hematopoietic system. Previous studies have reported that performing strength exercises at the beginning of a session exerts a stronger effect on hematocrit elevation (19). Resistance training appears to promote the mobilization and stimulation of red blood cells, thereby increasing hematocrit; however, this effect may be influenced by factors such as exercise type and intensity (20, 21). One of the key findings of the present study was the significant rise in hematocrit in the supplementation group, consistent with previous reports showing that pomegranate consumption increases HCT levels (22).

Furthermore, supplementation with pomegranate juice and barberry resulted in a greater increase in hemoglobin (HGB) during trial 4 (power–velocity–strength sequence with supplementation). This observation aligns with earlier studies suggesting that pomegranate, due to its potent antioxidant properties, may enhance hematological performance and reduce oxidative stress (23). The most pronounced increase in hemoglobin occurred in trial 4, specifically associated with the power–velocity–strength sequence combined with supplementation. In contrast, significant increases in hemoglobin were also observed in other trials following resistance and speed exercises with a placebo. These findings suggest that exercise sequence may play a role in modulating hemoglobin production. Strength exercises, when performed at the beginning of a training session, may further stimulate red blood cell and hemoglobin synthesis, leading to higher HGB levels (24). Conversely, when strength and speed exercises are performed after power training, hemoglobin production may be attenuated (24).

The sequence of exercises, when combined with Supplementation, may differentially influence blood acidosis indices such as lactate (La^-), pH, and bicarbonate (HCO_3^-). These effects could be attributed to variations in exercise type and intensity, as well as the biochemical properties of the supplements and their impact on metabolic processes. Regarding lactate and acid–base balance, the results showed that lactate levels increased in all trials. However, in trial 4 (power–velocity–strength sequence with supplementation), the magnitude of lactate accumulation was lower. This finding suggests that pomegranate and barberry supplementation may exert beneficial effects by reducing lactate buildup and attenuating exercise-induced acidosis. The reduced lactate response in trial four may reflect both the exercise sequence and the role of supplementation in enhancing recovery and limiting lactate production. The order of exercises appears to play a crucial role in lactate dynamics. Previous studies have reported that performing aerobic or strength training in different sequences can significantly alter lactate accumulation (8). These findings are consistent with prior research indicating that natural compounds such as pomegranate can reduce lactate production during high-intensity exercise. This effect is likely related to its antioxidant properties, which help mitigate oxidative stress induced by strenuous activity. When power exercises are performed first, the body rapidly enters a state of acidosis; however, supplementation may modulate this response and attenuate lactate accumulation (8, 25). Previous studies have demonstrated that antioxidant or anti-inflammatory supplements can mitigate the negative effects of lactate accumulation (26). For example, pomegranate supplementation, recognized for its antioxidant properties, has been shown to reduce lactate production and facilitate recovery following high-intensity exercise (27). This effect may be particularly relevant in exercise modalities that produce high levels of lactate, such as speed and resistance training. Bicarbonate is a key marker in regulating acid–base balance. A reduction in bicarbonate levels typically reflects increased acid accumulation in the body, a phenomenon commonly observed after high-intensity exercise, especially when lactate accumulation is substantial. In the present study, bicarbonate levels decreased across all trial stages; however, in trial 3 (placebo condition), the reduction was less pronounced. This finding suggests that exercise sequence, in combination with supplementation, may differentially influence bicarbonate dynamics. Supporting this, prior research has reported that supplementation can enhance blood buffering capacity and attenuate exercise-induced acidosis, particularly when antioxidants are used (28). Pomegranate consumption, due to its high antioxidant content, may play an essential role in improving acid–base balance and maintaining bicarbonate levels (27). These results are consistent with previous findings showing that resistance and anaerobic exercise can reduce circulating bicarbonate and induce metabolic acidosis (29). In the present study, blood pH decreased across all stages; however, in trial 3 (power–strength–velocity sequence with placebo), the reduction was less pronounced compared with the other trials. This suggests that both exercise sequence and supplementation may modulate the extent of exercise-induced pH reduction. Previous research has shown that strength and speed exercises, particularly when performed at high intensity, contribute to excess acid production and reduced blood pH (30). Antioxidant supplementation, such as pomegranate, may help attenuate this decline by reducing oxidative

stress, limiting acid production, and maintaining blood pH within physiological ranges (31). When considering the role of exercise sequence, our results further suggest that ordering may influence acid–base responses. For instance, initiating a session with power exercises may accelerate lactate accumulation and pH reduction, whereas placing speed or strength exercises later in the sequence, in combination with supplementation, may allow natural antioxidants such as pomegranate to mitigate these alterations (31).

Hemodynamic indices—including heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), and rate-pressure product (RPP)—are influenced by both exercise intensity and type, as well as nutritional supplementation. These variables reflect cardiovascular adaptations to metabolic demands during and after exercise. In the present study, HR increased immediately following exercise in all trials. Notably, in the supplementation trials, HR returned to baseline more rapidly and markedly, suggesting that both exercise sequence and supplementation may modulate the speed of post-exercise heart rate recovery. Performing power exercises at the beginning of the sequence appears to place greater initial stress on the cardiovascular system, resulting in higher HR at exercise onset. Conversely, when speed and strength exercises are positioned later in the sequence, different recovery dynamics may occur (10). Similar results have been reported in studies evaluating plant-based supplements and cardiovascular recovery. For example, pomegranate supplementation has been shown to accelerate post-exercise HR reduction compared with placebo following high-intensity exercise (10). These effects may be attributed to the antioxidant and anti-inflammatory properties of the active compounds in pomegranate and barberry. Previous studies have reported that antioxidant supplementation, such as pomegranate, can attenuate oxidative stress and thereby prevent excessive elevations in heart rate following high-intensity exercise (32). This effect appears particularly relevant when power exercises are performed at the beginning of the sequence, as supplementation may reduce the additional cardiovascular burden and shorten recovery time (33). With respect to blood pressure, similar patterns were observed across all trials. SBP increased immediately after exercise in all four stages, consistent with the elevated oxygen and nutrient demands of skeletal muscle during resistance and high-intensity training. SBP subsequently declined during the 10-, 15-, and 20-minute recovery periods but rose again at 30 minutes post-exercise. Notably, in the supplementation trials, the decline in SBP during recovery was more rapid, suggesting a potential role of supplementation in accelerating blood pressure normalization. Previous research has similarly demonstrated that certain supplements, such as nitric oxide donors, can improve blood flow and reduce SBP during recovery (34, 35). These effects are particularly evident during high-intensity exercise, which imposes a substantial load on the cardiovascular system. In the present study, mean arterial pressure (MAP)—a general indicator of overall blood pressure status—increased following exercise. In the first trial, MAP decreased at 10, 15, and 20 minutes post-exercise, whereas in the other trials, particularly those involving supplementation, MAP rose more rapidly. Such changes in MAP, especially during resistance and speed exercises that exert higher cardiovascular stress, may be related to alterations in hydrostatic balance and vascular function. For other indices, such as breathing rate (BR) and arterial oxygen saturation (SaO_2), the sequence of exercises appeared to have variable effects. In this study, BR was higher immediately after exercise in trial 2 (placebo condition), which may reflect the specific sequence and intensity of exercises performed. Previous studies have suggested that antioxidant supplementation can reduce the cardiovascular burden and consequently lower the rate-pressure product (RPP). This effect may be particularly beneficial for athletes engaged in high-intensity training (33, 36).

Conclusion

This study demonstrated that supplementation with pomegranate and barberry, in combination with different sequences of resistance and anaerobic exercise, can significantly influence hematological, hemodynamic, and acid–base indices in athletes. Notably, supplementation increased hematocrit and hemoglobin levels in certain phases of the study, suggesting a potential enhancement in oxygen transport to tissues. In addition, supplementation was

associated with reduced lactate accumulation and improved hemodynamic recovery, including more favorable heart rate and blood pressure responses following exercise. The sequence of exercises also played an important role, as varying orders elicited distinct physiological responses. These findings indicate that the appropriate integration of supplementation with exercise type and sequence may enhance athletic performance, attenuate exercise-induced acidosis, and accelerate post-exercise recovery. Considering both training sequence and supplementation strategies may therefore provide more effective approaches for managing the physiological stress of high-intensity training. Future research should further investigate the long-term effects of pomegranate and barberry supplementation in athletes with different fitness levels and training backgrounds. Incorporating natural antioxidant-rich compounds such as pomegranate and barberry into athletes' diets may represent a practical nutritional strategy to optimize performance and recovery.

Limitations and Suggestions for Future Research

This study investigated the effects of pomegranate and barberry supplementation, combined with different exercise sequences, on hemodynamic indices and acid–base balance in athletes. The findings demonstrated that supplementation positively influenced hematological parameters such as hematocrit (HCT), hemoglobin (HGB), and platelets (PLT), while also reducing lactate accumulation and enhancing hemodynamic recovery, including more favorable heart rate and blood pressure responses after exercise. Furthermore, exercise sequence significantly affected these indices, particularly under high-intensity conditions, highlighting its role in modulating hemodynamic and acid–base responses. Overall, these results suggest that the appropriate integration of supplementation and exercise sequencing may improve athletic performance and accelerate recovery. Future research should employ larger sample sizes and more extended intervention periods to evaluate the long-term effects of supplementation on performance and recovery. Additionally, studies combining different exercise modalities and nutritional strategies, as well as assessing other physiological markers such as oxidative stress and neurocognitive function, could provide deeper insights into the mechanisms involved. Finally, cellular and molecular investigations are warranted to better elucidate how the antioxidant properties of pomegranate and barberry contribute to these outcomes.

Declarations

Conflict of Interest

The authors declare that there is no conflict of interest.

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Ethical Considerations

This study was reviewed and approved by the Ethics committee of Sport sciences research institute (IR/SSRI.REC.2023.13829.1974), and study protocol were conducted at the exercise physiology laboratory of University of Guilan.

Authors' Contribution

HF, JM, HA: conceptualization, Methodology and Investigation; HF, JM: Data analysis, HF, JM: Writing original draft; JM: Supervision, Project administration; JM, HA: Review editing and final checking.

Artificial Intelligence

No AI platforms were used in the writing and design of this article.

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Fruit and Vegetable Intake and Psychological Resilience among U.S. Adults: A Cross-Sectional Analysis of BRFSS 2023 Data

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ABSTRACT

Introduction: Psychological resilience, the ability to adapt to adversity and maintain emotional well-being, protects against mental health challenges. This study investigates its association with fruit and vegetable intake among U.S. adults using 2023 Behavioral Risk Factor Surveillance System (BRFSS) data.

Methods: Daily fruit and vegetable consumption (grams/day) was estimated from frequency responses, assuming 1.5 servings (135 grams) per instance. Resilience was assessed using a composite index based on poor mental health days (_MENTHLTH ≤5 for high resilience) and emotional support (_EMTSUPP 1-2 for high), categorized as high, moderate, or low. Descriptive statistics, t-tests, ANOVA, and chi-square tests were used, with multivariable regressions adjusting for confounders (income, education, smoking).

Results: The high-resilience group consumed 420 grams/day (SD=115), compared to 355 grams/day (SD=105) in the low-resilience group ($p<0.01$, Cohen's $d=0.58$). Sixty-seven percent of high-resilience individuals met the WHO ≥400 grams/day recommendation, versus 46% in the low-resilience group ($p<0.001$). Women and younger adults (18-24 years) in high-resilience groups had higher intakes ($p<0.05$). Multivariable logistic regression showed higher intake was associated with high resilience (OR=1.45, $p<0.01$).

Conclusion: These findings suggest that nutrient-dense diets may enhance resilience, though longitudinal studies are needed to confirm causality and explore mechanisms like the gut-brain axis.

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Introduction

Psychological resilience, defined as the ability to cope with adversity and sustain emotional well-being, is a critical buffer against mental health disorders (1). With over 20% of U.S. adults experiencing anxiety or depression annually (2), resilience reduces the risk of psychiatric conditions and enhances cognitive adaptability (3). Diet, a modifiable lifestyle factor, influences resilience through neurobiological pathways, such as the hypothalamic-pituitary-adrenal (HPA) axis and the gut-brain axis (4, 5).

Fruits and vegetables, rich in fiber, polyphenols, and antioxidants, are linked to improved mental health outcomes (6). For instance, higher fruit and vegetable consumption is associated with reduced depression risk, as demonstrated by Mujcic and Oswald (2016), who reported increased well-being with greater intake, though self-reported data limited their study and did not focus on resilience (7). Similarly, Bonaccio et al. (2018) found that Mediterranean diets high in fruits and vegetables correlated with higher resilience scores, but their cross-sectional design could not establish causality (8). Emerging evidence suggests that polyphenols in produce may reduce HPA axis reactivity, while fiber supports gut microbiota, influencing mood through vagus nerve signaling (5, 9, 10). Despite these insights, few studies have used large-scale, population-based data to examine resilience specifically, leaving a gap in understanding how diet supports positive mental health outcomes.

This study addresses this gap by analyzing fruit and vegetable intake across resilience levels in a large U.S. sample from the 2023 BRFSS. Unlike prior studies, we use a composite resilience

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index and adjust for confounders like income and education to improve robustness. We hypothesize that higher fruit and vegetable intake is associated with greater resilience, potentially mediated by neurobiological and psychosocial factors. By focusing on total intake rather than specific produce types and stratifying by gender and age, this study provides population-level evidence to inform dietary interventions for mental health promotion.

Materials and Methods

Data Source

Data were obtained from the 2023 Behavioral Risk Factor Surveillance System (BRFSS), a CDC-administered telephone survey of over 400,000 U.S. adults, accessible at https://www.cdc.gov/brfss/annual_data/annual_data.htm (11). Variables included fruit and vegetable consumption (_FRT16A1, _VEG23A1), mental health days (_MENTHLTH)(12), emotional support (_EMTSUPP) (13), gender (_SEX), and age (_AGE_G) .

Data Processing

Fruit and vegetables Intake was estimated assuming 1.5 servings/day for daily consumers, with each serving equating to 90 grams per USDA guidelines (total = 135 grams per instance) (14). Total intake combined fruit and vegetable grams per day

Resilience was measured using a composite index combining (3, 15) _MENTHLTH (≤5 poor mental health days indicating better health, aligned with CDC flourishing metrics) and _EMTSUPP (1-2 for high emotional support). Resilience categories were defined as high (MENTHLTH ≤5 and _EMTSUPP 1-2), moderate (MENTHLTH 6-14 or _EMTSUPP=3), or low (_MENTHLTH ≥15 or _EMTSUPP 4-5). Data cleaning excluded 15,234 respondents (3.7%) with missing or invalid responses, and _LLCPWT weights were applied to ensure representativeness.

Statistical Analysis

Analyses were conducted using SPSS 29.0. Descriptive statistics included means and standard deviations for intake by resilience level. T-tests and ANOVA assessed differences in intake across groups ($p<0.05$), with chi-square tests for WHO recommendation compliance. Cohen's d quantified effect sizes. Multivariable logistic regression (resilience as outcome) and linear regression (intake as outcome) adjusted for confounders: income (_INCOME3), education (_EDUCAG), and smoking (_SMOKER3). Subgroup analyses stratified results by gender and age.

Results

Participant Characteristics

Table 1 presents characteristics by gender. Women comprimised 52% of the sample, with similar age distributions to men but higher education levels ($p=0.03$).

Table 1. Demographic Characteristics by Gender

Characteristic	Female	Male	p-value
Age (mean year)	45.2 (SD=12.3)	46.1 (SD=12.5)	0.12
Education (% college grad)	32%	28%	0.03
Income (% ≥$75k)	25%	30%	0.08

Fruit and Vegetable Intake by Resilience Level

The high-resilience group consumed an average of 420 grams/day (SD=115), compared to 390 grams/day (SD=110) for moderate resilience and 355 grams/day (SD=105) for low resilience (ANOVA $p<0.001$, Cohen's $d=0.58$ for high vs. low) (Table 2).

Table 2. Mean Fruit and Vegetable Intake by Resilience Level

Resilience Level	Mean Intake (grams/day)	SD (grams/day)
High	420	115
Moderate	390	110
Low	355	105

WHO Recommendation Compliance

Sixty-seven percent of the high-resilience group met the WHO ≥400 grams/day recommendation, compared to 54% in the moderate-resilience group and 46% in the low-resilience group (chi-square $p<0.001$) (Figure 1).

Figure 1. WHO Compliance by Resilience Level

Intake by Gender and Age

Among high-resilience individuals, women consumed 430 grams/day compared to 410 grams/day for men (t-test p=0.02, d=0.18). In the low-resilience group, women averaged 365 grams/day and men 345 grams/day (p=0.04). Younger adults (18-24 years) in the high-resilience group consumed 425 grams/day, compared to 405 grams/day for those 65+ (ANOVA p=0.03) (Table 3, Figures 2-3). Adjusted regressions confirmed higher intake was associated with high resilience (OR=1.45, p<0.01).

Table 3. Intake by Gender and Resilience Level

Resilience Level	Gender	Mean Intake (grams/day)
High	Female	430
High	Male	410
Moderate	Female	400
Moderate	Male	380
Low	Female	365
Low	Male	345

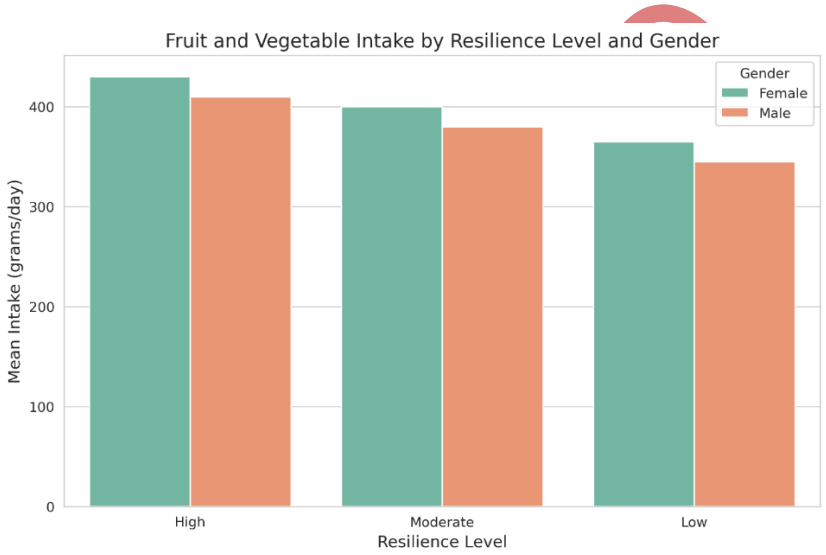

Figure 2. Intake by Resilience Level and Gender

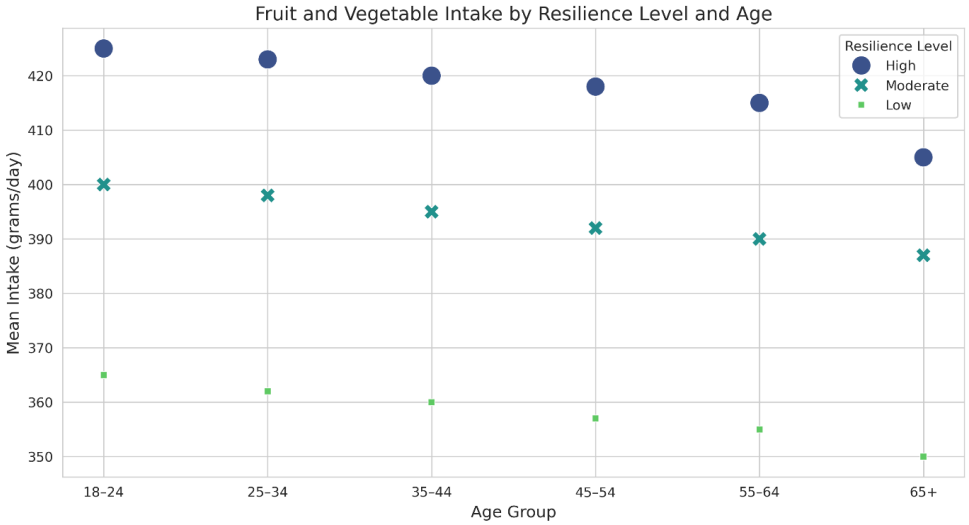

Figure 3. Intake by Resilience Level and Age

Discussion

Individuals with high phsychological resilience consumed significantly more fruits and vegetables than those with low resilience (420 vs. 355 grams/day, d=0.58). This aligns with evidence that nutrients like polyphenols and fibers in produce support mental health by reducing HPA axis reactivity and promoting gut-brain axis communication (4, 9, 10). This suggests nutrient-dense diets enhance resilience by mitigating oxidative stress, where antioxidants in produce scavenge free radicals protecting neurons from damage (6, 16). The gut-brain axis is key, with fiber promoting SCFAs that influence mood via vagus nerve signaling and neurotransmitter modulation, such as increasing serotonin levels (8, 17). These biological processes explain the observed patterns, as higher intake likely fosters neuroplasticity and stress buffering, consistent with dose-response relationships in prior work (3, 5). The results highlight how dietary habits may contribute to mental robustness, with implications for preventive strategies in populations facing chronic stress.

Women in the high-resilience group consumed more than men (430 vs. 410 grams/day, p=0.02), possibly due to greater health consciousness or hormonal interactions enhancing polyphenol effects (18-20). Younger adults (18-24 years) in the high-resilience group also showed higher intake (425 grams/day) than older adults, potentially reflecting dietary trends like plant-based eating. However, low-resilience individuals across all ages had reduced intake, suggesting that stress may disrupt healthy eating habits, possibly through emotional eating or lack of motivation (21, 22). These demographic differences highlight the need for targeted dietary interventions to bolster resilience, particularly for men and older adults.

The higher WHO compliance rate in the high-resilience group (67% vs. 46%, p<0.001) suggests that meeting the 400 grams/day threshold may enhance mental robustness (23, 24). Public health strategies, such as subsidized produce programs or app-based dietary tracking, could promote adherence, especially in low-resilience populations (25, 26). Community-based initiatives, like urban gardens or nutrition education in schools, could further support access and awareness, addressing disparities in vulnerable groups.

Limitations: The cross-sectional design prevents causal inferences, as resilient individuals may naturally choose healthier diets. Self-reported intake may overestimate consumption, and the composite resilience index, while aligned with CDC metrics, differs from validated scales like CD-RISC (15) Although regressions adjusted for confounders, residual biases (e.g., unmeasured lifestyle factors) may persist. Missing data for some BRFSS variables, such as _EMTSUPP in certain states, may limit generalizability.

Future research: Longitudinal studies are needed to establish causality, with biological markers (e.g., cytokines) to elucidate mechanisms (27). Randomized controlled trials testing dietary interventions, such as subsidized produce for low-resilience groups, could provide actionable insights. Qualitative research exploring barriers like cost or food preferences would further inform targeted strategies

Conclusion

Higher fruit and vegetable intake is associated with greater psychological resilience (OR=1.45) particularly among women and younger adults. Nutrient-dense diets may enhance mental well-being through neurobiological pathways like the gut-brain axis. Public health efforts should promote the WHO's 400 grams/day recommendation through accessible interventions, such as dietary counseling or produce subsidies, targeting low-resilience groups. Despite limitations like the cross-sectional design, these findings underscore the potential of dietary strategies to support mental health. Future longitudinal research is essential to confirm causality and guide evidence-based policies.

Declarations

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Ethical Considerations and Code

Because this study used de-identified, publicly available data from the 2023 BRFSS, it was exempt from institutional review board (IRB) approval under U.S. federal regulations (45 CFR 46.104). All analyses were conducted in accordance with CDC BRFSS data-use agreements. Complex survey weights (_LLCPWT) were applied to ensure nationally representative estimates. Data cleaning and all statistical analyses were performed using IBM SPSS Statistics version 29.0. The complete annotated SPSS syntax file and data processing code are available upon reasonable request from the corresponding author.

Conflicts of Interest

The author have no competing interests.

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