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The Effect of Acute Caffeine Consumption on Hemorheological and Antioxidant Responses to a High-Intensity Interval Training Session in Overweight Women

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Abstract

Aim: The present study aimed to investigate the acute effect of caffeine ingestion prior to a single session of high-intensity interval training (HIIT) on hemorheological responses and oxidative stress markers in overweight women

Method: In a randomized, double-blind, placebo-controlled clinical trial with a parallel design, forty-five overweight women (BMI: 25–30 kg·m⁻², aged 25–35 years) were randomly assigned to three groups: HIIT with caffeine (6 mg·kg⁻¹ body mass), HIIT with placebo, and a control group. Caffeine or placebo capsules were consumed 60 minutes before a single HIIT session (4×4 min at 85–90% HR_max with active recovery). Venous blood samples were collected at baseline and immediately post-exercise. Plasma viscosity, fibrinogen, total antioxidant capacity (TAC), and malondialdehyde (MDA) were measured. Data were analyzed using a mixed-model ANOVA (3 groups × 2 time points).

Results: A significant group × time interaction was observed for plasma viscosity, fibrinogen, and TAC ($p<0.05$). Plasma viscosity and fibrinogen significantly decreased, while TAC significantly increased following exercise only in the caffeine-HIIT group ($p<0.05$). No significant changes were observed in the placebo-HIIT or control groups. MDA levels did not change significantly in any group ($p>0.05$).

Conclusion: Acute caffeine ingestion prior to a single HIIT session improves hemorheological parameters and enhances antioxidant capacity without increasing lipid peroxidation in overweight women. These findings suggest that caffeine may serve as a short-term nutritional strategy to support vascular and oxidative responses during high-intensity exercise.

Keywords: Caffeine; High-Intensity Interval Training; Plasma Viscosity; Fibrinogen; Total Antioxidant Capacity; Malondialdehyde; Oxidative Stress.

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1. Introduction

Overweight and obesity are major public health concerns that substantially increase the risk of cardiovascular and metabolic disorders through multiple physiological mechanisms, including impaired vascular function, chronic low-grade inflammation, and altered blood rheology. Women with overweight are particularly susceptible to these abnormalities because excess adiposity is associated with increased plasma viscosity, elevated fibrinogen concentrations, and oxidative imbalance, all of which contribute to endothelial dysfunction and an increased risk of cardiovascular disease. Consequently, identifying effective and practical interventions capable of improving vascular health and reducing oxidative stress in this population has become an important objective in exercise and sports nutrition research.

High-intensity interval training (HIIT) has emerged as an effective time-efficient exercise strategy for improving cardiometabolic health and vascular function. Acute and chronic HIIT interventions have been shown to enhance endothelial function, improve blood flow, and stimulate antioxidant defense mechanisms. Likewise, caffeine, one of the most widely consumed ergogenic aids, has consistently demonstrated beneficial effects on exercise performance through central and peripheral physiological actions. In addition to its established ergogenic properties, several studies have suggested that acute caffeine ingestion may influence hemorheological characteristics and oxidative balance. However, previous findings remain inconsistent, with some investigations reporting improvements in antioxidant capacity and blood rheology, whereas others have observed no significant changes or even increases in oxidative stress markers following intense exercise combined with caffeine ingestion.

These inconsistencies may be attributed to differences in caffeine dosage, participants' training status, sex, nutritional status, exercise protocols, and the timing of supplementation. Furthermore, most previous studies have been conducted in trained male athletes or physically active individuals using supramaximal exercise protocols, such as the Wingate test or exhaustive exercise, which differ substantially from standardized high-intensity interval training in terms of metabolic demand and physiological responses. Evidence regarding the acute interaction between caffeine ingestion and a single session of standardized HIIT in overweight women remains limited. In particular, little is known about the combined effects of acute caffeine ingestion on hemorheological indices, including plasma viscosity and fibrinogen, together with oxidative stress biomarkers such as total antioxidant capacity and malondialdehyde in this population.

Therefore, the present study was designed to investigate the effect of acute caffeine ingestion before a single session of high-intensity interval training on hemorheological indices and oxidative stress responses in overweight women. We hypothesized that acute caffeine ingestion (6 $mg \cdot kg^{-1}$ body mass) before HIIT would improve hemorheological parameters by reducing plasma viscosity and fibrinogen concentrations, enhance total antioxidant capacity, and attenuate exercise-induced oxidative stress compared with placebo.

2. Methods

total of 45 overweight women volunteered to participate in this randomized, double-blind, placebo-controlled clinical trial. All participants completed the study, and no withdrawals or dropouts occurred during the intervention period. Participants were randomly assigned to one of three groups (n = 15 per group): high-intensity interval training plus caffeine (HIIT+Caffeine), high-intensity interval training plus placebo (HIIT+Placebo), and placebo control (Control).

Participants were 25–35 years of age and had a body mass index (BMI) between 25.0 and 30.0 $kg \cdot m^{-2}$. Baseline characteristics were comparable among the three groups. Mean age ranged from 28.7 ± 3.5 to 29.4 ± 3.1 years, body mass from 71.5 ± 5.2 to 73.1 ± 4.9 kg, height from 161.9 ± 4.9 to 163.2 ± 5.0 cm, and BMI from 27.3 ± 1.4 to 27.6 ± 1.3 $kg \cdot m^{-2}$. Body composition variables, including fat mass and fat-free mass, were not assessed. Participants were recreationally active and did not engage in structured exercise training more than twice per

week. None of the participants were competitive athletes.

Inclusion criteria were female sex, age between 25 and 35 years, BMI between 25.0 and 30.0 $kg \cdot m^{-2}$, good general health based on medical history, non-smoking status, and no regular participation in structured exercise training. Participants were also required to have abstained from antioxidant supplements and medications affecting cardiovascular function for at least six weeks before the study. Exclusion criteria included pregnancy or lactation, chronic metabolic, cardiovascular, inflammatory, or endocrine diseases, musculoskeletal disorders limiting exercise participation, consumption of caffeine-containing foods, beverages, or herbal products within 48 h before testing, failure to comply with dietary instructions, participation in strenuous physical activity outside the study protocol, or the occurrence of any medical condition preventing completion of the study.

Participants were instructed to maintain their habitual dietary intake throughout the study. To minimize dietary variability, an isocaloric diet was prescribed by a registered dietitian during the 24 h preceding the experimental trial, and compliance was monitored using dietary records. Participants were instructed to abstain from vigorous physical activity for 48 h and from caffeine-containing products for 24 h before testing. None of the participants reported habitual use of nutritional supplements during the study period.

All participants provided written informed consent before enrollment and was conducted in accordance with the principles of the Declaration of Helsinki.

This study was designed as a randomized, double-blind, placebo-controlled clinical trial with a parallel-group design and repeated measurements. Participants were randomly allocated in a 1:1:1 ratio to one of three experimental groups: high-intensity interval training plus caffeine (HIIT+Caffeine), high-intensity interval training plus placebo (HIIT+Placebo), or placebo control (Control). Randomization was performed by an independent researcher using a computer-generated randomization sequence with block randomization. Allocation concealment was ensured by coding identical capsules before participant enrollment. Caffeine and placebo capsules were identical in appearance, color, weight, and packaging. Both participants and investigators remained blinded to group allocation until completion of the statistical analyses.

Before the experimental trial, participants underwent screening to confirm eligibility according to the inclusion and exclusion criteria. They were instructed to refrain from strenuous physical activity for 48 h before testing and to avoid caffeine-containing foods, beverages, and supplements for 24 h before the experimental session. To minimize dietary variability, participants consumed a standardized isocaloric diet during the 24 h preceding the trial and arrived at the laboratory following an overnight fast.

On the experimental day, baseline venous blood samples were collected after participants arrived at the laboratory. Subsequently, participants in the HIIT+Caffeine group ingested caffeine capsules (6 $mg \cdot kg^{-1}$ body mass), whereas those in the HIIT+Placebo and Control groups received identical placebo capsules. All capsules were consumed with 250 mL of water 60 min before the exercise protocol to allow sufficient time for peak plasma caffeine concentration.

After the supplementation period, participants in the intervention groups completed a standardized high-intensity interval training (HIIT) session consisting of a 10-min warm-up, four 4-min running intervals performed at 85–90% of maximal heart rate (HRmax), interspersed with 3-min active recovery periods at 50–60% of HRmax, followed by a 5–10 min cool-down. Exercise intensity was continuously monitored using a Polar heart rate monitor (Polar Electro, Finland). Participants assigned to the Control group remained at rest under identical environmental conditions and did not perform

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any exercise. Immediately after completion of the intervention, post-exercise venous blood samples were collected for the assessment of hemorheological and oxidative stress biomarkers.

The required sample size was estimated a priori using G*Power software (version 3.1; Heinrich Heine University, Düsseldorf, Germany). The calculation was based on the primary outcome of plasma viscosity, considering the findings of previous studies investigating the effects of high-intensity interval training and acute caffeine supplementation on hemorheological responses. Assuming a medium effect size (Cohen's f = 0.25), a statistical power of 0.80, a two-sided significance level (α) of 0.05, and a repeated-measures analysis of variance (three groups × two time points), the minimum required sample size was calculated to ensure adequate statistical power.

To account for an anticipated dropout rate of approximately 10–15%, the target sample size was increased to 45 participants (15 participants per group). All enrolled participants completed the study, and no participants withdrew or were excluded during the intervention. Therefore, the final analyzed sample met and exceeded the minimum sample size required by the a priori power analysis.

Participants assigned to the HIIT+Caffeine group received caffeine capsules containing anhydrous caffeine at a dose of 6 $mg \cdot kg^{-1}$ body mass. The selected dose was based on previous evidence demonstrating its efficacy and safety as an ergogenic aid in exercise performance. The caffeine powder was of pharmaceutical/food-grade quality and was administered in capsule form.

Participants in the HIIT+Placebo and Control groups received identical placebo capsules containing corn starch. Placebo capsules were identical to the caffeine capsules in appearance, color, weight, and packaging to ensure successful blinding. All capsules were prepared, coded, and packaged by an independent researcher who was not involved in data collection or statistical analysis.

Participants consumed one capsule with 250 mL of water 60 min before the experimental session to coincide with the expected peak plasma caffeine concentration. Supplement ingestion was directly supervised by the research team to ensure full compliance with the intervention protocol. Because this investigation evaluated the acute effects of caffeine, supplementation was administered only once before the exercise session.

To minimize potential confounding effects, participants were instructed to refrain from consuming caffeine-containing foods, beverages, herbal products, or nutritional supplements for 24 h before the experimental trial and to avoid strenuous physical activity for 48 h before testing. Compliance with these instructions was verified verbally before the experimental session. No participant reported any adverse events or side effects associated with caffeine or placebo ingestion during the study.

Participants assigned to the intervention groups completed a standardized high-intensity interval training (HIIT) protocol on a motorized treadmill. The exercise session consisted of a 10-min warm-up, followed by four 4-min running intervals performed at 85–90% of maximal heart rate (HRmax). Each high-intensity interval was separated by 3 min of active recovery at 50–60% of HRmax, resulting in a work-to-recovery ratio of 4:3. The exercise session concluded with a 5–10 min cool-down at low intensity.

Exercise intensity was continuously monitored throughout the session using a heart rate monitor (Polar Electro, Finland) to ensure that participants maintained the prescribed target heart rate during both the high-intensity and active recovery phases. All exercise sessions were performed under the direct supervision of the research team to ensure protocol adherence and participant safety.

Participants assigned to the Control group remained seated under identical environmental conditions for a period equivalent to the exercise session and did not perform any physical activity.

dietary variability, an isocaloric diet was prescribed by a registered dietitian during the 24 h preceding the experimental trial, and compliance was monitored using dietary records. Participants were instructed to abstain from caffeine-containing foods, beverages, and nutritional supplements for 24 h before the experimental session.

Participants were also instructed to refrain from strenuous physical activity for 48 h before the experimental trial. Compliance with these instructions was confirmed before the experimental session.

Statistical analyses were performed using SPSS software (version 26.0; IBM Corp., Armonk, NY, USA). The normality of data distribution was assessed using the Shapiro–Wilk test. Data are presented as mean ± standard deviation (SD).

A two-way repeated-measures analysis of variance (ANOVA) was used to evaluate the effects of group (HIIT+Caffeine, HIIT+Placebo, and Control) and time (pre- and post-intervention), as well as the group × time interaction. When significant main or interaction effects were observed, Bonferroni post hoc tests were performed for pairwise comparisons. Statistical significance was set at $P < 0.05$.

3. Results

A total of 45 overweight women were enrolled in the study and randomly assigned to three groups (n = 15 per group). All participants completed the study, and no dropouts or exclusions occurred during the intervention. Baseline characteristics, including age, body mass, height, and body mass index (BMI), were comparable among the three groups (Table 1).

Compliance with the supplementation protocol was complete, as all participants consumed the assigned capsules under the direct supervision of the research team. Participants also adhered to the prescribed dietary and physical activity instructions before the experimental trial, and no protocol violations were reported.

3.2. Primary outcomes

The primary outcomes of the study included plasma viscosity, fibrinogen concentration, total antioxidant capacity (TAC), and malondialdehyde (MDA). Descriptive statistics for all outcome variables are presented in Table 2, and the results of the mixed-design analysis of variance are summarized in Table 3.

A significant group × time interaction was observed for plasma viscosity ($F = 5.12$, $P = 0.01$, partial $\eta^2 = 0.19$). Post hoc analysis revealed a significant reduction in plasma viscosity following the intervention in the HIIT+Caffeine group ($P < 0.05$), whereas no significant changes were observed in the HIIT+Placebo or Control groups ($P > 0.05$).

Similarly, a significant group × time interaction was found for fibrinogen concentration ($F = 4.26$, $P = 0.02$, partial $\eta^2 = 0.16$). Pairwise comparisons demonstrated that fibrinogen levels decreased significantly only in the HIIT+Caffeine group ($P < 0.05$), with no significant changes in the HIIT+Placebo or Control groups ($P > 0.05$).

For total antioxidant capacity (TAC), the mixed-design ANOVA showed a significant group × time interaction ($F = 8.43$, $P < 0.001$, partial $\eta^2 = 0.28$). Post hoc comparisons indicated a significant increase in TAC following the intervention in the HIIT+Caffeine group ($P < 0.05$), whereas no significant changes were observed in either the HIIT+Placebo or Control groups ($P > 0.05$).

No significant group × time interaction was observed for malondialdehyde (MDA) ($F = 1.15$, $P = 0.27$, partial $\eta^2 = 0.05$). Likewise, neither the main effect of time nor the main effect of group reached statistical significance ($P > 0.05$), indicating that the intervention did not significantly affect lipid peroxidation. Numerical

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data for all primary outcomes are presented in Tables 2 and 3.

Table 1. Basic characteristics of participants

Variable	HIIT + Caffeine (n=15)	HIIT + Placebo (n=15)	Control + Placebo (n=15)	p-value
Age (years)	29.1 ± 3.2	28.7 ± 3.5	29.4 ± 3.1	0.81
Body mass (kg)	72.3 ± 4.8	71.5 ± 5.2	73.1 ± 4.9	0.67
Height (cm)	162.4 ± 5.1	161.9 ± 4.9	163.2 ± 5.0	0.74
Body mass index (BMI, kg/m²)	27.5 ± 1.2	27.3 ± 1.4	27.6 ± 1.3	0.89
Maximum heart rate (HRmax, bpm)	189 ± 7	190 ± 6	188 ± 8	0.77

Table 2. Changes in hemorheological and oxidative indices

Variable	Time	Control + Placebo (n=15)	HIIT + Placebo (n=15)	HIIT + Caffeine (n=15)	Time × Group p-value	Partial η²
Plasma viscosity (mPa·s)	Pre	1.43 ± 0.07	1.41 ± 0.08	1.42 ± 0.09	—	—
	Post	1.42 ± 0.08	1.35 ± 0.07	1.29 ± 0.08*	0.01	0.19
Fibrinogen (g/L)	Pre	3.14 ± 0.22	3.12 ± 0.24	3.15 ± 0.21	—	—
	Post	3.13 ± 0.25	3.05 ± 0.21	2.95 ± 0.20*	0.02	0.16
Total antioxidant capacity (TAC, mmol/L)	Pre	1.11 ± 0.12	1.13 ± 0.13	1.12 ± 0.14	—	—
	Post	1.12 ± 0.13	1.26 ± 0.14	1.40 ± 0.16*	<0.001	0.28
Malondialdehyde (MDA, µmol/L)	Pre	3.14 ± 0.22	3.18 ± 0.27	3.21 ± 0.25	—	—
	Post	3.21 ± 0.23	3.17 ± 0.25	3.19 ± 0.26	0.27	0.05

Table 3. Mixed analysis of variance results

Variable	Time effect (F, p, η²)	Group effect (F, p, η²)	Time × Group interaction (F, p, η²)
Plasma viscosity	F=6.14, p=0.02, η²=0.15	F=1.21, p=0.31, η²=0.07	F=5.12, p=0.01, η²=0.19
Fibrinogen	F=5.01, p=0.03, η²=0.13	F=1.09, p=0.34, η²=0.06	F=4.26, p=0.02, η²=0.16
Total antioxidant capacity (TAC)	F=9.87, p<0.001, η²=0.23	F=2.14, p=0.09, η²=0.11	F=8.43, p<0.001, η²=0.28
Malondialdehyde (MDA)	F=0.89, p=0.37, η²=0.04	F=0.75, p=0.48, η²=0.03	F=1.15, p=0.27, η²=0.05

Figure 1A. Plasma Viscosity Changes

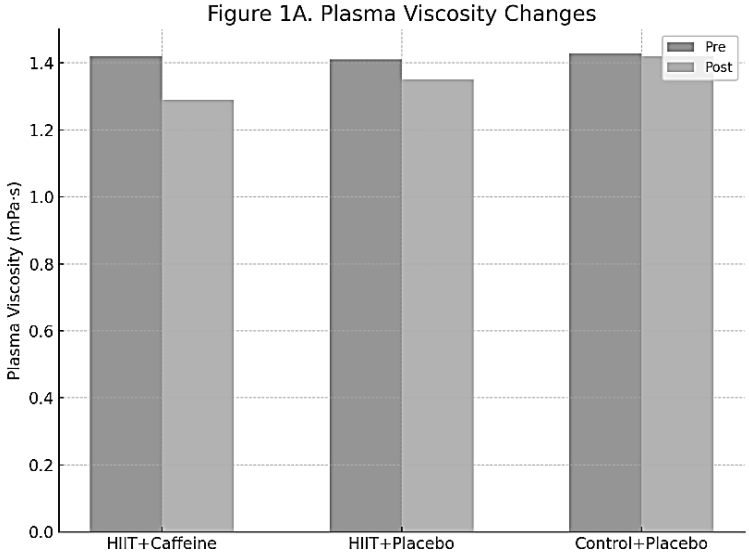


Figure 2A. Total Antioxidant Capacity

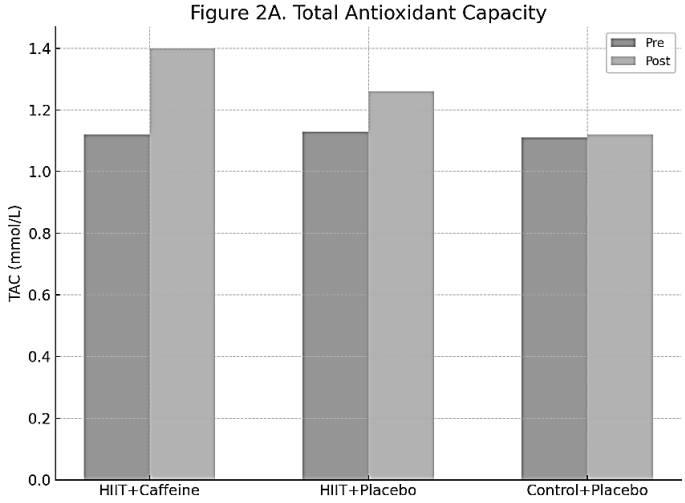
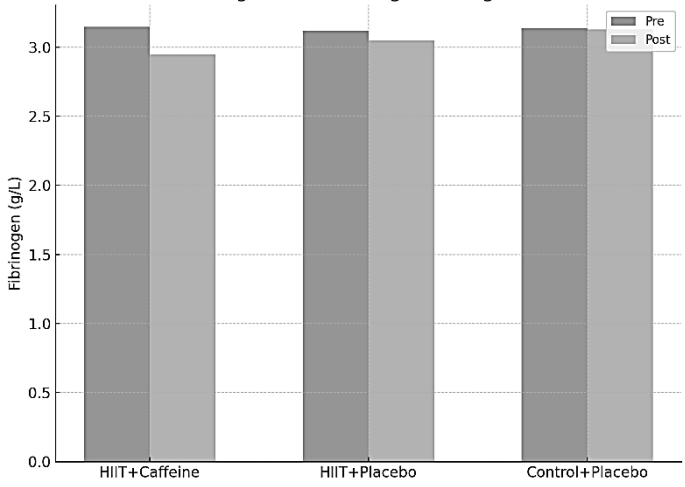


Figure 1B. Fibrinogen Changes



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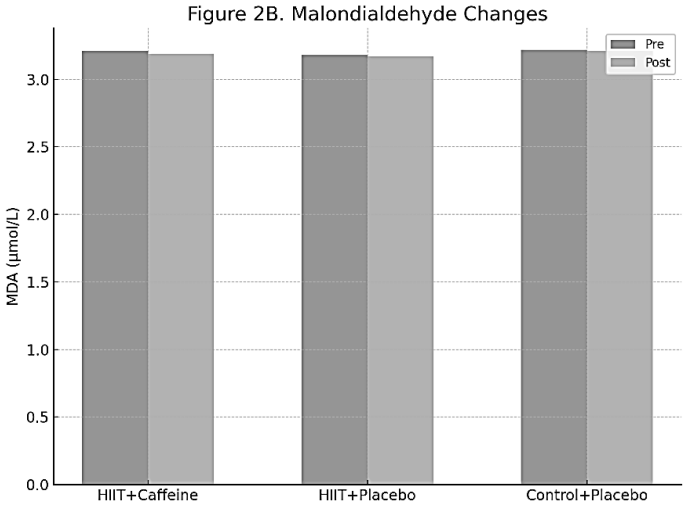

4. Discussion

The findings of the present study demonstrated that acute caffeine supplementation prior to a high-intensity interval training (HIIT) session significantly improved hemorheological parameters, including reductions in plasma viscosity and fibrinogen levels, and increased total antioxidant capacity (TAC) in overweight women, while malondialdehyde (MDA), as an indicator of lipid peroxidation, remained unchanged. This physiological response pattern suggests that the combination of caffeine intake and HIIT may induce beneficial vascular and rheological adaptations without promoting oxidative imbalance in this population, which is of considerable importance from the perspective of cardiovascular disease prevention and metabolic health.

The observed reductions in plasma viscosity and fibrinogen levels in the caffeine plus HIIT group are consistent with previous studies highlighting the modulatory effects of caffeine on blood rheological properties (9, 10). Caffeine may exert these effects through adenosine receptor antagonism, increased catecholamine release, and improved hemodynamic responses, thereby enhancing blood flow, reducing peripheral vascular resistance, and improving endothelial function. These mechanisms may contribute to decreased fibrinogen concentration and improved blood fluidity, ultimately reducing cardiovascular load and enhancing microcirculatory function, both of which are recognized as important indicators of cardiovascular health (1, 21).

Furthermore, HIIT has been recognized as one of the most effective exercise modalities for improving vascular function. The increased shear stress induced by HIIT stimulates nitric oxide-dependent pathways, enhances vascular compliance, and modulates endothelial responses (6). The absence of significant changes in hemorheological parameters in the HIIT plus placebo group suggests that a single bout of intense exercise alone may not be sufficient to induce acute alterations in these markers among overweight women. In this context, the synergistic interaction between caffeine supplementation and HIIT may explain the favorable responses observed in the present study, particularly in a population characterized by elevated blood viscosity and low-grade chronic inflammation (22).

The significant increase in TAC following caffeine supplementation observed in the present study is in agreement with existing evidence regarding the direct and indirect antioxidant properties of caffeine and its metabolites (12). Caffeine may enhance antioxidant defense by reducing reactive oxygen species (ROS) production, increasing antioxidant enzyme activity, and strengthening endogenous protective mechanisms, thereby shifting the oxidative balance toward a more favorable antioxidant state. Moreover, acute high-intensity exercise induces a controlled oxidative challenge that activates adaptive pathways, including NRF2-mediated antioxidant responses and other cellular defense mechanisms, ultimately enhancing antioxidant capacity (7). The simultaneous exposure to caffeine and HIIT may therefore explain the significant elevation in TAC without the development of oxidative damage.

The lack of a significant change in MDA levels indicates that despite the increased metabolic demand induced by HIIT and caffeine intake, lipid peroxidation was not exacerbated. This finding is consistent with previous studies demonstrating that oxidative damage markers do not necessarily increase when antioxidant capacity is simultaneously enhanced (13). However, some investigations have reported elevated MDA concentrations following intense exercise or caffeine consumption (14). These discrepancies may be attributed to differences in caffeine dosage, participants' training status, sex,

nutritional status, and blood sampling timing. In the present study, the use of a standardized caffeine dose (6 mg·kg^{-1}) and immediate post-exercise blood sampling may have allowed protective antioxidant responses to be activated before the occurrence of measurable lipid oxidative damage.

From a physiological perspective, the findings of this study suggest that the oxidative response induced by a single HIIT session likely remained within an adaptive range rather than reaching a damaging level. Recent evidence has proposed that a controlled increase in ROS production during intense exercise may function as an intracellular signaling mechanism that activates antioxidant defense pathways and improves mitochondrial function rather than acting solely as a detrimental factor (20). Within this framework, acute caffeine supplementation in the present study appears to have enhanced HIIT-induced adaptive responses without increasing lipid peroxidation. Moreover, differences between the present findings and studies employing supramaximal exercise tests, such as the Wingate test, may be explained by variations in metabolic demands, exercise duration, and the accumulation of oxidative metabolites. Standardized HIIT protocols generally produce a more controlled oxidative challenge and a more stable hemodynamic response compared with supramaximal exercise protocols (19, 21).

From a practical perspective, the findings of the present study suggest that acute caffeine supplementation prior to HIIT may serve as a short-term, accessible, and potentially safe nutritional strategy to improve vascular health and maintain oxidative balance in overweight women. This finding is particularly relevant for individuals seeking time-efficient exercise interventions while simultaneously being at increased risk of cardiovascular and metabolic disorders (2).

From a clinical and applied perspective, the current findings may have important implications for the development of exercise–nutrition interventions in overweight women. One of the major challenges in exercise prescription for individuals with excess body weight is achieving an optimal balance between maximizing metabolic benefits and preventing excessive physiological stress. The results of this study indicate that acute caffeine supplementation before a HIIT session not only failed to increase oxidative damage markers but was also associated with improvements in antioxidant capacity and hemorheological parameters.

These findings suggest that targeted caffeine supplementation, when administered at an appropriate dose and timing, may enhance physiological tolerance to high-intensity exercise in overweight individuals and may be considered as a component of preventive strategies aimed at improving cardiovascular health (11, 18).

The strengths of the present study include its randomized, double-blind, placebo-controlled design, strict control of caffeine intake, implementation of a standardized HIIT protocol, and simultaneous assessment of hemorheological and antioxidant markers. Nevertheless, several limitations should be acknowledged, including the assessment of only acute responses, the lack of inflammatory and molecular biomarker measurements, and the restriction of the sample population to overweight women. Therefore, caution should be exercised when generalizing these findings to other populations. Future studies should investigate the chronic effects of caffeine supplementation combined with HIIT, dose–response relationships, the influence of habitual caffeine consumption, and responses in different populations.

5. Conclusion

Overall, the findings of the present study indicate that acute caffeine supplementation prior to a high-intensity interval training (HIIT) session may improve hemorheological blood parameters and enhance antioxidant capacity in overweight women without exacerbating oxidative stress. These results support the potential role of caffeine as a supportive nutritional intervention alongside high-intensity exercise to promote vascular and metabolic health. Furthermore, the findings may provide a scientific basis for developing targeted exercise–nutrition strategies aimed at improving health outcomes in this population.

6. Highlights

The findings of this study indicate that acute caffeine supplementation prior to a high-intensity interval training (HIIT) session may improve hemorheological parameters and enhance antioxidant capacity in overweight women without exacerbating oxidative stress. These results highlight the importance of short-term nutritional strategies combined with high-intensity exercise to support vascular health.

Statements

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Conflict of Interest

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The authors declare that there are no financial or non-financial conflicts of interest associated with the conduct or publication of this study.

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Author Contributions

Shahin Ketabi: Study design, development of the research concept, statistical analysis of data, interpretation of findings, and preparation of the initial manuscript draft.

Mozhgan Ketabollahi: Data collection, implementation of research protocols, and scientific revision of the manuscript.

Bayan Feizi: Contribution to data collection, monitoring of study procedures, and manuscript revision.

Hasti Ghasem Karimi: Contribution to interpretation of results, critical revision of the manuscript, and approval of the final version.

All authors have read and approved the final version of the manuscript.

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