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Original Article

Can Probiotics Amplify the Vascular Benefits of High-Intensity Interval Training? A Randomized, Placebo-Controlled Trial in Women With Metabolic Syndrome

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Abstract

Introduction: Endothelial dysfunction and proatherogenic lipid abnormalities are central to metabolic syndrome (MetS). This trial examined whether combining high-intensity interval training (HIIT) with a multi-strain probiotic yields superior benefits in vascular cell adhesion molecule-1 (VCAM-1), plasminogen activator inhibitor-1 (PAI-1), and the lipid profile (total cholesterol [TC], triglycerides [TG], low-density lipoprotein (LDL)-cholesterol [LDL-C], low-density lipoprotein-C [HDL-C]) versus either alone.

Methods: In this randomized, placebo-controlled trial, forty women (25–35 years) with MetS were divided into (n = 10/group) to control, HIIT (8 weeks, 3 ×/week), probiotic (*Lactobacillus casei*, *L. bulgaricus*, *L. rhamnosus*, *L. acidophilus*, *Bifidobacterium longum*, *B. breve*, and *Streptococcus thermophilus*; 2×10^8 CFU/day; Lactol®), and HIIT + probiotic groups. VCAM-1, PAI-1, and lipid profile were assessed at baseline and 48 hour post-intervention (repeated-measures and change-score ANOVA).

Results: Significant time × group interactions occurred for all six outcomes ($P < 0.001$; η^2: 0.780–0.881), with the largest changes in the HIIT + probiotic group (VCAM-1: –118 ng/mL; PAI-1: –7.3 ng/mL; TC: –32.1 mg/dL; TG: –27.1 mg/dL; LDL-C: –21.5 mg/dL; HDL-C: + 5.8 mg/dL). Post-test, VCAM-1 was lower in HIIT + probiotic versus control ($P = 0.015$) and probiotic ($P = 0.002$) groups. TG, LDL-C, HDL-C were borderline after Bonferroni correction ($P = 0.07$–0.09), and PAI-1/TC were non-significant. Ultimately, Tukey's HSD on change scores demonstrated that HIIT + probiotic exceeded exercise alone for all six outcomes ($P \leq 0.004$).

Conclusion: HIIT plus multi-strain probiotics produced the highest vascular and lipid improvements in women with MetS, with change-score analysis confirming a significant additive probiotic benefit, supporting this non-pharmacological strategy pending larger trials.

Keywords: Metabolic syndrome, High-intensity interval training, Probiotics, Vascular cell adhesion molecule-1, Plasminogen activator inhibitor 1, Vascular diseases

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Introduction

Metabolic syndrome (MetS) affects over one-third of adults in developed nations and confers a twofold to threefold greater risk of cardiovascular disease and a fivefold increased risk of diabetes. The National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) defines MetS as the presence of at least three of five risk factors, including abdominal obesity, elevated triglycerides (TG), low high-density lipoprotein cholesterol (HDL-C), hypertension, and impaired fasting glucose (1). Central to this process is endothelial dysfunction, an early, reversible step marked by reduced nitric oxide bioavailability, a pro-adhesive/pro-thrombotic endothelial surface, and chronic inflammation (2). Vascular cell adhesion molecule-1 (VCAM-1), measured here as its soluble form, sVCAM-1, is induced by inflammatory cytokines and oxidative stress. It promotes monocyte transmigration into the vessel wall and is consistently elevated in MetS (3). Moreover, circulating levels of sVCAM-1 are consistently elevated in individuals with MetS and independently predict future cardiovascular events (4). Likewise, plasminogen activator inhibitor-1 (PAI-1), the principal inhibitor of fibrinolysis, is secreted by visceral adipocytes and endothelial cells; its overexpression favors atherothrombosis and is mechanistically linked to insulin resistance and visceral adiposity, thereby serving as both a marker and mediator of vascular risk (4).

The lipid irregularities that accompany MetS, namely, elevated triglyceride levels, reduced HDL-C, and a greater proportion of small, dense low-density lipoprotein (LDL) particles, further enhance endothelial activation and atherogenesis (5). Oxidized LDL particles up-regulate VCAM-1 expression via the lectin-like oxLDL receptor-1 and stimulate nuclear factor kappa B (NF-kB) signaling, while TG-rich remnant lipoproteins directly promote PAI-1 release from endothelial cells and hepatocytes. Total cholesterol (TC) and LDL-C, although occasionally within the normal range in MetS, are frequently elevated due to hepatic over-production of apolipoprotein lipoprotein B-containing lipoproteins driven by insulin resistance (6). This lipid triad creates a vicious cycle in which dyslipidemia aggravates endothelial dysfunction, thereby facilitating further lipid deposition and plaque progression.

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Lifestyle modification, particularly regular exercise, remains the cornerstone of MetS management (7). High-intensity interval training (HIIT) is a time-efficient exercise strategy that improves endothelial function, insulin sensitivity, and lipid metabolism (8). These benefits are primarily mediated through enhanced nitric oxide bioavailability (9), NF-κB signaling inhibition, increased lipoprotein lipase activity, and greater hepatic fatty acid oxidation (10).

The gut microbiome plays a pivotal role in the pathogenesis of MetS. Gut dysbiosis promotes metabolic endotoxemia, systemic inflammation, and lipid abnormalities through metabolites such as lipopolysaccharide (LPS) and short-chain fatty acids (SCFAs) (11). Furthermore, probiotics can improve gut barrier integrity, reduce circulating LPS, and favorably regulate lipid metabolism (12). Although probiotic supplementation has been shown to improve lipid profiles in individuals with MetS (13), its effects on endothelial biomarkers such as VCAM-1 and PAI-1 remain inconsistent (13). These discrepancies may reflect differences in probiotic strains, dosage, intervention duration, and participant characteristics, suggesting that probiotics may be more effective when combined with exercise rather than used alone (14).

Emerging evidence supports a bidirectional gut–muscle axis, whereby exercise enhances gut microbial diversity, increases SCFA-producing bacteria, and improves gut barrier function (15). This may augment the colonization and metabolic activity of probiotic strains, thereby enhancing their systemic effects. However, the combined effects of HIIT and multi-strain probiotics on endothelial biomarkers and lipid profile in women with MetS remain largely unexplored. Therefore, this randomized, placebo-controlled trial aims to investigate whether 8 weeks of HIIT combined with multi-strain probiotic supplementation would elicit greater improvements in cardiovascular risk markers than either intervention alone.

Materials and Methods

Study Design and Participants

The present study was a randomized, placebo-controlled trial employing a pre-test/post-test design. A total of 40 women aged 25–35 years, diagnosed with MetS according to the NCEP-ATP III criteria (presence of at least three of the following: waist circumference > 88 cm, fasting blood glucose ≥ 100 mg/dL, TG ≥ 150 mg/dL, HDL-C < 50 mg/dL, and blood pressure ≥ 130/85 mmHg), were enrolled from Tabriz, Iran. Participants were recruited through a combination of public announcements (posters and social media notices distributed in Tabriz, Iran) and referrals from local health centres/clinics. Then, interested women underwent initial screening for MetS at the study site, where eligibility was confirmed against the NCEP-ATP III criteria before enrolment. Nonetheless, individuals were excluded if they had engaged in regular exercise (two or more sessions per week) over the preceding six months, were taking lipid-lowering or antihypertensive medications, or had used probiotics or antibiotics during the previous four weeks. Similarly, they were excluded from the study if they had any gastrointestinal disorder, reported an allergy to probiotics, were pregnant or breastfeeding, or had any medical condition that would contraindicate high-intensity exercise. Next, written informed consent was obtained from all participants prior to enrolment. This manuscript reports a pre-specified subset of outcomes (VCAM-1, PAI-1, and the lipid profile) from a larger PhD thesis project; additional outcomes from the same trial, including total antioxidant capacity, are reported separately.

Randomization and Blinding

Using a computer-generated randomization sequence and sealed, opaque envelopes, the participants were assigned to four equal groups of ten individuals; they included a control arm receiving placebo (C), an exercise arm performing HIIT alongside placebo (E), a probiotic arm taking only the supplement (P), and a combined arm undertaking HIIT together with the probiotic regimen (E + P). Supplementation was double-blinded: probiotic and placebo capsules were identical in appearance, taste, and smell, and dispensed by a researcher not involved in outcome assessment. Additionally, laboratory personnel were blinded, whereas the blinding of the exercise intervention was not feasible.

Interventions

High-Intensity Interval Training Program (Exercise and Exercise + Probiotic Groups): An 8-week treadmill HIIT program was performed three times per week. Sessions began with a 15-minute warm-up (jogging at 50 % maximal aerobic velocity [MAV], strides, and dynamic stretching). In addition, the main phase consisted of 30-second runs at 100 % MAV alternated with 30-second active recovery at 50 % MAV, organized into 4 laps per set. Four sets were performed in weeks 1–2, increasing to six sets from weeks 3–8, with 5-minute passive rest between sets. Further, MAV was determined via the multi-stage fitness test and updated at week 4 (16). All sessions were supervised.

Probiotic Supplementation (Probiotic and Exercise + Probiotic Groups): Participants received two capsules daily, each containing 2×10^8 colony-forming units of a seven-strain probiotic blend (*Lactobacillus casei*, *L. bulgaricus*, *L. rhamnosus*, *L. acidophilus*, *Bifidobacterium longum*, *B. breve*, and *Streptococcus thermophilus*; Lactol®). Capsules were taken 1 hour after lunch. Groups C and E received identical placebo (dextrose) capsules, and compliance was monitored by capsule counts and weekly reminders (17).

Control Group: The control group maintained habitual lifestyle and received placebo capsules.

Dietary and Activity Monitoring

Participants maintained their usual diet; three-day food records at baseline, week 4, and week 8 confirmed no significant changes in energy or macronutrient intake within or between groups. Habitual physical activity (International Physical Activity Questionnaire–Short

Form) remained stable.

Anthropometric and Blood Pressure Measurements

Body weight, height, waist circumference, and resting blood pressure were measured at baseline and 48 hour post-intervention using standardized protocols (digital scale, stadiometer, non-elastic tape, and automated oscillometric device).

Blood Sampling and Biochemical Analyses

Venous blood samples were collected between 07:00 and 09:00 am, after a 10-hour overnight fast, at two time points: baseline and 48 hours following the final exercise session (or the corresponding time for non-exercising groups), to minimize circadian variation in the measured biomarkers. Serum concentrations of sVCAM-1 (RayBiotech, USA) and PAI-1 (MyBioSource, USA) were determined using commercially available enzyme-linked immunosorbent assay kits according to the manufacturers' instructions. Moreover, TC and TG were quantified through enzymatic colorimetric assays (Pars Azmoon, Tehran, Iran). Furthermore, HDL-C was measured after precipitation of apolipoprotein B-containing lipoproteins. Eventually, low-LDL-C was computed using the Friedewald equation, provided that triglyceride levels were below 400 mg/dL.

Statistical Analysis

An a priori sample size calculation indicated that a total of 36 participants would be needed, assuming a significance level of 0.05, a statistical power of 0.80, and a moderate effect size; accordingly, 40 women were enrolled to account for potential dropouts. All statistical analyses were conducted using SPSS software (version 28). The Shapiro-Wilk test was applied to verify normal distribution, and Levene's test was used to confirm the homogeneity of variances. Additionally, a two-way repeated-measures analysis of variance (ANOVA) was employed, with time (pre-intervention vs. post-intervention) and group assignment (C, E, P, and E + P) serving as the within-subjects factor and the between-subjects factor, respectively. The six dependent variables, including VCAM-1, PAI-1, TC, TG, LDL-C, and HDL-C, were examined together in a multivariate model, followed by the univariate analyses of the corresponding outcomes. When a significant time × group interaction was detected, Bonferroni-adjusted pairwise comparisons were performed to locate the differences. In addition, change scores (post-test minus pre-test) were calculated for each outcome and compared across the four groups using one-way ANOVA; where the omnibus test was significant, Tukey's honest significant difference (HSD) test was used for post-hoc pairwise comparisons. Further, effect sizes were expressed as partial eta-squared (η^2_p), and a two-tailed P value of less than 0.05 was considered statistically significant.

Results

All 40 participants completed the study with 100% adherence, with no adverse events. Participant flow through the trial is shown in Figure 1.

Based on the results (Table 1), baseline characteristics did not differ among the groups.

Multivariate and Univariate Analyses

The multivariate analysis revealed a significant time × group interaction [Pillai's Trace = 1.179, F (18,99) = 3.563, $P < 0.001$]. In univariate tests, this interaction was significant for all six outcomes: VCAM-1 [F (3,36) = 88.51, $P < 0.001$, $\eta^2_p = 0.881$], PAI-1 (F = 88.28, $\eta^2_p = 0.880$), TC (F = 68.99, $\eta^2_p = 0.852$), TG (F = 42.48, $\eta^2_p = 0.780$), LDL-C (F = 70.43, $\eta^2_p = 0.854$), and HDL-C (F = 55.90, $\eta^2_p = 0.823$).

Within-Group Changes

The simple effects of time within each group, extracted from the group × time pairwise comparisons, are presented in Table 2. All three active intervention groups showed significant improvements in every outcome ($P < 0.001$ for all comparisons in E, P, and E + P). The control group did not significantly change on any variable ($P > 0.05$). The largest absolute pre-to-post differences were consistently observed in the E + P group (Figure 2).

Each value represents the post-intervention minus pre-intervention difference (Δ = Post – Pre). A negative Δ indicates a decline, whereas a positive Δ denotes an elevation relative to baseline. Based on the results, the E + P group consistently displayed the highest decreases in inflammatory and atherogenic markers while showing the most pronounced increase in HDL-C.

Between-Group Comparisons at Post-Test

At baseline, no significant differences were found among the groups for any variable (all Bonferroni-adjusted $P > 0.999$). At post-test, the univariate tests of the simple effect of group at time 2 yielded these results: F (3,36) = 5.952, $P = 0.002$, $\eta^2_p = 0.332$ for VCAM-1, F = 1.134, $P = 0.348$, $\eta^2_p = 0.086$ PAI-1, F = 2.089, $P = 0.119$, $\eta^2_p = 0.148$ for TC, F = 3.462, $P = 0.026$, $\eta^2_p = 0.224$ for TG, F = 3.133, $P = 0.037$, $\eta^2_p = 0.207$ for LDL-C, and F = 2.912, $P = 0.048$, $\eta^2_p = 0.195$ for HDL-C.

Pairwise Comparisons (Bonferroni-Adjusted) at Post-Test

Regarding VCAM-1, the E + P group had significantly lower levels than the control group (mean difference [MD]: –109.6 ng/mL, $P = 0.015$) and the probiotic group (–131.2 ng/mL, $P = 0.002$). The difference between E + P and exercise groups was not significant ($P = 0.392$). Concerning TG, LDL-C and HDL-C, despite significant omnibus F-tests, no pairwise comparison survived Bonferroni correction: E + P vs. control for TG ($P = 0.071$), LDL-C ($P = 0.094$) and HDL-C ($P = 0.053$). Finally, PAI-1 and TC displayed no significant pairwise differences.

Change-Score Analysis (Tukey's Honest Significant Difference)

To provide a more accessible measure of the magnitude of change, change scores (post-test minus pre-test) were compared across the four groups using one-way ANOVA

Table 1. Baseline Demographic and Anthropometric Characteristics (Mean ± SD)

Variable	Control (n = 10)	Exercise (n = 10)	Probiotic (n = 10)	Exercise + Probiotic (n = 10)
Age (years)	31.3 ± 2.7	30.0 ± 2.8	29.9 ± 3.7	30.2 ± 3.0
Height (cm)	161.1 ± 3.6	163.5 ± 7.9	161.4 ± 5.4	161.6 ± 5.8
Weight (kg)	84.1 ± 6.6	84.6 ± 9.8	81.8 ± 7.4	83.4 ± 10.0
Body mass index (kg/m²)	32.8 ± 2.5	31.6 ± 2.2	31.4 ± 2.1	31.9 ± 2.3

Note. SD: Standard deviation; ANOVA: Analysis of variance. All between-group $P > 0.05$ (one-way ANOVA)

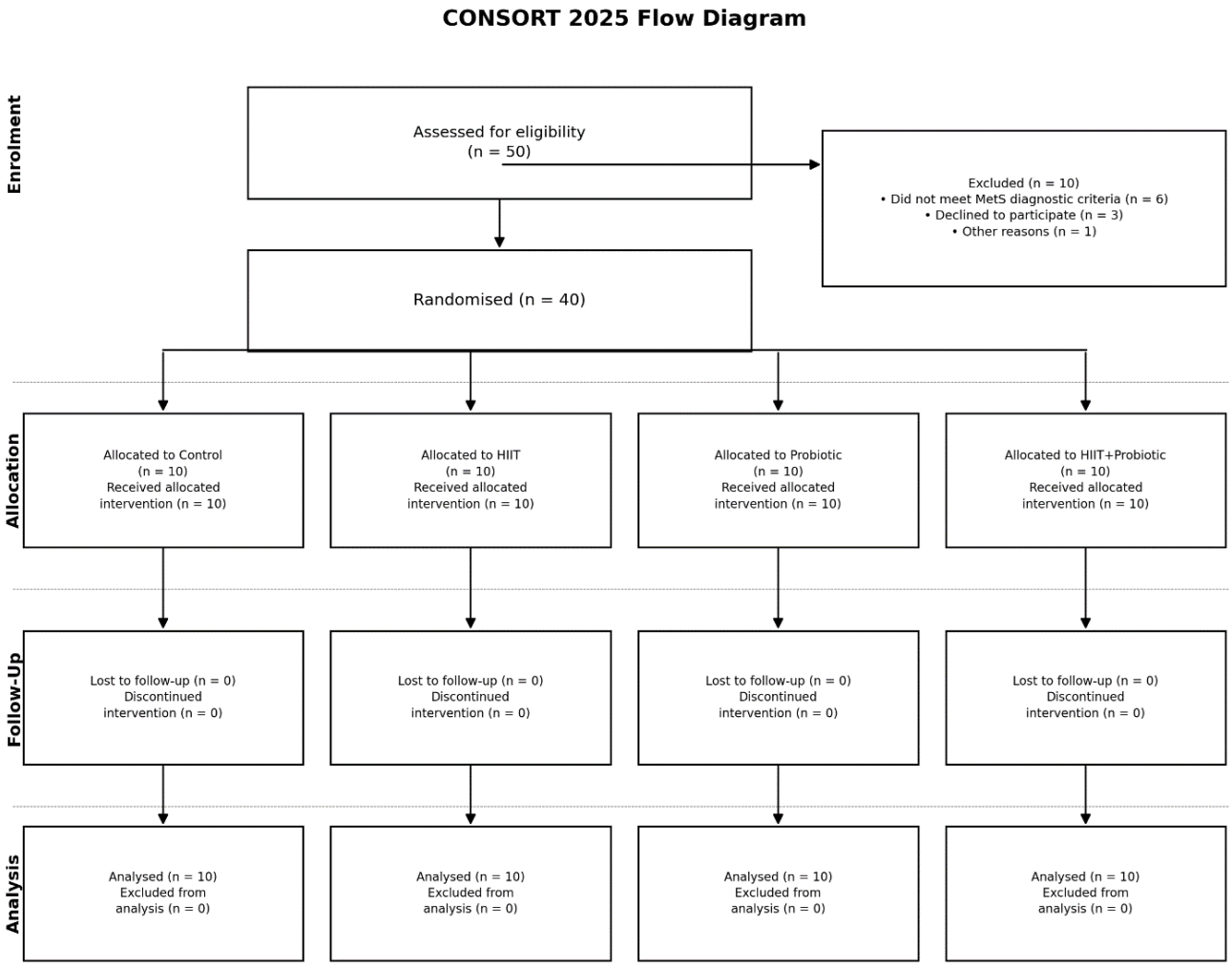

Figure 1. CONSORT 2025 Flow Diagram Displaying the Progress of Participants Through Enrolment, Randomization, Allocation, Follow-up, and Analysis in the Control, HIIT, Probiotic, and HIIT + Probiotic Groups
Note. CONSORT: Consolidated Standards of Reporting Trials; HIIT: High-intensity interval training

followed by Tukey's HSD post-hoc test. For all six outcomes, the HIIT + probiotic group showed significantly greater change scores than the control group (all $P < 0.001$) and then the probiotic-alone group (all $P < 0.001$). Compared with exercise alone, the HIIT + probiotic group depicted markedly higher improvement for VCAM-1 (MD: 44.27, $P = 0.002$), PAI-1 (MD: 4.28, $P = 0.004$), TC (MD: 16.08, $P < 0.001$), TG (MD: 11.61, $P < 0.001$), LDL-C (MD: 10.91, $P < 0.001$), and HDL-C (MD: 2.42, $P < 0.001$), indicating a consistent additive benefit of probiotic co-supplementation across every outcome. In contrast, exercise alone and probiotic alone did not remarkably differ from one another for PAI-1, HDL-C, TC, TG, or LDL-C ($P = 0.099$–0.841). However, they did significantly differ for VCAM-1 ($P = 0.001$), suggesting that HIIT exerted a stronger independent effect on VCAM-1 than probiotic supplementation alone. Overall, these change-score analyses corroborate and extend the time × group interaction findings from the repeated-measures ANOVA, providing more granular, Tukey-adjusted evidence that the combined intervention produced superior improvements over exercise alone across the full panel of vascular and lipid outcomes (Table 3).

Discussion

The findings of this randomized, placebo-controlled trial demonstrated that an 8-week HIIT programme combined with a multi-strain probiotic yielded the greatest improvements in circulating VCAM-1, PAI-1, TC, TG, LDL-C, and HDL-C in women with MetS. The

Table 2. Outcome Variables Before and After the 8-Week Intervention (Mean ± SD)

Variable	Group	Pre	Post	*P* (Within)†
VCAM-1 (ng/mL)	C	743.4 ± 84.2	746.4 ± 85.7	0.591
	E	770.5 ± 77.3	700.7 ± 70.4	0.001
	P	811.5 ± 59.5	768.0 ± 63.0	0.001
	E + P	754.8 ± 98.3	636.8 ± 79.7	0.001
PAI-1 (ng/mL)	C	46.7 ± 10.5	47.2 ± 10.2	0.107
	E	44.1 ± 5.9	40.6 ± 5.8	0.001
	P	46.1 ± 13.0	43.5 ± 12.2	0.001
	E + P	47.8 ± 9.2	40.5 ± 8.0	0.001
TC (mg/dL)	C	208.7 ± 21.9	208.9 ± 22.4	0.888
	E	211.2 ± 34.2	195.1 ± 28.6	0.001
	P	208.6 ± 20.6	197.8 ± 19.8	0.001
	E + P	216.7 ± 13.6	184.6 ± 14.2	0.001
TG (mg/dL)	C	192.3 ± 28.5	191.6 ± 27.8	0.666
	E	178.8 ± 22.0	163.3 ± 20.4	0.001
	P	198.8 ± 16.7	186.2 ± 12.2	0.001
	E + P	185.4 ± 49.3	158.2 ± 42.6	0.001
LDL-C (mg/dL)	C	137.6 ± 21.5	136.7 ± 21.3	0.336
	E	136.0 ± 21.7	125.4 ± 20.2	0.001
	P	146.9 ± 18.4	137.5 ± 17.2	0.001
	E + P	137.2 ± 17.7	115.7 ± 14.3	0.001
HDL-C (mg/dL)	C	40.0 ± 7.6	40.0 ± 7.3	0.883
	E	43.0 ± 5.0	46.5 ± 5.7	0.001
	P	42.2 ± 5.4	44.5 ± 5.8	0.001
	E + P	42.0 ± 6.2	47.8 ± 6.5	0.001

Note. C: Control; E: Exercise; P: Probiotic; E + P: Exercise + probiotic; VCAM-1: Vascular cell adhesion molecule-1; PAI-1: Plasminogen activator inhibitor-1; TC: Total cholesterol; TG: Triglycerides; LDL-C: Low-density lipoprotein cholesterol; HDL-C: High-density lipoprotein cholesterol
† Within-group *P* value from Bonferroni-adjusted simple effects of time (groups × time table)

highly significant time × group interactions for all six outcomes, with large effect sizes (partial η^2 ranging from 0.780 to 0.881), along with the consistent pattern of the largest absolute changes in the HIIT + probiotic group, could provide robust evidence for additive, and possibly synergistic, benefits. Importantly, the between-group differences after stringent Bonferroni correction reached significance for VCAM-1, while for the other variables, the omnibus tests were significant but pairwise comparisons were borderline, reflecting both the small sample size and the biological variability inherent in these markers. However, when directly quantified as change scores and compared using Tukey's HSD test, the E + P group substantially differed from the exercise-alone group for all six outcomes, providing more granular evidence of an additive benefit of probiotic co-supplementation (refer to change-score analysis in the Results section).

The marked reduction in VCAM-1 observed in the combined group is clinically relevant, as elevated VCAM-1 is an independent predictor of future cardiovascular events and vascular dysfunction (18). This improvement is likely mediated by multiple mechanisms. HIIT enhances endothelial nitric oxide bioavailability and suppresses NF-κB signaling (19) while reducing oxidative stress and pro-inflammatory cytokines that stimulate VCAM-1 expression (20). Concurrently, probiotics improve gut barrier integrity, decrease circulating LPS, and increase SCFA production, particularly butyrate, which may further inhibit endothelial activation (21). In addition, the concurrent improvements in LDL-C and TG may have contributed to the reduction in VCAM-1, as oxidized LDL and TG-rich lipoproteins are known stimulators of endothelial inflammation (22). Moreover, the greater reduction in VCAM-1 in the combined group compared with the control, probiotic, and

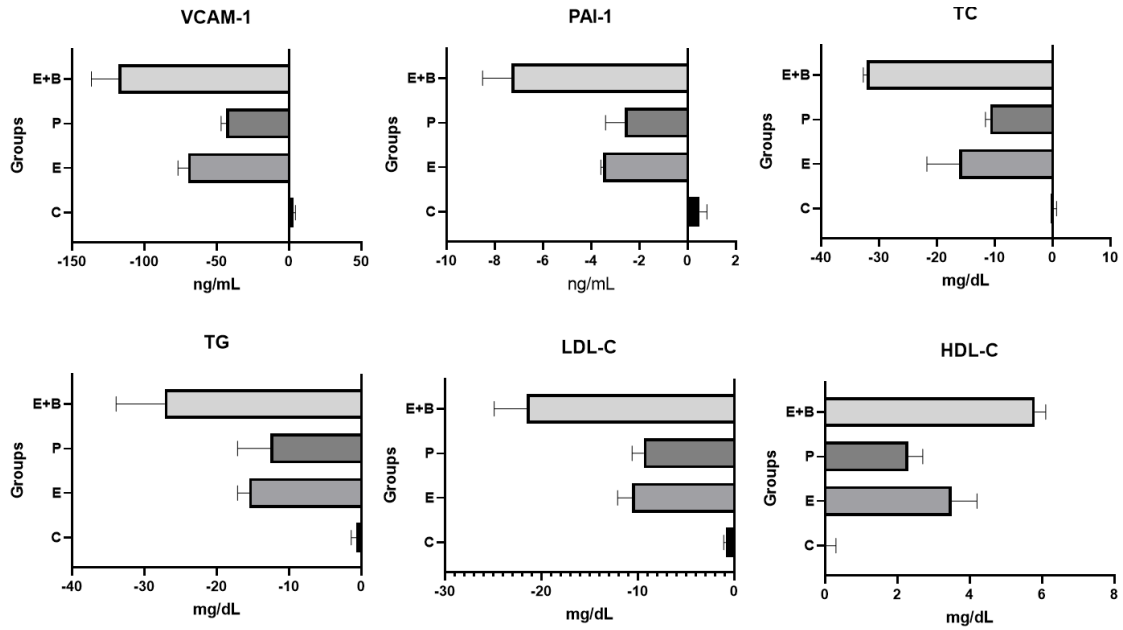

Figure 2. Pre-to-Post Changes (Δ) in Circulating Levels of sVCAM-1, PAI-1, TC, TG, LDL-C, and HDL-C Across Control (C), exercise (E), probiotic (P), and Exercise Plus Probiotic (E + P) Groups
Note. sVCAM-1: Soluble vascular cell adhesion molecule-1; PAI-1: Plasminogen activator inhibitor-1; TC: Total cholesterol; TG: Triglycerides; LDL-C: Low-density lipoprotein cholesterol; HDL-C: High-density lipoprotein cholesterol

Table 3. Pairwise Comparisons of Change Scores (Post-Test Minus Pre-Test) Between Groups for VCAM-1, PAI-1, and Lipid Profile Parameters Using Tukey's Honest Significant Difference Test

Variable	ANOVA *P*	Group	Groups	Mean Difference	Standard Deviation	*P*-Post Hoc
VCAM-1 (ng/mL)	.001	C	E	89.16	11.08	.001
			P	43.25	11.08	.002
			E+P	133.43	11.08	.001
		E	P	-45.91	11.08	.001
			E+P	44.27	11.08	.002
		P	E+P	90.18	11.08	.001
PAI-1 (ng/mL)	.001	C	E	7.88	1.15	.001
			P	6.22	1.15	.001
			E+P	12.16	1.15	.001
		E	P	-1.66	1.15	.48
			E+P	4.28	1.15	.001
		P	E+P	5.94	1.15	.001
TC (mg/dL)	.001	C	E	16.28	2.29	.001
			P	11.06	2.29	.001
			E+P	32.36	2.29	.001
		E	P	-5.21	2.29	.12
			E+P	16.08	2.29	.001
		P	E+P	21.29	2.29	.001
TG (mg/dL)	.001	C	E	14.81	2.35	.001
			P	11.82	2.35	.001
			E+P	26.41	2.35	.001
		E	P	2.98	2.35	.58
			E+P	11.6	2.35	.001
		P	E+P	14.59	2.35	.001
LDL-C (mg/dL)	.001	C	E	9.61	1.14	.001
			P	8.44	1.14	001
			E+P	20.52	1.14	001
		E	P	-1.17	1.14	.84
			E+P	10.9	1.14	.001
		P	E+P	12.08	1.14	.001
HDL-C (mg/dL)	.001	C	E	-3.47	.46	.001
			P	-2.37	.46	.001
			E+P	-5.98	.46	.001
		E	P	1.1	.46	.09
			E+P	-2.24	.46	.001
		P	E+P	-3.25	.46	.001

Note. C: Control; E: Exercise; P: Probiotic; E+P: Exercise+probiotic; VCAM-1: Vascular cell adhesion molecule-1; PAI-1: Plasminogen activator inhibitor-1; TC: Total cholesterol; TG: Triglycerides; LDL-C: Low-density lipoprotein cholesterol; HDL-C: High-density lipoprotein cholesterol

exercise groups suggests that probiotic co-supplementation provides additional vascular benefits beyond HIIT alone.

PAI-1 was markedly reduced following the combined intervention, representing an improvement in fibrinolytic balance. This effect is likely mediated by HIIT-induced reductions in visceral adiposity, insulin resistance, and circulating free fatty acids (23), along with the anti-inflammatory and lipid-lowering effects of probiotics (24). The concurrent decrease in TG may have further suppressed PAI-1 expression by reducing activator protein-1 (AP-1-mediated signaling (25). Although post-hoc comparisons were not consistently significant, change-score analysis demonstrated a greater decline in the combined group, supporting an additive benefit of probiotic supplementation beyond HIIT alone, which is consistent with previous findings (26).

The combined intervention markedly improved the lipid profile, indicating a clinically meaningful reduction in cardiovascular risk. These benefits are likely mediated by HIIT-induced enhancement of lipoprotein lipase activity and fatty acid oxidation, coupled with probiotic-mediated modulation of bile acid metabolism and SCFA production, which collectively improve lipid metabolism (20). Although post-hoc comparisons for some lipid variables were borderline, change-score analysis confirmed greater improvements in the combined group than in the exercise-only group, highlighting an additive effect of probiotic supplementation (27).

A notable observation was that probiotics consistently magnified the effects of HIIT alone, potentially via the gut-muscle axis: exercise increases gut microbial diversity and barrier integrity (20), which may allow supplemented strains to engraft more efficiently and produce higher levels of anti-inflammatory metabolites such as butyrate and propionate. This is consistent with recent animal data showing that exercise potentiates the metabolic benefits of probiotics (28); our trial provided early human evidence for this concept. The interconnection between improved lipids and reduced VCAM-1/PAI-1 further suggests that the combined intervention helped break the cycle between dyslipidaemia and endothelial activation characteristic of MetS.

Our findings are generally in line with those of previous studies while extending the current evidence. Unlike the results of Shahidi et al (29) that revealed no significant effect of HIIT on VCAM-1, our findings demonstrated a significant reduction, possibly due to greater baseline endothelial dysfunction and the higher exercise intensity used in our MetS cohort. Similarly, the vascular benefits observed with probiotic supplementation contradict the findings of a short-term study by Noshadi et al. (30), highlighting the importance of intervention duration and study population. Furthermore, the favorable effects on lipid profile corroborate previous trials and meta-analyses (13), while our findings further indicate that combining HIIT with probiotics may provide greater cardiometabolic benefits than either intervention alone (31).

From a clinical perspective, the combined intervention may contribute to cardiovascular risk reduction in women with MetS by improving endothelial function, fibrinolytic balance, and lipid metabolism. The combination of HIIT and multi-strain probiotics represents a promising non-pharmacological strategy for early cardiovascular prevention, since both interventions are safe, low-cost, and accessible interventions. Nevertheless, larger longitudinal studies are needed to confirm these benefits using clinical endpoints such as cardiovascular events, arterial stiffness,

and quality of life.

Several limitations should be acknowledged. The sample size was small, which limited statistical power for some post-hoc comparisons and may explain why between-group differences for TG, LDL-C and HDL-C did not survive Bonferroni correction despite significant omnibus tests. This limitation was partly mitigated by the change-score analysis, which detected significant differences between the E + P and exercise-alone groups for all six outcomes. Moreover, the violation of homogeneity of variance for TG should be considered, although equal group sizes and the robustness of the F-test mitigate this concern. Likewise, faecal microbiota analysis was not performed, so the direct link between probiotic engraftment and the observed benefits remains inferential. Finally, the findings are specific to young-to-middle-aged women with MetS and may not be generalizable to men or older populations.

Conclusion

In general, the combination of 8-week HIIT and a multi-strain probiotic produced the highest improvements in VCAM-1, PAI-1, and the entire atherogenic lipid profile in women with MetS. The pattern of results indicates additive, and possibly synergistic, benefits, likely mediated through complementary anti-inflammatory, lipid-modulating, and gut-barrier-reinforcing mechanisms. Accordingly, these findings support the integration of exercise and gut microbiome modulation as a powerful non-pharmacological strategy for cardiovascular risk reduction in MetS. Nonetheless, larger, longer-term trials with metagenomic profiling are warranted to confirm these effects and to elucidate the precise gut-muscle-vascular pathways underlying these effects.

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Artificial Intelligence Use Disclosure

During the preparation of this manuscript, an AI-based language model (large language model) was employed as an auxiliary tool for language editing, translation, statistical consultation, and reference formatting. All AI-generated suggestions were critically reviewed, verified for scientific accuracy, and edited by the authors. It is noteworthy that the authors take full responsibility for the entire content of the manuscript, including the AI-assisted sections. No AI tool was used to generate, fabricate, or manipulate the original research data.

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Competing Interests

The authors have no conflict of interests to disclose.

Ethical Approval

This article has been derived from a doctoral dissertation submitted to Islamic Azad University, Tabriz Branch (ethical code: IR.IAU.TABRIZ.REC.1404.384 and clinical trial code: IRCT20251116068018N1). The ethics approval covers a broader project examining total antioxidant capacity and vascular inflammatory markers; the present manuscript specifically focused on the vascular adhesion and lipid outcomes.

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