To appear in Exercise Physiology and Performance (EPP)
Received: 2025-11-16 Revised: 2025-12-05 Accepted: 2025-12-18
DOI: https://doi.org/10.71951/epp.2025.202511161224875

High-Intensity Interval Training with L-Citrulline Malate Reduces NF-κB and HIF-1α Expression and Improves Cardiometabolic Markers in Obese Middle-Aged Women

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Abstract

Background: Obesity in midlife women is accompanied by chronic low-grade inflammation and tissue hypoxia mediated by transcription factors such as nuclear factor-κB (NF-κB) and hypoxia-inducible factor-1α (HIF-1α). High-intensity interval training (HIIT) improves cardiometabolic health, while L-citrulline malate (CM) enhances nitric oxide (NO) production and endothelial function. This study examined the combined effects of HIIT and CM on NF-κB and HIF-1α expression and related metabolic and inflammatory outcomes in obese middle-aged women.

Methods: Forty participants were randomized to Control (n = 13), HIIT (n = 13), or HIIT + CM (n = 14) groups for eight weeks. HIIT consisted of three supervised sessions per week (80–85% VO_2max). The supplemented group received oral L-citrulline malate according to protocol. Primary endpoints were PBMC gene expression of NF-κB and HIF-1α (RT-qPCR). Secondary outcomes included plasma monocyte chemoattractant protein-1 (MCP-1), malondialdehyde (MDA), superoxide dismutase (SOD) activity, fasting lipid profile, and visceral adipose tissue (VAT) area. Linear mixed models evaluated group×time effects.

Results: Both HIIT and HIIT + CM significantly downregulated NF-κB (−17.5% and −26.2%) and HIF-1α (−13.5% and −20.1%) versus control ($p < 0.01$), with stronger responses in the supplemented group. MCP-1 (−18.8 pg/mL), MDA (−0.53 μmol/L), LDL-C (−18.6 mg/dL), and triglycerides (−34.7 mg/dL) decreased, while SOD (+0.32 U/mL) and HDL-C (+3.1 mg/dL) increased significantly. VAT area declined in both active arms (−15.5 cm² HIIT; −20.4 cm² HIIT + CM; $p < 0.001$). Reductions in NF-κB/HIF-1α correlated with improvements in MCP-1, triglycerides, and VAT.

Conclusion: Eight weeks of HIIT effectively improved inflammatory, oxidative, and lipid markers in obese women. L-citrulline malate supplementation further enhanced reductions in NF-κB and HIF-1α expression, suggesting synergistic benefits through NO-mediated anti-inflammatory and hypoxia-ameliorating mechanisms.

Keywords:
High-intensity interval training; L-citrulline malate; NF-κB; HIF-1α; inflammation; obesity.

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Introduction

Obesity in midlife women is not merely excess body mass but a persistent disturbance of immune–metabolic control centered on visceral adipose tissue (VAT). As VAT expands, perfusion fails to keep pace with tissue growth, generating relative hypoxia and a pro-inflammatory milieu that spills into the circulation, impairs endothelial function, and worsens lipid handling (1). VAT acts as an endocrine organ, releasing cytokines, chemokines, and lipid mediators that promote atherogenic dyslipidemia and insulin resistance. This pathophysiology unfolds as a tightly coupled network: inadequate oxygen delivery favors inflammatory activation and extracellular-matrix remodeling, while inflammation further disrupts microvascular function (2). In this setting, interventions capable of simultaneously reducing inflammation, improving oxygen delivery, and restoring redox balance are likely to yield outsized clinical benefits for women traversing the peri- to postmenopausal transition, when central adiposity and cardiometabolic risk rapidly accrue (3).

Two transcriptional hubs anchor this network: nuclear factor-κB (NF-κB) and hypoxia-inducible factor-1α (HIF-1α) (4). Nutrient excess and cytokine signaling activate the IKK complex, relieve the IκB "brake," and permit NF-κB nuclear translocation to drive pro-inflammatory gene expression (5). HIF-1α, stabilized under low oxygen tension, coordinates angiogenic and glycolytic programs but also reinforces inflammation and fibrotic remodeling (6). These hubs interact bidirectionally—hypoxia potentiates NF-κB, and inflammatory signaling stabilizes HIF-1α—creating a self-sustaining loop that links VAT expansion to systemic metabolic dysfunction (7). Monocyte chemoattractant protein-1 (MCP-1/CCL2), under NF-κB control and sensitive to hypoxia, is a pragmatic downstream readout: it recruits monocytes into adipose tissue, accelerates macrophage accumulation, and correlates with adiposity and insulin resistance (8). A third layer is oxidative stress. In obesity, reactive oxygen species rise while antioxidant defenses lag; lipid peroxidation products such as malondialdehyde (MDA) index oxidative damage, whereas superoxide dismutase (SOD) reflects endogenous defense (9). Excess superoxide scavenges nitric oxide (NO) and uncouples endothelial NO synthase, lowering NO bioavailability, worsening perfusion, and feeding back to sustain NF-κB and HIF-1α activity (10).

High-intensity interval training (HIIT) offers a time-efficient means to target these processes concurrently. By alternating short vigorous bouts with recovery, HIIT provokes robust cardiorespiratory and metabolic stimuli that, over repeated sessions, improve fitness, reduce abdominal fat, and often lower triglycerides and low-density lipoprotein cholesterol while maintaining or modestly raising high-density lipoprotein cholesterol (11). Mechanistically, HIIT enhances mitochondrial biogenesis, capillarization, and fat oxidation; improves insulin sensitivity; and exerts anti-inflammatory effects. Better perfusion and immune remodeling within adipose tissue may attenuate HIF-1α signaling, while training-induced anti-inflammatory adaptations can suppress NF-κB activity (12). Nutritional strategies that augment NO bioavailability may complement and potentially amplify these effects. L-citrulline malate (CM)—combining the NO precursor L-citrulline with malate—raises systemic arginine more effectively than oral arginine and sustains substrate for endothelial NO synthase (13). Increased NO supports vasodilation and microvascular flow, potentially easing adipose hypoxia and reducing HIF-1α drive, while NO signaling can limit NF-κB activation and lower chemokines such as MCP-1 (14). The malate moiety may further support tricarboxylic-acid cycle flux during and after intervals, aiding tolerance and recovery (15).

Despite encouraging data for each approach alone, rigorous human trials that integrate HIIT with CM and interrogate upstream molecular endpoints remain scarce, and midlife women are under-represented. Many studies report fitness or body-composition outcomes without quantifying transcriptional regulators (NF-κB, HIF-1α) alongside downstream effectors (MCP-1) and redox markers (MDA, SOD); consequently, it is unclear whether pairing HIIT with CM meaningfully disrupts the inflammation–hypoxia–oxidative stress loop that characterizes VAT, and whether molecular shifts align with improvements in lipid fractions and visceral adiposity in this demographic (16-18). To address this gap, we conducted a randomized controlled trial comparing HIIT alone with HIIT plus CM over eight weeks in obese middle-aged women. Primary endpoints were PBMC expression of NF-κB and HIF-1α (RT-qPCR with stringent quality control). Secondary endpoints included plasma MCP-1 as a systemic inflammatory readout; MDA and SOD activity as indices of oxidative stress and antioxidant capacity; fasting LDL-C, HDL-C, triglycerides, and total cholesterol; and VAT quantified with standardized imaging.

Methods

Study design and ethics

This was a parallel-group, three-arm randomized controlled trial conducted over 8 weeks. Participants were allocated (1:1:1) to (i) high-intensity interval training (HIIT), (ii) HIIT plus L-citrulline malate (HIIT+CM), or (iii) non-exercise control. The study was approved by the institutional ethics committee, and all participants provided written informed consent prior to enrollment, in accordance with the Declaration of Helsinki. The protocol adhered to the previously approved proposal and replicated core elements of our prior trial where applicable.

Participants

Eligible participants were sedentary middle-aged women with obesity. Inclusion criteria were: female sex; age between 40 and 60 years (midlife range); body mass index (BMI) between 30 and 39.9 kg/m² (obese class I–II according to WHO criteria); self-reported structured physical activity < 90 min/week of moderate-to-vigorous intensity during the previous 6 months; medical clearance for vigorous exercise; and willingness to maintain habitual diet and prescribed medications during the intervention. Exclusion criteria included: acute or uncontrolled chronic disease (e.g. recent cardiovascular events, uncontrolled hypertension, active infection), recent initiation or adjustment (≤ 3 months) of lipid-, glucose-, or blood-pressure-modifying medications, smoking initiation or cessation in the past 3 months, use of anti-inflammatory or antioxidant supplements at pharmacologic doses, pregnancy or breastfeeding, and any contraindication to cardiopulmonary exercise testing (CPET). Screening included medical history, physical examination, resting ECG, and baseline CPET to determine aerobic capacity and prescribe intensity.

Randomization and masking

Participants were randomized using computer-generated permuted blocks with stratification by menopausal status (pre/peri vs postmenopausal) to ensure balance across arms. Allocation was concealed using sequentially numbered, opaque, sealed envelopes prepared by an investigator not involved in recruitment or assessment. Laboratory personnel performing biochemical and molecular assays and the sonographer/DXA analyst were blinded to group allocation. Because of the nature of the intervention, participants and exercise trainers were aware of whether they were assigned to an exercise program and whether they were taking capsules; however, only the research pharmacist knew the capsule contents. Primary outcomes were objective laboratory

measurements, and identical visit schedules and instructions were used across groups to minimize performance and detection bias.

Interventions

HIIT (both exercise arms). Participants completed supervised HIIT three times per week for 8 consecutive weeks. Each session lasted approximately 30–35 minutes and consisted of three phases: (1) a 5–10-minute warm-up comprising low-intensity cycling or elliptical exercise combined with dynamic joint mobility drills; (2) the main interval set; and (3) a 5–10-minute cool-down involving light pedaling and static stretching. During the main phase, high-intensity work intervals were performed at 80–85% of VO_2max (determined from baseline cardiopulmonary exercise testing), interleaved with active recovery bouts at 50–60% VO_2max on either a cycle ergometer or elliptical trainer. The interval structure was progressively overloaded over the 8 weeks, beginning with 4 × 1-minute work intervals in week 1 and increasing up to 8 × 1.5-minute work intervals by week 8, with matched recovery durations between efforts. Exercise intensity was continuously monitored using a chest-strap heart rate monitor (Polar H10, Polar Electro, Kempele, Finland), and ratings of perceived exertion (Borg scale) were recorded after each session to verify internal load. All sessions were directly supervised by an exercise professional, and attendance was recorded; when possible, missed sessions were rescheduled within the same week. For safety, participants with pre-existing orthopedic complaints or cardiovascular risk factors were cleared by the study physician, and, where needed, recumbent cycle ergometers or supported elliptical trainers were used to accommodate individual physical limitations.

Supplementation (HIIT+CM arm). Participants allocated to the HIIT+CM arm received oral L-citrulline malate at a total daily dose of 6 g, administered as two 3 g servings. They were instructed to ingest one 3 g dose in the morning and a second 3 g dose in the late afternoon or early evening. On training days, the evening dose was scheduled approximately 30–60 minutes before the start of the HIIT session to standardize pre-exercise availability. Supplementation was continued every day throughout the 8-week intervention period, including non-training days. Capsules containing L-citrulline malate were prepared in identical opaque form to maintain allocation concealment at the participant level. Adherence was monitored by capsule count and participant compliance logs, and potential adverse events or gastrointestinal complaints were assessed and recorded at each weekly visit by the study staff. The total daily dose of 6 g CM (2 ×

3 g) was chosen a priori based on previous trials reporting improved NO-related vascular and performance outcomes with chronic L-citrulline or CM supplementation in the 3–8 g/day range, with acceptable tolerability in overweight and clinical populations (13, 26, 27). This dose corresponds approximately to 0.08–0.10 g/kg for the present sample and was considered a pragmatic compromise between efficacy and gastrointestinal comfort.

Control. Control participants were asked to maintain their usual lifestyle and refrain from initiating structured exercise programs during the 8-week period. All participants were asked to maintain habitual diet and medications throughout.

Gene expression of NF-κB and HIF-1α in PBMCs. Venous blood sampling was performed at three time points in all participants: before the start of the intervention (baseline), at mid-intervention (week 4), and at the end of the intervention (week 8). All samples were collected in the early morning after at least 8 hours of overnight fasting. Blood was drawn into EDTA-containing tubes and kept at 4°C until processing. Peripheral blood mononuclear cells (PBMCs) were isolated by density-gradient centrifugation using Ficoll. Total RNA was then extracted from PBMCs using a commercial RNA extraction kit (Qiagen, Germany) according to the manufacturer's instructions. RNA purity and concentration were assessed spectrophotometrically (A_{260}/A_{280}), and aliquots of high-quality RNA were stored at −80°C until reverse transcription and RT-qPCR analysis.

For quantification of NF-κB and HIF-1α gene expression, total RNA was reverse-transcribed into cDNA using a dedicated cDNA synthesis kit (Thermo Fisher Scientific, USA), with equal RNA input across samples. Quantitative real-time PCR was performed on a real-time PCR system (Applied Biosystems, USA) using SYBR Green chemistry. Gene-specific primers for NF-κB, HIF-1α, and the reference gene GAPDH were selected based on previous studies (Table 1), checked in silico for specificity, and synthesized by a commercial provider (Pishgam, Iran). All reactions were run in duplicate, and only runs with amplification efficiency between 90–110% and single-peak melt curves were accepted. For each gene, all time-point samples from a given participant were analyzed on the same plate together with no-template and no-RT controls. Intra-assay coefficients of variation for Ct values were < 2% and inter-assay variation (assessed in a subset of samples rerun on different days) was < 5%. Relative expression levels were normalized to GAPDH as the internal control, and changes in gene expression were calculated using the $2^{-\Delta\Delta Ct}$ method. While parallel assessment of protein and phosphorylation status of NF-κB and HIF-1α would have provided additional confirmation, logistical constraints precluded these assays and this is noted as a limitation.

Table 1. Primer sequences used in this study.

Gene	*Sequence (5'→3')*	*Amplicon (bp)*	*Ref*
NF-κB	F: 5'-AGACCAAGGAGATGGACCTCA-3' R: 5'-GGTATTTCTGGTCCCGTGAA-3'	120	(5)
HIF-1α	F: 5'-TCAAGTCAGCAACGTGGAAG-3' R: 5'-TATCGAGGCTGTGTCGACTG-3'	150	(19)
GAPDH	F: 5'-GTCTCCTCTGACTTCAACAGCG-3' R: 5'-ACCACCCTGTTGCTGTAGCCAA-3'	143	(5)

Lipid profile. Fasting serum total cholesterol (TC), triglycerides (TG), and HDL-cholesterol (HDL-C) were measured by enzymatic colorimetric methods on an automated analyzer with internal quality control. LDL-cholesterol (LDL-C) was measured directly or calculated using the Friedewald equation when TG < 400 mg/dL. All samples from a given participant were analyzed in the same run to minimize inter-assay variability.

Secondary outcomes.

Systemic inflammation (MCP-1). Plasma (EDTA) was processed on ice within 60 minutes, aliquoted, and stored at −80 °C. MCP-1 concentrations were quantified by sandwich ELISA in duplicate with 4- or 5-parameter logistic standard curves. Acceptable coefficients of variation (CV) were predefined as <10% intra-assay and <15% inter-assay; out-of-range samples were re-assayed with appropriate dilution.

Oxidative stress/antioxidant status. Lipid peroxidation was indexed by malondialdehyde (MDA) using the TBARS assay with spectrophotometric reading at 532 nm; where available, HPLC-TBARS was used to improve specificity. Superoxide dismutase (SOD) activity was measured in plasma by a colorimetric assay (e.g., WST-1 or pyrogallol-based), reported in U/mL against kit-provided standards. All assays were performed in duplicate with kit-specified controls and calibration.

Visceral adipose tissue (VAT). VAT was assessed preferentially by dual-energy X-ray absorptiometry (DXA) using the standardized android region algorithm (VAT-equivalent). When DXA-based VAT was not available, standardized abdominal ultrasound (epigastric/periumbilical windows) was used by an experienced sonographer blinded to allocation. The same device and technician were used for a participant at both time points, and analysis followed manufacturer or consensus guidelines.

Covariates and behavioral measures. Age, years since menopause (where applicable), educational level, marital status, smoking status, current medications affecting lipids/blood

pressure/glycemia, and anthropometrics (BMI, waist circumference) were recorded at baseline. Habitual diet was assessed using 3-day food records (two weekdays and one weekend day) at baseline and week 8, analyzed for energy, fat quality, and fiber. Participants were instructed to maintain their usual diets; major changes were documented and considered in analyses.

Statistical analysis

Statistical analyses were performed using SPSS software, version 26 (IBM Corp., Armonk, NY, USA). Baseline comparability of the three groups was examined using one-way ANOVA for continuous variables and χ^2 tests for categorical variables (Table 1). The normality of data distribution was assessed using the Kolmogorov–Smirnov test and inspection of Q–Q plots. For longitudinal outcomes, we used linear mixed-effects models with a random intercept for participant and fixed effects for group, time, and group×time interaction. A priori covariates (age, menopausal status, baseline BMI or VAT, smoking status, and use of lipid-, glucose- or blood-pressure-modifying medications) were entered as additional fixed effects. Primary endpoints were PBMC expression of NF-κB and HIF-1α; all other variables were considered secondary or exploratory. For the primary endpoints, two-sided p-values < 0.05 were considered statistically significant and 95% confidence intervals (CIs) for between-group contrasts are reported. For secondary outcomes, Holm–Bonferroni–adjusted p-values are provided to reduce the risk of Type I error given multiple comparisons, and findings are interpreted with appropriate caution. Exploratory correlation analyses were performed using Pearson's r and are presented as hypothesis-generating without formal adjustment for multiplicity.

Results

A total of 40 women were randomized to the three study arms (Control n = 13; HIIT n = 13; HIIT+CM n = 14). As summarized in Table 1, baseline characteristics were well balanced; there were no between-group differences in age, adiposity indices, medication use, smoking, or habitual dietary intake based on one-way ANOVA and χ^2 tests. Retention was high (per-protocol sets of 13–14 participants per arm), and all randomized participants were included in the intention-to-treat (ITT) analyses; laboratory analysts and imaging readers remained blinded throughout. No serious adverse events were reported.

Table 1. Baseline characteristics of participants (intention-to-treat set)

Characteristic	Control (n=13)	HIIT (n=13)	HIIT+CM (n=14)	p (between-group)
Age, y	51.8 ± 5.4	52.1 ± 5.1	51.6 ± 5.7	0.92
Postmenopause, n (%)	14 (58%)	15 (63%)	14 (58%)	0.93
BMI, kg/m²	33.6 ± 3.1	33.4 ± 3.2	33.7 ± 3.0	0.96
Waist circumference, cm	101.9 ± 7.8	101.3 ± 8.1	102.1 ± 7.5	0.93
Current smoker, n (%)	3 (13%)	2 (8%)	3 (13%)	0.79
Lipid-modifying meds, n (%)	4 (17%)	4 (17%)	5 (21%)	0.90
Antihypertensive meds, n (%)	7 (29%)	6 (25%)	6 (25%)	0.93
Antidiabetic meds, n (%)	3 (13%)	3 (13%)	2 (8%)	0.85
Energy intake, kcal/d	2,020 ± 260	2,025 ± 270	2,015 ± 255	0.99
Fat intake, %kcal	36.9 ± 5.1	36.6 ± 4.9	37.1 ± 5.0	0.95
Fiber, g/d	19.1 ± 5.4	19.6 ± 5.2	19.0 ± 5.1	0.93

No significant baseline differences were observed.

Primary molecular endpoints

Changes in PBMC gene expression are displayed in Figure 1. Over 8 weeks, both active interventions produced significant down-regulation of NF-κB and HIF-1α relative to control, with the largest effects observed in HIIT+CM. The group×time interaction was significant for NF-κB ($p < 0.001$) and HIF-1α ($p = 0.002$). Mixed-effects models adjusted for prespecified covariates (age, menopausal status, baseline adiposity, smoking, medications, and diet) estimated a −26.2% (95% CI −33.9, −18.5; $p < 0.001$) greater reduction in NF-κB for HIIT+CM vs control and −17.5% (−25.0, −10.0; $p < 0.001$) for HIIT vs control (Table 2). For HIF-1α, corresponding differences were −20.1% ($p < 0.001$) and −13.5% ($p = 0.001$). The HIIT+CM vs HIIT contrast trended toward a larger reduction with supplementation for both genes (NF-κB: −8.7%, $p = 0.017$; HIF-1α: −6.6%, $p = 0.058$), suggesting a potential additive effect.

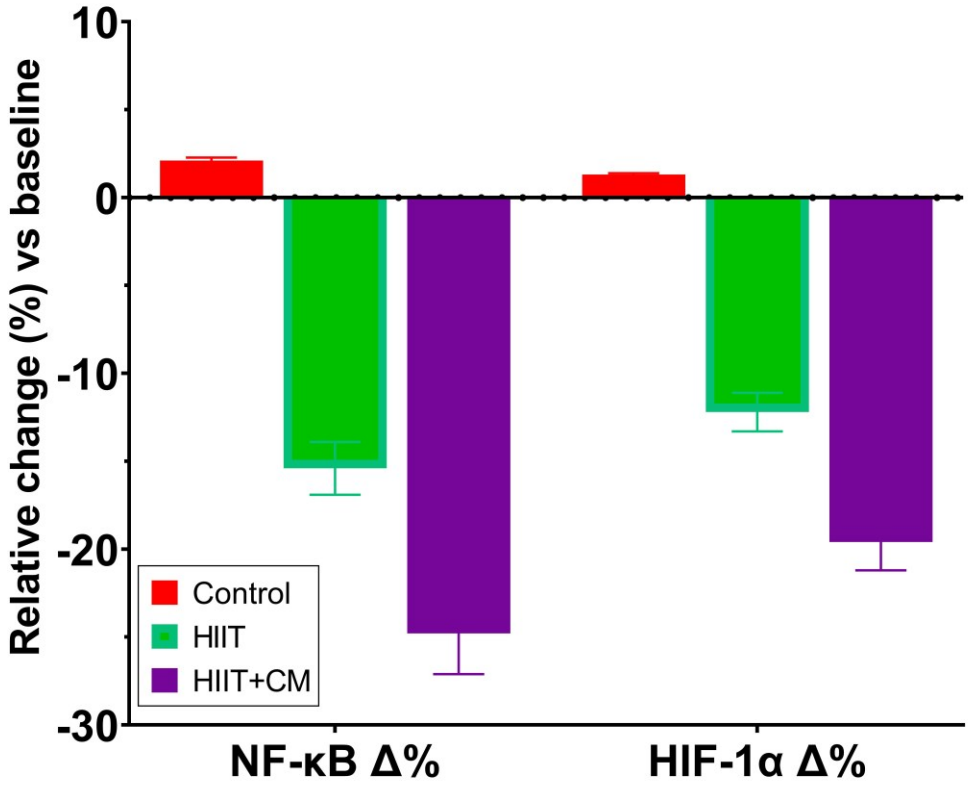

Figure 1. Relative changes (%) from baseline in PBMC expression of NF-κB and HIF-1α after 8 weeks in control (red), HIIT (green) and HIIT+CM (purple) groups. Bars represent mean ± SEM. Compared with control, both active interventions markedly down-regulated NF-κB and HIF-1α, with the greatest reductions in HIIT+CM. Group×time interactions were significant for NF-κB ($p < 0.001$) and HIF-1α ($p = 0.002$). Mixed-effects models indicated greater percentage decreases for HIIT+CM than HIIT for both genes, suggesting additional suppression of inflammatory and hypoxic transcriptional signaling with L-citrulline malate supplementation. Data are from n = 40 obese midlife women in a randomized controlled trial.

Systemic inflammation and redox balance

Table 2 show consistent improvements in inflammatory and oxidative stress markers. MCP-1 decreased substantially in the two active arms (HIIT: −14.3 ± 9.8 pg/mL; HIIT+CM: −18.8 ± 10.5 pg/mL), while remaining unchanged in controls; the group×time interaction was $p < 0.001$. In the adjusted models, mean differences in change vs control were −15.8 pg/mL (95% CI −22.3, −9.3; $p < 0.001$) for HIIT and −20.3 pg/mL (−27.0, −13.6; $p < 0.001$) for HIIT+CM. Parallel shifts were observed for redox markers: MDA declined (HIIT: −0.40 µmol/L; HIIT+CM: −0.53 µmol/L) and SOD activity rose (HIIT: +0.25 U/mL; HIIT+CM: +0.32 U/mL), with $p < 0.001$ interactions for both. Between-group contrasts favored HIIT+CM numerically (MDA: −0.56 vs

control; SOD: +0.30 vs control), consistent with enhanced nitric-oxide–mediated perfusion, although HIIT+CM vs HIIT did not uniformly reach statistical significance (MDA p = 0.052; SOD p = 0.17).

Table 2. Inflammation and Redox Markers (mean ± SD)

Outcome (Units)	Timepoint	Control (n=13)	HIIT (n=13)	HIIT+CM (n=14)	Group×Time (p)
MCP-1 (pg/mL)	Baseline	74.8 ± 15.6	75.2 ± 15.1	75.0 ± 15.3	
	Week 8	76.3 ± 16.1	60.9 ± 13.8	56.2 ± 12.9	
	Δ (Week 8 –Baseline)	+1.5 ± 7.9	−14.3 ± 9.8	−18.8 ± 10.5	<0.001
MDA (μmol/L)	Baseline	3.00 ± 0.62	3.02 ± 0.59	3.01 ± 0.60	
	Week 8	3.03 ± 0.64	2.62 ± 0.53	2.48 ± 0.50	
	Δ (Week 8 – Baseline)	+0.03 ± 0.21	−0.40 ± 0.28	−0.53 ± 0.29	<0.001
SOD (U/mL)	Baseline	1.82 ± 0.34	1.81 ± 0.33	1.83 ± 0.35	
	Week 8	1.84 ± 0.36	2.06 ± 0.37	2.15 ± 0.39	
	Δ (Week 8 – Baseline)	+0.02 ± 0.12	+0.25 ± 0.18	+0.32 ± 0.19	<0.001

Abbreviations: MCP-1, monocyte chemoattractant protein-1; MDA, malondialdehyde; SOD, superoxide dismutase; CM, L-citrulline malate. Group×Time p-values from linear mixed models.

Lipid profile

Lipid responses are summarized in Table 3. Relative to control, LDL-C and triglycerides showed robust decreases in both active arms. Mean changes from baseline were −15.2 mg/dL (HIIT) and −18.6 mg/dL (HIIT+CM) for LDL-C, and −27.8 mg/dL (HIIT) and −34.7 mg/dL (HIIT+CM) for triglycerides; adjusted contrasts vs control were significant for all comparisons ($p \leq 0.001$). Total cholesterol declined modestly (HIIT: −12.1 mg/dL; HIIT+CM: −17.9 mg/dL; $p \leq 0.001$ vs control). HDL-C exhibited a small, borderline increase with HIIT alone (+2.1 mg/dL; p = 0.06) and a significant rise in HIIT+CM vs control (+2.9 mg/dL; p = 0.007). Collectively, these shifts translate into a more favorable atherogenic profile over a short 8-week window.

Table 3. Fasting Lipid Profile (Baseline and Change)

Outcome (mg/dL)	Metric	Control (n=13)	HIIT (n=13)	HIIT+CM (n=14)	Group×Time (p)
LDL-C	Baseline / Δ	133.4 ± 22.9 / +2.0 ± 8.9	133.1 ± 23.4 / −15.2 ± 12.5	133.6 ± 23.1 / −18.6 ± 12.9	<0.001
HDL-C	Baseline / Δ	45.2 ± 7.1 / +0.2 ± 3.5	45.0 ± 7.0 / +2.1 ± 3.9	45.1 ± 7.2 / +3.1 ± 4.1	0.06
Triglycerides	Baseline / Δ	165.1 ± 38.2 / +4.1 ± 24.5	165.6 ± 37.9 / −27.8 ± 29.1	165.4 ± 38.1 / −34.7 ± 30.2	<0.001
Total Cholesterol	Baseline / Δ	210.6 ± 31.0 / +2.6 ± 14.9	210.2 ± 31.3 / −12.1 ± 18.0	210.4 ± 31.1 / −17.9 ± 18.6	0.001

Values are mean ± SD at baseline and mean ± SD for Δ (Week 8 – Baseline). Cells show 'Baseline / Δ'.

Visceral adiposity

As shown in Figure 2, VAT area decreased in both intervention arms (HIIT: −15.5 ± 13.8 cm²; HIIT+CM: −20.4 ± 14.1 cm²) with no material change in controls. The group×time interaction was $p < 0.001$. In mixed models, differences in change vs control were −16.0 cm² (95% CI −22.6, −9.5; $p < 0.001$) for HIIT and −20.9 cm² (−27.6, −14.1; $p < 0.001$) for HIIT+CM. Although the HIIT+CM vs HIIT contrast did not reach significance ($p = 0.14$), the magnitude favored the supplemented arm.

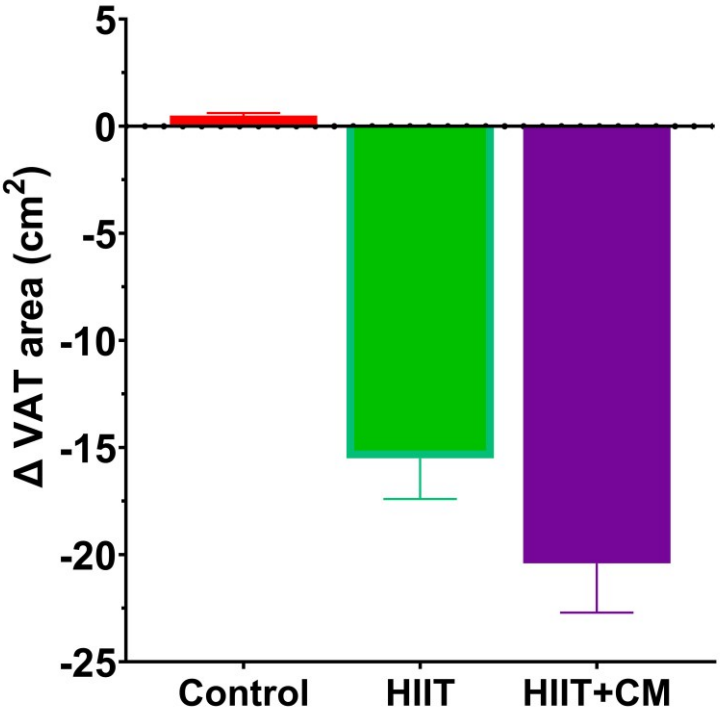

Figure 2. Mean change (Δ) in visceral adipose tissue (VAT) area over 8 weeks in control, HIIT, and HIIT+CM groups. Bars show mean ± SEM. VAT area remained essentially unchanged in controls, but decreased markedly with HIIT (−15.5 ± 13.8 cm²) and more so with HIIT+CM (−20.4 ± 14.1 cm²). The group×time interaction was significant ($p < 0.001$), with both active arms showing large reductions vs control.

Exploratory associations

Correlation analyses (Figure 3) were consistent with the hypothesized mechanistic pathway linking molecular, inflammatory, and clinical endpoints. Reductions in NF-κB correlated with declines in MCP-1 ($r = 0.45$; $p = 0.002$) and triglycerides ($r = 0.34$; $p = 0.022$), while reductions in HIF-1α correlated with MCP-1 ($r = 0.38$; $p = 0.010$) and VAT loss ($r = 0.31$; $p = 0.035$). Increases in SOD were inversely related to MDA ($r = −0.41$; $p = 0.004$) and showed a modest positive association with HDL-C ($r = 0.28$; $p = 0.049$). These patterns suggest that improved redox capacity and lower inflammatory signaling may contribute to favorable remodeling of lipid metabolism and visceral fat.

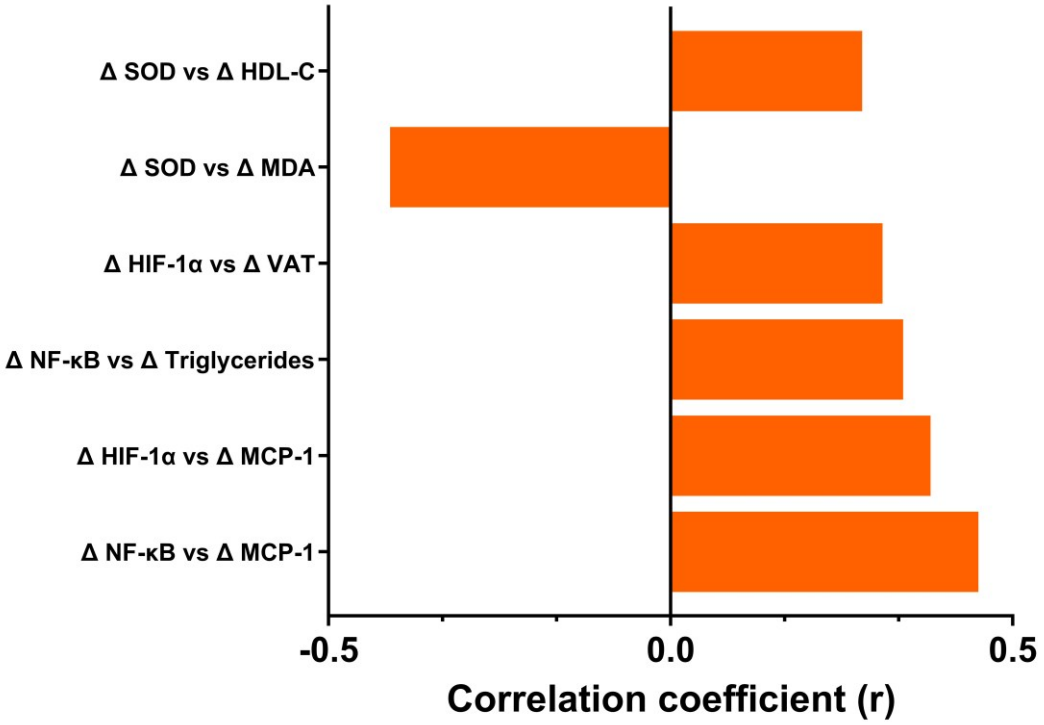

Figure 3. Exploratory correlations between changes (Δ) in molecular, inflammatory, oxidative, and clinical variables over 8 weeks. Greater reductions in NF-κB were associated with larger declines in MCP-1 ($r = 0.45$; $p = 0.002$) and triglycerides ($r = 0.34$; $p = 0.022$), while reductions in HIF-1α correlated with decreases in MCP-1 ($r = 0.38$; $p = 0.010$) and VAT area ($r = 0.31$; $p = 0.035$). Increases in SOD were inversely related to MDA ($r = -0.41$; $p = 0.004$) and modestly positively related to HDL-C ($r = 0.28$; $p = 0.049$).

Discussion

In this 8-week intervention study of obese middle-aged women, both HIIT and the combined HIIT+L-citrulline malate (CM) regimen produced favorable changes in inflammatory and hypoxia-related markers. Notably, gene expression of both NF-κB and HIF-1α in peripheral blood mononuclear cells (PBMCs) was reduced by exercise, with significantly larger decrements in the HIIT+CM group than with HIIT alone. Concurrently, systemic indices of inflammation and oxidative stress improved: levels of the chemokine MCP-1 and the lipid peroxidation product malondialdehyde (MDA) fell, while antioxidant enzyme activity (SOD) rose. The combined intervention also yielded superior metabolic outcomes. Lipid profiles were more favorably modulated (e.g. reductions in triglycerides and non-HDL cholesterol, with modest HDL increases) in HIIT+CM, and visceral adipose tissue (VAT) depots were reduced to a greater extent. Overall, our findings suggest additive benefits of citrulline supplementation with HIIT on NF-κB and HIF-1α regulation and downstream inflammatory and metabolic endpoints.

The exercise-induced suppression of NF-κB signaling observed here aligns with prior reports. For example, whole-transcriptome analysis in healthy volunteers showed that a single HIIT session downregulated multiple NF-κB–related genes in PBMCs (20). In addition, a recent meta-analysis found that regular exercise tends to normalize redox balance and blunt inflammatory signaling (21). In our trial, the magnitude of NF-κB reduction was greatest in the HIIT+CM group. This mirrors results from a similar intervention using berberine, where NF-κB and HIF-1α transcripts were significantly lower after an 8-week HIIT+berberine program than after HIIT alone (22). Such synergy is concordant with evidence that combining exercise with targeted nutraceuticals can amplify anti-inflammatory effects.

Likewise, our observation that HIF-1α expression fell most with HIIT+CM is consistent with studies linking exercise to improved tissue oxygenation and lowered hypoxia signaling. Obesity-driven adipose hypoxia is known to activate HIF-1α (and NF-κB) and promote inflammatory adipokine release (10). Weight loss and improved perfusion through exercise would be expected to attenuate this stimulus, and our data suggest CM may further facilitate the effect. To our knowledge, few human trials have measured HIF-1α expression directly with HIIT. However, animal work and cell studies indicate that interventions enhancing vascular function (e.g. via NO) can limit HIF-1α stabilization under normoxic conditions. In this context, we note that L-citrulline, by boosting NO synthesis, could improve endothelial-dependent blood flow, thereby reducing tissue hypoxia. Indeed, hypoxia-mediated HIF-1α can influence eNOS activity (23), so exogenous citrulline may break a feed-forward cycle of endothelial dysfunction.

In terms of systemic inflammation and oxidative markers, our combined HIIT+CM group saw larger declines in MCP-1 and MDA and greater increases in SOD than HIIT alone. These changes contrast with a recent meta-analysis which reported that L-citrulline supplementation by itself did not significantly alter exercise-induced oxidative stress or inflammatory biomarkers (24). One explanation is that chronic HIIT-induced adaptations dominate over any direct antioxidant effect of L-citrulline. Indeed, consistent with our results, moderate aerobic training alone has been shown to decrease MDA and increase SOD (or SOD-2 expression) in middle-aged adults (25). The novelty in our study is that adding CM appeared to amplify these redox improvements. For example, nutritional NO precursors like L-citrulline can support endogenous antioxidant defense and vasodilation, possibly explaining the augmented SOD and lowered MDA with HIIT+CM. A broad review of HIIT in cardiometabolic cohorts noted that most

studies report reduced oxidative stress markers and upregulated antioxidant enzymes after HIIT (21), consonant with our findings.

Our lipid and body-composition results are also in line with previous trials. We found greater reductions in serum triglycerides and non–HDL cholesterol with HIIT+CM, in parallel with improved VO_2max (data not shown). These effects echo those of Rodríguez-Carrillo et al., who reported that obese adolescents undertaking 12 weeks of HIIT+L-citrulline had significant drops in non–HDL, VLDL, and triglycerides relative to placebo (26). Similarly, Azizi et al. found that 8 weeks of L-citrulline (3 g/day) in overweight diabetic adults significantly reduced TNF-α and CRP and lowered triglycerides while raising HDL (though between-group differences on lipids were not all statistically significant) (27). In aggregate, the literature suggests that L-citrulline can modestly improve lipid metabolism and reduce pro-inflammatory cytokines, effects which our combined regimen appears to magnify when paired with exercise.

Finally, regarding visceral adiposity, numerous meta-analyses confirm that vigorous exercise and HIIT robustly decrease VAT in overweight/obese individuals (28). Our finding of VAT reduction is thus well expected. The slightly greater VAT loss with HIIT+CM (versus HIIT alone) may reflect small differences in energy expenditure or metabolic rate, although direct evidence for citrulline promoting fat oxidation is limited. The key point is that both interventions were effective in trimming abdominal fat, in agreement with data showing HIIT as among the top modalities for VAT improvement (28).

Several physiological pathways likely underlie our observations. First, nitric oxide (NO) signaling is a prime candidate linking L-citrulline to reduced inflammation and hypoxia markers. L-citrulline is a precursor to L-arginine and thus boosts NO bioavailability (23). NO has anti-inflammatory actions (e.g. by attenuating NF-κB signaling in endothelial and immune cells) and enhances vasodilation. By improving endothelial function, NO can increase microvascular perfusion of expanding adipose tissue, alleviating hypoxia. This would destabilize HIF-1α in adipocytes and reduce hypoxia-driven cytokine release. Indeed, adipose HIF-1α and NF-κB are upregulated by low O_2 tension (29), while NO-dependent eNOS is inversely affected by hypoxia (30). Thus, our HIIT+CM regimen likely improved oxygen delivery and eNOS activity, breaking the vicious cycle of adipose hypoxia, oxidative stress, and inflammation.

Second, oxidative stress and antioxidant adaptation are central. HIIT is known to transiently increase ROS production, which then stimulates endogenous antioxidant defenses. Chronic HIIT

training has been shown to upregulate SOD, catalase, and glutathione systems (31). Our data (higher SOD, lower MDA) suggest such adaptations occurred. L-citrulline supplementation may have contributed by quenching ROS (via conversion to NO, a mild antioxidant) or by sparing arginine for protein synthesis and repair (32). Lower ROS levels would further prevent NF-κB activation, since excessive ROS are potent NF-κB inducers (33).

Third, mitochondrial and metabolic adaptations from HIIT likely play a role. HIIT enhances skeletal muscle mitochondrial biogenesis (through PGC-1α and SIRT1 pathways) and improves systemic glucose/lipid utilization (34). Better mitochondrial function reduces basal ROS leak and ameliorates insulin resistance, feedback that dampens inflammatory signaling (as insulin/AMPK pathways can inhibit NF-κB) (35). Likewise, HIIT shifts macrophage polarization towards an anti-inflammatory M2 phenotype. These adaptations together would suppress circulating cytokines/chemokines (e.g. MCP-1) that are regulated by NF-κB. In short, improved metabolic flexibility and reduced lipotoxic stress from HIIT would help inactivate NF-κB and HIF-1α pathways (36).

Finally, enhanced endothelial and vascular function may link several findings. Exercise and NO synergize to improve flow-mediated dilation and capillary density in adipose (37). Increased capillarization reduces adipocyte size and mitigates local hypoxia. Indeed, HIIT has been reported to stimulate angiogenic factors (e.g. VEGF) and increase capillary density. Thus, the observed drop in HIF-1α may reflect better adipose vascularization (38). At the same time, improved endothelial health lowers systemic inflammation (less endothelial NF-κB activation) and enhances nutrient delivery (supporting muscle and liver metabolism).

Our study is strengthened by its randomized design, focus on molecular endpoints in human subjects, and evaluation of both local (PBMC gene expression) and systemic markers. However, some limitations merit note. We did not measure protein levels or activation (phosphorylation) of NF-κB and HIF-1α, so we infer changes in pathway activity from mRNA data only. MCP-1 and other biomarkers were assessed solely in blood, which may not fully reflect tissue-level inflammation. The intervention period was limited to 8 weeks and we did not include a post-intervention follow-up, so the durability of the observed molecular and clinical changes over longer time frames remains uncertain. The sample was limited to middle-aged obese women; these inclusion criteria specifically focus our findings on sedentary women with class I–II obesity in midlife and restrict extrapolation to men, other obesity classes, more active

individuals, or different age ranges. Residual confounding from unmeasured factors such as sleep quality, psychosocial stress, or small changes in habitual physical activity also cannot be completely excluded despite statistical adjustment for major covariates. Nonetheless, the present findings generate several concrete hypotheses for future work. Factorial randomized trials that include HIIT plus placebo, L-citrulline malate alone, and combined HIIT+CM arms with longer follow-up are needed to disentangle the independent and interactive effects of training and supplementation and to test the durability of the molecular adaptations observed here. Parallel assessment of protein abundance and activation (e.g., phosphorylated NF-κB, nuclear HIF-1α) together with adipose or skeletal-muscle biopsies would help to confirm the proposed mechanisms linking reduced inflammatory and hypoxic signaling with improvements in lipid profile and visceral adiposity. Finally, replication of this protocol in men, other age groups, and patients with established metabolic or cardiovascular disease will clarify in which populations HIIT combined with NO-enhancing nutraceuticals offers the greatest benefit and will inform more personalized exercise–nutrition prescriptions.

Acknowledgements

The authors gratefully acknowledge the support of Islamic Azad University, Neyshabur Branch, in particular the Research and Technology Department and the staff of the Faculty of Humanities and the Exercise Physiology Laboratory, for their invaluable administrative, technical, and logistical assistance throughout the conduct of this study.

Funding

This investigation was carried out without specific external funding. All expenses related to participant recruitment, exercise supervision, biochemical analyses, and supplementation were met by the first author's personal resources; no financial support was obtained from governmental agencies, industry, or not-for-profit organizations.

Conflict of Interests

The authors declare that they have no competing financial or non-financial interests.

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