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Effects of Curcumin and Ibuprofen on Redox and Inflammatory Responses to Exercise-Induced Muscle Damage in Competitive Female Rugby Players: A Randomized Controlled Trial

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Abstract

Background/Objectives: Exercise-induced muscle damage (EIMD) involves interconnected redox, inflammatory, and tissue-stress responses. Evidence on the effects of curcumin on these responses in trained female athletes, and on how these effects compare with those of non-steroidal anti-inflammatory drugs, remains limited. This study compared the effects of curcumin, ibuprofen, and placebo on biochemical responses to eccentric exercise in competitive female rugby players. **Methods:** In this single-center, triple-blind, randomized, placebo-controlled trial, 24 players were allocated to curcumin (CUR; $n = 8$; 760 mg curcumin plus 49.8 mg piperine/day), ibuprofen (IBP; $n = 8$; 400 mg/day), or placebo (PLA; $n = 8$) for 10 consecutive days before completing a standardized eccentric knee-extension protocol on the final day of supplementation. Malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), interleukin-6 (IL-6), aspartate and alanine aminotransferases (AST and ALT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) were assessed at baseline, immediately before exercise, and at 24, 48, and 72 h post-exercise. Data were analyzed using two-way mixed-design repeated-measures ANOVA followed by Bonferroni-corrected pairwise comparisons, as appropriate. **Results:** All participants completed the trial, with no adverse events reported. MDA showed a significant time × group interaction ($p = 0.005$, $\eta_p^2 = 0.22$), with Bonferroni-corrected comparisons showing significantly lower concentrations in CUR than in both IBP and PLA at 24 and 48 h post-exercise ($p < 0.05$), but not at 72 h. CAT, SOD, and IL-6 showed no significant interactions. AST showed a significant time × group interaction ($p = 0.008$, $\eta_p^2 = 0.21$), with significant increases from pre-exercise to 72 h and from 24 to 72 h within CUR ($p < 0.05$), but no significant between-group differences at individual time points. LDH showed a

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significant main effect of time ($p < 0.001$), with significant decreases from pre-exercise to 24 h in all groups and increases from 24 to 48 h in IBP and PLA ($p < 0.05$), without a significant time × group interaction, whereas ALT and ALP showed no significant effects. **Conclusions:** Curcumin was associated with a biomarker-specific biochemical response, primarily involving lipid peroxidation, rather than generalized changes across antioxidant, inflammatory, or tissue-stress biomarkers. Individual redox biomarkers should not be interpreted in isolation as evidence of enhanced overall recovery, and these findings do not establish therapeutic superiority or equivalence between curcumin and ibuprofen.

Keywords: curcumin; exercise-induced muscle damage; oxidative stress; lipid peroxidation; female athletes; sports medicine

1. Introduction

Exercise-induced muscle damage (EIMD) is a well-established physiological response to strenuous or unaccustomed exercise, particularly following high-intensity eccentric contractions, and is characterized by structural disruption of skeletal muscle and a transient impairment of muscle function [1,2]. Beyond these mechanical alterations, EIMD is accompanied by increased production of reactive oxygen species (ROS), which may disrupt redox homeostasis and promote oxidative damage when endogenous antioxidant defenses are insufficient [3]. Lipid peroxidation represents an important component of this response and can be assessed through circulating malondialdehyde (MDA), whereas antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) contribute to the endogenous defense against ROS. Oxidative and inflammatory responses are closely interconnected after muscle-damaging exercise, with cytokines such as interleukin-6 (IL-6) participating in the acute systemic response to exercise-induced tissue stress [3].

In addition to redox and inflammatory perturbations, strenuous eccentric exercise may induce transient changes in circulating enzymes associated with skeletal muscle and systemic tissue stress. Lactate dehydrogenase (LDH) is frequently assessed as a biochemical marker of exercise-induced tissue damage, reflecting alterations in cellular membrane integrity and the release of intracellular enzymes into the circulation [3,4]. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT), although traditionally regarded as indicators of hepatocellular injury in clinical practice, are also present in extrahepatic tissues, including skeletal muscle, and their circulating concentrations may increase following strenuous or muscle-damaging exercise [5]. Alkaline phosphatase (ALP), while considerably less specific to skeletal muscle, may provide complementary information within a broader biochemical assessment of the systemic response to exercise. Therefore, the simultaneous evaluation of oxidative stress, antioxidant defense, inflammation, and circulating enzymes associated with tissue stress may provide a more comprehensive characterization of the biological response to EIMD than the assessment of isolated biomarkers [4].

Given the multifactorial nature of EIMD, considerable attention has been directed toward pharmacological and nutritional strategies aimed at modulating the secondary responses to muscle-damaging exercise and facilitating post-exercise recovery. Among pharmacological approaches, non-steroidal anti-inflammatory drugs (NSAIDs), particularly ibuprofen, are frequently used by athletes because of their analgesic and anti-inflammatory properties, primarily mediated through inhibition of cyclooxygenase (COX) activity and subsequent prostaglandin synthesis [6,7]. However, the effects of NSAIDs on exercise-induced muscle damage and recovery remain inconsistent, while their repeated or inappropriate use may expose athletes to gastrointestinal, renal, and cardiovascular adverse effects [8,9]. Moreover, because inflammatory and redox signaling contribute not only to secondary

tissue responses but also to muscle repair and adaptation, indiscriminate suppression of these pathways may not necessarily translate into improved recovery or physiological adaptation [10]. These limitations have increased interest in nutritional compounds capable of modulating oxidative and inflammatory responses while potentially offering a more favorable safety profile for use in physically active populations.

Among nutritional strategies, curcumin, the principal bioactive polyphenol derived from *Curcuma longa* L., has attracted considerable interest because of its potential to modulate several biological processes involved in the response to muscle-damaging exercise [3,11]. Curcumin exhibits antioxidant and anti-inflammatory properties through multiple molecular pathways, including direct and indirect modulation of ROS, attenuation of lipid peroxidation, regulation of endogenous antioxidant defenses, and modulation of nuclear factor kappa B (NF-κB)-dependent signaling and pro-inflammatory mediators such as cyclooxygenase-2 (COX-2), 5-lipoxygenase (5-LOX), and inflammatory cytokines [3,11]. These pleiotropic actions provide a biological rationale for investigating curcumin as a nutritional strategy to modulate the oxidative and inflammatory responses associated with EIMD. Nevertheless, the clinical and physiological effects of orally administered curcumin may be limited by its relatively low systemic bioavailability, which is influenced by poor absorption and rapid metabolism and elimination. Co-administration with piperine has therefore been used to enhance curcumin bioavailability and may increase systemic exposure to curcuminoids [12].

Despite this biological rationale, the evidence supporting curcumin supplementation for exercise recovery remains heterogeneous. A recent systematic review of randomized, double-blind, placebo-controlled trials reported favorable effects in six of seven studies assessing oxidative stress, whereas findings for muscle damage and inflammation were less consistent; importantly, the certainty of evidence was rated as low for oxidative stress and very low for several other recovery-related outcomes [13]. Differences in participant training status, exercise protocols, curcumin dose and formulation, supplementation timing, biomarker selection, and assessment time points may contribute to this variability [13]. Moreover, evidence from highly trained athletes remains limited, and direct comparisons between curcumin and established pharmacological strategies such as ibuprofen are particularly scarce. This evidence gap is especially relevant in competitive female athletes, who remain underrepresented in exercise-recovery research despite being exposed to substantial training and competition demands. Accordingly, well-controlled trials comparing curcumin with both an active pharmacological comparator and placebo are needed to determine whether its proposed antioxidant and anti-inflammatory properties translate into measurable differences in the biochemical response to muscle-damaging exercise.

Therefore, the aim of this triple-blind, randomized, placebo-controlled trial was to compare the effects of curcumin, ibuprofen, and placebo on oxidative stress, antioxidant biomarkers, inflammatory response, and circulating enzymes associated with tissue stress following eccentric exercise in competitive female rugby players. We hypothesized that curcumin supplementation would attenuate post-exercise oxidative and inflammatory responses compared with placebo, whereas comparisons with ibuprofen were considered non-directional.

2. Materials and Methods

2.1. Participants

Twenty-four competitive female rugby players participated in this single-center randomized controlled trial. All participants had at least 2 years of rugby training and competitive experience and were actively competing in a regional rugby union league.

The study was conducted during the preseason period, between August and September 2026, before the beginning of the competitive season. During the competitive season, the

players' habitual weekly schedule comprised approximately 225 min of structured training, including one 85 min technical–tactical session, two 35 min endurance sessions, and two 35 min strength and conditioning sessions, in addition to one official match per week.

No experimental dietary regimen was imposed during the intervention. Players continued their usual dietary practices, which were routinely guided by the team nutritionist toward an approximate macronutrient distribution of 55% carbohydrate, 20% protein, and 25% fat [14].

Players were eligible for inclusion if they: (i) were women aged 18–40 years; (ii) had ≥2 years of rugby training and competitive experience; (iii) had participated in official competition during the preceding year; (iv) had medical clearance for participation and no chronic disease or pre-existing condition contraindicating participation; and (v) had not used any substance prohibited by the World Anti-Doping Agency during the previous 3 months. Of the 37 players assessed for eligibility, 13 were excluded, and 24 underwent randomization. Participant progression through enrollment, allocation, follow-up, and analysis is presented in the CONSORT flow diagram (Figure 1) [15].

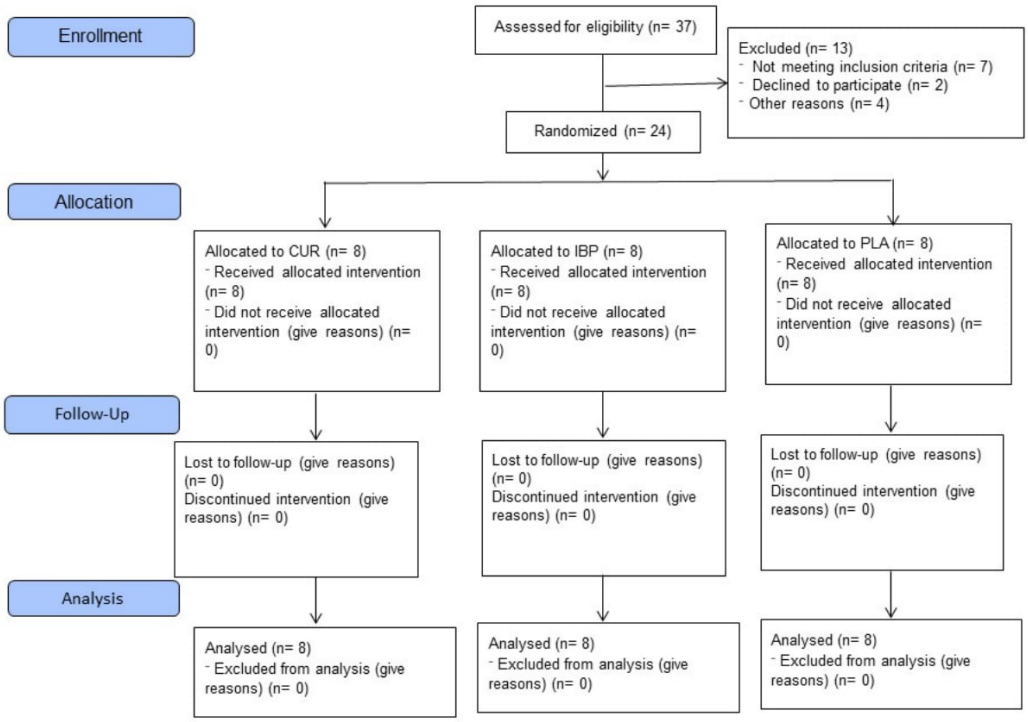


Figure 1. CONSORT flow diagram showing participant enrollment, allocation, follow-up, and analysis. Abbreviations: CUR, curcumin group; IBP, ibuprofen group; PLA, placebo group.

The study protocol was approved by the Ethics Committee of the University of León (ETICA-ULE-03-2025) and conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent before enrollment. The trial was registered with ClinicalTrials.gov (NCT07785986).

2.2. Experimental Design, Randomization, Blinding, and Pre-Experimental Procedures

The study treatments were administered in capsule form to reduce potential sensory cues related to treatment allocation, particularly those associated with the characteristic color and taste of curcumin. Capsules were visually similar and supplied in unlabeled metallic blister packs to further minimize treatment identification based on appearance or packaging. Participants were randomly assigned in a 1:1:1 ratio to receive curcumin (CUR),

ibuprofen (IBP), or placebo (PLA). An independent statistician generated the allocation sequence using Research Randomizer (version 4.0; Urbaniak & Plous). An independent research assistant who was not involved in outcome assessment or data analysis prepared and coded the study treatments and retained the concealed allocation code until completion of the statistical analyses. Participants, outcome assessors, and data analysts remained blinded to group allocation throughout the study.

Blinding effectiveness was assessed at T4 by asking participants to indicate which treatment group they believed they had been assigned to, with an additional "do not know" response option. Responses were classified as correct, incorrect, or "do not know" according to the actual treatment allocation, consistent with approaches used to assess blinding in randomized controlled trials [16].

The study is reported in accordance with the CONSORT 2025 statement [15]. An overview of the experimental protocol and timing of assessments is provided in Figure 2.

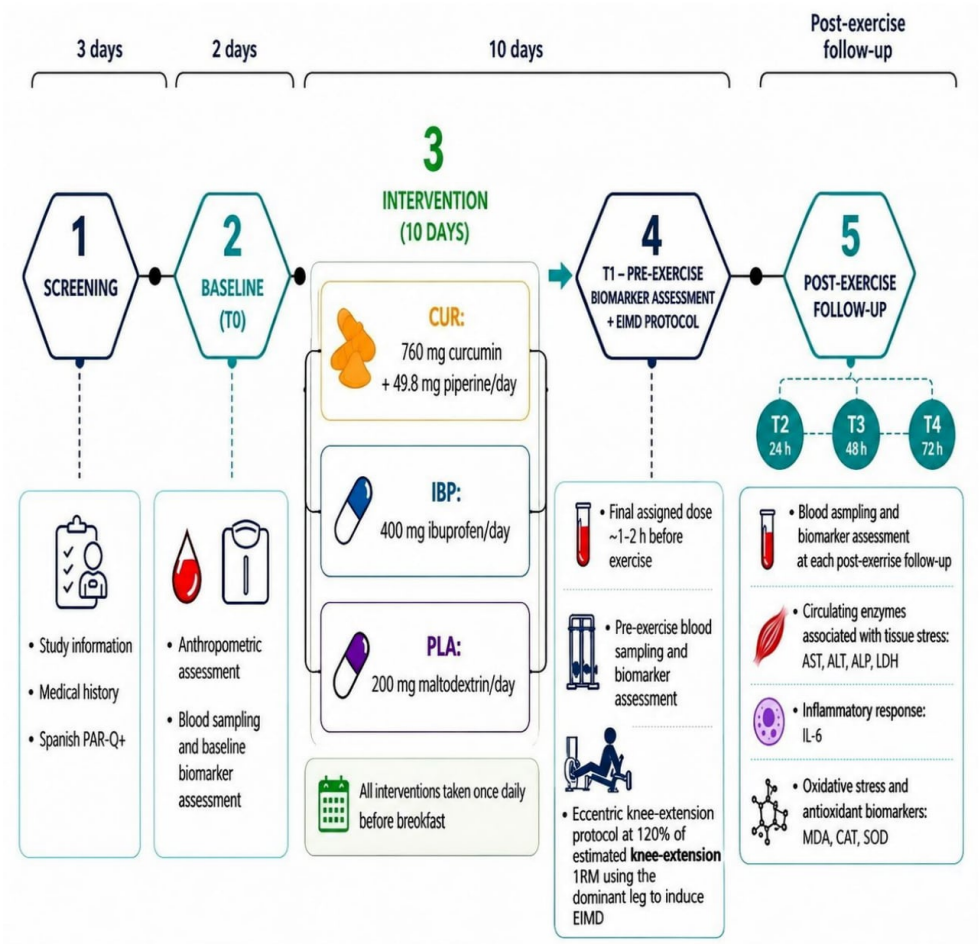


Figure 2. Experimental design and assessment timeline. The figure summarizes the sequence of screening, baseline assessment (T0), the 10-day intervention period, the exercise session with pre-exercise biomarker assessment (T1), and the post-exercise follow-up at 24 h (T2), 48 h (T3), and 72 h (T4). Detailed procedures, including supplementation timing, the eccentric knee-extension protocol, and blood sampling, are described in the Materials and Methods section. Abbreviations: 1RM, one-repetition maximum; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CAT, catalase; CUR, curcumin group; EIMD, exercise-induced muscle damage; IBP, ibuprofen group; IL-6, interleukin-6; LDH, lactate dehydrogenase; MDA, malondialdehyde; PAR-Q+, Physical Activity Readiness Questionnaire for Everyone; PLA, placebo group; SOD, superoxide dismutase.

Before baseline testing, participants attended an information and screening session during which the study procedures and potential risks and benefits were explained. Readiness to safely undertake the exercise protocol was assessed using the Spanish version of the Physical Activity Readiness Questionnaire for Everyone (PAR-Q+) [17]. Participants were instructed to avoid strenuous physical activity and medication use and to minimize exposure to stressful situations during the 72 h preceding baseline assessment. Three days after the screening session, participants attended the laboratory for baseline assessment (T0). During the intervention period, participants maintained their usual rugby training schedule, which was not standardized by the research team. No additional exercise restrictions were imposed during the intervention period beyond the study procedures. During the 72 h post-exercise follow-up period, participants were instructed to refrain from strenuous exercise and to avoid recovery interventions that could influence the response to muscle-damaging exercise, including cryotherapy, massage, intense stretching, and compression garments. The use of non-study NSAIDs was also prohibited during this period.

2.3. Supplementation Protocol

Participants received their assigned intervention once daily for 10 consecutive days. The CUR group received two capsules per day of a commercially available turmeric formulation (Vitalart Cúrcuma Complex; Natur Import, Barberà del Vallès, Barcelona, Spain). The daily dose contained 800 mg of *Curcuma longa* root dry extract standardized to 95% curcumin, providing 760 mg of curcumin, together with 52.4 mg of *Piper nigrum* dry extract standardized to 95% piperine, providing 49.8 mg of piperine. Participants assigned to the IBP group received two capsules per day providing a total daily dose of 400 mg of ibuprofen (Kern Pharma, Barcelona, Spain), whereas participants in the PLA group received two capsules per day containing a total of 200 mg of maltodextrin.

Piperine was co-administered to potentially enhance the oral bioavailability of curcumin, as previously described [12]; the daily piperine dose (49.8 mg) corresponded to the standardized composition of the commercially available formulation used in this study.

The ibuprofen regimen corresponded to a low total daily dose (400 mg/day). Consequently, the comparison with curcumin and placebo was limited to this specific ibuprofen regimen and was not designed to evaluate dose-dependent responses or the effects of higher therapeutic doses.

All interventions were taken before breakfast. On the day of the eccentric-exercise session (T1), the final assigned dose was administered approximately 1–2 h before the exercise protocol. Compliance with the assigned intervention and treatment tolerability were monitored through unannounced telephone contacts throughout the 10-day intervention period. At the end of the intervention, participants returned the blister packs, and any remaining capsules were counted to verify adherence. Participants were instructed to report any adverse events and to discontinue the assigned intervention if moderate or severe adverse effects occurred.

Throughout the 10-day intervention period, participants were instructed to refrain from using any additional medications, including non-study ibuprofen or other NSAIDs, and from consuming nutritional supplements other than the study treatments. Participants were also instructed to maintain their usual diet and habitual dietary patterns and to avoid making substantial changes to their food intake.

2.4. Baseline Assessment (T0)

At baseline (T0), body mass and height were measured using a digital scale (seca 877, seca gmbh & co. kg, Hamburg, Germany) and a stadiometer (seca 213, seca gmbh & co. kg, Hamburg, Germany), respectively, and body mass index (BMI) was calculated.

Information on menstrual-cycle timing was obtained by self-report, and study assessments were preferentially scheduled during the early follicular phase when feasible; menstrual-cycle phase was not confirmed using hormonal measurements. Venous blood sampling was subsequently performed, followed by estimation of the one-repetition maximum (1RM) for knee extension (Leg Extension/Curl L020B, BH Fitness, Vitoria-Gasteiz, Spain). The 1RM was estimated only at T0 and was used to individualize the load prescribed for the eccentric-exercise protocol. Two days after T0, participants began the 10-day supplementation period.

2.5. One-Repetition Maximum Estimation and Eccentric-Exercise Protocol

The 1RM for knee extension was estimated at T0 using the Brzycki method [18] and was used to individualize the load for the subsequent eccentric-exercise protocol. On the final day of the 10-day supplementation period, participants returned for the eccentric-exercise session (T1). Before exercise, blood sampling and biomarker assessment were repeated under the same standardized conditions as at T0.

The overall experimental sequence, including baseline assessment, the 10-day supplementation period, the eccentric-exercise session, and the post-exercise follow-up assessments at 24, 48, and 72 h, is summarized in Figure 2.

Participants first completed a standardized warm-up comprising 5 min of treadmill running at 6 km/h, lower-limb mobility exercises, five submaximal countermovement jumps, and a specific warm-up on the knee-extension machine. The eccentric-exercise protocol consisted of five sets of up to 15 repetitions performed through a full range of motion, with each eccentric repetition lasting 5 s and 1 min of recovery between sets, based on a previously described eccentric-exercise model [19]. If the prescribed load could no longer be maintained, it was reduced by 5 kg until fatigue or completion of 15 repetitions. EIMD was subsequently induced in the dominant leg by performing eccentric knee extensions at 120% of the estimated knee-extension 1RM.

2.6. Blood Sampling and Biochemical Analyses

Blood samples for the assessment of circulating enzymes associated with tissue stress, oxidative stress and antioxidant biomarkers, and inflammatory response were collected at T0, T1, T2, T3, and T4. All samples were obtained at 10:00 h following a 12 h overnight fast and 5 min of seated rest. Eight milliliters of venous blood were collected from an antecubital vein and centrifuged at 2500 rpm for 20 min at room temperature. Serum was subsequently divided into 250 µL aliquots and stored at −80 °C until biochemical analysis. All biomarker analyses were performed at Santa Bárbara Hospital, Soria, Spain, within the Castile and Leon Public Health Network, following the laboratory's established analytical quality-control procedures.

2.6.1. Biochemical Determination of Circulating Enzymes Associated with Tissue Stress

Serum AST, ALT, ALP, and LDH were determined using an automated clinical chemistry analyzer (Hitachi 917, Hitachi, Japan). AST, ALT, and LDH were measured using standard enzymatic methods, whereas ALP activity was determined using a kinetic colorimetric method with p-nitrophenyl phosphate (p-NPP) as substrate, with absorbance measured at 405 nm.

2.6.2. Inflammatory Response

Serum IL-6 concentrations were measured using a Human IL-6 ELISA Kit (Cat. No. KHC0061; Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA), with an analytical sensitivity of <2 pg/mL.

2.6.3. Oxidative Stress and Antioxidant Biomarkers

Serum MDA concentrations were estimated spectrophotometrically using the thiobarbituric acid reactive substances (TBARS) assay, following established methodology [20]. CAT and SOD were determined spectrophotometrically according to established assay principles [21]. SOD was assessed at a wavelength of 550 nm. CAT activity was expressed as U/L, whereas SOD concentrations were expressed as μg/mL.

2.7. Statistical Analysis

Sample size was determined a priori using G*Power version 3.1 [22]. MDA was selected as the reference biomarker for sample-size planning, based on previous randomized placebo-controlled research reporting a large between-group difference in exercise-induced MDA-TBARS concentrations following curcumin supplementation (effect size = 1.00) [23]. The calculation assumed a two-sided type I error (α) of 0.05 and a type II error (β) of 0.20, corresponding to a statistical power of 80%, and indicated a minimum total sample size of 23 participants across the three study groups. Recruitment planning additionally accounted for an anticipated non-participation rate of 20%. The final sample comprised 24 participants, with eight participants allocated to each group.

Statistical analyses were conducted using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation (SD), and normality was assessed using the Kolmogorov–Smirnov test, with no significant departures from normality detected. A two-way mixed-design repeated-measures analysis of variance (ANOVA), with time (T0–T4) as the within-subject factor and group (CUR, IBP, and PLA) as the between-subject factor, was used for each biochemical outcome. Main effects of time and group, as well as the time × group interaction, were examined. Effect sizes were expressed as partial eta squared (η_p^2). When significant main effects or time × group interactions were identified, follow-up pairwise comparisons were performed, as appropriate, to examine differences between assessment time points and between treatment groups. These comparisons included simple-effects analyses within individual treatment groups and between groups at specific assessment time points, with Bonferroni adjustment for multiple comparisons. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Participant Flow and Baseline Characteristics

Of the 37 competitive female rugby players screened for eligibility, 24 met the eligibility criteria and were randomized. Participants were equally allocated to the CUR, IBP, and PLA groups ($n = 8$ per group), and all completed the study protocol and were included in the final analysis (Figure 1). Baseline demographic and anthropometric characteristics of the participants are summarized in Table 1. Descriptively, the three groups showed similar mean body weight, height, and body mass index, although some variation in mean age was observed across groups (Table 1).

Table 1. Baseline demographic and anthropometric characteristics of participants.

Characteristics	CUR ($n = 8$)	IBP ($n = 8$)	PLA ($n = 8$)
Age (years)	22.7 ± 4.2	25.6 ± 3.7	21.0 ± 3.8
Weight (kg)	55.8 ± 5.4	56.0 ± 6.0	57.7 ± 7.1
Height (cm)	161.0 ± 3.9	162.3 ± 4.8	161.5 ± 4.1
BMI (kg/m^2)	21.5 ± 2.2	21.3 ± 2.4	22.1 ± 1.7

Data are presented as mean ± SD. Abbreviations: BMI, body mass index; CUR, curcumin group; IBP, ibuprofen group; PLA, placebo group; SD, standard deviation.

At T4, 21 of the 24 participants reported that they did not know their treatment allocation. Of the three participants who believed they knew their allocation, two correctly identified their assigned group and one did not.

No adverse events were reported during the 10-day intervention period. Adherence to the assigned intervention was high, based on monitoring through unannounced telephone contacts during the intervention and the absence of remaining capsules in the returned blister packs.

3.2. Lipid Peroxidation

A significant main effect of group was observed for serum MDA concentrations ($F = 17.93$, $p < 0.001$, $\eta_p^2 = 0.63$), together with a significant main effect of time ($F = 31.33$, $p < 0.001$, $\eta_p^2 = 0.60$) and a significant time × group interaction ($F = 3.02$, $p = 0.005$, $\eta_p^2 = 0.22$). Mean MDA concentrations increased from pre-exercise to 24 h post-exercise in all groups, with the highest mean values occurring at 24–48 h. Descriptively, mean MDA concentrations were consistently lower in the CUR group than in the IBP and PLA groups at all post-exercise time points (T2–T4) (Figure 3). Post hoc comparisons with Bonferroni correction revealed significant increases in MDA concentrations from T1 to T2, T3, and T4 within all three groups ($p < 0.05$). Between-group comparisons showed significantly lower MDA concentrations in CUR than in both IBP and PLA at T2 (24 h) and T3 (48 h) ($p < 0.05$), whereas these differences were not statistically significant at T4 (72 h).

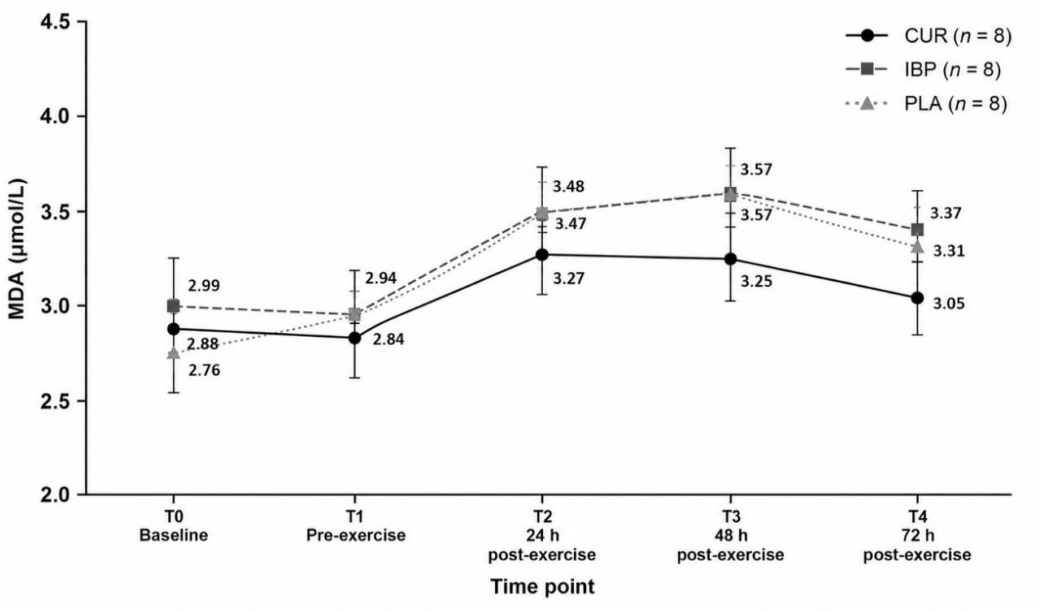


Figure 3. Changes in serum malondialdehyde concentrations following eccentric exercise. Serum MDA concentrations were assessed at baseline (T0), immediately before eccentric exercise (T1), and at 24 h (T2), 48 h (T3), and 72 h (T4) post-exercise. Data are presented as mean ± SD. Statistical analysis was performed using a two-way mixed-design repeated-measures ANOVA, with time as the within-subject factor and group as the between-subject factor. Abbreviations: CUR, curcumin group; IBP, ibuprofen group; MDA, malondialdehyde; PLA, placebo group; SD, standard deviation. The ANOVA revealed significant main effects of group ($p < 0.001$) and time ($p < 0.001$), as well as a significant time × group interaction ($p = 0.005$).

3.3. Antioxidant Biomarkers

No significant main effects or interactions were observed for CAT activity or SOD concentrations. For CAT, there were no significant main effects of group ($F = 1.93$, $p = 0.170$, $\eta_p^2 = 0.16$) or time ($F = 1.46$, $p = 0.222$, $\eta_p^2 = 0.07$), and the time × group interaction was

also not significant ($F = 0.63$, $p = 0.750$, $\eta_p^2 = 0.06$). Similarly, SOD showed no significant main effect of group ($F = 0.67$, $p = 0.522$, $\eta_p^2 = 0.06$) or time ($F = 2.22$, $p = 0.074$, $\eta_p^2 = 0.10$), and no significant time × group interaction ($F = 1.63$, $p = 0.129$, $\eta_p^2 = 0.13$) was observed (Table 2).

Table 2. Antioxidant and inflammatory biomarkers across the study time points.

Outcome	Group	T0 (Baseline)	T1 (Pre-Exercise)	T2 (24 h)	T3 (48 h)	T4 (72 h)
CAT (U/L)	CUR	42.33 ± 4.24	42.03 ± 2.94	43.56 ± 7.41	45.12 ± 8.04	50.76 ± 7.01
	IBP	46.47 ± 8.31	45.76 ± 9.41	50.14 ± 9.74	46.66 ± 9.23	47.54 ± 7.13
	PLA	46.44 ± 5.88	46.66 ± 8.71	46.08 ± 7.12	48.41 ± 7.65	46.31 ± 6.06
SOD (μg/mL)	CUR	4.96 ± 0.64	5.12 ± 0.87	5.02 ± 0.79	4.77 ± 0.57	4.68 ± 0.74
	IBP	5.21 ± 0.44	5.29 ± 0.77	5.04 ± 0.80	5.17 ± 0.92	5.07 ± 0.86
	PLA	5.11 ± 0.28	5.32 ± 0.63	5.09 ± 0.88	5.03 ± 0.81	5.14 ± 0.83
IL-6 (pg/mL)	CUR	3.26 ± 0.57	3.12 ± 0.44	2.79 ± 0.74	3.33 ± 0.68	3.01 ± 0.77
	IBP	3.16 ± 0.92	3.23 ± 0.93	3.21 ± 0.91	3.12 ± 0.98	2.87 ± 0.19
	PLA	2.88 ± 1.11	3.11 ± 0.97	2.93 ± 0.93	2.69 ± 0.93	2.83 ± 1.21

Data are presented as mean ± SD. Statistical analysis was performed using a two-way mixed-design repeated-measures ANOVA, with time as the within-subject factor and group as the between-subject factor. Abbreviations: CAT, catalase; CUR, curcumin group; IBP, ibuprofen group; IL-6, interleukin-6; PLA, placebo group; SD, standard deviation; SOD, superoxide dismutase.

3.4. Inflammatory Response

No significant main effect of group ($F = 0.10$, $p = 0.905$, $\eta_p^2 = 0.01$) or time ($F = 1.83$, $p = 0.131$, $\eta_p^2 = 0.08$) was observed for circulating IL-6 concentrations, and the time × group interaction was also not significant ($F = 1.14$, $p = 0.346$, $\eta_p^2 = 0.10$) (Table 2).

3.5. Circulating Enzymes Associated with Tissue Stress

For AST, there was no significant main effect of group ($F = 1.89$, $p = 0.176$, $\eta_p^2 = 0.15$), whereas a significant main effect of time ($F = 6.81$, $p < 0.001$, $\eta_p^2 = 0.25$) and a significant time × group interaction ($F = 2.83$, $p = 0.008$, $\eta_p^2 = 0.21$) were observed (Table 3). Descriptively, mean AST concentrations increased progressively in the CUR group from 16.83 ± 2.12 U/L at 24 h to 20.34 ± 5.56 U/L at 48 h and 25.36 ± 6.80 U/L at 72 h. In contrast, IBP and PLA exhibited their highest post-exercise mean concentrations at 24 h (23.47 ± 3.51 and 21.33 ± 4.34 U/L, respectively), followed by different temporal trajectories. Bonferroni-corrected simple-effects analyses revealed a significant increase in AST concentrations within the CUR group from T1 to T4 and from T2 to T4 ($p < 0.05$). No significant within-group changes were observed in IBP or PLA, and no between-group differences reached statistical significance at individual assessment time points after correction ($p > 0.05$).

For ALT, no significant main effect of group ($F = 2.48$, $p = 0.108$, $\eta_p^2 = 0.19$) or time ($F = 0.90$, $p = 0.468$, $\eta_p^2 = 0.04$) was observed, and the time × group interaction was also not significant ($F = 1.17$, $p = 0.327$, $\eta_p^2 = 0.10$).

Similarly, ALP showed no significant main effect of group ($F = 0.54$, $p = 0.591$, $\eta_p^2 = 0.05$) or time ($F = 1.72$, $p = 0.153$, $\eta_p^2 = 0.08$), and no significant time × group interaction was observed ($F = 1.08$, $p = 0.385$, $\eta_p^2 = 0.09$).

LDH showed no significant main effect of group ($F = 1.91$, $p = 0.173$, $\eta_p^2 = 0.15$). A significant main effect of time was observed ($F = 9.88$, $p < 0.001$, $\eta_p^2 = 0.32$), whereas the time × group interaction was not significant ($F = 1.52$, $p = 0.163$, $\eta_p^2 = 0.13$) (Table 3). Descriptively, mean LDH concentrations decreased at 24 h across all groups (CUR: 463.92 ± 82.33 U/L; IBP: 412.16 ± 79.94 U/L; PLA: 374.75 ± 95.35 U/L), followed by increases at 48–72 h in the IBP and PLA groups (e.g., IBP at 48 h: 528.25 ± 169.55 U/L; PLA at 48 h: 521.16 ± 118.19 U/L). Bonferroni-corrected simple-effects comparisons indicated a

significant decrease in LDH concentrations from T1 to T2 within all three groups ($p < 0.05$), followed by a significant increase from T2 to T3 in IBP and PLA ($p < 0.05$). No significant between-group differences were observed at individual assessment time points after correction ($p > 0.05$).

Table 3. Circulating enzymes associated with tissue stress across the study time points.

Outcome	Group	T0 (Baseline)	T1 (Pre-Exercise)	T2 (24 h)	T3 (48 h)	T4 (72 h)
AST (U/L)	CUR	17.76 ± 1.62	16.59 ± 3.04	16.83 ± 2.12	20.34 ± 5.56	25.36 ± 6.80
	IBP	18.85 ± 2.19	18.54 ± 3.47	23.47 ± 3.51	21.69 ± 2.57	22.60 ± 1.79
	PLA	18.34 ± 3.53	18.56 ± 3.08	21.33 ± 4.34	17.97 ± 5.46	20.37 ± 9.85
ALT (U/L)	CUR	10.79 ± 1.30	11.02 ± 1.33	11.84 ± 1.53	15.39 ± 2.74	13.40 ± 2.49
	IBP	12.28 ± 1.75	11.89 ± 1.45	15.27 ± 2.20	13.32 ± 2.25	13.45 ± 1.43
	PLA	11.14 ± 1.49	11.36 ± 1.39	16.28 ± 2.33	16.15 ± 4.89	13.30 ± 1.58
ALP (U/L)	CUR	108.01 ± 3.22	107.80 ± 3.11	107.93 ± 3.03	107.58 ± 3.28	107.97 ± 3.51
	IBP	108.03 ± 3.68	108.20 ± 3.43	109.90 ± 6.13	109.64 ± 4.33	107.80 ± 2.86
	PLA	107.95 ± 3.89	108.94 ± 4.39	108.08 ± 4.17	109.95 ± 4.95	109.50 ± 4.57
LDH (U/L)	CUR	467.58 ± 88.84	480.33 ± 94.16	463.92 ± 82.33	413.33 ± 84.76	443.00 ± 98.00
	IBP	448.41 ± 93.01	474.75 ± 84.70	412.16 ± 79.94	528.25 ± 169.55	513.33 ± 139.56
	PLA	452.75 ± 104.00	455.91 ± 93.07	374.75 ± 95.35	521.16 ± 118.19	530.16 ± 106.98

Data are presented as mean ± SD. Statistical analysis was performed using a two-way mixed-design repeated-measures ANOVA, with time as the within-subject factor and group as the between-subject factor. Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CUR, curcumin group; IBP, ibuprofen group; LDH, lactate dehydrogenase; PLA, placebo group; SD, standard deviation.

4. Discussion

This triple-blind, randomized, placebo-controlled trial investigated the effects of curcumin supplementation, compared with ibuprofen and placebo, on the biochemical response to eccentric exercise in competitive female rugby players. The main finding was that MDA exhibited significant effects of group and time, together with a significant time × group interaction. Post hoc comparisons identified significantly lower MDA concentrations in the curcumin group than in both the ibuprofen and placebo groups at 24 and 48 h post-exercise, but not at 72 h. In contrast, CAT activity, SOD concentrations, and circulating IL-6 concentrations showed no significant main effects of group or time × group interactions. Among the circulating enzymes associated with tissue stress, AST showed a significant time × group interaction, whereas LDH changed significantly over time without evidence of a differential response between groups; ALT and ALP showed no significant effects of group, time, or their interaction. Collectively, these findings suggest that the effects of curcumin were more apparent for the lipid peroxidation response than for circulating antioxidant biomarkers, IL-6, or the broader biochemical response to exercise-induced tissue stress.

The most prominent treatment-related finding of the present study was the differential MDA response following eccentric exercise. MDA concentrations increased during the 24–48 h recovery period in all three groups, consistent with an exercise-induced increase in lipid peroxidation. The significant time × group interaction was further characterized by Bonferroni-corrected comparisons showing significantly lower MDA concentrations in CUR than in both IBP and PLA at 24 and 48 h post-exercise, whereas the between-group differences at 72 h were not statistically significant. Several complementary mechanisms could plausibly contribute to this response pattern. Curcumin may exert direct antioxidant activity by scavenging reactive species and limiting the propagation of lipid peroxidation, while also indirectly enhancing cellular antioxidant responses through modulation of the Kelch-like ECH-associated protein 1 (Keap1)–nuclear factor erythroid 2-related factor 2 (Nrf2)/antioxidant response element (ARE) pathway [24]. Such mechanisms could contribute to

reduced oxidative damage to membrane lipids and, consequently, to lower generation of lipid peroxidation products such as MDA. Consistent with this mechanistic rationale, a meta-analysis of randomized controlled trials reported significantly lower MDA concentrations following curcumin supplementation, including in studies combining curcumin with piperine, although substantial between-study heterogeneity and the inclusion of clinical populations limit direct extrapolation to trained athletes [25]. More recent systematic evidence in exercise and sport settings has also reported predominantly favorable findings for oxidative-stress outcomes following curcumin supplementation, although the certainty of evidence remains low [13]. Therefore, the present findings extend the available evidence by showing a differential MDA response in competitive female rugby players following a standardized eccentric-exercise challenge and by including both placebo and an active pharmacological comparator. Nevertheless, because MDA reflects one component of oxidative damage rather than the overall redox state, these findings should not be interpreted as evidence of a generalized enhancement of antioxidant capacity.

In contrast to the differential MDA response, CAT activity and SOD concentrations showed no significant effects of group or time and no significant time × group interactions, indicating that the differential MDA response was not accompanied by detectable changes in these circulating antioxidant biomarkers during the 72 h recovery period. This dissociation is biologically plausible because lipid peroxidation can be modulated through multiple antioxidant mechanisms and does not necessarily require parallel changes in circulating antioxidant biomarkers [26]. Curcumin can exert direct radical-scavenging antioxidant effects and limit the propagation of lipid peroxidation, thereby potentially reducing oxidative damage to membrane lipids without requiring detectable parallel changes in circulating CAT activity or SOD concentrations [27]. Consistent with this interpretation, Drobnic et al. [28] reported no significant change in CAT activity following curcumin supplementation in an eccentric-exercise model, whereas Bańkowski et al. [29] found no significant effects of six weeks of curcumin supplementation on SOD or CAT activity in amateur endurance runners. Moreover, a randomized placebo-controlled trial in physically active women reported lower MDA concentrations following curcumin supplementation without a concomitant significant increase in total antioxidant capacity, further supporting the possibility that reductions in lipid peroxidation may occur without parallel changes across antioxidant defense markers [30]. Although curcumin can modulate Nrf2-dependent antioxidant signaling, the absence of changes in CAT activity and SOD concentrations in the present study indicates that such molecular effects, if present, were not reflected in these circulating antioxidant biomarkers within the 72 h assessment period. Accordingly, the combined MDA, CAT, and SOD findings support a biomarker-specific redox response associated with curcumin supplementation rather than a generalized enhancement of systemic antioxidant capacity under the present experimental conditions.

Circulating IL-6 concentrations showed no significant effects of group or time and no significant time × group interaction, providing no evidence that curcumin supplementation measurably modified the systemic IL-6 response under the present experimental conditions. This finding is consistent with previous exercise-based trials in which curcumin did not significantly modify circulating IL-6. In an eccentric-exercise model, Tanabe et al. [31] observed no significant changes in IL-6 after exercise and no differences between curcumin and placebo, whereas Sciberras et al. [32] reported an exercise-induced increase in IL-6 following prolonged cycling but no significant difference between curcumin and placebo.

An important consideration in interpreting the present findings is the rapid and transient nature of the exercise-induced IL-6 response [33]. Because the first post-exercise blood sample in the present study was obtained at 24 h, an earlier transient IL-6 response—and

any potential treatment-related differences in its magnitude or temporal profile—may have occurred outside the sampling window [34].

Furthermore, circulating IL-6 should not be interpreted exclusively as a marker of systemic inflammation in the exercise setting. Skeletal muscle-derived IL-6 acts as a myokine with context-dependent metabolic and immunoregulatory functions, including roles in substrate mobilization and inter-organ communication during exercise [35]. Accordingly, the absence of significant IL-6 changes in the present study should not be interpreted simply as evidence of a lack of anti-inflammatory activity of curcumin. Rather, we detected no differential systemic IL-6 response within the 24–72 h post-exercise period assessed. Given the pleiotropic and context-dependent physiological role of exercise-induced IL-6, modulating rather than indiscriminately suppressing this response may be more biologically relevant in the exercise setting [35]. Curcumin can modulate inflammatory signaling pathways involved in cytokine regulation, including NF-κB signaling [36]; however, whether supplementation preferentially attenuates excessive IL-6 signaling while preserving its physiological exercise-related functions remains hypothetical and cannot be determined from the present study.

The circulating enzyme responses observed in the present study should be interpreted within the broader context of exercise-induced tissue stress and its interaction with redox and inflammatory processes, rather than exclusively as indicators of hepatic injury. Strenuous eccentric exercise can perturb skeletal muscle cellular and membrane integrity, while secondary oxidative and inflammatory processes form part of the subsequent tissue response and may contribute to cellular stress and the appearance of intracellular constituents in the circulation [37]. In this context, skeletal muscle may contribute substantially to circulating AST and LDH after strenuous exercise, while exercise-induced increases in aminotransferases should not automatically be interpreted as evidence of hepatic injury; conversely, ALP appears to be less responsive to exercise-induced muscle damage [5].

In the present study, AST showed a significant time × group interaction; however, its temporal pattern, including the later increase observed in the CUR group, did not indicate a consistent attenuation of the AST response with curcumin. Bonferroni-corrected comparisons identified significant increases in AST within CUR from pre-exercise to 72 h and from 24 to 72 h, without significant between-group differences at individual time points. LDH changed significantly over time but showed no significant time × group interaction, indicating a temporal response without evidence of differential responses among the intervention groups. Follow-up comparisons showed a significant decrease in LDH from pre-exercise to 24 h in all groups, followed by a significant increase from 24 to 48 h in IBP and PLA. In contrast, ALT and ALP showed no significant effects of group, time, or their interaction. The absence of significant changes in ALT and ALP indicates that these circulating enzymes remained relatively stable across the assessment period. This observation is consistent with, although it does not independently establish, the generally favorable safety profile reported for curcumin at commonly investigated doses [38]. However, the present study was not designed to establish hepatic safety, and these findings should not be interpreted as excluding hepatotoxicity.

Importantly, the differential MDA response observed with curcumin was not accompanied by a generalized reduction in circulating markers associated with exercise-induced tissue stress. Redox balance, inflammatory signaling, membrane integrity, and intracellular enzyme release represent interconnected but biologically distinct components of the response to muscle-damaging exercise [39]; consequently, modulation of lipid peroxidation does not necessarily imply proportional preservation of membrane integrity or reduced release of AST and LDH. Taken together, these findings further support a biomarker-specific

response to curcumin rather than a generalized biochemical response across the domains assessed under the present experimental conditions.

When the biochemical findings are considered collectively, curcumin and ibuprofen did not produce a uniform pattern of response across the biomarkers assessed. The clearest differential response associated with curcumin was observed for MDA, whereas CAT activity, SOD concentrations, circulating IL-6, and most circulating enzymes associated with exercise-induced tissue stress showed no significant time × group interactions. Although AST showed a significant time × group interaction, its temporal pattern did not indicate a consistent attenuation of the AST response in the CUR group. Accordingly, the present findings do not support a generalized biochemical effect of curcumin across antioxidant, inflammatory, or tissue-stress biomarkers following eccentric exercise; rather, they suggest a biomarker-specific response involving lipid peroxidation under the conditions tested.

The inclusion of ibuprofen as an active pharmacological comparator provides an additional perspective for interpreting these findings. NSAIDs are widely used in athletic settings to manage exercise-related pain and inflammation, although their effects on the broader biological processes involved in post-exercise recovery remain heterogeneous [40]. In the present study, ibuprofen did not produce a biochemical response that consistently paralleled that observed with curcumin, particularly for MDA. Moreover, IBP and PLA exhibited broadly similar response patterns across several biochemical outcomes, with no significant between-group differences identified in the follow-up comparisons performed for MDA, AST, and LDH. This observation suggests that ibuprofen did not provide a detectable additional benefit over placebo for these specific biochemical outcomes under the present experimental conditions. However, it does not demonstrate that ibuprofen supplementation is unnecessary or ineffective for managing exercise-related symptoms. An alternative explanation is that the selected circulating biomarkers and assessment time points may not have adequately captured certain pharmacological effects of ibuprofen, particularly effects related to pain, prostaglandin-mediated pathways, or early inflammatory responses. This divergence is consistent with the multidimensional nature of the biological response to exercise-induced stress, in which redox, metabolic, inflammatory, and tissue-level processes represent interconnected but distinct components of the overall response [41]. However, the study was not designed to establish therapeutic equivalence or superiority between curcumin and ibuprofen, and the absence of significant treatment-related effects for individual biomarkers should not be interpreted as evidence of therapeutic equivalence or comparable efficacy.

From a clinical exercise physiology perspective, the present findings emphasize the importance of avoiding interpretation of isolated biochemical changes as surrogate evidence of global tissue recovery. A reduction in lipid peroxidation does not necessarily indicate attenuation of systemic inflammation, preservation of muscle-cell integrity, or enhanced overall biological recovery, as these processes are related but not interchangeable [39]. This distinction is particularly relevant when biochemical markers are used to evaluate responses to exercise, nutritional interventions, or pharmacological strategies in physically active individuals, because individual biomarkers capture specific components of a complex and temporally dynamic biological response rather than recovery as a whole [42]. Accordingly, the present results support a multidimensional interpretation of exercise-induced tissue stress in which redox, inflammatory, and tissue-stress biomarkers should be considered according to their specific biological meaning rather than combined into a single construct of "recovery."

The findings also have implications for the clinical use of recovery interventions. Although MDA showed a differential temporal response across groups, with significantly lower concentrations in CUR than in IBP and PLA at 24 and 48 h post-exercise, these data do

not establish a therapeutic advantage over ibuprofen and do not support replacing NSAIDs with curcumin when pharmacological treatment is clinically indicated. Conversely, the absence of a generalized biochemical benefit with ibuprofen provides no rationale for its routine prophylactic use solely to modify post-exercise biochemical responses. Nevertheless, the lack of detectable differences between IBP and PLA for the assessed biomarkers should not be extrapolated to clinical outcomes that were not measured, including exercise-related pain and functional recovery. Decisions regarding NSAID use should remain clinically justified and should consider the potential adverse effects associated with unnecessary exposure [9]. An alternative interpretation is that training-related adaptations may have influenced the athletes' physiological responses to the eccentric-exercise stimulus. However, the present biochemical findings cannot determine whether these responses remained entirely within their adaptive capacity or establish that curcumin or ibuprofen supplementation is unnecessary for recovery.

This study has several strengths. Its randomized, triple-blind, placebo-controlled design, together with the inclusion of ibuprofen as an active pharmacological comparator, provides a rigorous framework for evaluating the biochemical response to curcumin supplementation following a standardized eccentric-exercise challenge. Complete participant follow-up minimized the potential influence of attrition, while repeated biochemical assessments from baseline through 72 h of recovery enabled characterization of the temporal response across complementary biological domains, including lipid peroxidation, antioxidant biomarkers, inflammatory response, and circulating enzymes associated with exercise-induced tissue stress. The study is reported in accordance with the CONSORT 2025 statement, supporting transparent and standardized reporting. Furthermore, the exclusive inclusion of competitive female rugby players provides evidence from an athletic population that remains comparatively underrepresented in exercise and recovery research.

Several limitations should nevertheless be considered. First, although the sample size was determined a priori, the relatively small number of participants and single-center design limit statistical precision and the generalizability of the findings, particularly for modest between-group and interaction effects. Second, multiple biochemical outcomes were analyzed without adjustment for multiplicity; therefore, statistically significant findings should be interpreted cautiously, particularly in view of the modest sample size. Third, the biochemical markers assessed provide indirect and domain-specific information and cannot comprehensively characterize exercise-induced tissue stress, redox status, inflammation, or overall recovery. Because the eccentric-exercise stimulus was localized to the knee extensors, circulating biomarkers may not adequately reflect the magnitude of localized muscle damage or associated functional impairment. Furthermore, the absence of direct measures of pain, functional recovery, and prostaglandin-related inflammatory pathways limits the interpretation of the apparent similarities between IBP and PLA, particularly regarding potential clinical effects of ibuprofen that may not be reflected in the selected circulating biomarkers. In addition, creatine kinase (CK), a widely used indirect biomarker of exercise-induced muscle damage, was not assessed, limiting the characterization of the muscle damage response. MDA measured using the TBARS method should be interpreted as an indirect index of lipid peroxidation rather than a comprehensive measure of systemic oxidative stress. Fourth, because the first post-exercise blood sample was obtained 24 h after the eccentric-exercise session, transient early responses—particularly for IL-6—may have occurred outside the sampling window. Fifth, circulating CAT activity and SOD concentrations may not directly reflect antioxidant responses within skeletal muscle tissue. Sixth, circulating concentrations of curcumin and piperine were not determined; therefore, individual systemic exposure and bioavailability could not be related to the biochemical responses observed. Seventh, although menstrual-cycle timing was obtained by self-report

and assessments were preferentially scheduled during the early follicular phase when feasible, cycle phase was not hormonally confirmed or incorporated into the statistical analyses. Residual variability related to menstrual-cycle characteristics therefore cannot be excluded. Future studies in female athletes should incorporate more precise characterization of menstrual-cycle phase into study design and analysis. In addition, habitual rugby training and diet during the intervention period were not standardized or quantitatively monitored by the research team, which may have introduced additional variability in the biochemical responses. Finally, the short supplementation and follow-up periods preclude conclusions regarding longer-term recovery, repeated exercise exposure, or training adaptation, and the findings should be considered specific to the doses, formulation, population, and eccentric-exercise protocol investigated.

5. Conclusions

In competitive female rugby players exposed to a standardized eccentric-exercise challenge, curcumin supplementation was associated with a differential MDA response compared with ibuprofen and placebo, whereas this pattern was not accompanied by corresponding changes in circulating antioxidant biomarkers, IL-6, or most circulating enzymes associated with exercise-induced tissue stress. Although AST showed a significant time × group interaction, its temporal pattern did not indicate a consistent attenuation of the AST response with curcumin. Collectively, these findings support a biomarker-specific biochemical response associated with curcumin supplementation, primarily involving lipid peroxidation, rather than generalized effects across the antioxidant, inflammatory, and tissue-stress biomarkers assessed. From a clinical sports-medicine perspective, changes in individual redox biomarkers should not be interpreted in isolation as evidence of enhanced overall recovery or reduced exercise-induced tissue stress. Moreover, the present findings establish neither therapeutic superiority nor equivalence between curcumin and ibuprofen and do not support substituting one intervention for the other.

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Abbreviations

1RM, one-repetition maximum; 5-LOX, 5-lipoxygenase; ALP, alkaline phosphatase; ALT, alanine aminotransferase; ANOVA, analysis of variance; ARE, antioxidant response element; AST, aspartate aminotransferase; BMI, body mass index; CAT, catalase; CK, creatine kinase; COX, cyclooxygenase; COX-2, cyclooxygenase-2; CUR, curcumin group; EIMD, exercise-induced muscle damage; IBP, ibuprofen group; IL-6, interleukin-6; Keap1, Kelch-like ECH-associated protein 1; LDH, lactate dehydrogenase; MDA, malondialdehyde; NF-κB, nuclear factor kappa B; Nrf2, nuclear factor erythroid 2-related factor 2; NSAIDs, non-steroidal anti-inflammatory drugs; PAR-Q+, Physical Activity Readiness Questionnaire for Everyone; PLA, placebo group; ROS, reactive oxygen species; SD, standard deviation; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances.

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