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Original Scientific Article

Taking Astaxanthin Supplementation Attenuates MDA and HMGB1 Following Eccentric Exercise: A Randomized Controlled Trial in Recreationally Active Students

Roy Januardi Irawan[1ABCDE], Ratna Candra Dewi[1BDE], Ananda Perwira Bakti[1BDE], Nanda Rimawati[1BCD], Abdul Rohim Tualeka[2ACD], Putri Ayuni Allayannur[2BDE] and Mokhamad Nur Bawono[1BCD]

[1]Universitas Negeri Surabaya
[2]Airlangga University

Authors' Contribution: A – Study design; B – Data collection; C – Statistical analysis; D – Manuscript Preparation; E – Funds Collection

Corresponding Author: Roy Januardi, Irawan, e-mail: royjanuardi@unesa.ac.id
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Abstract

Background. Eccentric exercise induces mechanical stress and microstructural damage in skeletal muscle, triggering oxidative stress and inflammation that impair recovery. Malondialdehyde (MDA) and High Mobility Group Box-1 (HMGB1) are reliable biomarkers of lipid peroxidation and systemic inflammation. Astaxanthin, a marine-derived carotenoid, has strong antioxidant and anti-inflammatory properties that may mitigate these responses.

Objectives. This study aimed to investigate whether 14-day astaxanthin supplementation attenuates post-exercise increases in plasma MDA and HMGB1 levels following acute eccentric exercise in recreationally active male students.

Materials and Methods. In a randomized, double-blind, placebo-controlled trial, fifty-four recreationally active male students were assigned to receive either 12 mg/day of natural astaxanthin (AST, n = 27) or placebo (PLA, n = 27) for 14 days. Participants performed a standardized eccentric exercise protocol (10 × 10 drop jumps from 60 cm) on day 16. Venous blood samples were collected at 1 hour before exercise (T0), 1 hour after exercise (T1), and 24 hours after exercise (T2) to measure plasma MDA (TBARS assay) and HMGB1 (ELISA). Data were analyzed using repeated-measures ANOVA or Welch's t-test with Bonferroni adjustment ($p < 0.05$).

Results. Both groups exhibited significant increases in MDA and HMGB1 from T0 to T1 ($p < 0.001$). However, the AST group showed smaller increases in MDA (+151 % vs. +187 %, $p = 0.021$) and HMGB1 (+279 % vs. +367 %, $p = 0.014$) compared to PLA. At T2, both biomarkers declined towards baseline, with greater reductions observed in the AST group (MDA: −27 % vs. −15 %, $p = 0.018$; HMGB1: −55 % vs. −40 %, $p = 0.012$).

Conclusions. Fourteen days of astaxanthin supplementation attenuated oxidative stress and inflammatory responses following eccentric exercise, as evidenced by lower elevations of MDA and HMGB1 compared to placebo. These findings suggest that astaxanthin may be an effective nutritional strategy to protect against exercise-induced oxidative damage and modulate inflammation in physically active individuals.

Keywords: eccentric exercise, malondialdehyde, HMGB1, oxidative stress, antioxidant, inflammation.

Introduction

Intense or unaccustomed exercise—particularly those involving eccentric contractions—imposes substantial mechanical stress on skeletal muscle. This leads to microstructural damage and temporary declines in muscle function (Irawan, Sudijandoko, et al., 2024; Markus et al., 2021). Activities that require repeated muscle lengthening under load, such as downhill running or the eccentric phase of resistance training, are particularly damaging. They often cause disruption of sarcomeres and compromise the sarcolemma (Lemire et al., 2021; Stožer et al., 2020). This structural disruption triggers a cascade of local and systemic inflammatory responses that resemble acute injury (da Silva et al., 2018; Fleckenstein et al., 2021).

A key consequence of eccentric-induced muscle damage (EIMD) is the overproduction of reactive oxygen species

(ROS). When ROS production exceeds the capacity of endogenous antioxidant systems, lipid peroxidation can occur (Jomova et al., 2023; J. Wang et al., 2020). Malondialdehyde (MDA) is a stable end-product of lipid peroxidation and serves as a widely recognized biomarker for oxidative stress and cell membrane damage (Zhang, 2023).

In parallel, muscle cell damage results in the release of damage-associated molecular patterns (DAMPs) into the extracellular space (Tu & Li, 2023). Among these, High Mobility Group Box-1 (HMGB1) plays a central role in inflammation when passively released from the nucleus (Lin et al., 2022; Tang et al., 2022).

HMGB1 acts as a potent pro-inflammatory signal by activating innate immune receptors, recruiting immune cells, and upregulating cytokines such as IL-6 and TNF-α (Pattelongi et al., 2021). This heightened immune activity contributes to delayed-onset muscle soreness (DOMS), which typically peaks 24–72 hours post-exercise and is associated with impaired recovery (Ali et al., 2023; Chang et al., 2020; Sulistyarto et al., 2022). Sustained elevations in oxidative and inflammatory biomarkers, including MDA and HMGB1, may further impair recovery and contribute to long-term muscle pathology (Mas-Bargues et al., 2021; Xue et al., 2021).

Compared to traditional cytokine markers like IL-6 and TNF-α, HMGB1 is a more proximal indicator of cellular stress. It is passively released by necrotic or mechanically disrupted muscle cells. MDA, meanwhile, provides a reliable measure of lipid peroxidation and oxidative membrane damage. Together, these biomarkers offer a comprehensive snapshot of the oxidative and inflammatory responses to EIMD and can guide the development of interventions that target both pathways.

One promising intervention is astaxanthin—a red-orange carotenoid derived from microalgae—known for its exceptional antioxidant capacity, reportedly greater than that of vitamins C and E (Barker et al., 2023; Bjørklund et al., 2022). Its molecular structure allows it to integrate into cell membranes and protect against lipid peroxidation (Barker et al., 2023; Waldman, 2024). Astaxanthin also enhances endogenous antioxidant defenses by upregulating enzymes such as superoxide dismutase and glutathione peroxidase (Heng et al., 2021).

In addition to its antioxidant properties, astaxanthin exerts anti-inflammatory effects by inhibiting NF-κB activation—a central transcription factor in inflammation (Medoro et al., 2023). This pathway is implicated in the release of HMGB1 and the subsequent production of cytokines. Evidence from both animal and human studies suggests that astaxanthin can reduce inflammation in settings ranging from metabolic syndrome to physical overexertion (Eldessouki et al., 2024; Tsao et al., 2025). These dual antioxidant and anti-inflammatory effects make astaxanthin a compelling candidate to support muscle recovery and adaptation in physically active individuals.

Despite these promising attributes, research on the combined effects of astaxanthin on oxidative stress (MDA) and inflammation (HMGB1) following eccentric exercise remains limited. Most prior studies have focused on endurance or concentric exercise. Recreationally active populations, who are frequently exposed to unaccustomed eccentric loading, are underrepresented in antioxidant intervention research (Brown et al., 2018).

Recreationally active individuals are particularly vulnerable to eccentric-induced muscle damage due to limited neuromuscular adaptation. Unlike trained athletes, they often engage in irregular or unstructured high-intensity activities, increasing susceptibility to oxidative stress and inflammation (Owens et al., 2019). Since antioxidant supplementation is most beneficial for populations with suboptimal physiological adaptation, focusing on this group enhances the clinical and practical relevance of the study.

Therefore, this study investigated the effects of 14-day astaxanthin supplementation on plasma MDA and HMGB1 levels following acute eccentric exercise in recreationally active male students. To our knowledge, this is among the first randomized controlled trials to evaluate the dual antioxidant and anti-inflammatory properties of astaxanthin in this context. We hypothesized that astaxanthin supplementation would attenuate post-exercise elevations in MDA and HMGB1 compared to placebo.

We hypothesized that astaxanthin supplementation would attenuate post-exercise elevations in MDA and HMGB1 compared to placebo, thereby providing evidence of its antioxidant and anti-inflammatory potential in the context of eccentric exercise.

Materials and Methods

Study Design

This randomized, double-blind, placebo-controlled experimental trial employed a pretest-posttest control group design to investigate the effects of astaxanthin supplementation on oxidative stress and inflammation following eccentric exercise. Fifty-four recreationally active male participants were randomly assigned to two equal groups (n = 27 per group) using computer-generated block randomization (block size = 6) by an independent researcher.

The study lasted 17 days, beginning with a screening visit on Day 0 (D0) to evaluate eligibility and obtain written informed consent. On Day 1 (D1), baseline assessments were conducted during the first visit. This was followed by a 14-day supplementation period from Day 2 to Day 15 (D2–D15). Participants returned for the second visit on Day 16 (D16) to complete the eccentric exercise protocol and provide pre- and post-exercise blood samples. A final blood sample was collected during the third visit on Day 17 (D17) to capture delayed physiological responses. Throughout the study, Participants were instructed to maintain habitual dietary intake while refraining from high-intensity training, structured resistance exercise, and additional supplement or medication use.

Participants

Fifty-four recreationally active male undergraduate students from the Sports Science Program at Universitas Negeri Surabaya were recruited for this study. All participants met the predetermined inclusion criteria, which required engagement in moderate to high levels of recreational physical activity (defined as exercising 3–5 times per week), a normal body mass index (BMI) range, and good general health. Eligible participants were also required to be non-smokers and not have participated in structured resistance training within the four weeks prior to enrollment.

Exclusion criteria included a history of musculoskeletal injury, chronic illness, or any medical condition potentially affecting exercise performance or inflammatory responses. Participants who had consumed antioxidant or anti-inflammatory supplements within the previous month, reported excessive alcohol consumption (>14 units/week), or were current smokers were excluded. Individuals with known allergies or intolerances to astaxanthin or its components were also deemed ineligible. All participants received a detailed explanation of the study procedures and signed written informed consent before participating.

Participants completed a standardized health questionnaire prior to enrolment. The questionnaire included items regarding medical history (e.g., cardiovascular, metabolic, musculoskeletal disorders), current medication use, history of smoking or alcohol consumption, recent illness or injury, and other lifestyle factors relevant to eligibility. Only participants who reported no chronic disease, no recent injury, and no use of medications or supplements affecting oxidative stress or inflammation were included in the study.

Baseline physical activity was assessed using the International Physical Activity Questionnaire (IPAQ), which indicated that all participants were classified within the moderate physical activity category. Nutritional characteristics were evaluated through a brief dietary recall, showing a typical student dietary pattern consisting mainly of rice, vegetables, chicken, fish, and fruit, with moderate intake of natural antioxidants. None of the participants reported adherence to restrictive or specialized diets. All participants were instructed to maintain their habitual diet and avoid additional supplements or medications during the study.

Supplementation

The Astaxanthin Group (AST) received 12 mg/day of natural astaxanthin, a dosage based on previous studies by Xia (Xia et al., 2020) and Brendel (Brendler & Williamson, 2019), while the Placebo Group (PLA) received visually identical capsules containing sunflower oil. Both groups consumed one capsule daily with breakfast for 14 consecutive days. Blinding of both participants and study personnel was maintained until completion of data analysis.

The astaxanthin supplement used in this study was Micro Ingredients Astaxanthin (12 mg softgel; Micro Ingredients®, California, USA), derived from the natural microalgae Haematococcus pluvialis. The product is certified non-GMO, gluten-free, soy-free, dairy-free, and preservative-free, and has undergone third-party laboratory testing to verify purity, potency, and safety in compliance with U.S. dietary supplement regulations.

Study Organization

The study was structured into three phases: (1) screening and baseline assessment, (2) a 14-day supplementation period, and (3) an eccentric exercise challenge followed by biomarker evaluation.

On Day 0 (D0), participants completed screening procedures, including a health questionnaire and anthropometric measurements. Baseline data were collected during the first laboratory visit on Day 1 (D1), after which participants received instructions regarding supplementation, dietary control, and physical activity restrictions.

The supplementation phase commenced on Day 2 (D2) and continued daily through Day 15 (D15). Participants were instructed to maintain their habitual diet while avoiding high-intensity physical activity, structured resistance training, and consumption of additional supplements or medications. Compliance was monitored via weekly check-ins and capsule counts.

On Day 16 (D16), participants returned to the laboratory to perform an eccentric exercise protocol. Venous blood samples were collected at three time points: T0 (1 hour before exercise), T1 (1 hour after exercise), and T2 (24 hours after exercise on Day 17 [D17]). Detailed procedures for the exercise protocol and blood collection are presented in the following section.

Anthropometric indicators including height, weight, and body mass index (BMI) were measured using a stadiometer and a calibrated digital scale. Body fat percentage was assessed using a Tanita bioelectrical impedance analyzer (BIA BC-730; Tanita, Tokyo, Japan) under standardized conditions. Habitual physical activity was evaluated with the short form of the International Physical Activity Questionnaire (IPAQ), and results were expressed in MET-minutes per week. Maximal oxygen uptake (VO_2max) was estimated using the Multistage Fitness Test (MFT, also known as the 20-m shuttle run test), which is a validated and widely used field protocol for assessing cardiorespiratory fitness in young adults.

Exercise Protocol

To induce exercise-induced muscle damage (EIMD), participants completed a standardized eccentric exercise protocol adapted from the Newham drop-jump model. The protocol consisted of 10 sets of 10 drop jumps from a 60 cm platform, with 60 seconds of rest between sets. Each jump was performed with maximal effort and a controlled landing to emphasize eccentric loading of the quadriceps and gastrocnemius muscles. This model has been previously validated as an effective method to induce muscle damage and oxidative stress.

Prior to the exercise session, participants performed a standardized warm-up including 10 minutes of light jogging followed by dynamic stretching. The eccentric exercise was conducted under the supervision of certified trainers to ensure safety and correct technique. To monitor internal load, Rating of Perceived Exertion (RPE) was recorded at the end of each set.

Venous blood samples were collected at three time points:

T0 (second visit): 1 hour prior to exercise (after 14 days of supplementation), before any physical activity—serving as the post-supplementation baseline.

T1 (second visit): 1 hour after exercise to assess acute oxidative and inflammatory responses.

T2 (third visit, Day 17): 24 hours after exercise, to evaluate delayed systemic responses associated with muscle damage and early recovery.

A summary of the study design is illustrated in Figure 1.

This study adhered to ethical guidelines and was approved by the Health Research Ethics Committee of the

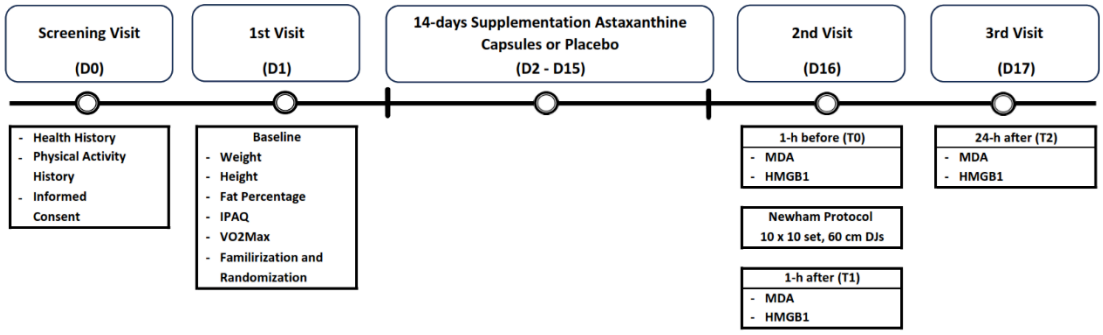

Fig. 1. Study Design

Faculty of Public Health, Airlangga University, receiving ethical clearance under the identification number 104/EA/KEPK/2023.

Outcome Measures

Venous blood samples were collected at three time points: T0 (1 houᵒᵒr before exercise, post-supplementation), T1 (1 hour after exercise), and T2 (24 hours after exercise). All samples were drawn into EDTA tubes, centrifuged at 3,000×g for 10 minutes at 4 °C, and the resulting plasma was aliquoted and stored at –80 °C until analysis.

Plasma malondialdehyde (MDA) levels, used as a biomarker of lipid peroxidation and oxidative stress, were assessed using the Thiobarbituric Acid Reactive Substances (TBARS) assay protocol. Plasma High Mobility Group Box 1 (HMGB1) protein, a marker of systemic inflammation, was quantified using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Elabscience HMGB1 ELISA Kit, Houston, TX, USA), in accordance with the manufacturer's instructions.

Venous blood samples were collected by a certified medical laboratory technologist. All analyses were conducted at the Educational Laboratory of Faculty of Public Health, which operates under standardized procedures for biomedical research and follows national laboratory safety and quality control guidelines.

All biochemical analyses were performed in duplicate to ensure reproducibility, and laboratory personnel were blinded to group assignments. To minimize confounding variables, participants were instructed to avoid intense physical activity, anti-inflammatory drugs, and additional supplementation throughout the study period.

Statistical Analysis

Normality of data distribution was assessed using the Shapiro–Wilk test, which confirmed that all variables were normally distributed. Homogeneity of variances was tested using Levene's test. Both MDA and HMGB1 met the assumptions of normality and homogeneity; therefore, between-group comparisons at each time point were performed using independent t-tests. For within-group and time–group interaction effects, two-way repeated-measures ANOVA was applied for MDA and HMGB1, followed by Bonferroni-adjusted post hoc tests when appropriate. Statistical significance was set at $p < 0.05$.

Results

Participant Characteristic

A total of 54 recreationally active male students (mean age = 19.29 ± 0.82 years; overall mean BMI = 20.72 ± 1.28) from the Faculty of Sport Sciences and Health, Universitas Negeri Surabaya, voluntarily participated in this study after providing written informed consent. Participants were randomly assigned to the astaxanthin group (n = 27) or placebo group (n = 27) using computer-generated block randomization (block size = 6), ensuring balanced allocation. Baseline characteristics were comparable between groups (Table 1), with the astaxanthin group showing a mean age of 19.07 ± 0.68 years and BMI of 22.52 ± 2.10, and the placebo group a mean age of 19.52 ± 0.89 years and BMI of 22.51 ± 0.70. Table 1 summarizes additional anthropometric and physiological variables.

Table 1. Baseline characteristics of participants in the Astaxanthin and Placebo groups

Variable	AST Mean ± SD (n=27)	PLA Mean ± SD (n=27)	p
Age (years)	19.07 ± 0.68	19.52 ± 0.89	0.130
Height (cm)	168.85 ± 4.87	169.30 ± 3.96	0.463
Weight (kg)	64.04 ± 4.78	64.70 ± 5.12	0.755
BMI	22.52 ± 2.10	22.51 ± 0.70	0.877
Fat Percentage (%)	17.04 ± 2.74	17.28 ± 2.84	0.746
IPAQ (MET-min/week)	1693.00 ± 336	1581.00 ± 360	0.250
VO_2max (mL·kg^{-1}·min^{-1})	36.95 ± 1.13	37.05 ± 1.14	0.839

Table 1 shows that both groups were comparable at baseline (all $p > 0.05$), suggesting that any subsequent differences are likely attributable to the intervention rather than pre-existing disparities. Homogeneity of variances were confirmed using Levene's test (all $p > 0.05$), supporting the validity of parametric analyses. Although minor numerical differences were observed (e.g., BMI, VO_2max), these were minimal and unlikely to be physiologically meaningful.

Data Distribution and Homogeneity Testing

Prior to the main statistical analyses, assumption testing was conducted to ensure the validity of subsequent parametric tests. Data normality was assessed using the Shapiro–

Table 2. Normality test result

Variable	Time Point	Astaxanthin Sig.	Conclusion	Placebo Sig.	Conclusion
MDA	1 h before (T0)	0.926	Normal	0.945	Normal
	1 h after (T1)	0.903	Normal	0.936	Normal
	24 h after (T2)	0.685	Normal	0.716	Normal
HMGB1	1 h before (T0)	0.178	Normal	0.544	Normal
	1 h after (T1)	0.122	Normal	0.704	Normal
	24 h after (T2)	0.069	Normal	0.634	Normal

Wilk test, which is recommended for small to moderate sample sizes ($n < 50$). In this study, the test was performed separately for each group (Astaxanthin, $n = 27$; Placebo, $n = 27$) and for each measurement time point—T0 (1 h before exercise), T1 (1 h after exercise), and T2 (24 h after exercise)—for both biomarkers: malondialdehyde (MDA) and High-Mobility Group Box 1 (HMGB1). Conducting the analysis per group and per time point ensured that the sample size criteria for appropriate application of the Shapiro–Wilk method were met.

As presented in Table 2, all p-values for the Shapiro–Wilk test exceeded the 0.05 threshold, indicating that the distributions of MDA and HMGB1 values did not deviate significantly from normality at any time point in either group. This finding supports the assumption of normality for both outcome variables throughout the study period.

Following confirmation of normality, the equality of variances between groups was assessed using Levene's test, a prerequisite for valid comparisons using independent t-tests and repeated-measures ANOVA.

Table 3. Homogeneity test result

Variable	1 h before (T0) Astaxanthin – Placebo Sig.	1 h after (T1) Astaxanthin – Placebo Sig.	24 h after (T2) Astaxanthin – Placebo Sig.
MDA	0.889	0.876	0.917
HMGB1	0.595	0.754	0.652

As shown in Table 3, all Levene's test p-values were greater than 0.05, indicating that the variances between the astaxanthin and placebo groups were statistically equivalent at all time points. Although certain values (e.g., HMGB1 at T2) approached the threshold, they remained within acceptable limits.

Together, the results of the Shapiro–Wilk and Levene's tests confirm that the dataset met the assumptions of normality and homogeneity of variances. This validation supports the appropriateness of applying parametric statistical methods, such as independent t-tests for between-group comparisons and repeated-measures ANOVA for within-subject analyses over time.

Assessment of Oxidative Stress Biomarker (MDA)

Malondialdehyde (MDA) is a widely recognized biomarker of lipid peroxidation and oxidative stress, which typically increases in response to eccentric or high-intensity exercise. In this study, plasma MDA concentrations were measured at three time points: 1 h before exercise (T0), 1 h after exercise (T1), and 24 h after exercise (T2). Descriptive statistics and pairwise comparisons of MDA levels in the astaxanthin and placebo groups are presented in Table 4.

As shown in Table 4, MDA levels increased significantly 1 h after exercise (T1) in both groups ($p < 0.001$). The astaxanthin group exhibited a smaller increase compared to the placebo group. At 24 h after exercise (T2), MDA levels decreased in both groups, with a greater reduction observed in the astaxanthin group ($p < 0.001$).

These results suggest that astaxanthin supplementation may attenuate exercise-induced oxidative stress and reduce MDA levels. Bonferroni-adjusted post-hoc tests confirmed significant differences between all-time points ($p < 0.001$),

Table 4. Changes in Plasma MDA Levels (μmol/L) Across Time Points in Astaxanthin and Placebo Groups

Group	Time Point	Mean ± SD (μmol/L)	Pairwise Comparison	Mean Difference (I-J)	P-value (Bonferroni adjusted)
Astaxanthin	1 h before (T0)	2.87 ± 1.45	T0 vs T1	-4.35*	< 0.001
	1 h after (T1)	7.22 ± 1.43	T0 vs T2	-2.36*	< 0.001
	24 h after (T2)	5.23 ± 1.41	T1 vs T2	1.99*	< 0.001
Placebo	1 h before (T0)	3.00 ± 1.42	T0 vs T1	-5.61*	< 0.001
	1 h after (T1)	8.61 ± 1.39	T0 vs T2	-4.32*	< 0.001
	24 h after (T2)	7.32 ± 1.38	T1 vs T2	1.29*	< 0.001

Notes: Mean differences are calculated as (I – J) for each pairwise comparison. Negative values indicate an increase from time point I to J (e.g., from before to after exercise). Asterisks (*) denote statistically significant differences after Bonferroni adjustment ($p < 0.05$). Data are presented as Mean ± SD (μmol/L). Post-hoc analyses were conducted using Bonferroni tests to compare time points within each group

Table 5. Changes in Plasma HMGB1 Levels (ng/mL) Across Time Points in Astaxanthin and Placebo Groups

Group	Time Point	Mean ± SD (ng/mL)	Pairwise Comparison	Mean Difference (I-J)	P-value (Bonferroni adjusted)
Astaxanthin	1 h before (T0)	1.45 ± 0.66	T0 vs T1	-4.05*	< 0.001
	1 h after (T1)	5.50 ± 0.67	T0 vs T2	-1.00*	< 0.001
	24 h after (T2)	2.45 ± 0.66	T1 vs T2	3.05*	< 0.001
Placebo	1 h before (T0)	1.52 ± 0.59	T0 vs T1	-5.57*	< 0.001
	1 h after (T1)	7.09 ± 0.61	T0 vs T2	-2.76*	< 0.001
	24 h after (T2)	4.28 ± 0.60	T1 vs T2	2.81*	< 0.001

Notes: Mean differences are calculated as (I – J) for each pairwise comparison. Negative values indicate an increase from time point I to J (e.g., from before to after exercise). Asterisks (*) denote statistically significant differences after Bonferroni correction ($p < 0.05$). Data are presented as Mean ± SD (ng/mL). Post-hoc analyses were performed using Bonferroni-adjusted pairwise comparisons within each group.

supporting the protective effect of astaxanthin in modulating oxidative stress responses after exercise.

Assessment of Inflammatory Biomarker (HMGB1)

High Mobility Group Box 1 (HMGB1) is a well-established biomarker used to evaluate inflammation and cellular stress, particularly in response to strenuous or unaccustomed eccentric exercise. In the present study, plasma HMGB1 concentrations were measured at three time points: 1 h before exercise (T0), 1 h after exercise (T1), and 24 h after exercise (T2), following a 14-day supplementation period with astaxanthin. The mean values and pairwise comparisons of HMGB1 levels in the astaxanthin and placebo groups are presented in Table 5.

As shown in Table 5, HMGB1 levels increased significantly 1 h after exercise (T1) in both groups ($p < 0.001$), with the astaxanthin group showing a smaller increase compared to the placebo group. By 24 h after exercise (T2), HMGB1 levels had decreased in both groups, with a greater reduction observed in the astaxanthin group ($p < 0.001$). The rise in HMGB1 from T0 to T1 was approximately 27% smaller in the astaxanthin group, indicating a difference that is both statistically significant and physiologically relevant. Bonferroni-adjusted post-hoc tests confirmed significant changes between all-time points ($p < 0.001$), supporting the role of astaxanthin in attenuating exercise-induced inflammation.

Discussion

Eccentric exercise is well known to induce muscle microdamage, triggering oxidative stress and inflammation that can impair recovery and performance (Nogueira et al., 2020; Sarkar et al., 2021). In the present study, a single bout of high-volume drop jumps markedly elevated plasma malondialdehyde (MDA) and High-Mobility Group Box 1 (HMGB1) levels in both the astaxanthin and placebo groups, confirming the sensitivity of these biomarkers to exercise-induced muscle damage (EIMD). Importantly, participants who consumed astaxanthin for 14 days exhibited smaller post-exercise increases in both biomarkers and a faster return toward baseline within 24 hours compared to placebo. These findings support the hypothesis that astaxanthin supplementation can attenuate oxidative and inflammatory responses following eccentric loading in recreationally active individuals.

During eccentric exercise, repeated lengthening contractions generate high mechanical tension on muscle fibers, leading to disruption of sarcomere alignment, damage to the sarcolemma, and disturbance of the cytoskeletal network (Stožer et al., 2020; Suzuki, 2021). This structural damage increases membrane permeability, allowing calcium influx into muscle cells, which activates calcium-dependent proteases such as calpains and phospholipases (Stožer et al., 2020; Uçar et al., 2024). These enzymes degrade contractile proteins and membrane phospholipids, amplifying cellular injury (Bouviere et al., 2021). Concurrently, mitochondrial dysfunction and activation of NADPH oxidase during intense contractions lead to excessive production of reactive oxygen species (ROS) such as superoxide anions (O_2^-) and hydrogen peroxide (H_2O_2) (Bouviere et al., 2021; Y. Wang et al., 2018). Elevated ROS levels trigger lipid peroxidation, protein oxidation, and DNA damage, while also serving as signaling molecules that activate nuclear factor kappa B (NF-κB) and other pro-inflammatory pathways (Bouviere et al., 2021; Hussein et al., 2021; Powers et al., 2010). Damaged fibers release damage-associated molecular patterns (DAMPs), including HMGB1, which bind to toll-like receptors (TLRs) on immune cells, promoting the release of cytokines such as TNF-α, IL-6, and IL-1β (Irawan, Wahyudi, et al., 2024; Langston & Mathis, 2024; Tu & Li, 2023). This cascade results in a sustained inflammatory response that can persist for 24–72 hours post-exercise, delaying recovery and impairing performance if not properly regulated (Farias-Junior et al., 2019; Minahan et al., 2020).

The need for supplementation arises because the body's endogenous antioxidant defenses, while effective under normal physiological conditions, are often insufficient to counteract the sharp surge in ROS and inflammatory mediators generated during and after intense exercise (Arazi et al., 2021; M.-C. Lee et al., 2021). During EIMD, excessive ROS production can overwhelm enzymatic antioxidants such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx), leading to lipid peroxidation, protein oxidation, and DNA damage (Ammar et al., 2020; Souissi et

al., 2020). Similarly, the heightened inflammatory response can persist for 24–72 hours post-exercise, delaying muscle recovery and potentially impairing subsequent performance (Farias-Junior et al., 2019). Nutritional supplementation with potent antioxidants such as astaxanthin can provide additional defense by directly (Sawada et al., 2023), stabilizing cell membranes, and modulating inflammatory pathways, thereby supporting faster and more complete recovery (E. Lee et al., 2023).

Astaxanthin's antioxidant effects are mediated through both direct and indirect mechanisms. Structurally, astaxanthin is a xanthophyll carotenoid with a unique polar–nonpolar–polar configuration that enables it to span the phospholipid bilayer of cell membranes (Rizzardi et al., 2022). This positioning allows it to neutralize ROS both at the membrane surface and within the lipid bilayer. Astaxanthin can directly scavenge singlet oxygen ($^1O_2$), superoxide anions (O_2^-), hydroxyl radicals (•OH), and peroxyl radicals (ROO•), thereby preventing the initiation and propagation of lipid peroxidation (Kumar et al., 2022). Its singlet oxygen quenching activity is reported to be up to 100 times greater than that of vitamin E (Yadollahi et al., 2021). In addition, astaxanthin enhances endogenous antioxidant defenses by upregulating enzymes such as SOD, catalase, and GPx through activation of the Nrf2–ARE signaling pathway (Davinelli et al., 2022). This dual mode of action provides both immediate and sustained protection against oxidative stress during and after exercise.

Astaxanthin also exerts anti-inflammatory effects through modulation of key signaling pathways. It inhibits activation of the NF-κB pathway, a central regulator of inflammation, by preventing phosphorylation and degradation of its inhibitor, IκBα (Davinelli et al., 2022). This suppression reduces the transcription of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β (Wu et al., 2024). Astaxanthin further downregulates the expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), thereby reducing the production of nitric oxide and prostaglandins, which contribute to vascular permeability, tissue swelling, and leukocyte infiltration (Dang et al., 2024). Moreover, astaxanthin may attenuate DAMP-mediated immune activation by limiting the extracellular release of HMGB1 from damaged muscle fibers, thus reducing toll-like receptor (TLR) activation and accelerating the resolution of inflammation (Nian et al., 2019; Xue et al., 2021).

Our results are consistent with these mechanisms. The placebo group showed substantial increases in MDA and HMGB1 after exercise, whereas the astaxanthin group experienced attenuated rises (~22% and ~27% smaller, respectively) and faster recovery toward baseline within 24 hours. The 14-day supplementation period used in this study was based on previous human trials indicating that plasma astaxanthin concentrations reach a steady state within 10–14 days of daily intake, allowing for sufficient incorporation into cell membranes and tissues (Bloomer, 2008). This timeframe is considered adequate to elicit measurable antioxidant and anti-inflammatory effects, as supported by studies showing significant reductions in exercise-induced oxidative markers after similar supplementation durations (Bazzucchi et al., 2019). Although longer supplementation periods (≥4 weeks) may further enhance tissue saturation and potentially amplify protective effects, the present findings demonstrate that even 14 days of astaxanthin intake can confer meaningful physiological benefits in attenuating oxidative stress and inflammation following eccentric exercise.

Overall, this study supports the potential of astaxanthin as a nutritional strategy to mitigate exercise-induced oxidative stress and inflammation. Given the growing interest in carotenoid-based recovery aids, astaxanthin may serve as a valuable alternative or adjunct to synthetic antioxidants for athletes and recreationally active individuals. Future research should aim to elucidate its molecular actions in human skeletal muscle, optimize supplementation protocols, and investigate its long-term effects on performance, adaptation, and health outcomes.

Conclusion

This study demonstrates that 14 days of astaxanthin supplementation attenuates exercise-induced oxidative stress and inflammation following a single bout of high-volume eccentric exercise in recreationally active males. Supplementation reduced post-exercise increases in plasma MDA and HMGB1 compared to placebo. Collectively, these findings suggest that astaxanthin may serve as an effective nutritional strategy to protect against oxidative damage and modulate inflammatory responses during strenuous eccentric loading. Further research is warranted to confirm these effects over longer supplementation periods, in different populations, and across various exercise modalities.

Limitation

Several limitations should be acknowledged in this study. First, the sample size was relatively small and limited to recreationally active young males, which may restrict the generalizability of the findings to other populations such as females, older adults, or elite athletes. Second, the supplementation period was limited to 14 days; although this duration is sufficient to achieve plasma saturation of astaxanthin, longer interventions may produce different or more pronounced effects. Third, only two biomarkers were assessed (MDA and HMGB1), which provide important but incomplete insights into the broader oxidative stress and inflammatory response. Including additional markers such as enzymatic antioxidants, cytokine profiles, and muscle damage indicators (e.g., creatine kinase) could yield a more comprehensive understanding. Lastly, dietary intake and physical activity outside the intervention were self-reported and not strictly controlled, which may have introduced variability in the results.

Strengths and Practical Implications

A major strength of this study is its randomized, placebo-controlled design, which reduces bias and increases the reliability of the results. The use of a standardized high-volume drop jump protocol provided a consistent and reproducible stimulus for inducing oxidative stress and inflammation, enabling a clear assessment of astaxanthin's effects. Measuring two key biomarkers—MDA for lipid peroxidation and HMGB1 for inflammation and tissue damage—offered direct insights into the body's oxidative and inflammatory responses to exercise. Although the

supplementation period was only 14 days, it was sufficient to produce measurable physiological benefits, showing that astaxanthin can be effective within a practical timeframe.

Practically, these findings suggest that astaxanthin supplementation can be incorporated into recovery strategies for athletes and active individuals, especially during periods of heavy training or repeated bouts of intense eccentric exercise. By reducing oxidative damage and inflammation, astaxanthin may shorten recovery time, limit performance loss, and support overall training adaptations—benefits that are particularly valuable in sports requiring frequent high-intensity efforts.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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Приймання добавок астаксантину зменшує рівень малонового діальдегіду (МДА) та амфотерину (HMGB1) після виконання ексцентричних вправ: Рандомізоване контрольоване дослідження студентів, які активно займаються рекреаційною діяльністю

Рой Джануарді Іраван[1ABCDE], Ратна Чандра Деві[1BDE], Ананда Первіра Бакті[1BDE], Нанда Рімаваті[1BCD], Абдул Рохім Туалека[2ACD], Путрі Аюні Аллайяннур[2BDE], Мохамад Нур Бавоно[1BCD]

[1]Державний університет Сурабая
[2]Університет Ерланґга

Авторський вклад: A – дизайн дослідження; B – збір даних; C – статаналіз; D – підготовка рукопису; E – збір коштів

Реферат. Стаття: 11 с., 5 табл., 1 рис., 56 джерел.

Історія питання. Ексцентричні вправи спричиняють механічне напруження та мікроструктурні пошкодження скелетних м'язів, що провокує окислювальний стрес та запалення, які погіршують процес відновлення. Малоновий діаль-

дегід (МДА) та білок групи високої рухливості 1 (амфотерин, HMGB1) є надійними біомаркерами ліпідної пероксидації та системного запалення. Астаксантин, каротиноїд морського походження, має сильні антиоксидантні та протизапальні властивості, які можуть пом'якшити зазначені реакції.

Мета дослідження. Мета цього дослідження полягала у вивченні того, чи 14-денне приймання добавок астаксантину послаблює підвищення рівнів МДА та HMGB1 у плазмі крові після інтенсивних ексцентричних вправ у рекреаційно активних студентів чоловічої статі.

Матеріали та методи. У рандомізованому, подвійно сліпому, плацебо-контрольованому дослідженні 54 студенти чоловічої статі, які активно займаються рекреаційною діяльністю, були розподілені на дві групи: одна група отримувала 12 мг/день натурального астаксантину (АСТ, n = 27), а інша — плацебо (ПЛА, n = 27) протягом 14 днів. На 16-й день учасники виконали стандартизований протокол ексцентричних вправ (10 × 10 стрибків з висоти 60 см). Зразки венозної крові було зібрано за 1 годину перед виконанням вправ (T0), через 1 годину після вправ (T1) та через 24 години після виконання вправ (T2) з метою вимірювання показників МДА (аналіз реактивних речовин тіобарбітурової кислоти, TBARS) та HMGB1 (імуноферментний аналіз, ELISA) у плазмі крові. Аналіз даних проводився з використанням дисперсійного аналізу з повторними вимірами або t-критерію Велча з поправкою Бонферроні ($p < 0.05$).

Результати. В обох групах спостерігалося значне підвищення рівнів МДА та HMGB1 від T0 до T1 ($p < 0.001$). Однак група АСТ продемонструвала менші підвищення рівнів МДА (+151% проти +187%, $p = 0.021$) та HMGB1 (+279% проти +367%, $p = 0.014$) порівняно з групою ПЛА. На етапі T2 обидва біомаркери знизилися до базового рівня, причому більші зниження показників спостерігалися в групі АСТ (МДА: −27% проти −15%, $p = 0.018$; HMGB1: −55% проти −40%, $p = 0.012$).

Висновки. Приймання добавок астаксантину протягом 14 днів сприяло зменшенню окислювального стресу та запальних реакцій після ексцентричних вправ, що підтверджується нижчими рівнями МДА та HMGB1 порівняно з плацебо. Отримані результати свідчать, що астаксантин може бути ефективною нутритивною стратегією щодо захисту від окислювального пошкодження, спричиненого фізичними навантаженнями, та регулювання запальних процесів у фізично активних осіб.

Ключові слова: ексцентричні вправи, малоновий діальдегід, HMGB1, окислювальний стрес, антиоксидант, запалення.

Information about the authors:

Irawan, Roy Januardi: royjanuardi@unesa.ac.id; https://orcid.org/0000-0002-0996-8718; Sports Science Department, Sports Science and Health Faculty, Universitas Negeri Surabaya, Kampus FIKK-Unesa Jl. Lidah Wetan, Surabaya 60213, East Java, Indonesia.

Dewi, Ratna Candra: ratnadewi@unesa.ac.id; https://orcid.org/0000-0003-0612-244X; Sports Science Department, Sports Science and Health Faculty, Universitas Negeri Surabaya, Kampus FIKK-Unesa Jl. Lidah Wetan, Surabaya 60213, East Java, Indonesia.

Bakti, Ananda Perwira: anandabakti@unesa.ac.id; https://orcid.org/0000-0002-8778-8114; Sports Science Department, Sports Science and Health Faculty, Universitas Negeri Surabaya, Kampus FIKK-Unesa Jl. Lidah Wetan, Surabaya 60213, East Java, Indonesia.

Rimawati, Nanda: nandarimawati@unesa.ac.id; https://orcid.org/0000-0001-8400-492X; Sports Science Department, Sports Science and Health Faculty, Universitas Negeri Surabaya, Kampus FIKK-Unesa Jl. Lidah Wetan, Surabaya 60213, East Java, Indonesia.

Tualeka, Abdul Rohim: abdul-r-t@fkm.unair.ac.id; https://orcid.org/0000-0002-8276-2441; Department of Occupational Safety and Health, Faculty of Public Health, Universitas Airlangga, Fakultas Kesehatan Masyarakat Kampus C Unair, Mulyorejo, Kec. Mulyorejo, Surabaya 60115, East Java, Indonesia.

Alayyannur, Putri Ayuni: putri.a.a@fkm.unair.ac.id; https://orcid.org/0000-0001-8701-6547; Department of Occupational Safety and Health, Faculty of Public Health, Universitas Airlangga, Fakultas Kesehatan Masyarakat Kampus C Unair, Mulyorejo, Kec. Mulyorejo, Surabaya 60115, East Java, Indonesia.

Bawono, Mokhamad Nur: mokhamadnur@unesa.ac.id; https://orcid.org/0009-0000-2298-5814; Sports Science Department, Sports Science and Health Faculty, Universitas Negeri Surabaya, Kampus FIKK-Unesa Jl. Lidah Wetan, Surabaya 60213, East Java, Indonesia.

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