



Article

A Single Dose of Microencapsulated Cocoa Supplementation Does Not Speed up Muscle Force Recovery after Eccentric Exercise-Induced Muscle Damage: A Placebo-Controlled, Double-Blind, Crossover Study

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Abstract: Exercise-induced muscle damage is associated with symptoms such as inflammation, delayed-onset muscle soreness, and impaired muscle performance. The intake of cocoa polyphenols has been suggested to improve muscle recovery due to their antioxidant and anti-inflammatory capacity. However, their bioavailability presents a challenge. Therefore, food microencapsulation may be an alternative to protect polyphenols, ensuring their biological effects. This study aimed to investigate the effect of a single dose of microencapsulated cocoa on the changes in muscle damage markers after eccentric exercise. In this randomized, double-blind, crossover study, fourteen healthy volunteers with previous resistance training experience performed 6×10 maximal isokinetic eccentric contractions of their elbow flexors using an isokinetic dynamometer after ingesting 25 g of microencapsulated cocoa or placebo. Peak isometric torque was measured using maximal voluntary isometric contractions, and pain was measured using a visual analogic scale both before and 24 h, 48 h, and 72 h after the damage protocol. Plasma glutathione and malondialdehyde levels were measured using high-performance liquid chromatography, and concentrations of myoglobin and C-reactive protein were determined using a fluorescence immunoassay analyzer. Significant decreases were seen in the peak isometric torque and pain measures from pre- to 72 h post-eccentric exercise. A significant main effect for time was found only for plasma myoglobin at 2 h, 48 h, and 72 h, and for C-reactive protein at 2 h, compared to the pre-eccentric exercise values. No significant time-treatment effects were observed (all $p > 0.05$). This study demonstrated that microencapsulated cocoa cannot improve muscle recovery after eccentric exercise, at least when a single dose is consumed.

Keywords: functional foods; cocoa polyphenols; food microencapsulation; muscle damage; exercise recovery



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1. Introduction

The appropriate recovery procedure after exercise-induced muscle damage (EIMD) is essential for athletes from all sports modalities since muscle damage can result in the impairment of various activities. EIMD usually occurs following strenuous exercise involving repetitive eccentric contractions (muscle lengthening) and is associated with symptoms such as inflammation, delayed-onset muscle soreness (DOMS), an increase in circulating skeletal muscle proteins, and impaired muscle performance [1–3]. Furthermore, daily tasks

such as sitting down/standing up from a chair, stair climbing, and even walking may also be difficult. Therefore, the use of nutritional strategies to assist in the process of muscle repair and recovery has been investigated.

The intake of polyphenols, compounds commonly found in fruits, is one of the nutritional strategies that has been investigated to improve muscle recovery after EIMD [4–6]. Of these compounds, flavanols from cocoa have been suggested to speed up muscle recovery by modulating the inflammatory response after EIMD [7,8]. However, a concern with respect to the recommendation of antioxidant supplementation for individuals engaged in physical exercise programs is that oxidative stress influences cellular processes that lead to the increased expression of antioxidants. Thus, a diet supplemented with exogenous antioxidants such as cocoa appears to prevent the health-promoting effects of physical exercise in humans [9]. Evidence from investigations of short-term periods of supplementation (i.e., a single dose) has not shown that cocoa polyphenols have a clear effect on overall post-exercise recovery parameters [7].

Furthermore, it is important to point out that the effects of phenolic compounds on muscle recovery following EIMD may depend upon their bioaccessibility (i.e., the fraction of an ingested compound that is available for absorption in the gut) and, hence, their bioavailability (i.e., the fraction of an ingested compound that reaches the systemic circulation and tissues to exert its biological action) [10–14]. Cocoa polyphenols are subject to extensive metabolism once introduced into the gastrointestinal tract [15], and most of them yield phase II conjugated derivatives and cannot reach systemic circulation in their natural form. This may compromise their bioavailability and, hence, their potential action at the target tissue [15]. This may be one of the possible reasons for the lack of effect of cocoa on the variables of muscle recovery following EIMD [7,8].

Food microencapsulation is a technological process widely used in the food industry to preserve bioactive compounds [16]. In this process, microspheres are formed in which the bioactive compounds present in food are protected by a capsule [17]. Evidence demonstrates that enhanced flavanol can be delivered into the gut from microencapsulated cocoa polyphenols [18,19]. Therefore, the microencapsulation of cocoa could be an adequate food technological process to provide effective protection for the polyphenols against their degradation throughout the gastrointestinal tract, ensuring their safe delivery and biological effect. Therefore, the microencapsulation process could increase the bioavailability of the polyphenols, enhancing their ability to increase muscle recovery without the need to ingest antioxidant supplements for a longer period (>7 days) when compared with a single dose.

Since there are conflicting results concerning the acute effect of polyphenols from cocoa on markers of muscle recovery after eccentric resistance exercise associated with the lack of studies using encapsulated formulations of cocoa, the purpose of the present study is to investigate the effect of a single dose of microencapsulated cocoa on markers of inflammation, oxidative stress, and muscle recovery parameters after exercise-induced muscle damage. The study hypothesis is that microencapsulated cocoa can attenuate muscle damage, oxidative stress, and inflammation and improve muscle recovery.

2. Material and Methods

2.1. Participants

Fourteen healthy male and female volunteers (26 ± 5 years old) with previous resistance training experience (at least 3 months) were recruited to participate in this study. All participants were fully informed of the nature and purpose of the investigation and gave their written consent to participate. The physical characteristics of the participants are described in Table 1. They were instructed not to deviate from their current training regimen during the study, except for refraining from exercise 24 h prior to each testing day. The exclusion criteria for participation in this study were any known cardiovascular, pulmonary, or metabolic diseases (i.e., asthma, diabetes *mellitus*, hypertension, dyslipidemia, smoking), upper limb injury, and/or the use of nutritional supplement (i.e., creatine,

caffeine, and vitamins and minerals complexes), anabolic steroids, and anti-inflammatory drugs six months prior to the beginning of the study. All experimental procedures were performed in accordance with the ethical standards of the Declaration of Helsinki and were approved by the Institutional Ethics Committee of the Federal University of Rio de Janeiro—Macaé Campus, Rio de Janeiro, Brazil (protocol CAAE: 36846720.7.0000.5699). The study was registered in the Brazilian Registry of Clinical Trials (RBR-3bc3rnb).

Table 1. Physical characteristics of the participants.

Characteristic	Male	Female
N	7	7
Age (years)	28 ± 5	27 ± 4
Body mass (kg)	75.7 ± 8.3	58.6 ± 7.5
Height (m)	1.72 ± 0.06	1.60 ± 0.06
Body Mass Index (kg/m ²)	24.7 ± 2.8	22.6 ± 1.8
Resistance training experience (years)	6 ± 2	3 ± 1

Values are expressed as mean ± SD.

2.2. Experimental Design

This study was a randomized, double-blind, placebo-controlled, crossover trial. Participants were randomized to establish their first supplementation condition. Participants were required to come to the laboratory for nine days, the first visit being baseline testing and familiarization with the EIMD protocol. During the familiarization with the exercise protocol, the participants were instructed to perform minimal effort to prevent unintentional muscle damage and to ensure they did not induce an adaptative repeated bout effect [20].

After one week, the volunteers came back to the lab for four consecutive days, and measures were taken in the following order: 1. Blood samples, 2. subjective perception of muscle pain through visual analogic scale (VAS), and 3. maximal voluntary isometric contractions (MVIC), before and 2 h, 24 h, 48 h, and 72 h post-EIMD. Participants returned to the laboratory after a 1-month washout period and followed the same procedures described above with the second intervention (Figure 1).

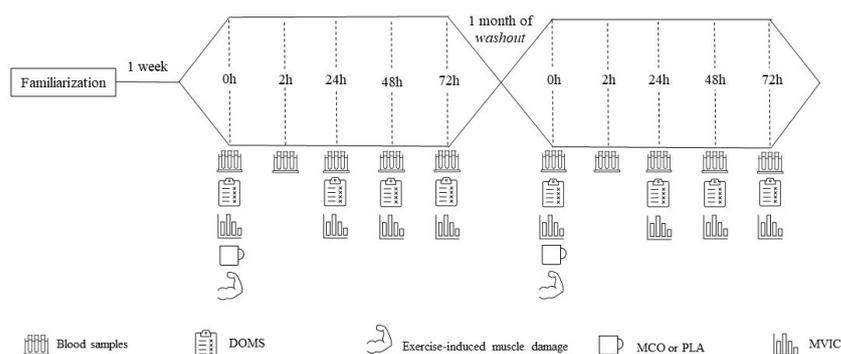


Figure 1. Summary of the experimental design of the study.

2.3. Nutritional Supplementation and Dietary Control

Participants consumed 25 g of microencapsulated cocoa (containing 75 mg of total flavonoids) or a similar amount of malt drink chocolate flavor as a placebo (PLA, containing 8 mg of total flavonoids), which were diluted in 200 mL of water and offered to the participants 2 h before the exercise-induced muscle damage protocol. Evidence has shown that a greater plasma concentration of cocoa flavonoid metabolites is observed 90–120 min after cocoa intake [7,21]. The dose of microencapsulated used in the present study was chosen based on a previous study [22] that demonstrated improved functional recovery (countermovement jump) following intensive knee extension exercise after consuming flavanol-rich cocoa mucilage juice (containing 154 mg of polyphenols and 86 mg of flavonoids).

For the microencapsulation process, maltodextrin (as a wall material) was mixed with 100% cocoa powder (Nestlé®, Vevey, Switzerland (1:1) and diluted in water. The mixture was spray dried using a mini spray dryer (Model B-290, Büchi, Flawil, Switzerland) with a 1.0 mm standard diameter nozzle and an evaporation capacity of 1.0 L/h. The equipment was operated at an inlet temperature of 160 °C, feed rate of 70%, and airflow of 30%.

The total phenolic and flavonoid contents of microencapsulated cocoa and placebo drinks were evaluated as described above [23,24]. In microencapsulated cocoa, total phenolics and flavonoids were 240 mg of gallic acid equivalents/100 g and 75 mg of quercetin/100 g, respectively. The placebo drink's total phenolic and flavonoid content was 9 mg of gallic acid equivalents/100 g and 8 mg of quercetin/100 g. The nutritional composition of the microencapsulated cocoa and placebo drink is found in Table 2.

Table 2. Nutritional composition in placebo (PLA) and microencapsulated cocoa (MCO) products (25 g).

Nutrient	Value		%DR *	
	PLA	MCO	PLA	MCO
Energy content (Kcal/kJ)	93/387	49/205	4.6	2.5
Carbohydrates (g)	21	11	7.1	3.7
Proteins (g)	1.0	2.5	1.3	3.3
Total fat (g)	0	1.2	0	2.2
Dietary fiber (g)	0.9	3.4	3.5	14
Sodium (mg)	35	3.8	1.5	0.2
Total flavonoids	8	75	-	-
Total polyphenols	9	240	-	-

* DR = Daily reference values based on a diet of 2.000 Kcal or 8.400 kJ.

Participants completed a food diary 24 h before the first visit (i.e., familiarization) and were required to follow the same diet during the entire study. They were required to abstain from alcohol, caffeine, polyphenol-containing foods, and heavy exercise for 72 h before each test visit and to have a rest day on the day immediately before each testing visit. It was also required that participants should not take any mineral or vitamin supplement or any other antioxidant supplements during the study period. They were provided a food-exclusion list to ensure they avoided high polyphenol-containing foods during the entire study period.

2.4. Exercise-Induce Muscle Damage Protocol

The participants performed a non-dominant elbow flexion and extension exercise with an isokinetic dynamometer (Humac Norm, CSMi Medical Solutions, Stoughton, MA, USA) in the eccentric (flexion)—concentric (flexion) mode. Each subject lay down in a supine position, with the elbow flexion-extension adapter adjusted to the semi-prone position, according to the body dimensions of each participant. The body was stabilized in the chair and strapped with Velcro to minimize movements other than the elbow flexion and extension. These adjustments were recorded to be repeated accurately in each subsequent visit. The exercise movement was performed with a joint range of motion from 0° to 90°, beginning with concentric elbow flexion, followed by eccentric elbow extension. The subjects performed 6 sets of 10 maximal voluntary contractions at a velocity of 30°·s⁻¹ in both the extension (active movement) and flexion (passive movement) phases, with a recovery period of 1 minute between sets. Verbal encouragement was given to ensure maximal resistance throughout each repetition.

2.5. Maximal Voluntary Contraction Measurement

During the familiarization visit, the dynamometer (Humac Norm, CSMi Medical Systems, Inc., Stoughton, MA, USA) was set up for each participant, and settings were recorded to ensure that participants were in the same position for each subsequent testing

visit. To measure maximal isometric strength, participants completed 4×3 s MVIC at a 70° angle with 30-s rest between each contraction. The higher MVIC value recorded for the four contractions was used for statistical analysis. This procedure was repeated at 24 h, 48 h, and 72 h after supplementation and EIMD protocol.

2.6. Blood Sample Analysis

Blood was drawn from the antecubital vein, collected in EDTA-containing tubes, and immediately centrifuged at 3000 g for 10 min at 4°C to separate the plasma before storage at -80°C for subsequent analysis. Blood samples were used to analyze plasma concentrations of reduced glutathione (GSH), malondialdehyde (MDA), myoglobin (Mb), and C-reactive protein (CRP).

As previously described, plasma GSH levels were quantified using a high-performance liquid chromatography (HPLC) system [25]. Briefly, blood samples were treated with N-ethylmaleimide and 15% tripotassium ethylenediaminetetraacetic acid. Then, vortexed to homogenize the tubes and centrifuged at room temperature at $14,000 \times g$ for 2 min. The supernatant was collected and used for analysis. The HPLC system was equipped with an analytical C18 column (L \times I.D. 15 cm \times 4.6 mm; Kromasil[®], Bohus, Sweden) and a photodiode array detector (SPD-M20A, Shimadzu[®], Kyoto, Japan) monitoring the wavelength at 265 nm. The run was performed isocratically at 1.1 mL/min with the mobile phase consisting of 0.25 % acetic acid (pH 3.1) and acetonitrile.

For plasma MDA levels, the HPLC system was performed as previously described [26]. In a microtube, 100 μL of the sample was mixed with 700 μL 1% ortho-phosphoric acid and vortexed. Subsequently, 200 μL of 42 mM 2-Thiobarbituric (TBA) was added, and the mixture was heated in a water bath for 60 min at 100°C and then cooled on ice. In total, 200 μL of the sample was transferred to another microtube containing 200 μL of sodium hydroxide:methanol (1:12) and immediately vortexed and centrifuged at $13,000 \times g$ for 3 min. The supernatant was used for analysis in HPLC. The HPLC system was equipped with an analytical C18 column (L \times I.D. 25 cm \times 4.6 mm; ACE 3), a guard C18 column (L \times I.D. 1 cm \times 4.6 mm; Nucleosil[®], Düren, Germany) and a photodiode array detector (SPD-M20A; Shimadzu[®], Kyoto, Japan) monitoring the wavelength at 532 nm. The run was performed isocratically at 0.5 mL/min with the mobile phase consisting of 10 mmol monopotassium phosphate (pH 6.8) and methanol.

Plasma concentrations of Mb and CRP were determined by a fluorescence immunoassay analyzer (Finecare Plus[®], Celer Biotecnologia SA., Belo Horizonte, Brazil) using specific testing strips for each analysis.

2.7. Delayed Onset Muscle Soreness (DOMS) Measurement

Muscle soreness was measured before and after 24 h, 48 h, and 72 h of the exercise-induced muscle damage protocol. Participants were asked to self-rate a 10-point-validated visual analog scale (VAS) indicating a line from 0 (no pain) to 10 (extreme pain) [27] during a passive elbow extension.

2.8. Statistical Analysis

An a priori power analysis was conducted (G*Power version 3.0.1) for a two-way repeated measure. Based on statical power ($1 - \beta$) of 0.80, an effect size of 0.25, and an overall level of significance of 0.05, at least twenty-four (twelve for each group) participants were needed to detect a statistical difference. Analysis of variance was used to identify differences in maximal voluntary contraction, plasma GSH, MDA, myoglobin, and CRP and perceived muscle soreness between MCO and PLA before and after 2 h, 24 h, 48 h, and 72 h of the exercise-induced muscle damage protocol. Multiple comparisons were conducted using the Bonferroni test when the F-ratios indicated a rejection of the null hypothesis. The significance α level was set at 0.05, and data were presented as means \pm standard deviation.

3. Results

3.1. Isometric Muscle Performance

A significant main effect for time ($p < 0.001$) was found for MVIC. Post hoc analysis revealed a significant decrease in MVIC at 24 h, 48 h, and 72 h following EIMD compared to pre-exercise values. No significant interaction effect regarding supplementation per time ($p = 0.270$) was observed for MVIC (Table 3 and Figure 2).

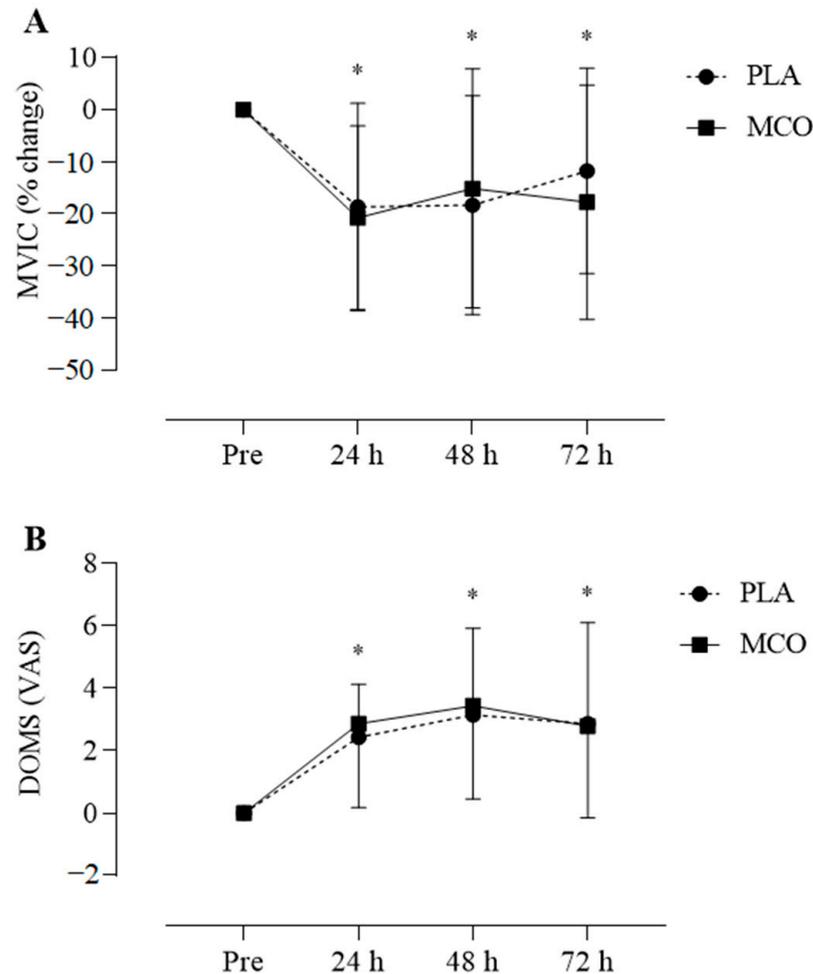


Figure 2. Changes in maximal voluntary isometric contractions (A) and delayed onset muscle soreness (B) on MCO (microencapsulated cocoa group) and PLA (placebo group) before (Pre) and 24 h, 48 h, and 72 h post-EIMD. * Denotes significant difference compared to pre-EIMD values ($p < 0.05$).

3.2. Blood Markers

No significant main effect for time was found for both plasma GSH ($p = 0.431$) and MDA ($p = 0.133$), and no significant interaction effect regarding supplementation per time ($p > 0.05$) was observed. A significant main effect for time was found for plasma myoglobin ($p < 0.001$). Post hoc analysis revealed a significant increase in plasma myoglobin at 2 h, 48 h, and 72 h following EIMD compared to pre-exercise values. No significant interaction effect between supplementation per time ($p = 0.270$) was observed for plasma myoglobin (Table 3 and Figure 3). A significant main effect for time ($p < 0.001$) was found for plasma CRP. Post hoc analysis revealed a significant increase in plasma CRP only at 2 h following EIMD compared to pre-exercise values ($p = 0.03$). No significant interaction effect between supplementation per time ($p = 0.960$) was observed for plasma CRP (Table 3 and Figure 3).

Table 3. Blood markers, isometric muscle performance, and muscle soreness before and following exercise-induced muscle damage (EIMD) in both placebo (PLA) and microencapsulated cocoa (MCO) conditions.

	PLA					MCO				
	Pre-EIMD	2 h	24 h	48 h	72 h	Pre-EIMD	2 h	24 h	48 h	72 h
Blood markers										
C-reactive protein (mg·L ⁻¹)	0.9 ± 0.4	2.1 ± 1.3 *	1.6 ± 1.4	1.4 ± 1.2	1.2 ± 1.1	1.0 ± 0.6	2.3 ± 1.6 *	1.5 ± 1.2	1.4 ± 1.5	1.2 ± 1.3
Malondialdehyde (μmol·L ⁻¹)	23.1 ± 9.8	24.3 ± 16.9	19.5 ± 10.4	24.6 ± 17.0	26.1 ± 11.1	26.5 ± 12.8	27.2 ± 11.1	28.0 ± 19.1	20.4 ± 8.1	20.8 ± 7.9
Myoglobin (ng·mL ⁻¹)	18.6 ± 3.3	36.4 ± 25.8 *	28.6 ± 20.8	84.9 ± 119.1 *	97.8 ± 130.8 *	19.4 ± 5.2	32.0 ± 17.1 *	29.1 ± 29.6	108.1 ± 129.1 *	120.6 ± 156.7 *
Reduced glutathione (μmol·L ⁻¹)	0.7 ± 0.4	0.8 ± 0.3	0.8 ± 0.3	0.8 ± 0.4	0.8 ± 0.3	0.8 ± 0.4	0.8 ± 0.3	0.7 ± 0.3	0.8 ± 0.4	0.8 ± 0.3
Isometric muscle performance										
MVIC (Nm)	54.4 ± 24.7	-	45.9 ± 27.0 *	45.9 ± 26.6 *	48.6 ± 26.1 *	53.4 ± 22.0	-	44.9 ± 24.8 *	48.4 ± 28.5 *	47.6 ± 29.0 *
MVIC (% change)	100 ± 0.0	-	82.2 ± 20.5 *	80.3 ± 22.5 *	87.9 ± 21.4 *	100 ± 0.0	-	79.3 ± 17.7 *	82.9 ± 23.9 *	80.7 ± 22.7 *
Muscle soreness										
DOMS (VAS)	0 ± 0	-	2 ± 2 *	3 ± 3 *	3 ± 3 *	0 ± 0	-	3 ± 2 *	3 ± 3 *	3 ± 3 *

Values are mean ± SD. DOMS = delayed onset muscle soreness; MVIC = maximal voluntary isometric contraction; VAS = visual analogic scale. The symbol * denotes significantly different from Pre-EIMD ($p < 0.05$).

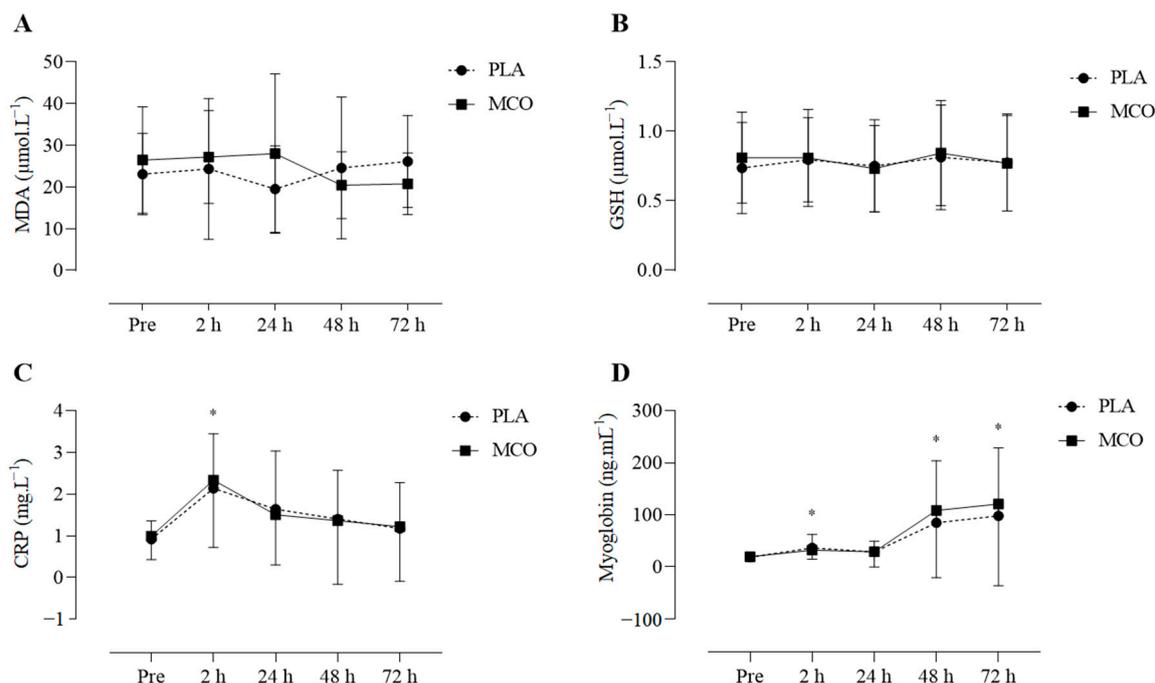


Figure 3. Changes in plasma concentrations of malondialdehyde (MDA) (A), reduced glutathione (GSH) (B), C-reactive protein (CRP) (C), and myoglobin (D) on MCO (microencapsulated cocoa group) and PLA (placebo group) before (Pre) and 2 h, 24 h, 48 h, and 72 h after post-EIMD. * Denotes significant difference compared to pre-EIMD values ($p < 0.05$).

3.3. Muscle Soreness

A significant main effect for time ($p < 0.001$) was found for DOMS. Post hoc analysis revealed a significant increase in DOMS at 24 h, 48 h, and 72 h following EIMD compared to pre-EIMD values. No significant interaction effect between supplementation and time ($p = 0.270$) was observed for DOMS (Table 3 and Figure 2).

4. Discussion

The key findings from the present study are that acute microencapsulated cocoa intake did not affect (1) isometric muscle strength recovery (MVIC), (2) muscle damage recovery (plasma myoglobin), (3) perceived muscle soreness (DOMS), (4) inflammation (plasma CRP); and (5) redox balance (plasma GSH and MDA) markers following the eccentric exercise protocol. These findings suggest that a single dose of microencapsulated cocoa (containing 75 mg of total flavonoids) does not improve muscle recovery or biomarkers of inflammation and oxidative stress following exercise-induced muscle damage in resistance-trained individuals.

The muscle damage caused by eccentric exercise stems at least partly from inflammation and excess reactive oxygen species (ROS) generation that leads to oxidative stress [28]. The accumulation of inflammatory cells (i.e., leukocytes, macrophages, and neutrophils) in the muscle tissue produces large amounts of ROS to lyse cellular debris and begin regeneration. However, it has been proposed that during this process, ROS may also induce lipid peroxidation in nearby healthy tissues [29]. Furthermore, a large amount of ROS produced has been demonstrated to impair calcium handling and sensitivity, resulting in reduced contractile force development [30–32]. Therefore, the increased inflammation and ROS production provoked by strenuous eccentric exercise may intensify muscle damage and at least partly explain why decrements in muscle function and increased muscle soreness can persist for several days after exercise [9].

Polyphenols from cocoa have been demonstrated to modulate inflammation supposedly by influencing signaling cascades via an alteration to eicosanoid production [33] and

reducing the activation of certain inflammatory transcription factors (e.g., nuclear factor kappa-beta) [34]. This may attenuate some symptoms caused by the EIMD, such as muscle soreness and decreases in force development [35,36].

In the present study, a single dose of microencapsulated cocoa did not promote significant changes in the oxidative stress (i.e., MDA and GSH) and inflammatory (i.e., CRP) markers in the days following EIMD. These observations agreed with Decroix et al. [37] study in that CF did not affect plasma MDA concentration after an exhaustive cycling time trial exercise. Wiswedel et al. [38] also found no significant difference in a single dose of CF on plasma MDA levels following cycling exercise in healthy, untrained men. On the other hand, Fraga et al. [39] observed decreases in plasma MDA after 14 days of supplementing a food containing cocoa flavanols (186 mg) in football players. Taub et al. [40] demonstrated an increased ratio of reduced versus oxidized glutathione and decreased protein carbonylation in untrained men after 3 months of supplementing with 175 mg of cocoa flavanols.

It is important to point out that the eccentric exercise protocol used in the present study did not increase the oxidative stress parameters analyzed in the participants. This may explain the lack of effect of microencapsulated cocoa on plasma GSH and MDA after the exercise-induced muscle damage protocol. Therefore, polyphenols may not effectively improve antioxidants' status in conditions where ROS production from exercise does not outweigh their neutralization due to an efficient endogenous antioxidant defense system. In such circumstances, it may not be possible to demonstrate an antioxidant effect after cacao supplementation. Furthermore, there is considerable variation across studies investigating plasma oxidative stress markers levels in response to strenuous exercise—with some studies demonstrating evidence of increase [39,41–43] and others finding no significant changes [22,40,44]. It is, therefore, difficult to make a strong conclusion about the effect of cocoa on the redox balance.

Exercise-induced muscle damage has been associated with increases in inflammatory markers, including c-reactive protein (CRP) [45], which typically increases for several hours following exercise and may persist for several days, depending on the severity of the damage [46]. Furthermore, exercise-induced inflammation has been associated with muscle function loss, suggesting that the acute inflammatory response plays a role in the recovery after exercise [47]. In the present study, the plasma levels of CRP increased 2 h following the exercise protocol, and the microencapsulated cocoa intake was not able to blunt the exercise-induced increases in plasma CRP. Currently, the only study using the EIMD protocol to investigate the effect of cocoa-based food on inflammation was conducted by Morgan et al. [22]. The authors did not observe significant differences between groups in IL-6 and CRP after performing 100 maximal leg extensions. The low dose (74 mg) of cocoa flavanols used in this study may be the main reason for the lack of significant effect. The limited number of studies demonstrating a significant reduction in inflammation following EIMD suggests no anti-inflammatory effect of cocoa, irrespective of delivery systems used to improve cocoa polyphenols bioavailability (i.e., food microencapsulation).

In the present study, muscle strength was negatively impacted by the EIMD protocol, with significant reductions in muscle force being evident at 24 h, 48 h, and 72 h following exercise. Although a previous study has found that cocoa supplementation enhances muscle function as evaluated by improved recovery of countermovement jump height [22], a single dose of microencapsulated cocoa (75 mg of total flavonoids) did not improve MIVC after 24 h, 48 h, and 72 h of the eccentric exercise. Our observation corroborates with other studies that investigated the effects of cocoa supplementation on exercise-induced changes in maximal voluntary contraction [22,44,48,49], besides one study [49] which found large effect sizes in MVC after an acute high dose of cocoa (1245 mg) compared to the control at 24 and 48 h post-exercise.

Myoglobin is released after strenuous exercise due to the degradation of protein structures within the muscle. Therefore, myoglobin has been used as a useful biochemical marker for monitoring muscle damage after EIMD [50]. Microencapsulated cocoa sup-

plementation did not affect the exercise-induced increases in the muscle damage marker myoglobin (Mb). Furthermore, muscle soreness increased following EIMD and persisted until 72 h post-EIMD, probably caused by the microtrauma of myofibers. However, microencapsulated cocoa was not able to reduce DOMS, corroborating with the existing literature demonstrating that cocoa polyphenols did not attenuate the exercise-induced DOMS [22,44,48,49].

In conclusion, this study demonstrated that microencapsulated cocoa (containing 75 mg of total flavonoids) was not enough to promote anti-inflammatory and antioxidant effects and did not speed muscle strength recovery after exercise-induced muscle damage in healthy and physically active individuals, at least when consumed in a single dose. Long-term studies with higher polyphenol doses are warranted to investigate whether food encapsulation may be a valuable technological procedure to ensure proper nutrient delivery and biological effects.

Experimental Considerations

Experimental variation across studies may, in part, explain the lack of significant effect and/or differences between studies, such as the type of exercise performed (i.e., resistance exercise versus cycling), frequency of exercise stimulus (i.e., acute versus chronic), the training status of participants (i.e., sedentary versus physically active), and the use of a variety of biomarkers to detect oxidative stress, inflammation, and muscle damage. It is also possible that the polyphenol dose (i.e., 75 mg of total flavonoids) and the delivery system (i.e., microencapsulation of cocoa using maltodextrin to protect the active ingredient) provided in our study had been not sufficient to induce antioxidant or anti-inflammatory effects. Furthermore, the elbow flexors were relatively refractory to muscle damage (participants had an average of 20% reduction in isometric strength), which may contribute to the lack of polyphenol effects in this population. Also, we cannot rule out a possible repeated bout effect, which may have provoked muscle adaptations and protected the participants against muscle damage from the subsequent eccentric exercise. However, we provided the participants with a 1-month washout period between visits in order to eliminate the lingering effects of the prior exercise bout that might confound the results.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in this study.

Data Availability Statement: Data are contained within the article.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

CF	Cocoa Flavanol
CRP	C-reactive protein
DOMS	Delayed onset muscle soreness
EIMD	Exercise-induced muscle damage
GSH	Reduced glutathione
HPLC	High-performance liquid chromatography
Mb	Myoglobin
MCO	Microencapsulated cocoa
MDA	Malondialdehyde
MVIC	Maximal voluntary isometric contractions
PLA	Placebo
ROS	Reactive oxygen species
TBA	2-Thiobarbituric
VAS	Visual analogic scale

References

- Clarkson, P.M.; Hubal, M.J. Exercise-induced muscle damage in humans. *Am. J. Phys. Med. Rehabil.* **2002**, *81* (Suppl. 11), S52–S69. [[CrossRef](#)] [[PubMed](#)]
- Owens, D.J.; Twist, C.; Cobley, J.N.; Howatson, G.; Close, G.L. Exercise-induced muscle damage: What is it, what causes it and what are the nutritional solutions? *Eur. J. Sport Sci.* **2019**, *2*, 71–85. [[CrossRef](#)] [[PubMed](#)]
- Stožer, A.; Vodopivec, P.; Bombek, L.K. Pathophysiology of exercise-induced muscle damage and its structural, functional, metabolic, and clinical consequences. *Physiol. Res.* **2020**, *26*, 565–598. [[CrossRef](#)] [[PubMed](#)]
- Carey, C.C.; Lucey, A.; Doyle, L. Flavonoid Containing Polyphenol Consumption and Recovery from Exercise-Induced Muscle Damage: A Systematic Review and Meta-Analysis. *Sports Med.* **2021**, *9*, 1293–1316. [[CrossRef](#)] [[PubMed](#)]
- Sorrenti, V.; Fortinguerra, S.; Caudullo, G.; Buriani, A. Deciphering the role of polyphenols in sports performance: From nutritional genomics to the gut microbiota toward phytonutritional epigenomics. *Nutrients* **2020**, *29*, 1265. [[CrossRef](#)] [[PubMed](#)]
- Bowtell, J.; Kelly, V. Fruit-Derived Polyphenol Supplementation for Athlete Recovery and Performance. *Sports Med.* **2019**, *22*, 3–23. [[CrossRef](#)] [[PubMed](#)]
- Marika, M.; Scoditti, E.; Carluccio, M.A.; Kaltsatou, A.; Cicchella, A. Effect of cocoa products and its polyphenolic constituents on exercise performance and exercise-induced muscle damage and inflammation: A review of clinical trials. *Nutrients* **2019**, *28*, 1471.
- Corr, L.D.; Field, A.; Pufal, D.; Clifford, T.; Harper, L.D.; Naughton, R.J. The effects of cocoa flavanols on indices of muscle recovery and exercise performance: A narrative review. *BMC Sports Sci. Med. Rehabilitation.* **2021**, *14*, 90. [[CrossRef](#)]
- Steinbacher, P.; Eckl, P. Impact of oxidative stress on exercising skeletal muscle. *Biomolecules* **2015**, *5*, 356–377. [[CrossRef](#)]
- Manach, C.; Williamson, G.; Morand, C.; Scalbert, A.; Rémésy, C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am. J. Clin. Nutr.* **2005**, *81* (Suppl. 1), 230S–242S. [[CrossRef](#)]
- D'Archivio, M.; Filesi, C.; Vari, R.; Scazzocchio, B.; Masella, R. Bioavailability of the polyphenols: Status and controversies. *Int. J. Mol. Sci.* **2010**, *31*, 1321–1342. [[CrossRef](#)] [[PubMed](#)]
- Bohn, T.; McDougall, G.J.; Alegría, A.; Alminger, M.; Arrigoni, E.; Aura, A.M.; Brito, C.; Cilla, A.; El, S.; Karakaya, S.; et al. Mind the gap—deficits in our knowledge of aspects impacting the bioavailability of phytochemicals and their metabolites—a position paper focusing on carotenoids and polyphenols. *Mol. Nutr. Food Res.* **2015**, *3*, 1307–1323. [[CrossRef](#)] [[PubMed](#)]
- Bento-Silva, A.; Koistinen, V.M.; Mena, P.; Bronze, M.R.; Hanhineva, K.; Sahlstrøm, S.; Sahlstrøm, S.; Kitrytė, V.; Moco, S.; Aura, A.-M.; et al. Factors affecting intake, metabolism and health benefits of phenolic acids: Do we understand individual variability? *Eur. J. Nutr.* **2020**, *21*, 1275–1293. [[CrossRef](#)]
- Landberg, R.; Manach, C.; Kerckhof, F.M.; Minihane, A.M.; Saleh, R.N.M.; De Roos, B.; Tomas-Barberan, F.; Morand, C.; Van de Wiele, T. Future prospects for dissecting inter-individual variability in the absorption, distribution and elimination of plant bioactives of relevance for cardiometabolic endpoints. *Eur. J. Nutr.* **2019**, *23*, 21–36. [[CrossRef](#)] [[PubMed](#)]
- Mena, P.; Bresciani, L.; Brindani, N.; Ludwig, I.A.; Pereira-Caro, G.; Angelino, D.; Llorach, R.; Calani, L.; Brighenti, F.; Clifford, M.N.; et al. Phenyl- γ -valerolactones and phenylvaleric acids, the main colonic metabolites of flavan-3-ols: Synthesis, analysis, bioavailability, and bioactivity. *Nat. Prod. Rep.* **2019**, *36*, 714–752. [[CrossRef](#)] [[PubMed](#)]
- Polia, F.; Pastor-Belda, M.; Martínez-Blázquez, A.; Horcajada, M.N.; Tomás-Barberán, F.A.; García-Villalba, R. Technological and Biotechnological Processes To Enhance the Bioavailability of Dietary (Poly)phenols in Humans. *J. Agric. Food Chem.* **2022**, *23*, 2092–2107. [[CrossRef](#)] [[PubMed](#)]
- Calderón-Oliver, M.; Ponce-Alquicira, E. The Role of Microencapsulation in Food Application. *Molecules* **2022**, *23*, 1499. [[CrossRef](#)]
- Fang, Z.; Bhandari, B. Encapsulation of polyphenols—A review. *Trends Food Sci. Technol.* **2010**, *21*, 510–523. [[CrossRef](#)]
- Vitaglione, P.; Barone Lumaga, R.; Ferracane, R.; Sellitto, S.; Morelló, J.R.; Reguant Miranda, J.; Shimoni, E.; Fogliano, V. Human bioavailability of flavanols and phenolic acids from cocoa-nut creams enriched with free or microencapsulated cocoa polyphenols. *Br. J. Nutr.* **2013**, *28*, 1832–1843. [[CrossRef](#)]

20. Chen, T.C. Variability in muscle damage after eccentric exercise and the repeated bout effect. *Res. Q. Exerc. Sport* **2006**, *77*, 362–371. [[CrossRef](#)]
21. Sansone, R.; Ottaviani, J.I.; Rodriguez-Mateos, A.; Heinen, Y.; Noske, D.; Spencer, J.P.; Crozier, A.; Merx, M.W.; Kelm, M.; Schroeter, H.; et al. Methylxanthines enhance the effects of cocoa flavanols on cardiovascular function: Randomized, double-masked controlled studies. *Am. J. Clin. Nutr.* **2017**, *105*, 352–360. [[CrossRef](#)] [[PubMed](#)]
22. Morgan, P.T.; Wollman, P.M.; Jackman, S.R.; Bowtell, J.L. Flavanol-rich cacao mucilage juice enhances recovery of power but not strength from intensive exercise in healthy, young men. *Sports* **2018**, *6*, 159. [[CrossRef](#)] [[PubMed](#)]
23. Sánchez-Rangel, J.C.; Benavides, J.; Heredia, J.B.; Cisneros-Zevallos, L.; Jacobo-Velázquez, D.A. The Folin-Ciocalteu assay revisited: Improvement of its specificity for total phenolic content determination. *Anal. Methods* **2013**, *5*, 5990–5999. [[CrossRef](#)]
24. Wabaidur, S.M.; Obbed, M.S.; Alothman, Z.A.; Alfari, N.A.; Badjah-Hadj-ahmed, A.Y.; Siddiqui, M.R.; Altamimi, J.Z.; Aldayel, T.S. Total phenolic acids and flavonoid contents determination in yemeni honey of various floral sources: Folin-ciocalteu and spectrophotometric approach. *Food Sci. Technol.* **2020**, *40*, 647–652. [[CrossRef](#)]
25. Giustarini, D.; Dalle-Donne, I.; Milzani, A.; Fanti, P.; Rossi, R. Analysis of GSH and GSSG after derivatization with N-ethylmaleimide. *Nat. Protoc.* **2013**, *1*, 1660–1669. [[CrossRef](#)] [[PubMed](#)]
26. Nielsen, F.; Mikkelsen, B.B.; Nielsen, J.B.; Andersen, H.R.; Grandjean, P. Plasma malondialdehyde as biomarker for oxidative stress: Reference interval and effects of life-style factors. *Clin. Chem.* **1997**, *43*, 1209–1214. [[CrossRef](#)]
27. Carlsson, A.M. Assessment of chronic pain. I. Aspects of the reliability and validity of the visual analogue scale. *Pain* **1983**, *16*, 87–101. [[CrossRef](#)]
28. Gomez-Cabrera, M.C.; Carretero, A.; Millan-Domingo, F.; Garcia-Dominguez, E.; Correas, A.G.; Olasso-Gonzalez, G.; Vina, J. Redox-related biomarkers in physical exercise. *Redox Biol.* **2021**, *42*, 101956. [[CrossRef](#)]
29. Morillas-Ruiz, J.M.; Villegas García, J.A.; López, F.J.; Vidal-Guevara, M.L.; Zafrilla, P. Effects of polyphenolic antioxidants on exercise-induced oxidative stress. *Clin. Nutr.* **2006**, *25*, 444–453. [[CrossRef](#)]
30. Reid, M.B. Redox interventions to increase exercise performance. *J. Physiol.* **2016**, *15*, 5125–5133. [[CrossRef](#)]
31. Moopanar, T.R.; Allen, D.G. Reactive oxygen species reduce myofibrillar Ca²⁺ sensitivity in fatiguing mouse skeletal muscle at 37 °C. *J. Physiol.* **2005**, *564*, 189–199. [[CrossRef](#)] [[PubMed](#)]
32. Powers, S.K.; Jackson, M.J. Exercise-induced oxidative stress: Cellular mechanisms and impact on muscle force production. *Physiol. Rev.* **2008**, *88*, 1243–1276. [[CrossRef](#)] [[PubMed](#)]
33. Schramm, D.D.; Wang, J.F.; Holt, R.R.; Ensunsa, J.L.; Gonsalves, J.L.; Lazarus, S.A.; Schmitz, H.H.; German, J.B.; Keen, C.L. Chocolate procyanidins decrease the leukotriene-prostacyclin ratio in humans and human aortic endothelial cells. *Am. J. Clin. Nutr.* **2001**, *1*, 36–40. [[CrossRef](#)]
34. Vázquez-Agell, M.; Urpi-Sarda, M.; Sacanella, E.; Camino-López, S.; Chiva-Blanch, G.; Llorente-Cortés, V.; Tobias, E.; Roura, E.; Andres-Lacueva, C.; Lamuela-Raventos, R.M.; et al. Cocoa consumption reduces NF-κB activation in peripheral blood mononuclear cells in humans. *Nutr. Metab. Cardiovasc. Dis.* **2013**, *23*, 257–263. [[CrossRef](#)] [[PubMed](#)]
35. Decroix, L.; Soares, D.D.; Meeusen, R.; Heyman, E.; Tonoli, C. Cocoa Flavanol Supplementation and Exercise: A Systematic Review. *Sports Med.* **2018**, *3*, 867–892. [[CrossRef](#)] [[PubMed](#)]
36. Vlachojannis, J.; Erne, P.; Zimmermann, B.; Chrubasik-Hausmann, S. The Impact of Cocoa Flavanols on Cardiovascular Health. *Phytother. Res.* **2016**, *30*, 1641–1657. [[CrossRef](#)] [[PubMed](#)]
37. Decroix, L.; Tonoli, C.; Soares, D.D.; Descat, A.; Drittij-Reijnders, M.J.; Weseler, A.R.; Bast, A.; Stahl, W.; Heyman, E.; Meeusen, R. Acute cocoa flavanols intake has minimal effects on exercise-induced oxidative stress and nitric oxide production in healthy cyclists: A randomized controlled trial. *J. Int. Soc. Sports Nutr.* **2017**, *14*, 28. [[CrossRef](#)] [[PubMed](#)]
38. Wiswedel, I.; Hirsch, D.; Kropf, S.; Gruening, M.; Pfister, E.; Schewe, T.; Sies, H. Flavanol-rich cocoa drink lowers plasma F₂-isoprostane concentrations in humans. *Free Radic. Biol. Med.* **2004**, *37*, 411–421. [[CrossRef](#)]
39. Fraga, C.G.; Actis-Goretta, L.; Ottaviani, J.I.; Carrasquedo, F.; Lotito, S.B.; Lazarus, S.; Schmitz, H.H.; Keen, C.L. Regular consumption of a flavanol-rich chocolate can improve oxidant stress in young soccer players. *Clin. Dev. Immunol.* **2005**, *12*, 11–17. [[CrossRef](#)]
40. Taub, P.R.; Ramirez-Sanchez, I.; Patel, M.; Higginbotham, E.; Moreno-Ulloa, A.; Román-Pintos, L.M.; Phillips, P.; Perkins, G.; Ceballos, G.; Villarreal, F. Beneficial effects of dark chocolate on exercise capacity in sedentary subjects: Underlying mechanisms. A double blind, randomized, placebo controlled trial. *Food Funct.* **2016**, *7*, 3686–3693. [[CrossRef](#)]
41. Allgrove, J.; Farrell, E.; Gleeson, M.; Williamson, G.; Cooper, K. Regular dark chocolate consumption's reduction of oxidative stress and increase of free-fatty-acid mobilization in response to prolonged cycling. *Int. J. Sport Nutr. Exerc. Metab.* **2011**, *21*, 113–123. [[CrossRef](#)] [[PubMed](#)]
42. Davison, G.; Callister, R.; Williamson, G.; Cooper, K.A.; Gleeson, M. The effect of acute pre-exercise dark chocolate consumption on plasma antioxidant status, oxidative stress and immunoendocrine responses to prolonged exercise. *Eur. J. Nutr.* **2012**, *5*, 69–79. [[CrossRef](#)] [[PubMed](#)]
43. Decroix, L.; Tonoli, C.; Lespagnol, E.; Balestra, C.; Descat, A.; Drittij-Reijnders, M.J.; Blackwell, J.R.; Stahl, W.; Jones, A.M.; Weseler, A.R.; et al. One-week cocoa flavanol intake increases prefrontal cortex oxygenation at rest and during moderate-intensity exercise in normoxia and hypoxia. *J. Appl. Physiol.* **2018**, *1*, 8–18. [[CrossRef](#)] [[PubMed](#)]

44. De Carvalho, F.G.; Fisher, M.G.; Thornley, T.T.; Roemer, K.; Pritchett, R.; de Freitas, E.C.; Pritchett, K. Cocoa flavanol effects on markers of oxidative stress and recovery after muscle damage protocol in elite rugby players. *Nutrition* **2019**, *62*, 47–51. [[CrossRef](#)] [[PubMed](#)]
45. Donia, T.; Khamis, A. Management of oxidative stress and inflammation in cardiovascular diseases: Mechanisms and challenges. *Environ. Sci. Pollut. Res.* **2021**, *8*, 34121–34153. [[CrossRef](#)] [[PubMed](#)]
46. Dimitriou, L.; Hill, J.A.; Jehnali, A.; Dunbar, J.; Brouner, J.; McHugh, M.P.; Howatson, G. Influence of a montmorency cherry juice blend on indices of exercise-induced stress and upper respiratory tract symptoms following marathon running—A pilot investigation. *J. Int. Soc. Sports Nutr.* **2015**, *12*, 22. [[CrossRef](#)]
47. Phillips, T.; Childs, A.C.; Dreon, D.M.; Phinney, S.; Leeuwenburgh, C. A Dietary Supplement Attenuates IL-6 and CRP after Eccentric Exercise in Untrained Males. *Med. Sci. Sports Exerc.* **2003**, *35*, 2032–2037. [[CrossRef](#)]
48. Peschek, K.; Pritchett, R.; Bergman, E.; Pritchett, K. The effects of acute post exercise consumption of two cocoa-based beverages with varying flavanol content on indices of muscle recovery following downhill treadmill running. *Nutrients* **2013**, *20*, 50–62. [[CrossRef](#)]
49. Corr, L.D.; Field, A.; Pufal, D.; Killey, J.; Clifford, T.; Harper, L.D.; Naughton, R.J. Acute Consumption of Varied Doses of Cocoa Flavanols Does Not Influence Exercise-Induced Muscle Damage. *Int. J. Sport Nutr. Exerc. Metab.* **2020**, *1*, 338–344. [[CrossRef](#)]
50. Brancaccio, P.; Lippi, G.; Maffulli, N. Biochemical markers of muscular damage. *Clin. Chem. Lab. Med.* **2010**, *1*, 757–767. [[CrossRef](#)]

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