Effect of microencapsulated cocoa intake on muscle recovery, inflammation, and oxidative stress after exercise-induced muscle damage

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Abstract

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

1. Introduction

The appropriate recovery after exercise-induced muscle damage (EIMD) is essential for athletes from all sports modalities since muscle damage can initiate the impairment of various activities. EIMD usually occurs following a strenuous exercise involving repetitive eccentric contractions (muscle lengthening) and is associated with some symptoms such as inflammation, delayed onset muscle soreness (DOMS), increased 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 impaired. Therefore, the use of nutritional strategies has been investigated to assist in the process of muscle repair and recovery.

The intake of polyphenols, compounds commonly found in fruits, has been investigated as one of the nutritional strategies to improve muscle recovery after EIMD (4–6). Of these compounds, flavanols from cocoa have been suggested to speed muscle recovery by modulating inflammatory response after EIMD (7, 8). However, evidence remains to show a clear effect of cocoa polyphenols on overall post-exercise recovery parameters (7).

It is important to point out that the effects of phenolic compounds on muscle recovery following EIMD may depend upon their bioaccessibility (i.e., a fraction of an ingested compound that is available for absorption in the gut) and, hence their bioavailability (i.e., a fraction of an ingested compound that reaches the systemic circulation and tissues to exert its biological action) (9–13). Cocoa polyphenols are subject to extensive metabolism once introduced into the gastrointestinal tract (14), and most of them yield phase II conjugated derivates and cannot reach the systemic circulation in their natural form, which may compromise their bioavailability and hence, their potential action at the target tissue (14). 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 (15). In this process, microspheres are formed in which the bioactive compounds present in food are protected by a capsule (16). Evidence demonstrates enhanced flavanol delivered into the gut from microencapsulated cocoa polyphenols (17, 18). Therefore, microencapsulated cocoa would 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.

Since there are conflicting results on 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 was to investigate the effect of a single dose of microencapsulated cocoa intake 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 the 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 the 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).

Table 1
Physical characteristics of the participants

Characteristic	Value
Age (years)	26 ± 5
Weight (kg)	66.32 ± 11.57
Height (cm)	1.67 ± 0.08
BMI (kg/m2)	23.58 ± 3.19
Resistance training experience (years)	4 ± 2
Values are expressed as mean ± SD	

2.2. Experimental Design

The study was a randomized, double-blind, and placebo-controlled trial. Participants were randomized into a microencapsulated cocoa (MCO) or placebo (PLA). 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 (19).

After a 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 2h, 24h, 48h, and 72h post-EIMD. Participants returned to the laboratory after a 1-month washout period and followed the same procedures described above with the second intervention (Fig. 1).

2.3. Nutritional supplementation and dietary control

Participants consumed 25 g of microencapsulated cocoa (containing 75 mg of total flavonoids) or the same amount of milk chocolate as a placebo (PLA, containing 32.5 mg of total flavonoids), which were diluted in 200 mL of water and offered to the participants 2 h before the exercise-induce muscle damage protocol. Evidence has shown that a greater plasma concentration of cocoa flavonoid metabolites is observed 90–120 minutes after cocoa intake (7, 20).

For the microencapsulation process, maltodextrin was mixed with 100% cocoa powder (Nestlé®) (1:1) and diluted in water. The mixture was spray dried using a mini spray dryer (Model B-290, Büchi) with 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 milk chocolate were evaluated as described above (20, 21). In microencapsulated cocoa, the content of total phenolics and flavonoids were respectively 961 mg of gallic acid equivalents/100 g and 300 mg of quercetin/100 g. The total phenolic and flavonoid content for milk chocolate was 144 mg of gallic acid equivalents/100 g and 130 mg of quercetin/100 g. The nutritional composition of the microencapsulated cocoa and milk chocolate is found in Table 2.

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

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	130	300	-	-
Total polyphenols	144	961	-	-
*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 products, 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, 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 $30°\cdot s^{-1}$ in both the extension (active movement) and flexion (passive movement) phases, with a recovery period of 1 min between sets. To ensure maximal resistance throughout each repetition, verbal encouragement was given.

2.5. Maximal voluntary contraction measurement

During the familiarization visit, the dynamometer (Humac Norm, CSMi Medical Systems, Inc., 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 x 3s 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 3,000g 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).

Plasma GSH levels were quantified using a high-performance liquid chromatography (HPLC) system as previously described (21). 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 g for 2 minutes. The supernatant was collected and used for analysis. The HPLC system was equipped with an analytical C18 column (L x I.D. 15 cm x 4.6 mm; Kromasil®) and a photodiode array detector (SPD-M20A, Shimadzu®) 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 (22). 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 minutes at 100°C and then cooled on ice. 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 g for 3 minutes. The supernatant was used to analysis in HPLC. The HPLC system was equipped with an analytical C18 column (L x I.D. 25 cm x 4.6 mm; ACE 3), a guard C18 column (L x I.D. 1 cm x 4.6 mm; Nucleosil®) and a photodiode array detector (SPD-M20A; Shimadzu®) 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 24h, 48h, and 72h of the exercise-induced muscle damage protocol. Participants were asked to self-rate a 10- point-validated visual analog scale (VAS) indicating on a line from 0 (no pain) to 10 (extreme pain) (23), 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 − β) of 0.80, an effect size of 0.25 and overall level of significance of 0.05, at least twenty-four (twelve 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 2h, 24h, 48h, and 72h 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 ± 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 Fig. 2).

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

3.2. Blood markers

No significant main effect for time was found for both plasma GSH ($p = 0.431$) and MDA ($p = 0.133$), as well 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 Fig. 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 Fig. 3).

3.4. 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 Fig. 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 (24). 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 (25). Furthermore, a large amount of ROS produced has been demonstrated to impair calcium handling and sensitivity resulting in reduced contractile force development (26–28). 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 (29).

Polyphenols from cocoa have been demonstrated to modulate inflammation supposedly by influencing signaling cascades via an alteration to eicosanoid production (30) and reducing the activation of certain inflammatory transcription factors (e.g., nuclear factor kappa-beta) (31). This may attenuate some symptoms caused by the EIMD, such as muscle soreness and decreases in force development (32, 33).

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. (33) study in that CF did not affect plasma MDA concentration after an exhaustive cycling time trial exercise. Wiswedel et al. (34) also found no significant difference of a single dose of CF on plasma MDA levels following cycling exercise in healthy untrained men. On the other hand, Fraga et al. (35) observed decreases in plasma MDA after 14 days of supplementing a food containing cocoa flavanols (186 mg) in football players, and Taub et al. (37) demonstrated an increased ratio of reduced versus oxidized glutathione and decreased protein carbonylation after 3 months supplementing with 175 mg cocoa flavanols in untrained men.

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, it seems that 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 large variation across studies investigating plasma oxidative stress markers levels in response to strenuous exercise, with some studies demonstrating evidence of increase (36, 38–40) and others finding no significant changes (37, 41, 42), becoming it 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) (43), which are typically increased for several hours following exercise and may persist for several days depending on the severity of the damage (44). Furthermore, the exercise-induced inflammation has been associated with muscle function loss, suggesting the acute inflammatory response plays a role in the recovery after exercise (45). In the present study, the plasma levels of CRP increased 2h 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. (42). 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 been evident at 24h, 48h, and 72h following exercise. Although a previous study has found that cocoa supplementation enhances muscle function as evaluated by improved recovery of countermovement jump height (42), a single dose of microencapsulated cocoa (75 mg of total flavonoids) did not improve MIVC after 24h, 48h, and 72h 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 (41, 42, 46, 47), besides one study (47) has found large effect sizes in MVC after as 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 (48). Microencapsulated cocoa supplementation did not affect the exercise-induced increases in the muscle damage marker myoglobin (Mb). Furthermore, muscle soreness increased following EIMD and persisted until 72h post-EIMD, probably caused by the microtrauma of myofibers and subsequent inflammation. 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 (41, 42, 46, 47).

In conclusion, this study demonstrated that microencapsulated cocoa was not enough to promote anti-inflammatory and antioxidant effect 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 are warranted to investigate whether food encapsulation may be a useful technological procedure to ensure proper nutrient delivery and biological effect.

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.

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

Declarations

Author contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

Ethical approvement

The study was 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).

Conflict of interest

The authors declare that there is no conflict of interest with the content of the present study.

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Figures

Figure 1.
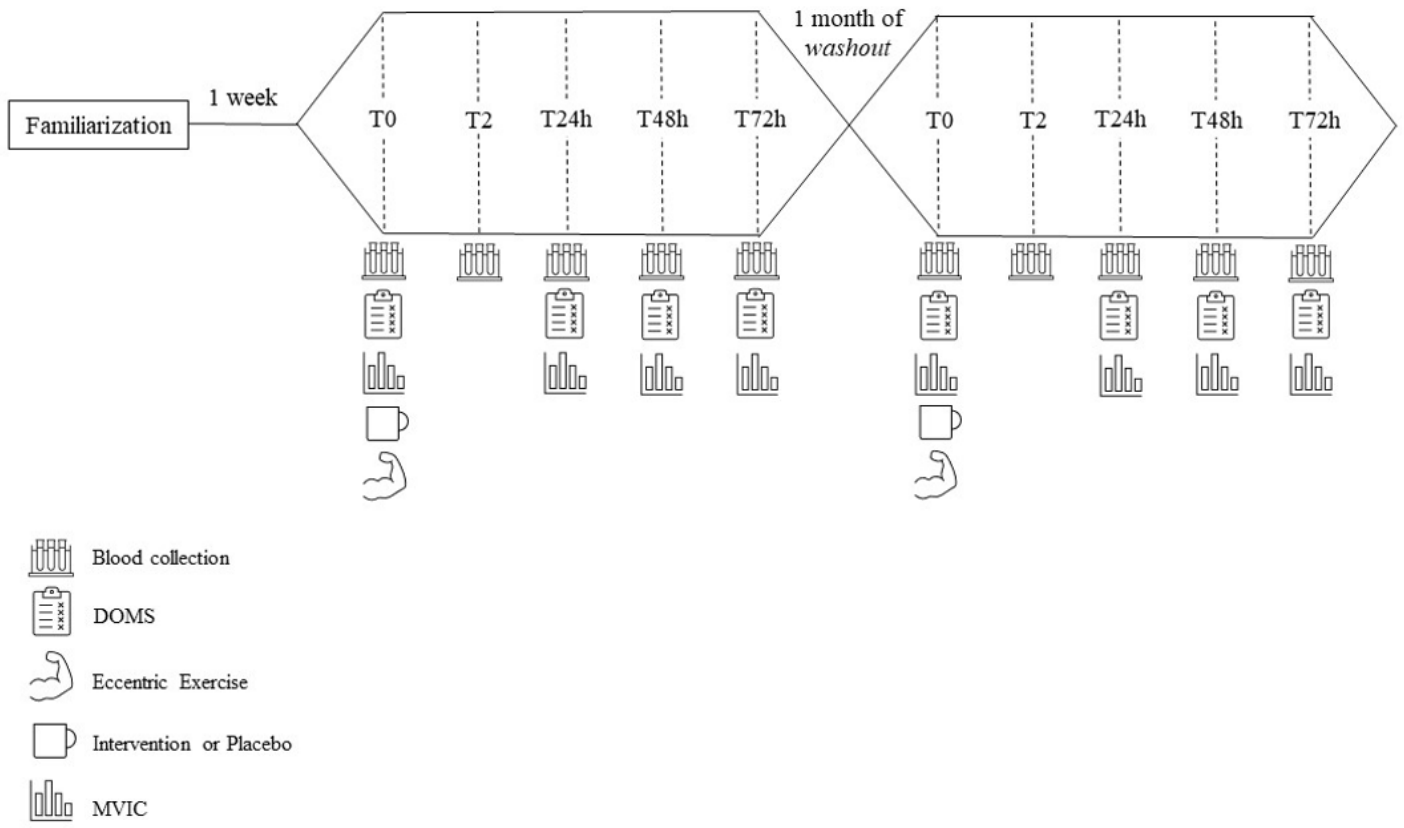
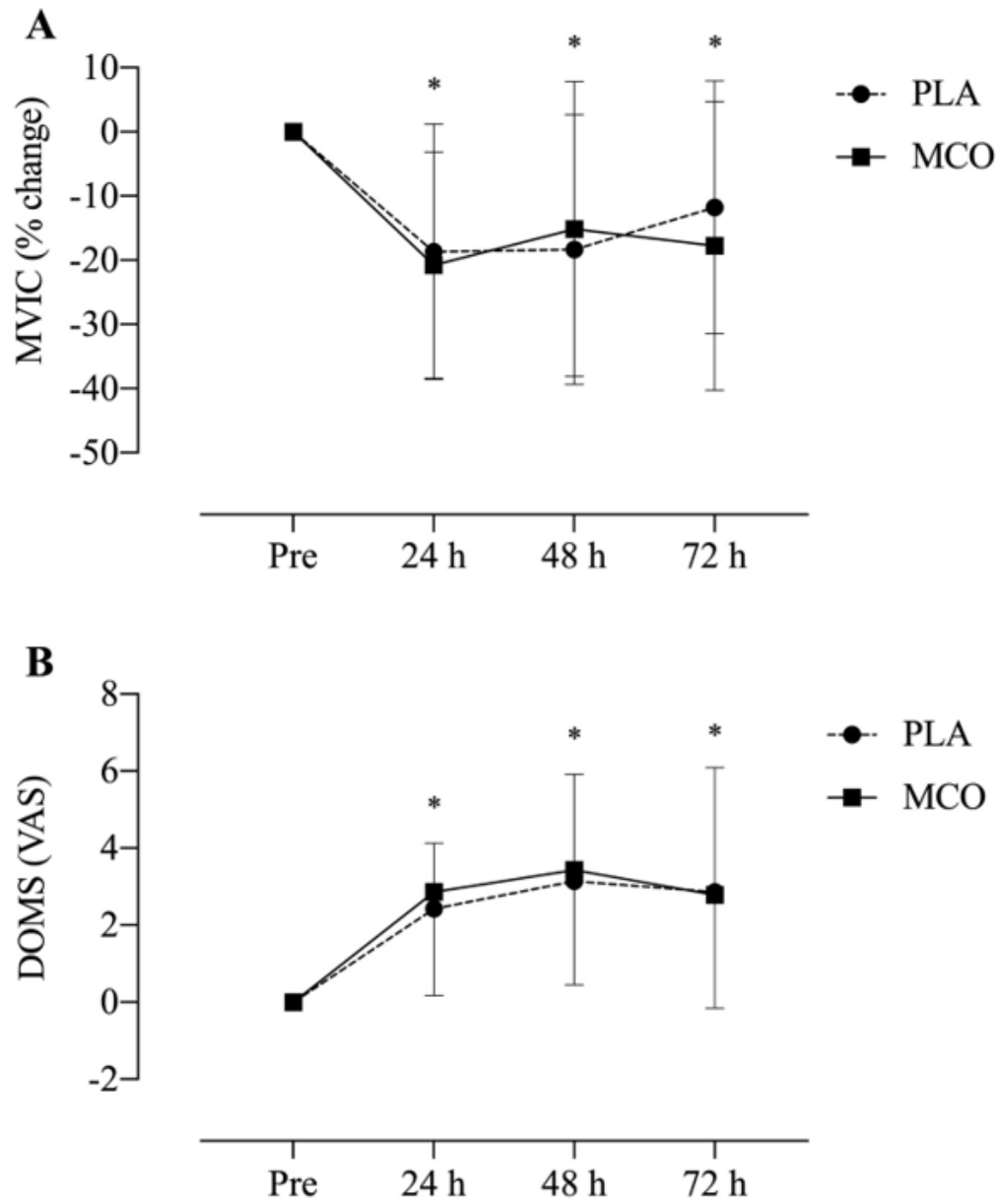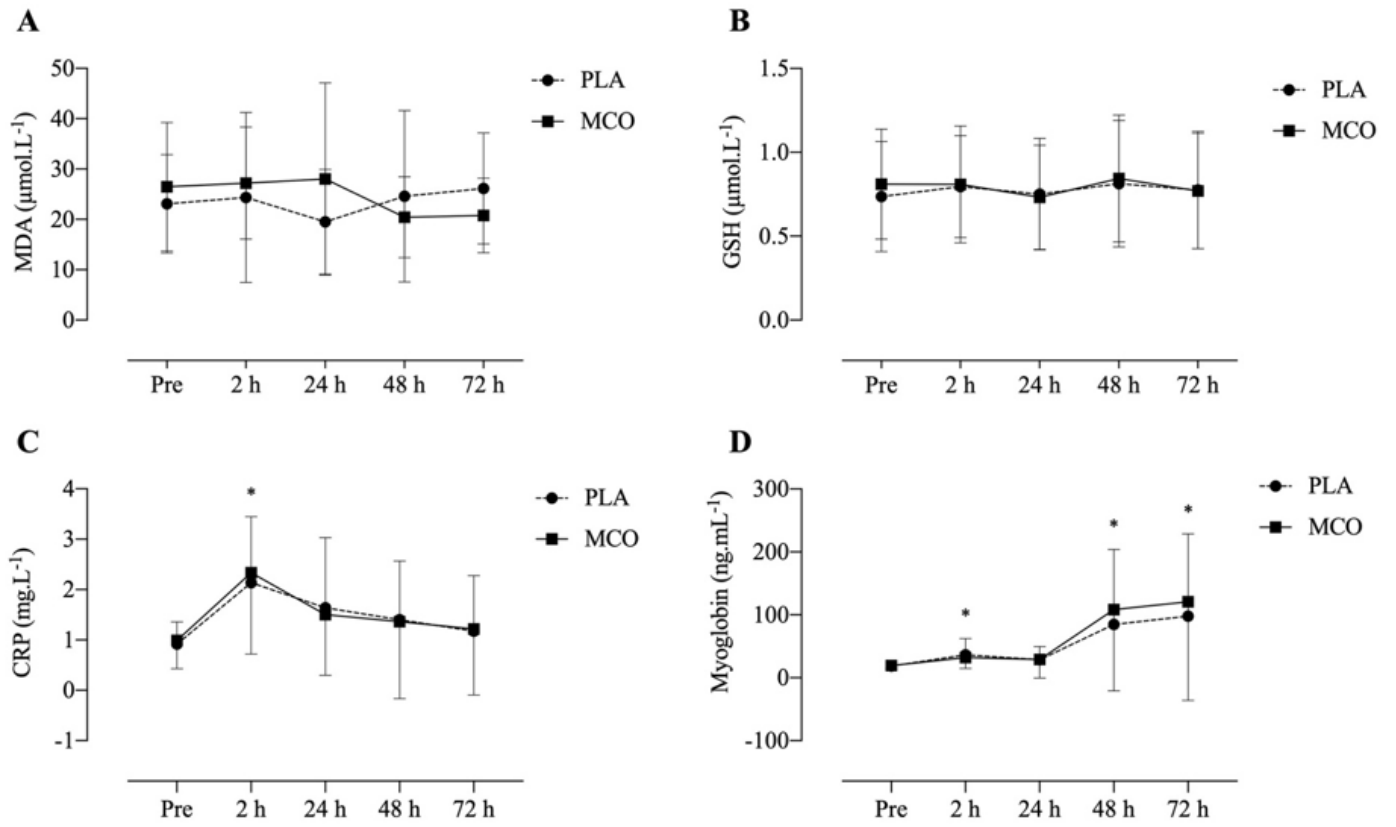Figure 1

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Figure 2.

Figure 2

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Figure 3.

Figure 3

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