

Original Research Article

Association between serum biomarkers of muscle damage after isokinetic eccentric exercise in trained individuals

Abstract

Eccentric exercise is well-known to induce muscle damage, and biomarkers may be related to the magnitude of impact on muscle force and muscle soreness after exercise. However, this phenomenon still needs to be better investigated. The present study aimed to analyze if a correlation between muscle force, soreness and biomarkers after eccentric exercise exists in healthy, trained individuals. Eleven volunteers with previous resistance training performed 6 x 10 maximal isokinetic eccentric contractions of the elbow flexors. Peak torque was measured by maximal voluntary isometric contractions (MVIC) and muscle soreness by visual analogic scale (VAS) before, 24h, 48h, and 72h after the isokinetic eccentric exercise protocol. A fluorescence immunoassay analyzer determined Myoglobin and C-reactive protein levels. The MVIC significantly decreased while muscle soreness increased 24h, 48h, and 72h post eccentric exercise. Myoglobin significantly increased 48h and 72h after exercise. Muscle soreness was inversely correlated with MVIC 24h, 48h, and 72h, and positively correlated with myoglobin 48h and 72h after eccentric exercise. The myoglobin was not correlated with muscle force. No significant correlation between C-reactive protein and muscle soreness and MVIC was found. In conclusion, our data suggest that myoglobin could be a valuable biomarker to be assessed alongside exercise induced-muscle soreness.

Keywords: Eccentric exercise; Inflammation; Muscle recovery; Muscle soreness; Myoglobin.

1. Introduction

Exercise-induced muscle damage (EIMD) usually occurs following a strenuous exercise involving unusual exercises and/or repetitive eccentric contraction (muscle lengthening) during exercise. Eccentric muscle contraction is a well-known phase of muscle contraction that induces more robust muscle damage¹. Muscle damage involves myofibrillar ruptures and

is associated with inflammatory response and abnormal production of reactive oxygen species (oxidative stress), resulting in muscle soreness and reduced muscle force and power after a session of high-intensity exercise. In this context, inflammatory, oxidative stress, and muscle damage biomarkers are utilized to assess muscle recovery after a high-intensity exercise session².

Several studies have investigated the effect of eccentric exercise on muscle damage over 96 hours after a single session of eccentric exercise²⁻⁴. It has been shown a reduced muscle force and increased muscle soreness up to 48-72 hours after eccentric exercise⁵⁻⁷. Speeding up muscle recovery after exercise is important to exercise performance, given that most sports demand a high muscle performance over days, weeks, and even months.

Many studies have shown that some serum biomarkers, such as myoglobin (muscle damage biomarker) and C-reactive protein (inflammatory biomarker), increase with eccentric exercise, which is utilized as indications of muscle damage. For example, a previous study demonstrated increased myoglobin and C-reactive protein from 24 hours to 96 hours after eccentric exercise⁸⁻¹¹. In this sense, myoglobin and C-reactive protein are typically utilized as serum biomarkers of muscle damage to better evaluate muscle recovery after intense exercise (i.e., eccentric exercise). Analyzing serum biomarkers would be a faster strategy for evaluating muscle recovery after exercise. However, if a correlation between these serum biomarkers and muscle force and muscle soreness exists, evidence needs to be shown to reinforce the utilization of myoglobin and C-reactive protein as indicative of muscle damage. Therefore, this study aimed to investigate whether a correlation between serum biomarkers and muscle force and soreness over 72 h after eccentric exercise exists. We hypothesized that a significant correlation between muscle force declines and muscle soreness would be correlated with serum biomarkers.

2. Methods

2.1. Participants

Eleven healthy male and female volunteers (26 ± 5 years old) with previous resistance training experience (at least three 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. 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). All individuals involved gave written informed consent. The study was registered in the Brazilian Registry of Clinical Trials (ReBEC) (RBR-3bc3rnb).

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/m ²) | 23.58 ± 3.19 |
| Resistance training experience (years) | 4 ± 2 |

Values are expressed as mean ± SD

2.2. Experimental Design

Participants were required to come to the laboratory for five days, the first visit being baseline testing and familiarization with the eccentric exercise 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 effect¹².

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. participant's perception of muscle pain through visual analogic scale (VAS), and 3. maximal voluntary isometric contractions (MVIC), before, 24h, 48h, and 72h post eccentric exercise protocol.

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, and heavy exercise for 72 h before each test visit and to have a rest day on the day immediately before each testing visit.

2.3. 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 individual 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 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 six sets of 10 maximal voluntary contractions at a velocity of $30^{\circ}\cdot\text{s}^{-1}$ 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.

2.4. 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 5s 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 the eccentric exercise protocol.

2.5. 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 myoglobin (Mb) and C-reactive protein.

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

2.6. Delayed onset muscle soreness (DOMS) measurement

Muscle soreness was measured before and after 24h, 48h, and 72h eccentric exercise protocol. Participants were asked to self-rate a 10- point-validated visual analogue

scale (VAS) indicating a line from 0 (no pain) to 10 (extreme pain)¹³ during a passive elbow extension.

2.7. Statistical analysis

An *a priori* power analysis was conducted (G*Power version 3.0.1) for a two-way repeated measure. Based on statistical power ($1 - \beta$) of 0.80, an effect size of 0.25 and an overall level of significance of 0.05. Analysis of variance was used to identify differences in maximal voluntary contraction, myoglobin and perceived muscle soreness before and after 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. To detect correlations between muscle soreness, MVIC, Myoglobin and C-reactive protein a Pearson correlation test was performed. Data were presented as means \pm standard deviation. All analyses were performed using a commercially available statistical package (IBM SPSS Statistics version 22 for Mac, Armonk, N.Y., USA). The graphics were designed using GraphPad Prism 5.

3. Results and discussion

Table 2. shows significant differences between the blood markers, isometric muscle force, and muscle soreness before and after eccentric exercise. A significant main effect for time ($p < 0.001$) was found for MVIC. Post hoc analysis revealed a significant decrease in MVIC at 24h ($p < 0.001$), 48h ($p < 0.001$), and 72h ($p < 0.005$) after eccentric exercise compared to pre-exercise values. In contrast, a significant increase in muscle soreness at 24h, 48h, and 72h was found ($p < 0.001$).

Table 2. Blood markers, isometric muscle performance, and muscle soreness before and following exercise-induced muscle damage (EIMD)

| | Pre-EIMD | 24 h | 48 h | 72 h |
|---|----------------|-----------------|-------------------|-------------------|
| Blood markers | | | | |
| C-reactive protein (mg. L ⁻¹) | 0.9 \pm 0.4 | 1.6 \pm 1.4 | 1.4 \pm 1.2 | 1.2 \pm 1.1 |
| Myoglobin (ng. mL ⁻¹) | 18.6 \pm 3.3 | 28.6 \pm 20.8 | 84.9 \pm 119.1* | 97.8 \pm 130.8* |

Isometric muscle performance

| | | | | |
|-----------------|-----------|------------|------------|------------|
| MVIC (Nm) | 54.4±24.7 | 45.9±27.0* | 45.9±26.6* | 48.6±26.1* |
| MVIC (% change) | 100±0.0 | 82.2±20.5* | 80.3±22.5* | 87.9±21.4* |

Muscle soreness

| | | | | |
|------------|-----|------|------|------|
| DOMS (VAS) | 0±0 | 2±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 ($p < 0.05$).

For biochemical marks, a significant increase in plasma myoglobin ($p < 0.001$) was observed after eccentric exercise. Post hoc analysis revealed a significant increase at 48h, 72h ($p < 0.002$) compared to pre-exercise values. Moreover, no significant change for C-reactive protein after eccentric exercise ($p > 0.05$) was observed.

Table 3. shows the correlation data. A significant correlation was observed between the MVIC and muscle soreness in 24h ($r = -0.841$; $P = 0.001$), 48h ($r = -0.843$; $P = 0.001$), 72h ($r = -0.734$; $p = 0.01$) (Figure.1 A-C) post eccentric elbow flexors protocol. A positive significance between plasma myoglobin and muscle soreness at 48h ($r = 0.837$; $p = 0.001$) and 72h ($r = 0.895$; $p = 0.001$) was found (Figure.2 A-B). No significant correlation between myoglobin and MVIC at 24h ($r = -0.680$; $p = 0.765$), 48h ($r = -0.334$; $p = 0.129$), 72h ($r = -0.265$; $p = 0.233$) was found. No significant correlation between C-reactive protein and MVIC and muscle soreness was found in relation to pre- and post-eccentric exercise ($P > 0.05$).

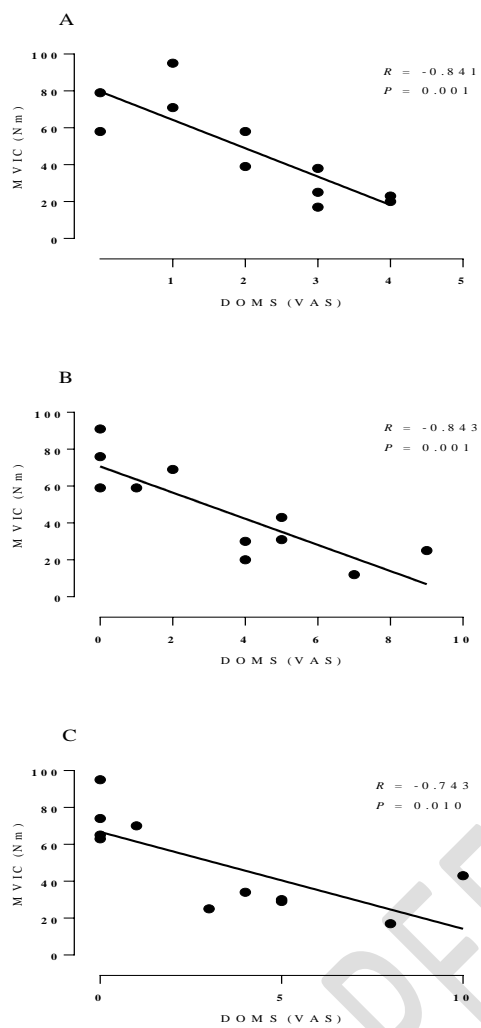


Figure 1. Correlation between delayed-onset muscle soreness (DOMS) and maximal voluntary isometric contraction (MVIC) at 24 h (A), 48 h (B), 72 h (C) post-eccentric exercise in healthy adult individuals.

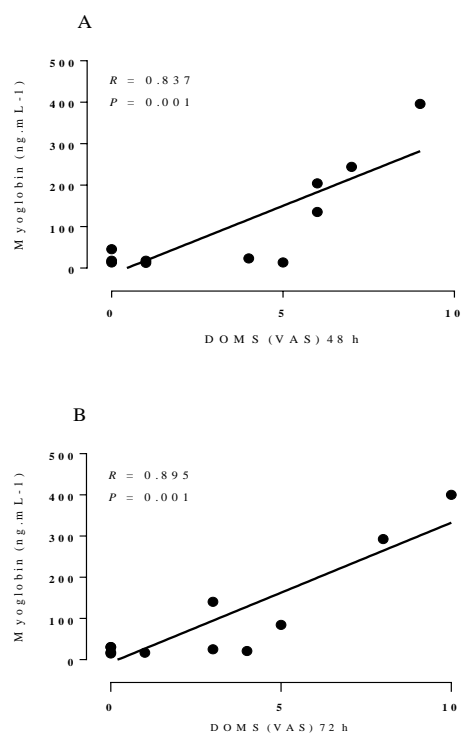


Figure 2. Correlation between delayed-onset muscle soreness (DOMS) and myoglobin at 48 h (A), 72 h (B) post-eccentric exercise in healthy adult individuals.

Table 3. Correlations between muscle damage parameters of the participants before and following Exercise-induced muscle damage. ($n = 11$)

| Relationship | 24 h | 48 h | 72 h |
|--|--------|--------|--------|
| Between DOMS (VAS) and MVIC (Nm) | | | |
| r | -0.84 | -0.84 | -0.73 |
| P | 0.01** | 0.01** | 0.01* |
| Between DOMS (VAS) and Mb (ng.mL ⁻¹) | | | |
| r | 0.29 | 0.83 | 0.89 |
| P | 0.19 | 0.01** | 0.01** |
| Between MVIC (Nm) and Mb (ng.mL ⁻¹) | | | |
| r | -0.68 | -0.33 | -0.26 |
| P | 0.76 | 0.12 | 0.23 |
| Between CRP (mg.L ⁻¹) and MVIC (Nm) | | | |
| r | 0 | -0.03 | 0.03 |
| P | 1 | 0.99 | 0.87 |
| Between CRP (mg.L ⁻¹) and DOMS (VAS) | | | |

| | | | |
|---|-------|------|-------|
| r | -0.30 | 0.5 | 0.25 |
| P | 0.17 | 0.80 | 0.25 |
| Between CRP (mg.L ⁻¹) and Mb (ng.mL ⁻¹) | | | |
| r | -0.32 | -0.9 | -0.19 |
| P | 0.14 | 0.68 | 0.37 |

MVIC = maximal voluntary isometric contraction; DOMS = delayed onset muscle soreness; VAS = visual analogic scale. Myoglobin = Mb; C-reactive protein = CRP; Statistical significance: * $P < 0.05$, ** $P < 0.01$

The aim of the present study was to investigate whether a correlation between muscle serum biomarkers (C-reactive protein and myoglobin) and muscle force and soreness over 72h after eccentric exercise exists. It was shown a positive correlation between myoglobin and muscle soreness, but not muscle force. Moreover, no significant correlation between C-reactive protein and muscle soreness and force was observed. We also observed a significant reduction in muscle force, as well as a significant increase in muscle soreness and myoglobin over 72h after eccentric exercise.

We demonstrated a significant reduction in muscle force over 72h after eccentric exercise. In addition, a significant increase in muscle soreness was also observed after the eccentric exercise protocol at the same time course. It is well established that muscle soreness impairs exercise performance due to muscle microinjury induced by high-intensity exercise¹⁴. In this perspective, we observed a negative correlation between DOMS and MVC 24h, 48h, and 72h after exercise.

Our findings are in line with previous studies^{15,16} demonstrating a decrease in muscle force associated with an increase in DOMS over 72h post eccentric exercise in elbow flexor muscles. For example, Tanabe et al. (2015) observed a reduced muscle force (isometric) 24h, 48h, 72h after 1 set of 50 eccentric contractions in elbow flexor muscles in untrained young men. The authors also found a significant increase in DOMS at the same time points; it was suggested that muscle force declined after exercise due to the DOMS, which impaired muscle performance. In addition, Chen et al. (2020) investigated the effect of eccentric exercise on DOMS and muscle force in three different intensities (10%, 50%, and 100% of MVIC) in sedentary young men's elbow flexor muscles. The authors found that changes in DOMS were more pronounced in those individuals that performed exercise at a high intensity (100% of MVIC); the muscle force recovery period was longer when performing a higher-intensity exercise. These findings suggested that DOMS is closely associated with muscle performance. In line, our study revealed a correlation between DOMS and muscle force, reinforcing that DOMS negatively affects muscle capacity to generate force. Therefore, when

intense and unusual exercise is performed, higher will be muscle soreness and, consequently, the impact on the course of muscle recovery.

In addition, our study also evaluated biochemical parameters normally analyzed when investigating the effect of eccentric exercise on muscle soreness and muscle force. We found an increase in myoglobin at 48h and 72h after eccentric exercise. Typically, myoglobin is released after high-intensity exercise due to the disruption of muscle cells leaking into the extracellular space after cell membrane damage¹. A previous study compared changes in myoglobin after eccentric exercise in the elbow flexor muscles among the exercises consisting of different numbers of eccentric contractions (30, 50, and 70 repetitions) in athletes (i.e. soccer, swimming, shooting, and track and field)¹⁷. The myoglobin concentration showed a significant increase at 72h after eccentric exercise, being more pronounced in the group with more repetitions¹⁷. Lavender et al.¹⁸ investigated changes in myoglobin after 6 sets of five eccentric contractions in elbow flexor muscles, comparing middle-aged and young men. In both groups, myoglobin concentration significantly increased in plasma 96h after exercise¹⁸. The myoglobin peak occurred at 72h post-exercise with no significant difference between the young and middle-aged groups.

Despite the fact that a significant reduction in MVIC was observed up to 72h after eccentric exercise, we failed to show a correlation between myoglobin and muscle force. However, a significant positive correlation between myoglobin and DOMS at 48h and 72h after eccentric exercise was observed. These data suggest that myoglobin appears to be interesting when investigating muscle soreness. The absence of a significant correlation between myoglobin and muscle force can be related to the many physiological factors that drive muscle force, such as neural and mechanical aspects of musculature. Furthermore, no significant changes in C-reactive protein over 72h after eccentric exercise was found. A previous study has demonstrated a significant increase in serum concentration of C-reactive protein 48h after 300 maximal eccentric repetitions (30 sets of 10 repetitions) with muscle quadriceps in healthy young individuals¹⁰. Considering that the C-reactive protein is an acute-phase protein induced by an inflammatory response and has been used to quantify the muscle damage generated after strenuous exercise¹⁹, it is likely that the eccentric exercise protocol utilized in the present study (6 sets of 10 repetitions) was not enough to induce a significant increase in serum concentration of C-reactive protein, given the eccentric exercise protocol utilized in the study above mentioned (300 repetitions). In contrast, in untrained individuals, there was a peak increase in C-reactive protein and DOMS at 72 h after performing eccentric exercises of elbow flexors (3 x 10 repetitions)⁹. In our study, C-reactive protein did not increase in trained individuals since the level of training of volunteers seems to impact the sensitivity of post-exercise C-reactive protein. Moreover, no significant

correlation between C-reactive protein and muscle force or soreness was observed, possibly for that reason.

4. Conclusion

The present study demonstrated that eccentric exercise significantly reduced muscle force and increased muscle soreness and myoglobin over 72h after exercise. The myoglobin was a positive correlation with muscle soreness but not muscle force. It suggests that myoglobin could be a valuable biomarker to be assessed alongside exercise induced-muscle soreness.

Ethical approval

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). Participants were informed about the study and gave their written informed consent.

5. References

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Abbreviations

| | |
|------|--|
| CRP | C-reactive protein |
| DOMS | Delayed onset muscle soreness |
| EIM | Exercise-induced muscle damage |
| Mb | Myoglobin |
| MVIC | Maximal voluntary isometric contractions |
| VAS | Visual analogic scale |