




## Effect of static stretching pre- pliometric exercise on indirect markers of muscle damage

### Efeito do alongamento estático pré-exercício pliométrico sobre marcadores indiretos de danos musculares

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#### ABSTRACT

**Introduction:** Static stretching is commonly used as part of routine exercise preparation, however, its influence on inhibition and/or reduction of muscle damage caused by eccentric exercise is still controversial. **Aim:** To analyze the effect of the use of a static stretching protocol with duration of 5 min (5 x 60 s) pre-exercise on the response of creatine kinase concentration ([CK]) and delayed onset muscle soreness (DOMS) 24 and 48 hours after plyometric jumping exercise. **Methods:** This is a counter-balanced quasi-experimental study of repeated measures and the sample consisted of ten healthy volunteers who underwent two experimental sessions: 1) without pre-exercise stretching (control); 2) with static stretching prior to exercise (static stretching). In both sessions the participants underwent a plyometric jumping exercise to induce muscle damage. Prior to the experimental sessions and 24- and 48-hours post-exercise the [CK] was measured, as well as DOMS 24- and 48-hours post-exercise. **Results:** There was no significant difference for [CK] and DMIT when compared to the experimental sessions, control and static stretching, ( $p > 0.05$ ). In addition, both had peak [CK] 24 hours post-exercise, and DMIT was classified as irritant for both sessions. **Conclusion:** The protocol of static stretching with a duration of five minutes (5 x 60s) prior to the exercise of plyometric jumps did not generate minimization or inhibition of muscle damage associated with the eccentric actions evaluated by [CK] and DMIT.

**Keywords:** muscle stretching exercises; exercise; creatine kinase.

#### RESUMO

**Introdução:** O alongamento estático é comumente utilizado como parte da rotina de preparação para o exercício físico, no entanto ainda é controversa a sua influência sobre a inibição e ou redução dos danos musculares ocasionados pelo exercício excêntrico. **Objetivo:** Analisar o efeito do uso de um protocolo de alongamento estático com duração de 5 min (5 x 60 s) pré-exercício na resposta da concentração de creatina quinase ([CK]) e dor muscular de início tardio (DMIT) 24 e 48 horas após exercício de saltos pliométricos. **Métodos:** Trata-se de um estudo quase-experimental contrabalanceado de medidas repetidas e a amostra foi composta por 10 voluntários saudáveis que foram submetidos a duas sessões experimentais: 1) sem alongamento prévio ao exercício (controle) e; 2) com alongamento estático prévio ao exercício (alongamento estático). Em ambas as sessões os participantes foram submetidos a um exercício de saltos pliométricos para indução de dano muscular. Previamente às sessões experimentais e 24 e 48 horas pós-exercício foi mensurada a [CK], bem como a DMIT 24 e 48 horas pós-exercício. **Resultados:** Não houve diferença significativa para [CK] e DMIT quando comparadas as sessões experimentais, controle e alongamento estático, ( $p > 0,05$ ). Além disso, ambas obtiveram pico da [CK] 24 horas pós-exercício, e a DMIT foi classificada como irritante para ambas as sessões. **Conclusão:** O protocolo de alongamento estático com duração de cinco minutos (5 x 60s) prévio ao exercício de saltos pliométricos não minimizou ou inibiu os danos musculares associados às ações excêntricas avaliadas pela [CK] e DMIT.

**Palavras-chave:** exercícios de alongamento muscular; exercício físico; creatina quinase.

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## Introduction

Muscle lesion affection is one of the major factors of interference to the continuity of a physical training program [1]. Muscle stretching, until these days used as part of the routine of preparation for physical exercise, is taken as a strategy to prevent muscle lesions in physical training programs for sports performance, or even previously to recreational practice [2,3].

Different stretching methods can be used in order to prepare the muscles for physical exercise, such as static, dynamic stretching and proprioceptive neuromuscular facilitation [4,5]; moreover, the first two methods can be performed either actively or passively [6,7]. Static stretching is highlighted mainly because it is easy to perform and is usually part of the preparation strategies for physical exercise [7]. However, the effectiveness of static stretching prior to exercise in preventing muscle injuries is still controversial [3].

The main divergences between the researchers are the duration period for each stretching technique, the control of the correct position by the volunteer during the stretching, and the performance or not of muscle warm-up prior to stretching [3,8]. It is believed that the short duration of the technique performed (15, 20, 30, 45s) may not be enough to decrease passive resistance, as well as periods of total stretching of 1.5 and 2 min (2 x 45 and 4 x 30 s, respectively) also seem inefficient [8]. Nevertheless, periods of 5 and 6 min (5 x 60 and 4 x 90 s, respectively) significantly reduce passive resistance and have their effect prolonged for about 10 min after stretching session, which suggests a possible preventive effect of muscle damage [9].

In addition, investigations of the effect of static stretching on the occurrence of muscle damage have used damage induction protocols from eccentric actions in isolated movements, such as isokinetic devices [10]. However, in the practice of daily sports training exercises for performance or recreational level, it is unlikely that subjects will be exposed to this type of exercise. In this way, the investigation of static stretching prior to exercises with eccentric actions similar to those performed during daily training is still little explored.

On the other hand, plyometric jumping exercises are widely used in training routines in order to increase the production of muscle strength from the induction of muscle damage [11-13]. During eccentric actions, such as with counter-movement jumps, there is a greater propensity to induce muscle damage, due to the increased time on tension generating a greater overload imposed on muscle fiber, causing the extravasation of enzymes, among them creatine kinase ([CK]), from the intracellular medium, to the bloodstream and reflected in the increase in serum concentration in subsequent days, with a peak occurring between 24 and 48 hours after physical demand [14-16].

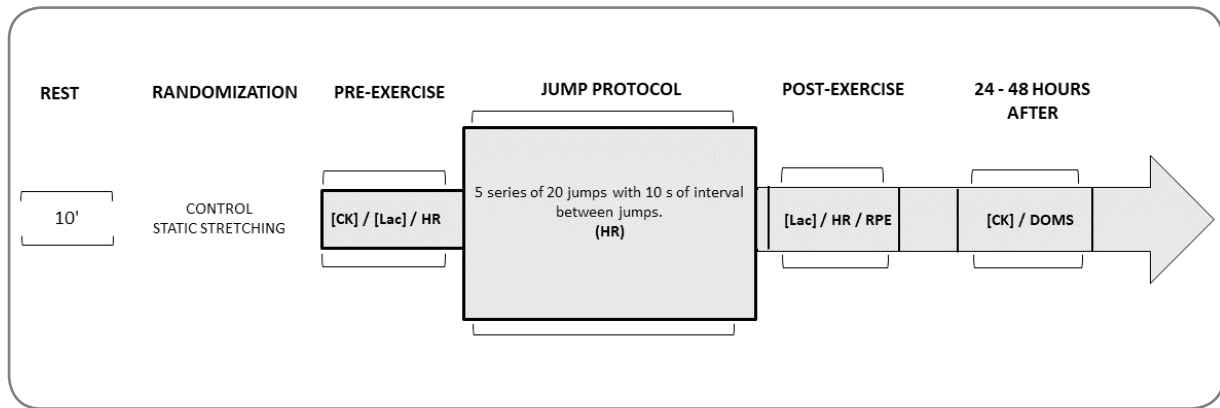
Muscle damage also directly interferes with the sensation of muscle pain in the days after exercise [17], an effect known as delayed-onset muscle soreness (DOMS), which also presents a peak variation between 24-48 hours post-exercise [18-20]. The increase in serum [CK] concentration and DOMS are indirect markers of muscle damage [21-23], and can be considered easy to assess and have useful practical application, since they make it possible to estimate muscle damage without the need for muscle biopsy techniques after exercise, which are costly and invasive to the subject. Therefore, the aim of the present study was to verify the effect of a pre-exercise static stretching protocol on the response of [CK] and DOMS after plyometric jumping exercise.

## Methods

### *Experimental design*

This is a quasi-experimental study balanced with repeated measures and the sample was composed of ten healthy volunteers who underwent two experimental sessions, being: 1) without stretching prior to exercise (control) and; 2) with static stretching prior to exercise (static stretching). A minimum interval of one week was respected between sessions, which were always held at the same time of the day. Both sessions were developed in the laboratory ( $25 \pm 3^\circ\text{C}$  at room temperature and  $51 \pm 2\%$  relative humidity). The displacement of volunteers to the research location was standardized. One week before the experimental sessions, an anthropometric evaluation of each volunteer was carried out, as well as familiarization with the test procedures and anchoring to the psychometric scales of rating of perceived exertion (RPE) and visual analog scale (VAS), to assess the perceived effort and DOMS respectively.

The tasks performed in each of the experimental sessions are shown in Figure 1 and followed the following order: when the volunteer arrived at the laboratory, he remained at rest for ten minutes. Subsequently the CK concentration ([CK]) and blood lactate concentration were measured ([Lac]), then according to the counter-balance of the sessions, the volunteers performed a static stretching session of the quadriceps femoris and hamstrings (5 sets of 60 s) or remained at rest, sitting for ten minutes prior to the plyometric jumps. Heart rate (HR) was recorded constantly throughout the session. After performing the last series of plyometric jumps, [LAC] and RPE were recorded and, 24 and 48 hours after the jumping protocol, the volunteers returned to the laboratory for evaluation of [CK] and DOMS.



[CK] = Creatine kinase concentration, [Lac] = Blood lactate concentration, HR = Heart rate, RPE = Rating of perceived exertion, DOMS = Delayed-onset muscle soreness

**Figure 1** - Experimental design

### Participants

Ten male university students volunteers ( $23.9 \pm 3.8$  years of age;  $73.9 \pm 8.6$  kg;  $176.4 \pm 5.2$  cm;  $14.9 \pm 3.4\%$  fat) were recruited, selected according to the following inclusion criteria: 1) male; 2) between 18 and 30 years old; 3) physically inactive (not practicing systematic physical activity for at least two or more days a week) and; 4) have little or no experience with strength training and activities that involve jumping.

After agreeing to participate on a voluntary basis, they signed an informed consent form in accordance with the guidelines established by the National Health Council (Res.466/12) involving research with human beings. While participating in the study, they were asked to avoid vigorous or unusual activities, use of medication, dietary supplementation and alcohol. This study was approved by the Human Research Ethics Committee of the Universidade Federal do Recôncavo da Bahia (number 1,577,899).

To estimate the sample size, a previous study was used, in which changes in [CK] were compared after inducing muscle damage in knee flexors and extensors [10]. Based on the eta-square of 0.681 for the difference in [CK] up to 2 days after muscle damage induction protocol, with an alpha level of 0.05 and a power ( $1 - \beta$ ) of 0.80 (G \* Power 3.1.9.2; Heinrich - Heine - Universitat Dusseldorf, Dusseldorf, Germany; <http://www.Gpower.hhu.de/>) showed that at least four participants were required for the present study.

### Procedures

#### Stretching protocol

The volunteers were submitted to a static stretching protocol of the quadriceps femoris and hamstrings in the static stretching session or remained at rest for 10 min during the control session. To stretch the quadriceps, the volunteers lay in prone position and had their knees flexed by the researcher to a position of mild discomfort that was indicated by the participant himself (Figure 2A). Five repetitions were performed with periods of 60 seconds in duration and 10 seconds of interval between them. Following the same protocol regarding repetitions and duration, for stretching the hamstring muscles, the volunteer standing and with the upper limbs hanging

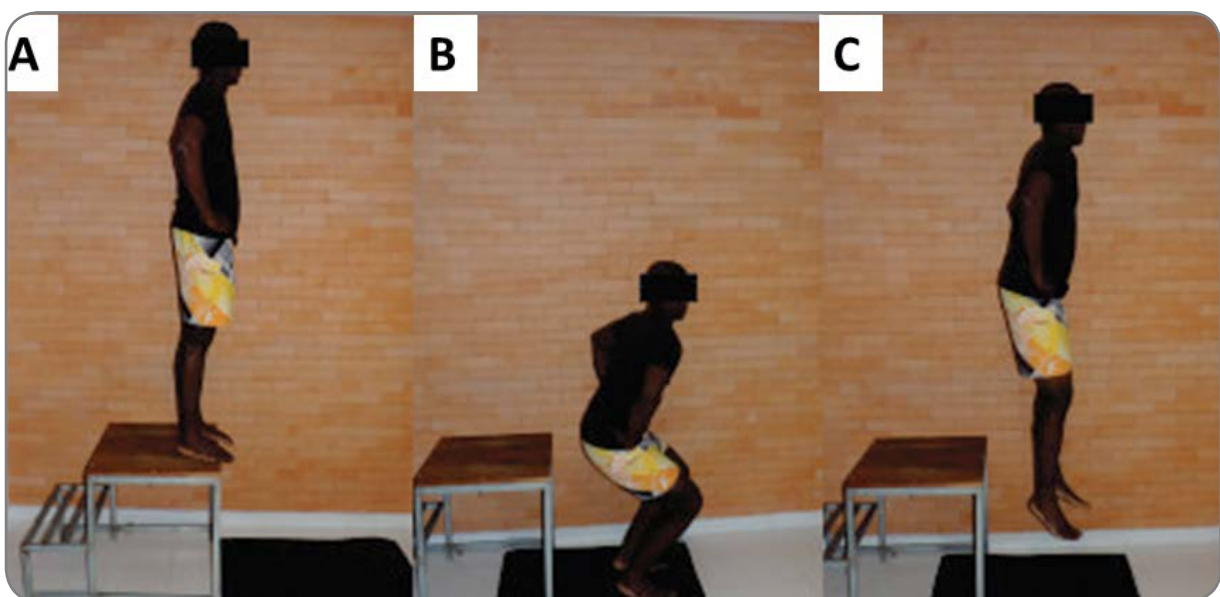
in front of the lower limbs by flexing the hip to a position of mild discomfort in the posterior thigh muscles (Figure 2B).

#### *Muscle damage induction protocol*

The protocol of plyometric jumps aimed to induce eccentric actions in the muscles of the lower limbs. The volunteers performed 5 sets of 20 deep jumps with a 10-second interval between jumps (to get back up on the platform) and 2 minutes of passive rest between sets [16]. The jumps started on a 60 cm high platform, with the participant keeping their hands on their hips throughout the movement. The volunteers fell off the platform and, immediately after landing on the ground, performed a quick knee flexion (approximately  $90^\circ$ ) followed by a new jump in order to reach the highest possible height (Figures 3A, 2B and 2C). To climb the platform, a 30 cm high step was used. The interval time between jumps was controlled by an evaluator and the subjects were encouraged to jump as high as possible during the entire session.



**Figure 2** - Quadriceps (2A) and hamstring (2B) stretching exercise



**Figure 3** - Jump exercise with counter movement (2A, 2B and 2C). Start position of the jump (2A), landing (2B) and jump after landing (2C) protocol

### *Assessment of CK and Lac concentrations*

After arriving at the laboratory, the volunteers remained at rest for ten minutes to assess [CK] and [Lac] before exercise. There was asepsis with alcohol from the volunteer's right index finger and, using a disposable lancet, a small hole was made from which two blood samples were taken. After discarding the first drop, 10  $\mu$ L were used to assess [Lac] by reflectance photometry on Accutrendr<sup>®</sup> Plus (Roche Diagnostics, Germany) and 30  $\mu$ L to analyze [CK] also by reflectance photometry using Reflotron<sup>®</sup> Plus (Roche Diagnostics, Germany), both devices properly calibrated. In both situations, the samples were placed in specific reagent strips for each equipment. [Lac] was evaluated again at the end of the muscle damage induction protocol and [CK] also at 24 and 48 hours after the session.

### *Monitoring exercise intensity*

HR was monitored every five seconds throughout the session. The values for rest and the end of each series of the plyometric jumping protocol were recorded (Polar RC3 GPS HR - Polar Electro Oy, Kempele, Finland). Immediately after the last series of jumps, RPE was also recorded on the 6 to 20 Borg scale [24].

### *DOMS Assessment*

The DOMS assessment took place 24 and 48 hours after exercise, subsequently measured by [CK]. The level of muscle pain perceived in the quadriceps was assessed from the sensation of pain in an eccentric action (movement of squatting slowly until approximately a 90° angle of knee flexion and returning to the initial position) [20,25,26]. The volunteer indicated which level of muscle pain he was feeling from a visual analog scale with a score from 0 to 10, with 0 being nothing; 2 discomfort; 4 irritating; 6 horrible; 8 terrible and; 10 agonizing [12].

### *Statistical analysis*

All data were submitted to the *Shapiro-Wilk* test to verify normality. Normally distributed data were expressed as mean values  $\pm$  standard deviation (SD) and non-parametric data were expressed as medians and quartiles. HR was analyzed through analysis of variance (ANOVA) with two variation factors (time and experimental group) with repeated measures for the time factor, followed by the *Bonferroni post hoc* test to identify the pairs of difference. To compare the behavior of [CK] between sessions and as a function of time, the *Friedman* test was used, followed by the *Wilcoxon* test. The same was adopted for DOMS. PSE was compared between sessions using the *Wilcoxon* test. The level of significance was set at  $p < 0.05$  and the statistical software used was SPSS 22.0 (SPSS, Inc., Chicago, IL). The effect size ( $r$ ) for nonparametric comparisons from the *Wilcoxon* test was calculated according to Pallant [25], in which the  $z$  value was divided by the square root of  $N$ , where  $N$  was the number of observations (20 in each comparison). The effect size was classified using the *Cohen* criteria [27], with 0.2 = small effect, 0.5 = medium effect and 0.8 = wide effect.

## Results

The *Wilcoxon test* revealed a significant increase in [CK] for participants in the control situation ( $z = -2.29$ ;  $p = 0.02$ ) with a medium effect ( $r = 0.51$ ); similar behavior was also observed when the subjects performed static stretching prior to the jumping protocol ( $z = -2.29$ ;  $p = 0.02$ ;  $r = 0.51$  medium effect). There was also a reduction in [CK] when comparing the 24 and 48 h post induction of muscle damage for the control situation ( $z = -2.19$ ;  $p = 0.02$ ,  $r = 0.49$  small effect size) as for the static stretching session ( $z = -2.70$ ;  $p < 0.01$ ;  $r = 0.60$  medium effect). In both sessions, [CK] showed a similar behavior with a peak 24 hours after the plyometric jumping protocol (Table I).

DOMS was classified as irritant 24 and 48 hours after plyometric jumping protocol for both sessions. No significant difference was observed between the 24 and 48 hours or between the control and static stretching sessions (Table I).

**Table I** - Effect of the plyometric jumping protocol on the concentration of creatine kinase (U / L) and delayed-onset muscle soreness (points) 24 h and 48 h after protocol

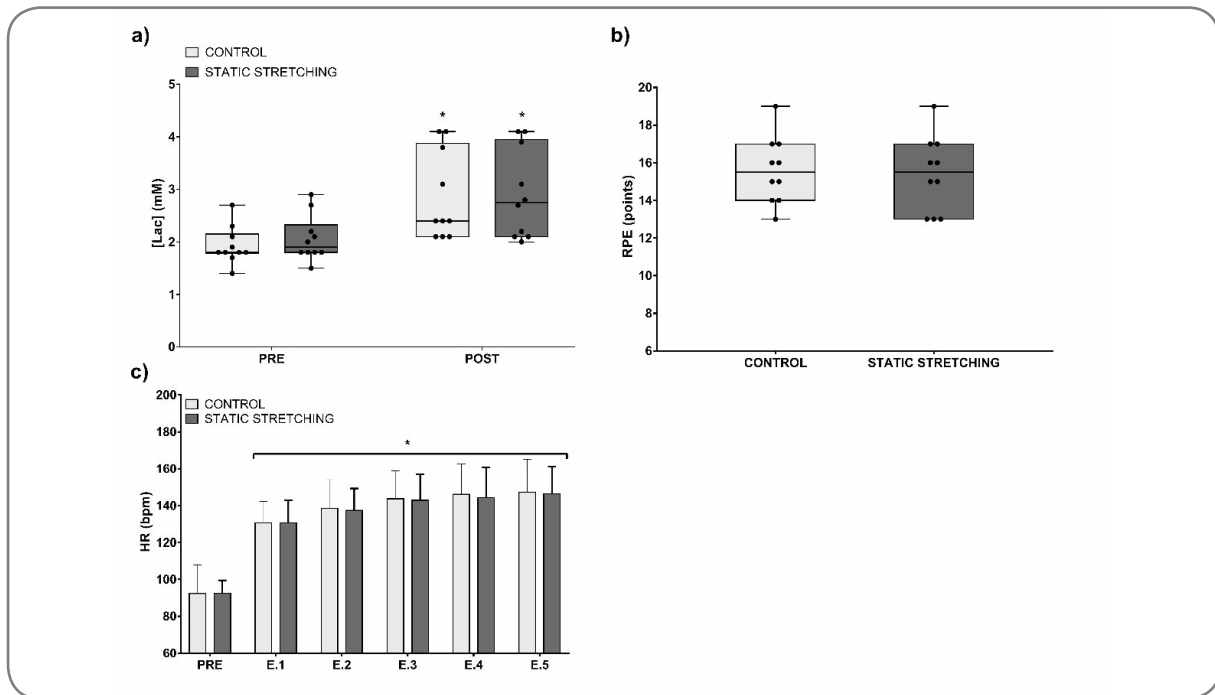
	Control			Static stretching		
	Pre	24 h	48 h	Pre	24 h	48 h
CK	228 (165 - 337)	344* (246 - 248)	205# (144 - 411)	186 (106 - 226)	326* (260 - 382)	258# (225 - 311)
DOMS	-----	4.5 (2.5 - 7.0)	5,5 (3.7 - 7.5)	-----	4.5 (2.7 - 6.0)	4.5 (2.0 - 5.7)

CK = Creatine kinase concentration, DOMS = delayed-onset muscle soreness; \*  $P < 0.05$  in relation to the Pre moment of the same session; #  $P < 0.05$  in relation to the 24h moment of the same session

The exercise of plyometric jumps induced a significant increase ( $z = -2.29$ ;  $p = 0.03$ ,  $r = 0.51$  medium effect) of [Lac] when comparing the pre and post moments in the control situation [Pre = 1.8 (1.8 - 2.2 mM) vs. Post = 2.4 (2.1 - 3.9 mM)]. In addition, there was a similar increase in [Lac] in the static stretching situation Pre = 1.9 (1.8 - 2.3 mM) vs. Post = 2.7 (2.1 - 3.9 mM),  $z = -2.09$ ;  $P = 0.02$ ;  $r = 0.47$  small effect (Figure 4a). Due to the similarity of the lactacidemic response, no difference was found when comparing the experimental situations, control and static stretching.

The RPE of the volunteers did not differ between experimental sessions [control = 15.5 (14 - 17 points) vs. static stretching = 15.5 (13 - 17 points),  $P = 0.08$ ]. The jumping protocol inducing muscle damage was classified as “tiring” in both sessions (Figure 4b).

As expected, resting HR differed significantly for all stages of exercise ( $P < 0.01$ ). A plateau was observed throughout the five series of jumps in both experimental sessions and the average HR for the control session was  $142 \pm 8$  bpm and for static stretching of  $141 \pm 6$  bpm. In addition, no significant difference occurred between sessions ( $P > 0.05$ ) (Figure 4c).



\* P < 0.05 in relation to the Pre

**Figure 4** - a) concentration of blood lactate ([Lac]) Pre and Post exercise of plyometric jumps, b) rating of perceived exertion (RPE) post exercise of plyometric jumps, c) heart rate (HR) Pre and at the end of each stage of plyometric jumps

## Discussion

The main finding of this study was that the performance of static stretching lasting five min (5 x 60s) before exercise did not minimize the responses associated with the induction of muscle damage promoted by the protocol of plyometric jumps, assessed through the variables [CK] and DOMS.

This result can be attributed to possible microlesions caused in muscle fibers, due to the increase in time under tension induced by eccentric muscle actions, resulting in possible ruptures, prolongations and/or enlargements located in the z lines of the sarcomeres, resulting in a consequent leakage of [CK] to the blood flow, however, damage to T tubules and myofibrils can also withstand the results found [8,10,17]. Furthermore, possible damage to the myofibrillar structure, greater protein degradation, autophagy and local inflammation with leukocyte infiltration and accumulation in the region could explain DOMS [17,19].

The eccentric exercise used in the present study was effective in inducing muscle damage, with an increase [CK] in the days following its performance (Table I), an effect also demonstrated by Miyama and Nosaka [16]. In addition, Ferreira-Junior *et al.* [20] identified that the referred plyometric jumping protocol was able to induce muscle damage resulting in increased perception of muscle pain in the days after exercise.

Both methods of indirect assessment ([CK] and DOMS) of the appearance of muscle damage are widely used, confirming their applicability in assessing the response to damage caused by eccentric exercise [28,29]. In the present study, the



responses of [CK] and DOMS suggest that the protocol was similarly effective in inducing muscle damage in the two compared sessions.

The similar induction of muscle damage between the two experimental sessions, assessed by varying the [CK] concentration and by DOMS, corroborates the findings of Smith *et al.* [28], who observed that static stretching prior to eccentric exercise was not able to mitigate muscle damage assessed by [CK]. However, the results of the present study contradict the findings of Chen *et al.* [2], methodological differences between the studies may explain the divergence in the results, mainly the profile of the volunteers and the protocols of stretching and eccentric exercise adopted.

The study by Chen *et al.* [2] used a protocol of maximum eccentric contractions performed on an isokinetic dynamometer, while the present study used a protocol with movements similar to the movements performed in training routines for physical exercise practitioners. Another relevant issue to be highlighted in the study by Chen *et al.* [2] was the use of five minutes of warm-up in the stretching groups, which may have favored the observed effect of preventing muscle damage, since it has been shown that muscle warm-up is effective in preventing muscle damage [30,31].

The ineffectiveness of static stretching prior to exercise has also been reported in studies investigating exercise-induced DOMS sensation. High *et al.* [32] used 2 sets of 50 seconds of stretching and Johansson *et al.* [33] used 4 sets of 20 seconds with a 20-second interval and did not identify a reduction in DOMS. It is possible that these stretching protocols have not been effective in reducing DOMS. As, according to McHugh and Cosgrave [8], approximately five minutes of static stretching are necessary to observe the effects of reduction in the passive resistance presented by the muscle, an effect which hypothetically could mediate the reduction in the induction of muscle damage, as shown in chronic studies with the same duration [3]. However, even respecting this minimum duration of static stretching, the present study did not find a reduction in DOMS in the session with stretching prior to exercise.

It is noteworthy in the present study that the use of static stretching seeking to decrease muscle stiffness in an acute way, did not differentiate the intensity of the exercise in the two experimental situations. HR monitoring throughout the jumping protocol demonstrated that in the control and static stretching sessions the volunteers exercised at similar intensities, with no significant difference. Furthermore, [Lac] and RPE's responses did not differ between the two experimental sessions, which enables asserting that besides the external load, the internal load of the jumping protocol (muscle damage inducer) was similar between the experimental conditions.

Although no volunteer reported having a limited range of hip flexion, one of the limitations of the present study was the non-use of experimental sessions with: a) only warm-up and, B) warm-up and stretching prior to the exercise of plyometric jumps. Another limitation of our study was the failure to perform an isolated stretch for the sural triceps, a muscle group also required during plyometric jumps. However, given the limitations regarding the real effectiveness of static stretching to reduce

passive resistance and its maintenance for a longer period than 10 min [34], we chose to perform only two stretching exercises in the experimental design of the present study with a focus on the largest muscle groups involved.

## Conclusion

The static stretching protocol lasting five minutes (5 x 60s) prior to the exercise of plyometric jumps did not minimize or inhibit the muscle damage associated with the eccentric actions evaluated by creatine kinase and delayed onset muscle soreness.

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### Conflict of interest

No conflicts of interest have been reported for this article.

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### Authors' contributions

Conception and design of the research: Santos GA, Teixeira-Coelho F. Data collection: Santos DFC, Santos FR, Santos GA. Statistical analysis and data interpretation: Santos DFC, Santos GA, Teixeira-Coelho F. Writing of the manuscript: Santos GAS, Moreira SR. Critical review of the manuscript: Moreira SR, Santos FR. Final revision of the manuscript: Santos FR, Teixeira-Coelho F.

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