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Sex differences in delayed onset muscle soreness induced by fatigue and measured by different methods

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HIGHLIGHTS
- DOMS assessed with a NRS is comparable between men and women.
- Absolute values of pressure pain thresholds were lower in women.
- Pressure pain thresholds data require normalization for sex-comparisons.

ABBREVIATIONS

CPGS	Chronic Pain Grade Scale
DOMS	Delayed onset muscle soreness
GEE	Generalized estimating equation
MPQ	McGill Pain Questionnaire
NRS	Numeric pain rate scale
PPT	Pressure pain thresholds
RF	Rectus femoris
VAS	Visual Analog Scale
VL	Vastus lateralis

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BACKGROUND: Controversial outcomes from different methods for assessment of delayed onset muscle soreness (DOMS) in male and female may influence clinical decisions.
AIM: In this study, we determine sex differences in pain perception and pain thresholds in a DOMS condition resultant of a fatigue protocol.
METHOD: 11 male and 15 female healthy adults were submitted to an exercise fatigue protocol to induce DOMS in the quadriceps muscles. Pain perception was determined using a numeric pain rate scale (NRS) and pressure pain thresholds (PPT) were determined by mechanical pressure in the vastus lateralis (VL) and rectus femoris (RF) regions. Data were compared between methods and sexes at baseline, immediately after (0h), and 48 h after DOMS induction.
RESULTS: Results showed normalized lower PPT and higher NRS outcomes after fatigue, without sex differences. Absolute values of PPT showed lower values in females comparing both time and sex (VL and RF, baseline $p = 0.002$ and $p = 0.009$; 0h $p = 0.002$ and $p = 0.001$; 48h $p<0.0001$ and $p<0.0001$) with a mean difference for females and males on 0h and 48h of from baseline VL 16,52% and 19.7%; -15.64% and -10.89%; RF 12.18% and 20.7%; -9.18% and -1.97%. No correlations were found between the number of repetitions of exercise nor the rate of perceived effort and DOMS outcomes.
CONCLUSION: Men and women show similar DOMS when NRS and normalized PPT outcomes are considered. Absolute PPT values may lead to a confusing analysis of fatigue exercise-induced DOMS if merging both sexes in the sample.

KEYWORDS: Muscle damage | Physical exercise | Pain perception | Pain thresholds

INTRODUCTION

Delayed onset muscle soreness (DOMS) is often experienced after intense physical exercise, an abrupt increase in exercise load, or performance of exercises involving unusual movement amplitude and speed [1]. DOMS involves a painful sensation associated with joint and muscle stiffness that appears when muscles are stretched or palpated, muscle swelling, and strength deficits [2,3]. These symptoms become significant from 6 to 12 h after exercise, with peak values observed between 48 and 72 h after exercise [2]. Despite its general progression to a condition of full recovery, the time course of DOMS can cause functional limitations and negatively impact training and rehabilitation protocols.

Methods for assessment of pain intensity are diverse and results can be controversial, which can difficult clinical decisions. Pressure algometry is a valid method to assess experimental pain. It involves the application of a known magnitude of force at specific points to stimulate nociceptors and cause a painful sensation, allowing to determine the pressure pain threshold [4]. However, the need for specialized instrumentation and time required for the assessment of pain thresholds often result in DOMS being assessed by a unidimensional scale, such as a numeric rate scale (NRS). Using an NRS, the patient is requested to self-report the perceived pain. NRS results show a strong correlation with results from other tools for pain assessment [5]. Other tools rely on subject report as well as the Visual Analog Scale (VAS), McGill Pain Questionnaire (MPQ) and Chronic Pain Grade Scale (CPGS) [6]. However, sex differences [7] and small variations in the perceived pain are difficult to identify when using unidimensional scales [5,8].

Additionally, there are physiological differences between sexes related to exercise adaptations and pain. Males are more susceptible to experiencing exercise-induced muscle damage or fatigue than women [9]. Females can be less fatigable at higher intensity isometric exercise, they can sustain a long time to task failure, and may have more type I fibers and a higher density of capillaries per unit of skeletal muscle in the vastus lateralis than males [10]. Additionally, females show a higher prevalence and sensitivity for pain compared to males [11–13]. Hormonal differences between males and females may also account for differences in pain outcomes, such as the higher levels of androgen hormones and testosterone in males that appear to be protective against chronic pain [14]. On the other hand, estrogen hormones have analgesic and hyperalgesia effects in females [15].

These physiological differences may suggest that pain perception outcomes could differ between males and females depending on the measure tool, and baseline conditions. However, sex differences regarding exercise-induced pain are considered in a few studies. It appears that when pain outcomes are compared between sex, even healthy females show lower pressure pain thresholds considering absolute values [16] and higher perception of pain using a visual analog scale [17]. This could be relevant information to be considered when comparing sexes, since the absolute values could lead to a misinterpretation on results. For instance, studies are not clear regarding data normalization considering baseline measures, which would be sound considering sex differences [18]. In this study, we determine whether there are differences in pain outcomes measured using an NRS and between absolute and normalized values using pressure algometry in females and males submitted to physical exercise to induce DOMS in the lower limbs. We hypothesized that females would report higher pain intensity and exhibit lower pressure pain thresholds than males. Also, we suggest that sex-based differences in pain perception may be influenced by the methods used to normalize pressure pain measurements.

METHODS

Participants and experimental design

This observational study was approved by the local institution's Ethics Committee. Participants were recruited from the local community through flyers posted in social media and signed a consent term agreeing to participate. Twenty-six participants, 15 females, and 11 males were included (see Table 1 for participant characteristics). They answered an anamnesis questionnaire to collect information about their physical activity routine. Leg preference was assessed by the Waterloo questionnaire [19]. Participants were adults of age between 18 and 40 years old, enrolled with recreational physical exercise, without lower limb injuries at least six months before the assessment, and not performing plyometric training. Data collection involved two visits to the laboratory. Participants were requested to refrain from physical exercise 24 h before each visit to the laboratory and not use any medication with anti-inflammatory properties or techniques for analgesia during the study participation. In the first visit, the pain was measured before and immediately after the exercise to induce the fatigue and delayed onset muscle soreness considering pain perceived using an NRS, and pressure pain thresholds using a digital algometer. After 48 h they visited the laboratory again to be evaluated for the presence of DOMS considering pain perceived using the NRS, and pressure pain thresholds using the digital algometer. Figure 1 illustrates the experimental design.

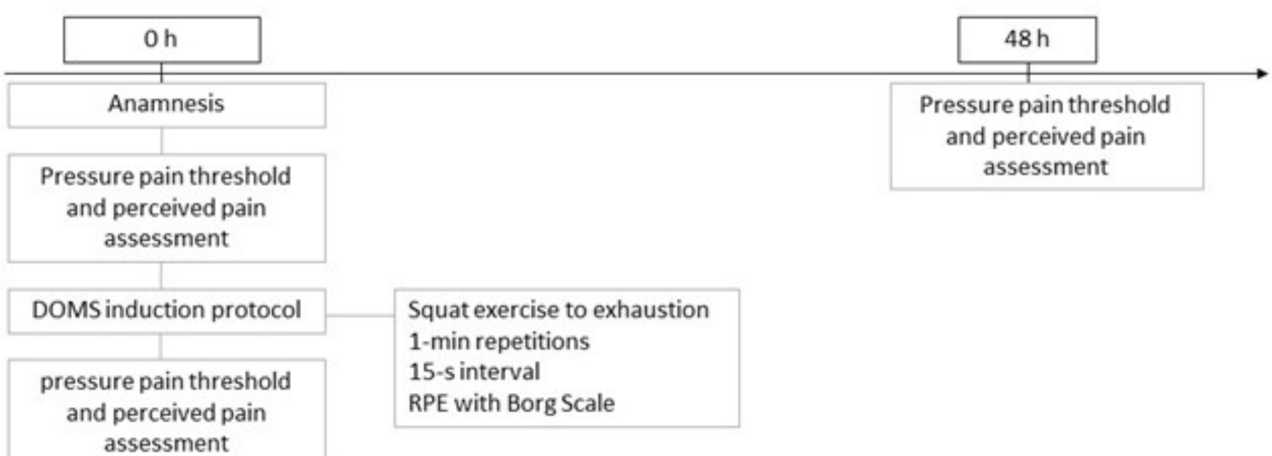

Figure 1. Experimental design.

DOMS induction protocol

To induce quadriceps DOMS, participants completed an exercise protocol for fatigue with maximum squat repetitions to exhaustion with their body weight as workload [20]. A researcher demonstrated how squat movements should be performed in terms of amplitude and movement speed. From the upright posture, the squat movement should be performed by flexing the knees to 90° and returning to the upright position again. To ensure that the participants performed a 90º degree knee flexion, an adjustable height bench was used, and whenever they felt the bench touching their thighs, they should return to the standing position. Participants were instructed to maintain their feet and knees aligned with the shoulders and avoid a knee valgus movement. They performed the maximal number of squats, as fast as possible, for repeated 1-min sets. The 1-min sets were repeated with a 15-s interval in between, until exhaustion. Exhaustion was defined by the participant being no longer able to perform the squat movements or when movement technique deteriorated, for example, reducing the range of motion. The rate of perceived exertion was reported at the end of the exercise using the Borg scale of 6-20 [21].

DOMS assessment

DOMS was estimated using a numeric rating scale ranging from 0 (left side, absence of pain) to 10 (right side, most intense pain) presented to participants in the pre, immediately after (0 h), 24 h, and 48 h post-DOMS induction. For NRS assessment the participants were seated, at rest, and should consider the pain only in the lower limbs as a result of the exercise.

DOMS was also determined by the pressure pain threshold (PPT) measured in the pre (baseline), immediately after (0 h), and 48 h post-DOMS induction, using a digital algometer (Instrutherm DD-200, Portable Digital Dynamometer) with a resolution of 0.01 N and a flat tip with an area of 1 cm^2 [22]. The PPT assessment was done in two days because of the peak DOMS occurs between 48 and 72 hours after the exercise [2], and we chose just two days with presential assessment because of the difficulty to bring the participants to the lab. For bilateral assessment of PPT, patients were in the supine position, with muscles relaxed. The algometer was pressed perpendicularly to the skin surface over the muscle belly of the rectus femoris (RF) and vastus lateralis (VL). For familiarization with the assessment, the algometer was first pressed on the anterior region of the deltoid, a non-exercised body region evaluated to reduce sensitization by systemic factors [23]. The algometer was gradually pressed against the sore region causing discomfort, and the participants were instructed to notify the evaluator when this discomfort became painful [22,23]. Participants were previously advised on how to report the moment when the sensation of pressure became uncomfortable pain. The PPT was recorded in N/cm^2, and the same researcher performed all assessments. Pre-exercise PPT measurement (baseline) was considered as a reference to normalize the subsequent PPT measures. Absolute and normalized values (%baseline) were considered.

Statistical analysis

The normality of data distribution was checked by the Shapiro-Wilk test. Levene's test was used to check the homogeneity. Nonparametric data are presented as median and interquartile intervals and parametric as mean and standard deviation. Qualitative data are reported by frequency and percentage among participants. The age, height, body mass index, the number of squat repetitions, and rate of perceived effort were compared between males and females using the Mann-Whitney U test. Body mass was compared using an independent t-test. Leg preferences were compared with a Pearson Chi-square test. PPT was compared between the preferred and non-preferred legs using a paired t-test. As leg differences were not found, the legs average was used for posterior PPT analyses. A generalized estimating equation (GEE) was used to compare sexes (males vs. females) and time (pre vs. 0 h vs. 48 h) for normalized and non-normalized values of PPT at vastus lateralis, rectus femoris and deltoid and NRS points. A delta was calculated considering the difference between baseline and the 48h absolute and normalized measures of PPT, and NRS. Mann-Whitney U test was used to compare this delta between males and females, except for the Vastus Lateralis normalized PPT compared by independent t-test. Pearson or Spearman's correlations were used to verifying the correlations between the rate of perceived exertion, squat repetitions, and PPT (normalized/non-normalized) and NRS outcomes, considering a pool of data from all participants. All analyzes considered a significance level of 0.05 and were performed using a commercial statistical package (version 26.0. IBM SPSS Statistics for Windows Armonk, NY: IBM Corp).

RESULTS

Table 1 summarizes the participants' characteristics and the exercise outcomes. Females showed lower body mass, body mass index, and height than males.

Numerical rate scale outcomes

NRS values (points, Figure 2) did not differ between females and males at baseline (0.80 ± 0.31 and 0.64 ± 0.34, $p = 0.727$, for females and males, respectively), 0 h (4.77 ± 0.52 and 5.27 ± 0.70, $p = 0.567$) and 48 h post DOMS induction (4.0 ± 0.78 and 3.47 ± 0.84, $p = 0.649$; Figure 2). The time course of NRS did not differ between females and males, with lower values in baseline compared to

0 h (0.72 ± 0.23 and 5.02 ± 0.43, p < 0.001, for females and males, respectively) and 48 h post DOMS induction (0.72 ± 0.23 and 3.74 ± 0.57, p < 0.001), without differences between 0 h and 48 h (5.02 ± 0.43 and 3.74 ± 0.57, p = 0.177).

Table 1. Characteristics of the participants of the study and the outcomes of the fatigue protocol and report of leg preference. Results are presented as median (interquartile interval), mean ± standard. The p-values are for the between-sex comparisons.

	Total (N=26)	Men (n=11)	Women (n=15)	p-value
Age (years)	26 (22-29)	24 (22-27)	27 (22-29)	0.443
Body mass (kg)	66.3 ± 12.5	73.2 ± 70-80	60 ± 55-62	<0.01
Height (cm)	169.2 (160-172)	172 (170.5-180)	160 (157-165.5)	<0.001
BMI (kg/m2)	22.9 (21.7-25.0)	23.39 (22.9-26.3)	22.36 (12.3-23.5)	0.047
Squat repetitions	238 (168-337)	243 (168-307)	233 (174.5-361.5)	0.959
RPE (points)	17 (15-17)	17 (16-17.5)	15 (15-17)	0.281
Leg preference				
Right n (%)	18 (69.2)	5 (45.4)	13 (86.7)	
Left n (%)	2 (7.7)	2 (18.9)	0 (0)	0.057
Both n (%)	6 (23.1)	4 (36.4)	2 (13.4)	

BMI: body mass index; RPE: rate of perceived exertion.

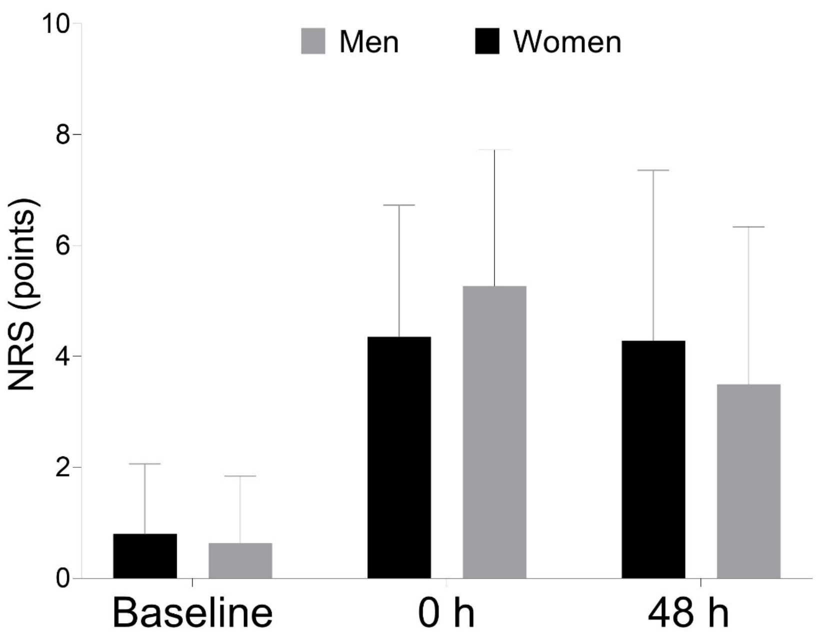

Figure 2. Numeric rate scale (NRS) outcomes for men and women before DOMS induction (Baseline), immediately after DOMS induction (0 h), and 48 h post DOMS induction (48 h).

Absolute pressure pain thresholds

Females presented lower magnitudes of absolute (N/cm2) PPT than males at all times of measure (Figure 3b and 3d). The lower absolute PPT in females were found for the vastus lateralis at baseline (23.45 ± 2.20 and 36.13 ± 3.37, p = 0.002, for females and males, respectively), 0 h (26.98 ± 2.47 and 43.44 ± 4.59, p = 0.002) and 48 h post DOMS induction (19.44 ± 1.99 and 31.66 ± 2.71, p < 0.0001). Similar differences were found for the rectus femoris, with lower absolute PPT in females than males in the baseline (28.94 ± 2.63 and 40.23 ± 3.44, p = 0.009, for females and males, respectively), 0 h (32.20 ± 2.98 and 47.47 ± 3.38, p = 0.001) and 48 h post (25.22 ± 1.88 and 38.58 ± 2.94, p < 0.0001).

The time course for changes in absolute PPT (N/cm2) for the vastus lateralis (Figure 3b) revealed a lower baseline threshold compared to 0 h (29.79 ± 2.01 and 35.21 ± 2.60, p < 0.001) and a higher threshold compared to 48 h (29.79 ± 2.01 and 25.55 ± 1.68, p < 0.0001). Absolute PPT on 0 h was higher than 48 h post (35.21 ± 2.60 and 25.55 ± 1.68, p < 0.0001). Rectus femoris absolute PPT

(Figure 3d) was lower at baseline compared to 0 h (34.59 ± 2.16 and 39.84 ± 2.25, $p < 0.001$) and higher in 0 h than 48 h (39.84 ± 2.16 and 31.90 ± 1.74, $p < 0.001$). No differences were found in absolute rectus femoris PPT between baseline and 48 h (34.59 ± 2.16 and 31.90 ± 1.74, $p = 0.072$). A similar time course was found for each sex, except rectus femoris for females that showed higher values of baseline compared to 48 h post (28.94 ± 2.63 and 25.22 ± 1.88 $p = 0.039$).

Normalized pressure pain thresholds

Normalized PPT (%baseline, Figure 3b and 3d) showed differences between time, without differences between sexes. For vastus lateralis normalized PPT (Figure 3b) no differences were found between females and males on 0 h (116.52 ± 5.84 and 119.70 ± 4.56, $p = 0.668$, for females and males, respectively) and 48 h (84.35 ± 6.06 and 89.10 ± 4.44 $p = 0.528$). The normalized PPT for vastus lateralis at baseline (100%) was lower when compared to 0 h (118.11 ± 3.71 $p < 0.001$, respectively) and higher than 48 h (86.72 ± 3.76, $p = 0.001$). Normalized PPT on 0 h was higher than 48 h post DOMS induction (118.11 ± 3.71 and 86.72 ± 3.76, $p < 0.0001$). Rectus femoris normalized PPT (% baseline, Figure 3d) did not differ between females and males on 0 h (112.18 ± 4.86 and 120.70 ± 5.21, $p = 0.232$, females and males, respectively) and 48 h after DOMS induction (90.81 ± 4.86 and 98.02 ± 5.25, $p = 0.314$). The normalized rectus femoris PPT was lower in the baseline compared to 0 h (116.44 ± 3.56, $p < 0.001$), and higher than in 48 h (116.44 ± 3.56 and 94.42 ± 3.58, $p < 0.001$, for females and males, respectively). Regarding the delta of NRS and PPT, both normalized/non-normalized, there were no differences between males and females (Table 2).

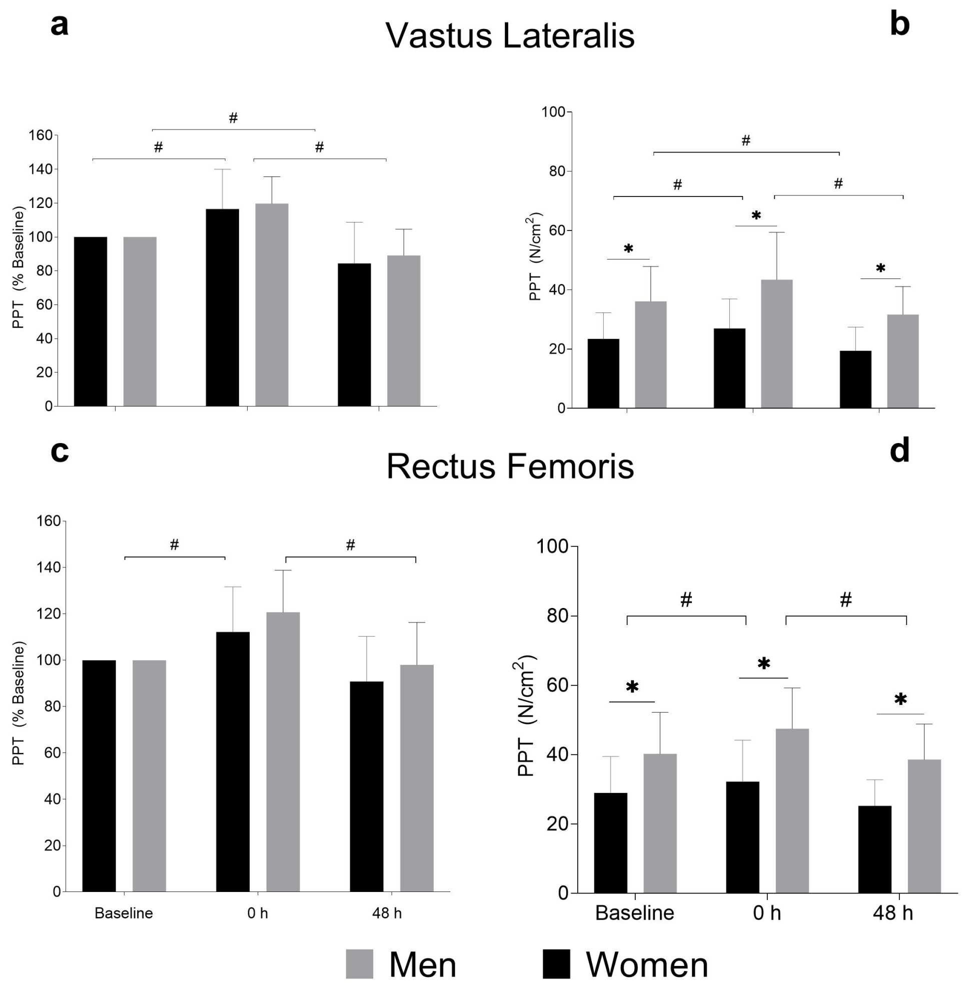

Figure 3. Results for men and women before DOMS induction (Baseline), immediately after DOMS induction (0 h) and 48 h after DOMS induction (48 h). Left column shows results with values normalized by baseline and right column shows absolute values. A-B PPT vastus lateralis; C-D PPT rectus femoris. # Represents the significant difference between times; * represents a significant difference between the sexes. Significant difference p< 0.05.

Table 2. Sex comparison of deltas for NRS and absolute and normalized (%) PPT in Vastus Lateralis (VL) and Rectus Femoris (RF). Data are presented as median (interquartile interval) and mean ± standard deviation. PPT VL: Pressure pain threshold of Vastus lateralis; PPT RF: Pressure pain threshold of Rectus femoris; NRS: Numeric Rate Scale.

	Men (n=11)	Women (n=15)	p-value
ΔPPT VL (N/cm²)	3.14 (1.71-9.89)	3.83 (1.87-9.19)	0.959
ΔPPT VL (%)	-10.89 ± 15.47	-15.64 ± 24.32	0.576
ΔPPT RF (N/cm²)	2.14 (0.50-9.96)	4.59 (3.50-7.78)	0.237
ΔPPT VL (%)	-0.30 ([-22.13] – 9.25)	-18.17 ([-11.35] – 1.34)	0.384
ΔNRS (points)	2.0 (0.5-4.0)	4.0 (0.5-6.0)	0.507

Correlation outcomes

The number of squat repetitions and RPE scores in the exercise protocol to induce fatigue and DOMS did not differ between females and males. Furthermore, these exercise characteristics did not show significant correlations with DOMS outcomes. We found no significant relationship between number of squat repetitions and: NRS on 48 h (r = -0.173 , p = 0.420), absolute PPT on 48 h (r = 0.012, p = 0.953, and r = -0.073 , p = 0.725, for vastus lateralis and rectus femoris, respectively), and normalized PPT on 48 h (r = 0.120, p = 0.558, and r = -0.021, p = 0.917, for vastus lateralis and rectus femoris, respectively). No significant correlation was found between RPE and NRS on 48 h (r = -0.103, p = 0.632) and absolute PPT on 48h (r = 0.025, p = 0.716, and r = - 0.119, p = 0.564, for vastus lateralis and rectus femoris, respectively). No significant correlation was found between RPE and normalized PPT on 48 h (r = 0.092, p = 0.654, and r = 0.144, p = 0.484 for vastus lateralis and rectus femoris, respectively).

DISCUSSION

DOMS assessment is part of the daily routine in contexts of sports performance and rehabilitation, but the different methods available for DOMS assessment may influence outcomes. Here we compared two different methods commonly used in clinical practice for assessing pain in a condition of exercise-induced DOMS in males and females. Our main findings show that numerical rate scale outcomes are comparable between females and males, but absolute values of pressure pain threshold are lower in females than males despite of DOMS. To compare PPT considering patient sex, a normalization of the PPT value considering the baseline measure could be recommended, which result in no difference between sex. We consider these results relevant to guide the clinical evaluation and patient management, especially regarding the importance of a proper baseline measure.

All participants performed the same fatigue protocol for inducing DOMS and achieved a similar number of repetitions and similar RPE. Although physical exercise may have different acute and chronic physiological effects in males and females [18], the volume of work performed, estimated by the number of repetitions performed, did not differ between the males and females participants. These exercise characteristics did not correlate with DOMS outcomes. Therefore, we argue that the sex difference in DOMS measured by PPT is the result of sensitivity for the pressure pain threshold in females rather than the exercise configuration. Furthermore, the lack of difference in the delta of changes in DOMS outcomes between males and females further supports this assumption and reinforces the need for proper normalization of PPT values considering a baseline measure.

The time course of changes in DOMS was similar for the males and females included in our study. DOMS is known to reach peak values between 48 and 72 h after its induction [2]. In our study, the acute effect of the squat-to-exhaustion protocol reduced the pressure pain threshold 48 h after exercise, but the magnitude of the DOMS considering PPT requires normalization for a proper comparison. When sex comparison is discussed, one could always argue about the influence of the menstrual cycle. Indeed, hormonal fluctuations during the menstrual cycle, as well as estrogen levels, can affect exercise-induced muscle damage causing DOMS and reducing muscle strength [24] so we suggest caution when considering these results.

As we tried to mimetize a clinical condition in which the phase of the menstrual cycle for a patient can not be controlled, the influences of this variable on DOMS outcomes may require an experiment aimed specifically at this research question. Despite this, the baseline measures indicated sex differences in pain thresholds, which reinforces that sex differences might be present regardless of the exercise and therefore there is a need to normalize PPT values, especially when aiming to compare both sexes. Finally, the normalization also makes PPT outcomes similar those observed with NRS, suggesting that if the aim of the study is just control magnitude of pain maybe there is no need to add more than NRS.

Our study has some limitations. The limited sample size without randomization is assumed, although we understand that our results present interesting directions for the guidance of the DOMS assessment. The presence of muscle damage due to fatigue protocol is securely based in the DOMS outcomes, but we lack in measuring the baseline muscle damage condition in the participants.

CONCLUSION

Numerical rate scale and normalized pressure pain threshold outcomes are comparable between females and males subjected to a fatigue protocol of exercise to induce delayed onset muscle soreness. Absolute values of pressure pain thresholds show higher pain sensitivity in females regardless of exercise. Baseline measures can be used to normalize pain outcomes and allow sex comparisons in the time course of DOMS.

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