TITLE

Effects of experimentally induced muscle pain on endurance performance: a proof-of-concept study assessing neurophysiological and perceptual responses

AUTHORS

Raul Canestri[1], Paulo Estevão Franco-Alvarenga[1,2,3], Cayque Brietzke[1,2], Ítalo Vinícius[1], Samuel A. Smith[4], Alexis R. Mauger[4], Márcio Fagundes Goethel[1,5,6], Flávio Oliveira Pires[1,2]

INSTITUTION AND AFFILIATIONS

1. Exercise Psychophysiology Research Group, School of Arts, Sciences and Humanities, University of São Paulo, São Paulo, Brazil

2. Human Movement Science and Rehabilitation Program, Federal University of São Paulo, Santos, Brazil

3. Estácio de Sá University (UNESA), Resende, Brazil

4. School of Sport & Exercise Sciences, University of Kent, Kent, United Kingdom

5. Centre of Research, Education, Innovation and Intervention in Sport, Faculty of Sport, University of Porto, Porto, Portugal

6. Porto Biomechanics Laboratory (LABIOMEP), University of Porto, Porto, Portugal

RUNNING TITLE

Muscle pain effects on endurance performance

CORRESPONDING AUTHOR:

Flávio Oliveira Pires, PhD, Associate Professor at the School of Arts, Sciences and Humanities, University of São Paulo, São Paulo, Brazil. Rua Arlindo Béttio, 1000 - Ermelino

Matarazzo, 03828-000, São Paulo - SP, Phone number: +55-11-995335777. E-mail: piresfo@usp.br

IMPACT STATEMENT

Intense exercise induces muscle pain, which potentiates muscle fatigue and reduces performance. We investigated neurophysiological and perceptual responses to exercise after inducing muscle pain by a model that separates muscle pain from muscle fatigue. Pain induced by hypertonic saline injection shifted the activation in pain cortical areas at rest and during exercise, altered the motor command to peripheral muscles assessed with EMG and reduced performance. Muscle pain also influenced perceived exertion and displeasure during exercise.

ABSTRACT

Pain arising from exercise potentiates fatigue and impairs the performance of endurance exercise. We assessed neurophysiological and perceptual responses to endurance exercise performed under experimentally-induced muscle pain by a model that separates muscle pain from muscle fatigue. After a series of pilot studies investigating different hypertonic saline volumes, 17 healthy males performed a preliminary VO_{2PEAK} test before performing a familiarization of the cycling time-to-exhaustion exercise (80% of the peak power output in the VO_{2PEAK} test). Participants, performed a baseline exercise session before the sessions with hypertonic and isotonic saline injections in the vastus lateralis of both legs, in a crossover and counterbalanced design. Neurophysiological and perceptual responses such as electroencephalography (EEG) in frontal, prefrontal, parietal and motor cortex, electromyography (EMG) of the vastus lateralis and biceps femoris muscles, ratings of perceived exertion (RPE), pain sensation and affective valence were measured at rest and during exercise. The hypertonic injection reduced the resting EEG alpha-beta ratio in the frontal and prefrontal cortex. When compared to exercise performed after the isotonic injection (430.5 ± 152.6s), hypertonic injection shortened the time-to-exhaustion (357.5 ± 173.0s), reduced the EMG of the assessed muscles, and increased the muscle co-contraction during exercise. The hypertonic injection also reduced the EEG alpha-beta ratio in the prefrontal and parietal cortex, increased RPE and pain sensation, and reduced affective valence during exercise. This proof-of-concept study showed that hypertonic injection-induced muscle pain reduced endurance performance, promoting centrally mediated alterations in motor command and cortical activation, as well as an interplay of perceptual responses.

KEYWORDS: EEG, endurance performance, fatigue, hypertonic saline, perceived exertion

1 INTRODUCTION

Performance in strenuous whole-body endurance exercise has been associated with the ability to tolerate unpleasant sensations such as pain while regulating motor output as fatigue progresses during exercise (Arendt-Nielsen & Graven-Nielsen, 2008; Astokorki & Mauger, 2017a; Meeusen et al., 2016). Overall, it has been suggested that exercise-induced muscle pain (or exercise-associated pain) frequently described as “aching”, “cramping” and “burning”, may potentiate fatigue and accelerate the reduction of the exercise capacity (Mauger, 2014).

Most studies have investigated the effects of pain on endurance exercise performance either through an exercise-induced muscle pain model in which pain arises from the exercise (Astokorki & Mauger, 2017b, 2017a) or a delayed onset muscular soreness model (Plattner, Lambert, Tam, & Baumeister, 2012; Plattner, Lambert, Tam, Lamberts, & Baumeister, 2014). Although these models may be useful to investigate muscle pain effects on exercise capacity in different contexts, they may be inappropriate to investigate the interplay between muscle pain and muscle fatigue during exercise. For example, although the exercise-induced muscle pain model is appropriate to investigate how pain arises from the exercise in progression, it does not separate the exercise-induced muscle pain from the exercise-induced muscle fatigue. Moreover, the presence of pain before the onset of exercise, rather than induced by the exercise in progression, may be clinically relevant for exercise medicine scenarios. Whereas the delayed onset muscular soreness model is sound to investigate long-term muscle pain effects, it also changes the muscle fiber electrophysiological properties and central command responses, thereby leading to a reduced exercise capacity irrespective of the muscle pain (Plattner et al., 2012, 2014). Consequently, a challenge for studies investigating the muscle pain-muscle fatigue relationship is the induction of a stable and controlled tonic pain at the onset of exercise, free from changes in fiber membrane properties, to investigate the muscle pain effects before the muscle fatigue is set in. Such a pain model should also induce a pain sensation qualitatively sensed as “aching” and “burning”.

The intramuscular injection of hypertonic saline is a well-established pain model that mimics the manifestation of clinical pain, which is often described as “aching”, “cramping” and “burning” (Graven-Nielsen, Arendt-Nielsen, Svensson, & Jensen, 1997). This model induces muscle pain without affecting muscle fiber electrophysiological properties, as the noxious stimulus is promoted by altering the intramuscular sodium concentrations (Farina, Arendt-Nielsen, & Graven-Nielsen, 2005; Graven-Nielsen & Mense, 2001). Hence, this model allows studying the centrally mediated muscle pain effects in the healthy neuromuscular system irrespective of muscle fatigue, while sodium and potassium accumulation is negligible during exercise. Although a number of studies have demonstrated that hypertonic saline-induced muscle pain reduces muscle strength in isometric voluntary contractions (Graven-Nielsen, Lund, Arendt-Nielsen, Danneskiold-Samsoe, & Bliddal, 2002; Graven-Nielsen, Svensson, & Arendt-Nielsen, 1997; Smith, Micklewright, Winter, & Mauger, 2020) through changes in cortical activation and central command (Chang, Arendt-Nielsen, Graven-Nielsen, & Chen,

2003; Farina et al., 2005), as well as agonist-antagonist muscles' electromyography (EMG) distribution (Falla, Farina, Dahl, & Graven-Nielsen, 2007; Farina et al., 2005; Poortvliet, Tucker, Finnigan, Scott, & Hodges, 2019), no study has investigated the hypertonic-induced muscle pain effects on endurance exercise performance. We performed a systematic search for pain-endurance exercise articles and found that no study used the hypertonic saline model to study the muscle pain-muscle fatigue interplay during endurance exercise (Supplementary table 1 and table 2), so that a comprehensive description of this pain model in exercise such as cycling has yet to be provided.

A "proof-of-concept" (Kendig, 2016) of the hypertonic-induced muscle pain effects on endurance exercise performance should report a variety of neurophysiological responses to exercise. Besides changes in agonist-antagonist muscle activation (co-contraction) during exercise (Falla et al., 2007), the assessment of cortical alterations to hypertonic-induced muscle pain may reveal how pain is centrally mediated, as a previous study showed that pain induced by repeated hypertonic injections at rest was associated with slower-to-faster electroencephalography (EEG) frequency band shift in frontal and posterior cortex areas (Chang et al., 2003). Results obtained with different pain models also showed that EEG alpha and beta waves were sensitive to muscle pain (Plattner et al., 2012, 2014). Therefore, as central processing of pain involves activation of frontal and posterior cortex areas, it is hypothesized that hypertonic saline-induced muscle pain induces a slower-to-faster EEG frequency band shift in areas of the cortex involved with integrating movement perception and proprioception (Chang, Arendt-Nielsen, Graven-Nielsen, Svensson, & Chen, 2001; Plattner et al., 2014).

A description of the hypertonic-induced muscle pain effects on endurance exercises also requires the inclusion of perceptual responses such as pain sensation, ratings of perceived exertion (RPE) and affective valence. According to a three-dimensional model of pain (Melzack & Wall, 1965) and exercise regulation (Venhorst, Micklewright, & Noakes, 2018), pain and exercise fatigability is interdependently governed by sensory-discriminative, affective-motivational and cognitive-evaluative dimensions. While pain sensation and RPE may inform about a sensory-discriminative dimension, affective valence may indicate the affective-motivational dimension (Moayedi & Davis, 2013; Venhorst et al., 2018). Together with behavioural changes such as exercise performance, these perceptual variables could provide a comprehensive picture of the muscle pain-muscle fatigue interplay effects on endurance exercis.

Therefore, we investigated the hypertonic-induced muscle pain effects on the time-to-exhaustion cycling exercise performance, assessing agonist-antagonist muscle EMG distribution, cortical activation and perceptual variables associated with sensory-discriminative and affective-motivational dimensions of pain and exercise regulation. We hypothesized that muscle pain induced prior to exercise would shorten the time-to-exhaustion, shifting agonist-antagonist muscle EMG distribution and EEG bands frequency. This pain model would further reveal the interplay between different dimensions of pain and fatigability during exercise.

2 METHOD

2.1 Participants

Following a prior sample size calculation (as detailed in the Statistics section), physically active males (n = 17) volunteered to participate in the full experimental procedures. Participants had no history of chronic pain or neuromuscular disorders, and were instructed to abstain from stimulants (coffee, energy drink, etc.), painkillers, and alcoholic beverages as well as from intense exercise for the 48 h before the sessions. Participants were informed about the risks and benefits of the experimental procedures, thereafter written informed consent was obtained. The experimental protocol was previously approved by the local Ethics Committee (#3.390.457) and conformed to the Declaration of Helsinki.

2.2 Study design

To support some methodological decisions of a proof-of-concept study, we first conducted a preliminary study in 37 participants to investigate: a) the effects of different volumes of hypertonic saline solution on muscle pain sensation; b) the pain sensation time-course of the selected volume; c) and the reliability of the time-to-exhaustion exercise. Readers can access preliminary results (investigation “a” and “b”) in supplementary figure 1 (panel a and b) and Methods (investigation “c”). Then, we conducted the main study to investigate the hypertonic-induced muscle pain effects on a variety of neurophysiological and perceptual responses during endurance exercise (Figure 1). Eligible participants (“see section 2. Induced muscle pain”) attended additional sessions to perform: 1) a maximal incremental cycling test to assess peak oxygen uptake (VO2PEAK) and peak power output (WPEAK), and a familiarization with the cycling time-to-exhaustion exercise and perceptual scales; 2) a baseline time-to-exhaustion exercise and a second familiarization with scales; 3 and 4) a time-to-exhaustion exercise after hypertonic or isotonic saline injection. Sessions 1 and 2 were performed in sequential order, while sessions 3 and 4 were performed in a counterbalanced order. The baseline session served only as an experimental procedure without induced muscle pain, so that participants could properly get acquainted with the experimental setup of exercise before the exercise sessions with saline injections (i.e. hypertonic and isotonic). All the sessions were conducted in a controlled and quiet environment (21º C temperature and 60% humidity) at the same time of the day.

*** FIGURE 1 ***

2.3 Induced muscle pain

Given that different studies reported a broad range of hypertonic volumes injected in a variety of muscles (Graven-Nielsen, 2006; Graven-Nielsen et al., 2002), we initially studied the effects of different hypertonic volumes on the muscle pain sensation time-course. Four participants sat comfortably on a chair, having their legs flexed at 90º. Different hypertonic volumes (2.0, 2.5, and 3.0 mL) were simultaneously injected into the vastus lateralis (VL) muscle belly (medial portion) of both legs, in a randomized order (3 to 7 days apart). Injections were synchronously administered over a 20 s timeframe by two researchers through a 5 mL syringe (20 G x 30 mm stainless steel needle) in a single bolus, as this was the shortest possible time that did not cause excessive discomfort to participants. Immediately after the injection (i.e. time zero), intensity of pain sensation was measured through a 0 to 10 points numerical scale (Cook, O'Connor, Eubanks, Smith, & Lee, 1997) at 1 min intervals, until a no-pain sensation has been rated (i.e. a pain sensation of "0"). We observed that different hypertonic volumes produced comparable muscle pain responses across time (Supplementary figure 1), therefore we used the lowest volume (i.e. 2.0 mL) in the main study. Importantly, we aimed to provide evidence with high internal validity for the use of hypertonic saline injection-induced muscle pain model in endurance exercise while assessing different variables, thus we reduced the between-subjects variability on muscle pain responses by recruiting to the main study only participants rating a peak pain sensation > 7 (very strong pain). Furthermore, this muscle pain threshold allowed us to assess resting EEG in a 3 min post-saline injection period, preserving a strong pain effect size for the exercise bout.

Muscle pain effects on agonist rather than synergic muscles involved in cycling were the objective of the main investigation, as we were interested in the agonist-antagonist muscle distribution. Therefore, 2.0 mL of hypertonic (6% NaCl) or isotonic (0.9% NaCl) solution were injected into the VL muscle belly of both legs, a muscle primarily recruited in cycling. Before the injections, participants performed a standard warm-up (5 min cycling at 100 W and 80 rpm). While they were still on the bicycle, they positioned both feet on steps specially built to keep their knees at 90º. The solutions were then injected, and participants were instructed to rate their muscle pain sensation every minute through the 11-point numerical scale. To minimize the pain sensation variability at the onset of the time-to-exhaustion exercise bout after the hypertonic injection, eligible participants (peak pain sensation > 7) started exercising only when they rated a pain sensation equal 5 (4.9 ± 0.3 a.u.), but they were unaware of this pain sensation threshold. In contrast, participants started the exercise bout after the post-injection EEG sampling in the isotonic saline condition (i.e. 3 min). This standardization produced small differences in time from the injections to the cycling exercise bout commencement between hypertonic (3.9 ± 1.3 min) and isotonic condition (3.0 ± 0.0 min).

Considering that hypertonic saline injection alters EEG and EMG responses (Chang et al., 2003; Graven-Nielsen & Arendt-Nielsen, 2008), baseline EEG signal was obtained during a 3 min rest period before the injections. Baseline EMG signal was continuously obtained during the standard warm-up (5 min cycling at 100 W and 80 rpm), before the injections. Hence, both EEG and EMG data used for normalization were obtained before injections of hypertonic and isotonic saline.

2.4. Cycling tests

All cycling tests were conducted on a bicycle (Giant®, United States) adjusted with comfortable saddle and pedals, attached to a cycle-simulator (CompuTrainer™ RacerMate® 8000, EUA), and calibrated before every test according to manufacturer's recommendations. Eligible participants performed a maximal incremental test for VO2PEAK and WPEAK assessment. They warmed up for 5 min at 100 W while maintaining a pedal cadence of 80 rpm, then they immediately started the incremental test. Power output was increased by 25 W·min−1 until exhaustion, defined as the inability to maintain 80 rpm pedal cadence despite three strong verbal encouragements. The WPEAK was defined as the highest power output recorded during the test.

The time-to-exhaustion cycling tests were set at 80% of the WPEAK. Participants warmed up for 5 min at 100 W (80 rpm pedal cadence), then they immediately started the time-to-exhaustion exercise bout. Participants were instructed to exercise to their limit of tolerance, but no verbal encouragement was provided during the time-to-exhaustion cycling tests. Participants had no available exercise feedback such as time, power output, or cadence, but a researcher verbally informed them to up or down the pedal cadence when it deviated 3 rpm or more from the 80 rpm cadence. The performance was recorded as time-to-exhaustion (s), defined as the inability to maintain the target pedal cadence despite three verbal encouragements. Feedback of performance was provided only at the study completion.

We calculated the time-to-exhaustion cycling test reliability and minimal worthwhile change (Weir, 2005) in 8 eligible participants. Before the experimental sessions with saline injections, these participants performed an extra baseline exercise 72 h from the first baseline exercise. We observed an intra-individual coefficient of variation of 3.3% (range from 1.5% to 4.9%) in performance when expressed as time-to-exhaustion, so that a worthwhile change caused by the induced muscle pain would require a difference in time greater than 29.5 s from control (isotonic injection).

2.5 Instruments, Measures and Data Analysis

Cortical activation was continuously obtained at rest and throughout the exercise bout through an EEG unit (Emsa®, EEG BNT 36, TiEEG, Rio de Janeiro - Brazil) at Fp1, F3, Cz, and P3 positions, according to the international EEG 10–20 system (Maurits, 2011). These positions were determined according to frontal and sagittal planes and referenced to the mastoid. The EEG was recorded at a 600 Hz sampling frequency, through active electrodes

(Ag-AgCl) with resistance ~10 KΩ. After exfoliation and cleaning, electrodes were fixed with a conductive gel, adhesive tape, and medical strips. The EEG signal was recorded during a 3 min rest, immediately before and after the injections, as well as throughout the exercise period. The resting EEG was recorded when participants were completely calm with eyes closed, avoiding head and trunk movements. The EEG signal was amplified (gain of 1 × 103) and filtered with a digital notch (60 Hz). Moreover, a signal showing spectral leakage (defined as ≥ 100μv) was considered as an artefact (n= 1–4, depending on the moment of the experimental setup) and excluded from analysis (Maurits, 2011). The resting EEG data recorded during the first and last 30 s of a 3 min time window were removed to avoid noise associated with the eventual body movements when participants were expecting the start and stop of EEG recording. EEG data were analysed in frequency domains through a fast-Fourier transformation so that the total power spectral density (tPSD) of alpha (8-13 Hz) and beta (14-30 Hz) waves were calculated over the steadiest (i.e. lowest SD) 15 s window during the remaining 120 s time. Accordingly, the exercise alpha and beta waves tPSD were calculated over a 15 s window every 25% of the total exercise duration. Considering that the exercise per se may increase EEG alpha and beta bands within a varied magnitude (Ftaiti, Kacem, Jaidane, Tabka, & Dogui, 2010; Pires et al., 2016; Robertson & Marino, 2015), we used an alpha-beta ratio to provide a clearer index of a slower-to-faster EEG band frequency shift, as suggested elsewhere (Chang et al., 2003). Thus, we obtained the exercise alpha-beta ratio index by dividing exercise alpha by exercise beta, after correcting exercise EEG data by baseline EEG data (prior to injection). This calculation reduced the interindividual and day-to-day variability and provided a clearer slower-to-faster EEG band frequency shift (Ftaiti et al., 2010; Nielsen, Hyldig, Bidstrup, Gonzalez-Alonso, & Christoffersen, 2001; Nybo & Nielsen, 2001).

The EMG was continuously assessed throughout the exercise bout through a surface bipolar active electrode placed over the VL and biceps femoris (BF) muscles, following recommendations by SENIAM (Hermens, Freriks, Disselhorst-Klug, & Rau, 2000). Before the electrode placement, the skin was shaved, cleaned, and exfoliated, then the electrode position was marked with a surgical pen on the skin to ensure the same electrode placement on the following testing session. The EMG signal was amplified (gain of 1 x 103) and sampled at 2 kHz (EMG Systems®, São José dos Campos - Brazil), thereafter the EMG signal was filtered by a bandpass 4th order recursive Butterworth filter having cut-off frequencies between 20 and 500 Hz. The exercise EMG data were used to calculate the root mean square (RMS) over the last 15 s window every 25% of the total cycling exercise bout. Afterward, the exercise EMG data were expressed as a percentage of the warm-up EMG signal obtained before the injections. The VL-BF co-contraction was calculated through the equation proposed by Winter (1990), after rectifying and enveloping the signal with a cut-off frequency at 6 Hz. We applied the following equation: %co-contraction = 2 x (common area A & B / area A + area B) x 100, where %co-contraction is the percentage of co-contraction between two antagonistic muscles, common area A & B is the common area of activity between two antagonistic muscles, and area A and B is the area under the enveloped muscle VL and BF EMG curve, respectively.

Participants wore a mask (Hans Rudolph®, Lenexa, KS, United States) connected to an open-system gas analyser for breath-by-breath measurements of the gaseous exchange such as VE and VO2 during exercise (maximal incremental test and time-to-exhaustion exercises). The gas analyser (Cortex Metalyzer 3B®, Germany) was calibrated according to

the manufacturer's recommendaton, using a 3 L syringe (Quinton Instruments®, Milwaukee, WI, United States) before each test. In addition, a cardio belt (Polar®, Finland) assessed HR beat-to-beat. The VE, VO2, and HR data were simultaneously collected during the maximal incremental test, thereafter VO2 data were smoothed to 10 s intervals to determine the VO2PEAK (average of the highest values in the test). The raw VE and VO2 data collected throughout the time-to-exhaustion exercise bouts were filtered to 8-breath moving averages and values higher than three standard deviations from the local mean were replaced by the local mean. A cubic spline interpolation technique provided VE, VO2, and HR data at 1 Hz frequency, before averaging the data within the last 15 s at every 25% of the total cycling time-to-exhaustion exercise bout.

Muscle pain sensation was assessed at rest, before and after the injections, as well as throughout the time-to-exhaustion exercises through an 11-point numerical scale that rates "no pain at all" as 0 and "extremely intense pain" as 10 (Cook et al., 1997). Participants were oriented to consider "extremely intense pain" as the worst pain they ever experienced. Moreover, they were instructed to consider the effort to produce motor output (i.e. to drive the limb) when classifying their RPE, thus avoiding that sensations of pain were mixed with perceived exertion. We asked them to rate how hard, heavy, and strenuous the physical task was by using a 15-point Borg scale, as suggested elsewhere (Staiano, Bosio, de Morree, Rampinini, & Marcora, 2018). Furthermore, affective valence was obtained through an 11-point feeling scale (Hardy & Rejeski, 1989), having descriptors as "neutral" (zero), "very good" (+5) and "very bad" (−5). The researcher showed the scales every 60 s during exercise in random order, and participants rated the intensity of pain sensation, RPE, and affective valence. To provide psychological responses paired with neurophysiological ones at every 25% of the time-to-exhaustion exercise bout (i.e. 25%, 50%, 75% and 100%), we used linear regressions to estimate pain sensation ($R2 = 0.90$ to 0.99) and RPE ($R2 = 0.93$ to 0.99), and polynomial regression to estimate affective valence values ($R2 = 0.92$ to 0.98).

2.6 Statistics

Data are presented as mean and standard deviation (±SD), after checking the Gaussian distribution. Briefly, we estimated the required sample size (G-power software 3.1.2, Germany) based on the hypertonic-induced muscle pain effect size (ES) reported by Graven-Nielsen et al. (2002), assuming pain main effects with $\eta2 = 0.24$ and pain by time interaction effects with $\eta2 = 0.199$. A sample size of 10 and 12 participants should be recruited if assuming a power > 0.80 ($p < 0.05$) in a repeated-measures design, having pain main effects and pain by time interaction effects, respectively. However, we expected a considerable sample loss due to the nature of experimental procedures, thus we enlarged the sample size to 17 individuals. The time-to-exhaustion exercise performance as well as EEG alpha-beta ratio were compared between hypertonic and isotonic conditions through a paired T-student test. A repeated-measures mixed model ANOVA compared EMG, %co-contraction, EEG alpha-beta ratio, VO2, VE, HR, RPE, pain sensation and affective valence responses to the time-to-exhaustion exercise bout between hypertonic and isotonic conditions, having pain condition (hypertonic vs isotonic) and time (25%, 50%, 75% and

100% of the exercise) as fixed factors, and participants as the random one. Importantly, we were interested in relative rather than systematic changes caused by non-random effects (e.g. interindividual variability), thus we expressed neurophysiological and perceptual responses relative to the total exercise duration (Hopkins, 2000). Bonferroni corrections were used for multiple comparisons in cases of significant F-values. We further checked the accuracy of the prior sample size estimation in a post-hoc calculation by using the most appropriate equation according to the statistical test family (Cohen's d and f2 to paired-T test and mixed model design, respectively). However, to make comparisons with previous literature easier we expressed all ES as Cohen's d, and interpreted values as small ($d \leq 0.1$), moderate ($0.1 > d < 0.3$), large ($0.3 > d < 0.5$), very large ($0.5 > d < 0.9$) and extremely large ($d > 0.9$) (Hopkins, Marshall, Batterham, & Hanin, 2009).

3 RESULTS

3.1 Descriptive Results

Eligible participants were 22.5 ± 3.7 years old, 73.3 ± 7.8 kg body mass, 176 ± 6.0 cm height, and 11.0 ± 3.9 % body fat. They achieved a WPEAK of 241.5 ± 31.2 W and a VO2PEAK of 44.1 ± 4.8 mL·kg-1·min-1 during the maximal incremental cycling test. In the time-to-exhaustion exercise baseline session, the average time-to-exhaustion was 449.6 ± 141.1 s. Other baseline results are shown in figures 2 to 5 of the Supplementary files.

3.1 Hypertonic saline-induced muscle pain effects

Hypertonic-induced muscle pain caused a significantly shorter time to exhaustion by 16.9% (357.5 ± 173.0 s; $t = 2.51$ $p = 0.02$; $d = 0.44$ large ES) when compared to isotonic condition (430.5 ± 152.6 s).

Regarding the cardiopulmonary responses to exercise after injection of hypertonic and isotonic saline solution, no pain by time interaction effect was observed in VO2 ($F = 0.21$; $p = 0.89$; $d = 0.18$ moderate ES), VE ($F = 0.20$; $p = 0.89$; $d = 0.18$ moderate ES) and HR ($F = 0.25$; $p = 0.85$; $d = 0.21$ moderate ES). Important main effects of pain and time were observed, as the reduced endurance cycling performance with hypertonic injection-induced muscle pain was accompanied by lower cardiopulmonary responses throughout the exercise bout, such as VO2 (pain main effect, $F = 4.15$; $p = 0.04$; $d = 0.82$ very large ES; time main effect, $F = 67.07$; $p < 0.001$; $d = 3.29$ extremely large ES), VE (pain main effect, $F = 5.49$; $p = 0.02$; $d = 0.94$ extremely large ES; time main effect, $F = 114.15$; $p < 0.001$; $d = 4.3$

extremely large ES) and HR (pain main effect, $F = 29.85$; $p < 0.001$; $d = 2.33$ extremely large ES; time main effect, $F = 177.53$; $p < 0.001$; $d = 5.70$ extremely large ES). Figure 2 depicts cardiopulmonary results during the time-to-exhaustion exercise bouts.

*** FIGURE 2 ***

Furthermore, we observed no pain by time interaction effect in muscle activation, either assessed as VL ($F = 0.57$; $p = 0.63$; $d = 0.39$ large ES) or BF muscle EMG ($F = 0.49$; $p = 0.68$; $d = 0.36$ large ES). However, the hypertonic saline-induced muscle pain led to a lower muscle activation throughout the exercise session, either assessed as VL (pain main effect, $F = 4.05$; $p = 0.04$; $d = 1.05$ extremely large ES; time main effect, $F = 3.67$; $p = 0.01$; $d = 1.00$ extremely large ES) or assessed as BF EMG (pain main effect, $F = 4.22$; $p = 0.04$; $d = 1.07$ extremely large ES; time main effect, $F = 4.87$; $p = 0.005$; $d = 1.15$ extremely large ES). Accordingly, we did not find a pain by time interaction effect in %co-contraction during exercise ($F = 0.23$; $p = 0.87$; $d = 0.23$ moderate ES), although the higher VL-BF co-contraction after hypertonic than isotonic saline injection (pain main effect, $F = 5.47$; $p = 0.02$; $d = 1.14$ extremely large ES; time main effect, $F = 0.34$; $p = 0.79$; $d = 0.28$ moderate ES). Figure 3 presents these EMG results.

*** FIGURE 3 ***

Regarding the muscle pain-induced cortical alterations at rest, hypertonic injection reduced the EEG alpha-beta ratio in Fp1 ($t = -2.33$; $p = 0.04$; $d = 0.68$ very large ES), F3 ($t = -2.67$; $p = 0.02$; $d = 1.07$ extremely large ES) and Cz positions ($t = -2.18$; $p = 0.05$; $d = 0.84$ very large ES), but no pain effect was observed in P3 ($t = -1.31$; $p = 0.21$; $d = 0,56$ very large ES). Figure 4 depicts these resting EEG results.

The exercise-derived EEG data revealed no pain by time interaction effect in derivations such as Fp1 ($F = 0.15$; $p = 0.92$; $d = 0.15$ moderate ES), F3 ($F = 0.60$; $p = 0.61$; $d = 0.32$ large ES), P3 ($F = 1.59$; $p = 0.19$; $d = 0.54$ very large ES) and Cz ($F = 0.71$; $p = 0.54$; $d = 0.34$ large ES). In contrast, the hypertonic saline-induced muscle pain lowered the cortical activation throughout the cycling exercise bout, given the lower EEG alpha-beta ratio in Fp1 (pain main effect, $F = 4.28$; $p = 0.04$; $d = 0.84$ very large ES; time main effect, $F = 4.52$; $p = 0.006$; $d = 0.87$ very large ES) and P3 positions (pain main effect, $F = 9.95$; $p = 0.002$; $d = 1.35$ extremely large ES; time main effect, $F = 3.68$; $p = 0.01$; $d = 0.82$ very large ES). No pain main effect was detected in Cz ($F = 3.71$; $p = 0.06$; $d = 0.78$ very large ES), although the time main effect ($F = 3.47$; $p = 0.02$; $d = 0.76$ very large ES). Accordingly, neither pain main effect ($F = 2.59$; $p = 0.11$; $d = 0.67$ very large ES) nor time main effect was found in F3 ($F = 2.62$; $p = 0.06$ $d = 0.68$ very large ES). Figure 5 depicts the EEG responses during exercise.

*** FIGURE 4 ***

*** FIGURE 5 ***

Regarding the perceptual variables, a pain by time interaction effect was observed in muscle pain sensation when the exercise started in the presence of induced muscle pain ($F = 6.62$; $p = 0.001$; $d = 0.91$ extremely large ES), as participants rated a higher pain sensation at 25% ($p < 0.001$) and 50% ($p = 0.01$) of the cycling exercise duration. However, there were no pain by time interaction effects in RPE ($F = 1.76$; $p = 0.15$; $d = 0.46$ large ES) and affective valence ($F = 2.18$; $p = 0.09$; $d = 0.52$ very large ES). Importantly, pain main effects were observed in perceptual variables, as there was a higher pain sensation (pain main effect, $F = 17.99$; $p < 0.001$; $d = 1.50$ extremely large ES) and RPE (pain main effect, $F = 9.64$; $p = 0.002$; $d = 1.09$ extremely large ES), and a lower affective valence (pain main effect, $F = 10.63$; $p = 0.001$; $d = 1.15$ extremely large ES) throughout the time-to-exhaustion exercise bout after hypertonic injection. A time main effect was also observed in pain sensation ($F = 35.12$; $p < 0.001$; $d = 2.09$ extremely large ES), RPE ($F = 290.32$; $p < 0.001$; $d = 6.02$ extremely large ES) and affective valence ($F = 47.40$; $p < 0.001$; $d = 2.43$ extremely large ES) likely as a result of the exercise in progression, regardless of painful conditions (Figure 6).

*** FIGURE 6 ***

4 DISCUSSION

Through a number of neurophysiological and perceptual responses, we observed that hypertonic saline injection was an effective model to study muscle pain effects on endurance exercise performance. Similar to the outcome observed in strength exercise (Graven-Nielsen et al., 2002), we found that this experimental muscle pain model reduced endurance exercise performance through centrally mediated factors such as motor command and cortical activation. Moreover, perceptual responses revealed an important interplay between sensory-discriminative, affective-motivational and cognitive-evaluative dimensions during exercise under muscle pain.

We observed a 16.9% mean reduction in time to exhaustion when participants completed the cycling exercise bout having hypertonic saline-induced muscle pain. The reduced exercise capacity in the hypertonic condition was accompanied by a lower cardiopulmonary response to exercise (e.g. VO_2, VE, and HR). This was not unexpected, given that cardiopulmonary variables respond to the magnitude of motor output during dynamc whole-body exercises (Pires et al., 2016, 2011). Interestingly, the hypertonic saline-induced muscle pain produced a mismatch in pain sensation between painful and non-painful conditions during the first half of the exercise bout, but not at any further time-point. This result is discussed ahead (“Methodological Aspects” section).

From a neurophysiological perspective, our results suggest an altered motor command to peripheral muscles likely associated with an increased afferent activity with muscle pain. Since the hypertonic saline solution changes neither muscle electrophysiological properties,

nor muscle conduction velocity and neuromuscular transmission (Falla et al., 2007; Farina et al., 2005; Graven-Nielsen et al., 2002; Khan, McNeil, Gandevia, & Taylor, 2011), this model is suggested to reduce corticomotor output to painful muscles as a result of the increased type III and IV afferents-inhibited α-motoneuron excitation (Graven-Nielsen et al., 2002). Indeed, it has been proposed that motor command as measured as EMG in painful muscles (Taylor, Amann, Duchateau, Meeusen, & Rice, 2016) is reduced due to pain-related signaling to pain processing cerebral regions (Graven-Nielsen, 2006). In the present study, we found that a higher pain sensation in hypertonic condition paralleled a reduced activation of the VL and BF during exercise, corroborating results obtained in different exercise modes after an induced muscle or joint pain (Ervilha, Farina, Arendt-Nielsen, & Graven-Nielsen, 2005; Falla et al., 2007; Farina et al., 2005; Rice, Mannion, Lewis, McNair, & Fort, 2019). The finding that pain reduced the VL and BF EMG although the power-matched constant cycling is not necessarily a contradiction, as the experimentally induced muscle pain may have increased the recruitment of other agonist or synergist muscles (not measured in the present study) not affected by the hypertonic injection in order to maintain the target power output. Indeed, as suggested elsewhere (Ervilha et al., 2005), we found an increased VL-BF muscles co-contraction throughout the exercise bout under muscle pain, showing a greater activation in non-painful muscle (BF muscle) relative to painful muscle (VL muscle). Together, these EMG results suggest that reductions in endurance cycling performance with experimentally induced muscle pain involved alterations in motor command.

Regarding the cerebral activation, it has been suggested that induced muscle pain decreases EEG alpha band mainly in the parietal and occipital cortex, but increases EEG beta band in most cortical areas, including the frontal cortex (Chang et al., 2003, 2001). Accordingly, analysis of resting EEG in the present study indicated that hypertonic-induced muscle pain significantly shifted the cortical activation from slower to faster frequencies in the prefrontal (Fp1) and frontal (F3) cortex. Despite the complexity of cerebral responses to painful stimuli, our resting alpha and beta wave results indicate less synchronized activation of a large number of neurons (Robertson & Marino, 2015; von Stein & Sarnthein, 2000) relative to an increased nociceptive input to pain processing cortical areas under muscle pain, respectively (Chang et al., 2003; Plattner et al., 2014).

The slower-to-faster frequencies shift in the prefrontal and parietal cortex during exercise (time effect) likely reflected the cortical work to process the increasing exercise-derived information such as fatigue (Craig, 202; Pires et al., 2016; Robertson & Marino, 2015). However, the hypertonic injection induced a greater EEG frequency shift when compared to isotonic injection (pain main effect), suggesting an increased cortical work to process fatigue and pain during exercise. Assuming that activation in prefrontal cortex plays a role in the exercise regulation when processing unpleasant and painful sensations during exercise, the lower EEG alpha-beta ratio with intramuscular hypertonic injection would indicate that this cortical region was in a greater demand to integrate peripheral stimuli such as pain and fatigue into emotionally relevant messages for the exercise regulation (Meeusen et al., 2016; Plattner et al., 2012, 2014). Given that prefrontal cortex also plays a role in cognitive and emotional functions, this altered activity in prefrontal cortex during exercise with muscle pain may also be associated with an integration of afferent signals into different dimensions of pain such as sensory-discriminatory and affective-motivational, as suggested by results of RPE and affective valence, respectively (Melzack & Wall, 1965; Venhorst et al., 2018).

According to the three-dimensional model of pain (Melzack & Wall, 1965) applied to goal-directed exercise behaviour (Venhorst et al., 2018), the disengagement from exercise encompasses dimensions such as cognitive-evaluative, sensory-discriminative and affective-motivational, with the cognitive-evaluative dimension exerting a control over the other two. In this sense, RPE and pain sensation may be a representation of the sensory-discriminative dimension while affective valence may represent the affective-motivational ones. The interplay between these two dimensions provides relevant information to the cognitive-evaluative dimension regarding the exercise decision. Due to the hypertonic-derived painful stimulus transmitted through type III and IV afferent fibers to pain processing cerebral regions, participants performed the first half of the power-matched exercise bout perceiving higher muscle pain. The hypertonic-derived painful stimulus also led to a higher RPE likely due to an increased recruitment of agonist and synergist muscles not assessed in the present study, as discussed earlier. Somehow, this hypertonic saline-induced alteration in muscle pain and RPE lowered the affective valence, leading participants to a judgment of disutility in performing a power-matched exercise with induced muscle pain, resulting in a decision to shorten the exercise and disengage from unpleasant sensations. This action required a higher involvement of the prefrontal cortex during the cognitive-evaluative process regarding the exercise decision, reflecting the integration between hypertonic-derived painful stimulus and different dimensions of pain.

A recent study challenged the role of pain sensation as a cardinal stopper and suggested RPE as the determinant variable for endurance exercise performance, as participants stopped exercising at maximal RPE levels but only submaximal pain levels during a cycling time-to-exhaustion exercise bout (Staiano et al., 2018). Although participants also stopped exercising at maximal RPE levels in the present study, they ceased exercising at comparable levels of pain sensation and affective valence in both the painful and non-painful conditions. It is important to note that RPE, pain sensation, and affective valence progressed more steeply during exercise after hypertonic injection, reaching comparable endpoint values at the exercise termination although the differences in time-to-exhaustion. Given the association between pain, RPE and affective valence durig exercise (Astokorki & Mauger, 2017a; Ramalho Oliveira, Viana, Pires, Junior Oliveira, & Santos, 2015), it is more plausible to argue that the interplay between different perceptual variables, rather than a single variable in isolation, was important to provide relevant information to the cognitive-evaluative process of reducing the time-to-exhaustion with induced muscle pain. Therefore, the decision to disengage from exercise seems to consider different variables equally important to provide information regarding sensory-discriminative and affective-motivational dimensions, during goal-directed exercise behaviour (Venhorst et al., 2018).

4.1 Methodological Aspects

To the best of our knowledge, we were the first to verify the viability of the hypertonic-induced muscle pain model to investigate muscle pain-muscle fatigue interplay during endurance exercise. The cycling exercise bout started under controlled conditions of muscle pain, when participants rated a very strong pain sensation corresponding to 5 of 10. In the

preliminary study, we observed that the hypertonic solution-induced muscle pain peaked within 1 min, but progressively decreased until reaching a negligible pain sensation from 7 min in participants reporting a varied pain sensation (supplementary figure 1). To provide unequivocal muscle pain effects on a number of neurophysiological and perceptual responses at rest and during exercise, we potentiated the muscle pain effect size by selecting participants rating a peak pain sensation > 7 (a pain sensation of 7.7 ± 1.8). This allowed us to assess resting EEG measures in a 3 min post-injection period and ensure that strong muscle pain effects were still present when the exercise was commenced. Despite the internal validity necessary for a proof-of-concept study, this experimental approach reduced the external validity, as we are obviously unaware of muscle pain effects in those participants rating a mild-to-strong pain response to hypertonic injection. However, this group of non-eligible participants also reported a considerable muscle pain sensation (4 ± 1.3 pain sensation) so that we have no reason to believe that this pain model is unreliable to study muscle pain effects in this particular group of individuals. We acknowledge that future studies are necessary to verify the effect size of the hypertonic-induced muscle pain in mild-to-strong pain responders.

Based on our preliminary results, the pain time course of the hypertonic injection was 7 min. The time between hypertonic saline injections and 50% of the mean exercise duration (~179 s of exercise) was 6 min (358.8 s). Assuming that both painful and non-painful stimuli are transmitted through group III and IV small muscle afferents (Laurin, Pertici, Dousset, Marqueste, & Decherchi, 2015) and that a muscle-derived metabolites accumulation peak from 40% of the exercise duration (Pires et al., 2011), we may argue that this experimental muscle pain model was effective to induce muscle pain effects regardless of muscle fatigue effects mainly during the first half of the exercise bout. This explained the higher muscle pain sensation in hypertonic condition observed in the first part of the exercise bout.

5 CONCLUSION

The hypertonic saline-induced muscle pain impaired endurance exercise performance through centrally mediated alterations in motor command and pain processing cortical areas, as well as through an interplay between cognitive-evaluative, sensory-discriminative and affective-motivational dimensions of pain. The hypertonic saline injection is an effective experimental model to study the effects of muscle pain on endurance exercise performance irrespective of muscle fatigue, mainly during the first half of a short exercise bout.

REFERENCES

Arendt-Nielsen, L., & Graven-Nielsen, T. (2008). Muscle pain: sensory implications and interaction with motor control. The Clinical Journal of Pain, 24(4), 291–298. https://doi.org/10.1097/AJP.0b013e31815b608f

Astokorki, A. H., & Mauger, A. R. (2017a). Tolerance of exercise-induced pain at a fixed rating of perceived exertion predicts time trial cycling performance. Scandinavian Journal of Medicine & Science in Sports, 27(3), 309–317. https://doi.org/10.1111/sms.12659

Astokorki, A. H., & Mauger, A. R. (2017b). Transcutaneous electrical nerve stimulation reduces exercise-induced perceived pain and improves endurance exercise performance. European Journal of Applied Physiology, 117(3), 483–492. https://doi.org/10.1007/s00421-016-3532-6

Chang, P. F., Arendt-Nielsen, L., Graven-Nielsen, T., & Chen, A. C. N. (2003). Psychophysical and EEG responses to repeated experimental muscle pain in humans: pain intensity encodes EEG activity. Brain Research Bulletin, 59(6), 533–543. https://doi.org/10.1016/s0361-9230(02)00950-4

Chang, P. F., Arendt-Nielsen, L., Graven-Nielsen, T., Svensson, P., & Chen, A. C. (2001). Different EEG topographic effects of painful and non-painful intramuscular stimulation in man. Experimental Brain Research, 141(2), 195–203. https://doi.org/10.1007/s002210100864

Cook, D. B., O'Connor, P. J., Eubanks, S. A., Smith, J. C., & Lee, M. (1997). Naturally occurring muscle pain during exercise: assessment and experimental evidence. Medicine and Science in Sports and Exercise, 29(8), 999–1012. https://doi.org/10.1097/00005768-199708000-00004

Craig, A. D. (2002). How do you feel? Interoception: the sense of the physiological condition of the body. Nature Reviews. Neuroscience, 3(8), 655–666. https://doi.org/10.1038/nrn894

Ervilha, U. F., Farina, D., Arendt-Nielsen, L., & Graven-Nielsen, T. (2005). Experimental muscle pain changes motor control strategies in dynamic contractions. Experimental Brain Research, 164(2), 215–224. https://doi.org/10.1007/s00221-005-2244-7

Falla, D., Farina, D., Dahl, M. K., & Graven-Nielsen, T. (2007). Muscle pain induces task-dependent changes in cervical agonist/antagonist activity. Journal of Applied Physiology, 102(2), 601–609. https://doi.org/10.1152/japplphysiol.00602.2006

Farina, D., Arendt-Nielsen, L., & Graven-Nielsen, T. (2005). Experimental muscle pain decreases voluntary EMG activity but does not affect the muscle potential evoked by transcutaneous electrical stimulation. Clinical Neurophysiology, 116(7), 1558–1565. https://doi.org/10.1016/j.clinph.2005.03.009

Ftaiti, F., Kacem, A., Jaidane, N., Tabka, Z., & Dogui, M. (2010). Changes in EEG activity before and after exhaustive exercise in sedentary women in neutral and hot environments. Applied Ergonomics, 41(6), 806–811. https://doi.org/10.1016/j.apergo.2010.01.008

Graven-Nielsen, T. (2006). Fundamentals of muscle pain, referred pain, and deep tissue hyperalgesia. Scandinavian Journal of Rheumatology. Supplement, 122, 1–43. https://doi.org/10.1080/03009740600865980

Graven-Nielsen, T., & Arendt-Nielsen, L. (2008). Impact of clinical and experimental pain on muscle strength and activity. Current Rheumatology Reports, 10(6), 475–481. https://doi.org/10.1007/s11926-008-0078-6

Graven-Nielsen, T., Arendt-Nielsen, L., Svensson, P., & Jensen, T. S. (1997). Experimental muscle pain: A quantitative study of local and referred pain in humans following injection of hypertonic saline. Journal of Musculoskeletal Pain, 5(1), 49–69. https://doi.org/10.1300/J094v05n01_04

Graven-Nielsen, T., Lund, H., Arendt-Nielsen, L., Danneskiold-Samsoe, B., & Bliddal, H. (2002). Inhibition of maximal voluntary contraction force by experimental muscle pain: a centrally mediated mechanism. Muscle Nerve, 26(5), 708–712. https://doi.org/10.1002/mus.10225

Graven-Nielsen, T., & Mense, S. (2001). The peripheral apparatus of muscle pain: evidence from animal and human studies. The Clinical Journal of Pain, 17(1), 2–10. https://doi.org/10.1097/00002508-200103000-00002

Graven-Nielsen, T., Svensson, P., & Arendt-Nielsen, L. (1997). Effects of experimental muscle pain on muscle activity and co-ordination during static and dynamic motor function. Electroencephalography and Clinical Neurophysiology, 105(2), 156–164. https://doi.org/10.1016/S0924-980X(96)96554-6

Hardy, C. J., & Rejeski, W. J. (1989). Not what, but how one feels: the measurement of affect during exercise. Journal of Sport and Exercise Psychology, 11(3), 304–317. https://doi.org/10.1123/jsep.11.3.304

Hermens, H. J., Freriks, B., Disselhorst-Klug, C., & Rau, G. (2000). Development of recommendations for SEMG sensors and sensor placement procedures. Journal of Electromyography and Kinesiology, 10(5), 361–374. https://doi.org/10.1016/s1050-6411(00)00027-4

Hopkins, W. G. (2000). Measures of reliability in sports medicine and science. Sports Medicine, 30(1), 1–15. https://doi.org/10.2165/00007256-200030010-00001

Hopkins, W. G., Marshall, S. W., Batterham, A. M., & Hanin, J. (2009). Progressive statistics for studies in sports medicine and exercise science. Medicine and Science in Sports and Exercise, 41(1), 3–13. https://doi.org/10.1249/MSS.0b013e31818cb278

Kendig, C. E. (2016). What is proof of concept research and how does it generate epistemic and ethical categories for future scientific practice? Science and Engineering Ethics, 22(3), 735–753. https://doi.org/10.1007/s11948-015-9654-0

Khan, S. I., McNeil, C. J., Gandevia, S. C., & Taylor, J. L. (2011). Effect of experimental muscle pain on maximal voluntary activation of human biceps brachii muscle. Journal of Applied Physiology, 111(3), 743–750. https://doi.org/10.1152/japplphysiol.00603.2011

Laurin, J., Pertici, V., Dousset, E., Marqueste, T., & Decherchi, P. (2015). Group III and IV muscle afferents: role on central motor drive and clinical implications. Neuroscience, 290, 543–551. https://doi.org/10.1016/j.neuroscience.2015.01.065

Mauger, A. R. (2014). Factors affecting the regulation of pacing: current perspectives. Open Access Journal of Sports Medicine, 5, 209–214. https://doi.org/10.2147/OAJSM.S38599

Maurits, N. (2011). From neurology to methodology and back: an introduction to clinical neuroengineering (1st ed.). https://doi.org/10.1007/978-1-4614-1132-1

Meeusen, R., Pires, F. O., Pinheiro, F. A., Lutz, K., Cheung, S. S., Perrey, S., … Hettinga, F. (2016). Commentaries on viewpoint: A role for the prefrontal cortex in exercise tolerance and termination. Journal of Applied Physiology, 120(4), 467–469. https://doi.org/10.1152/japplphysiol.00967.2015

Melzack, R., & Wall, P. D. (1965). Pain mechanisms: a new theory. Science (New York, N.Y.), 150(3699), 971–979. https://doi.org/10.1126/science.150.3699.971

Moayedi, M., & Davis, K. D. (2013). Theories of pain: from specificity to gate control. Journal of Neurophysiology, 109(1), 5–12. https://doi.org/10.1152/jn.00457.2012

Nielsen, B., Hyldig, T., Bidstrup, F., Gonzalez-Alonso, J., & Christoffersen, G. R. (2001). Brain activity and fatigue during prolonged exercise in the heat. Pflugers Archiv : European Journal of Physiology, 442(1), 41–48. https://doi.org/10.1007/s004240100515

Nybo, L., & Nielsen, B. (2001). Perceived exertion is associated with an altered brain activity during exercise with progressive hyperthermia. Journal Applied Physiology, 91(5), 2017–2023. https://doi.org/10.1152/jappl.2001.91.5.2017

Pires, F. O., Dos Anjos, C. A., Covolan, R. J., Pinheiro, F. A., St Clair Gibson, A., Noakes, T. D., … Ugrinowitsch, C. (2016). Cerebral Regulation in Different Maximal Aerobic Exercise Modes. Frontiers in Physiology, 7, 253. https://doi.org/10.3389/fphys.2016.00253

Pires, F. O., Noakes, T. D., Lima-Silva, A. E., Bertuzzi, R., Ugrinowitsch, C., Lira, F. S., & Kiss, M. A. (2011). Cardiopulmonary, blood metabolite and rating of perceived exertion responses to constant exercises performed at different intensities until exhaustion. British Journal of Sports Medicine, 45(14), 1119–1125. https://doi.org/10.1136/bjsm.2010.079087

Plattner, K., Lambert, M. I., Tam, N., & Baumeister, J. (2012). The response of cortical alpha activity to pain and neuromuscular changes caused by exercise-induced muscle damage. Scandinavian Journal of Medicine & Science in Sports, 24(1), 166–178. https://doi.org/10.1111/j.1600-0838.2012.01486.x

Plattner, K., Lambert, M. I., Tam, N., Lamberts, R. P., & Baumeister, J. (2014). Changes in cortical beta activity related to a biceps brachii movement task while experiencing exercise induced muscle damage. Physiology & Behavior, 123, 1–10. https://doi.org/10.1016/j.physbeh.2013.08.022

Poortvliet, P. C., Tucker, K. J., Finnigan, S., Scott, D., & Hodges, P. W. (2019). Experimental pain decreases corticomuscular coherence in a force but not a position control task. The Journal of Pain, 20(2), 192–200. https://doi.org/10.1016/j.jpain.2018.08.012

Ramalho Oliveira, B. R., Viana, B. F., Pires, F. O., Junior Oliveira, M., & Santos, T. M. (2015). Prediction of Affective Responses in Aerobic Exercise Sessions. CNS & Neurological Disorders Drug Targets, 14(9), 1214–1218. https://doi.org/10.2174/1871527315666151111121924

Rice, D. A., Mannion, J., Lewis, G. N., McNair, P. J., & Fort, L. (2019). Experimental knee pain impairs joint torque and rate of force development in isometric and isokinetic muscle activation. European Journal of Applied Physiology, 119(9), 2065–2073. https://doi.org/10.1007/s00421-019-04195-6

Robertson, C. V, & Marino, F. E. (2015). Prefrontal and motor cortex EEG responses and their relationship to ventilatory thresholds during exhaustive incremental exercise. European Journal of Applied Physiology, 115(9), 1939–1948. https://doi.org/10.1007/s00421-015-3177-x

Smith, S. A., Micklewright, D., Winter, S. L., & Mauger, A. R. (2020). Muscle pain induced by hypertonic saline in the knee extensors decreases single-limb isometric time to task failure. European Journal of Applied Physiology. https://doi.org/10.1007/s00421-020-04425-2

Staiano, W., Bosio, A., de Morree, H. M., Rampinini, E., & Marcora, S. (2018). The cardinal exercise stopper: Muscle fatigue, muscle pain or perception of effort? Progress in Brain Research, 240, 175–200. https://doi.org/10.1016/bs.pbr.2018.09.012

Taylor, J. L., Amann, M., Duchateau, J., Meeusen, R., & Rice, C. L. (2016). Neural contributions to muscle fatigue: from the brain to the muscle and back again. Medicine and Science in Sports and Exercise, 48(11), 2294–2306. https://doi.org/10.1249/MSS.0000000000000923

Venhorst, A., Micklewright, D., & Noakes, T. D. (2018). Towards a three-dimensional framework of centrally regulated and goal-directed exercise behaviour: a narrative review. British Journal of Sports Medicine, 52(15), 957–966. https://doi.org/10.1136/bjsports-2016-096907

von Stein, A., & Sarnthein, J. (2000). Different frequencies for different scales of cortical integration: from local gamma to long range alpha/theta synchronization. International Journal of Psychophysiology, 38(3), 301–313. https://doi.org/10.1016/S0167-8760(00)00172-0

Weir, J. P. (2005). Quantifying test-retest reliability using the intraclass correlation coefficient and the SEM. Journal of Strength and Conditioning Research, 19(1), 231–240. https://doi.org/10.1519/15184.1

Winter, D. (1990). Biomechanics and motor control of human movement. Wiley-Interscience: Toronto-Ontario.

ADDITIONAL INFORMATION SECTION

Ethics approval

The study was approved by the ethics committee of the School of Arts, Sciences and Humanities of the University of São Paulo (# 3.390.457).

Consent for publication

All authors have read the final version of the manuscript and consented to its submission.

Competing interests

All authors declare that they have no conflict interests.

Funding

This study was supported by FAPESP-Brazil (2016/16496-3).

Authors' contributions

RC, SAS, ARM and FOP designed the study. RC, PEFA, CB, IV collect the data. RC, MFG and FOP analysed and processed the data. RC and FOP wrote the first version of the manuscript. PEFA, CB, IVFP, SAS, ARM and MFG wrote and critically reviewed the manuscript.

Acknowledgments

R.C. and I.V. are grateful to CAPES-Brazil for their scholarships (#001) and F.O.P. is grateful to CNPq-Brazil for his researcher scholarship (#310355/2019-2). We are thankful to Professor Ulysses F. Ervilha for methodological assistance.

Data Availability Statement

Data are available upon request.

FIGURE CAPTIONS

Figure 1. Design of the preliminary study and proof-of-concept study

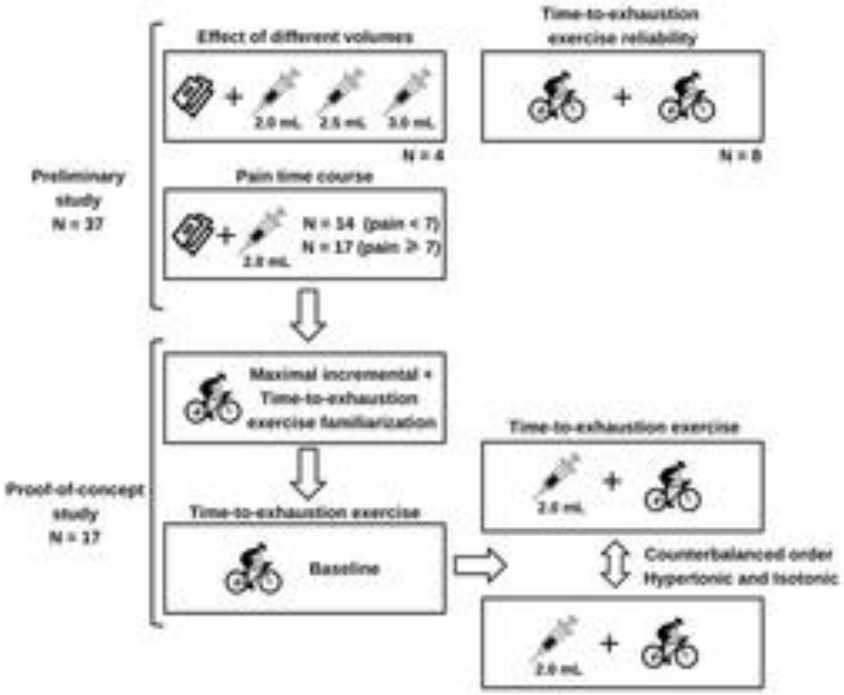
Figure 2. VO2 (panel a), VE (panel b) and HR (panel c) responses during the time-to-exhaustion exercise bout in hypertonic and isotonic saline solution conditions. Symbols indicate pain (#) and time (*) main effects

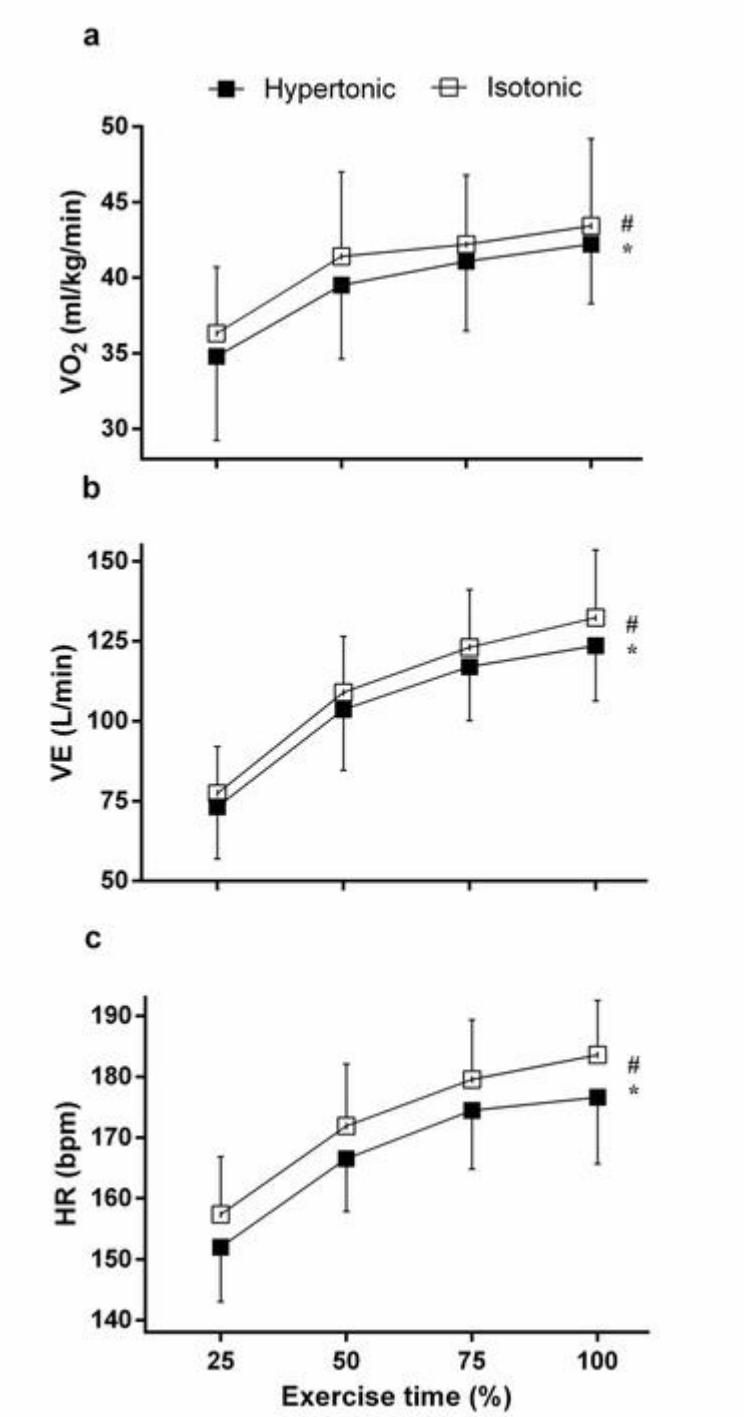
Figure 3. VL muscle (panel a), BF muscle (panel b) EMG and %co-contraction (panel c) responses during the time-to-exhaustion exercise bout in hypertonic and isotonic saline solution conditions. Symbols indicate pain (#) and time (*) main effects

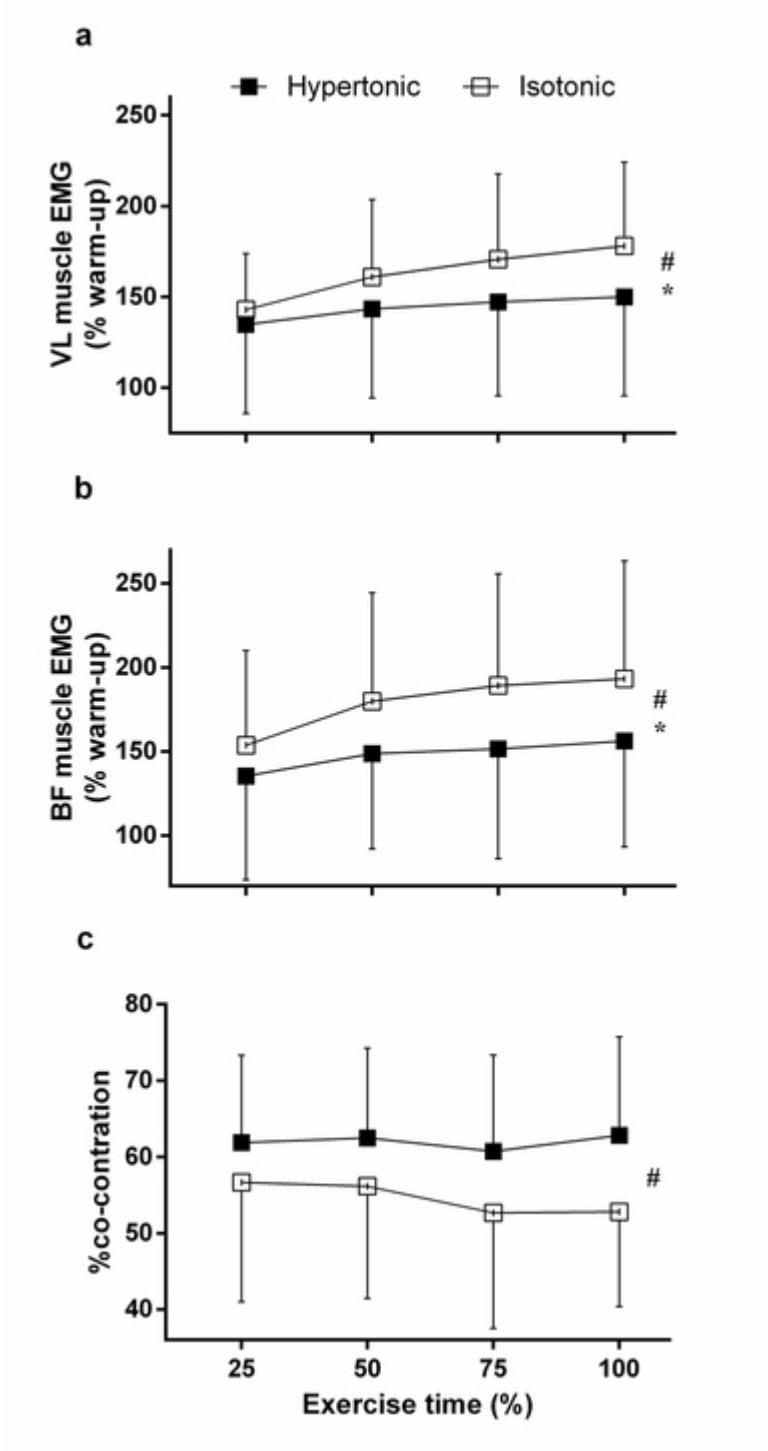
Figure 4. Resting EEG alpha-beta ratio in Fp1 (panel a), F3 (panel b), Cz (panel c) and P3 (panel d) positions before and after the hypertonic and isotonic saline solution injections. * indicates significant difference between conditions

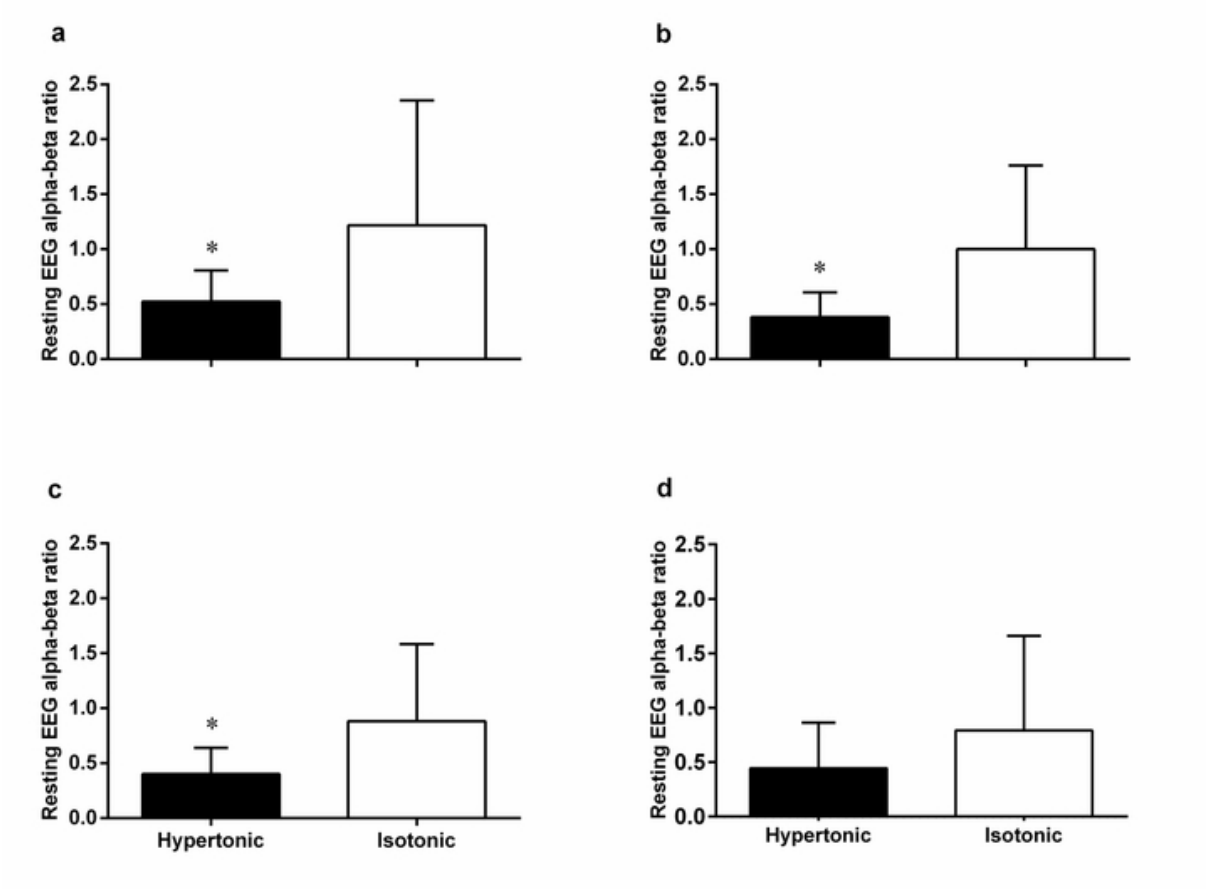
Figure 5. EEG alpha-beta ratio in Fp1 (panel a), F3 (panel b), Cz (panel c) and P3 (panel d) positions during the time-to-exhaustion exercise bout in hypertonic and isotonic saline solution conditions. Symbols indicate pain (#) and time (*) main effects

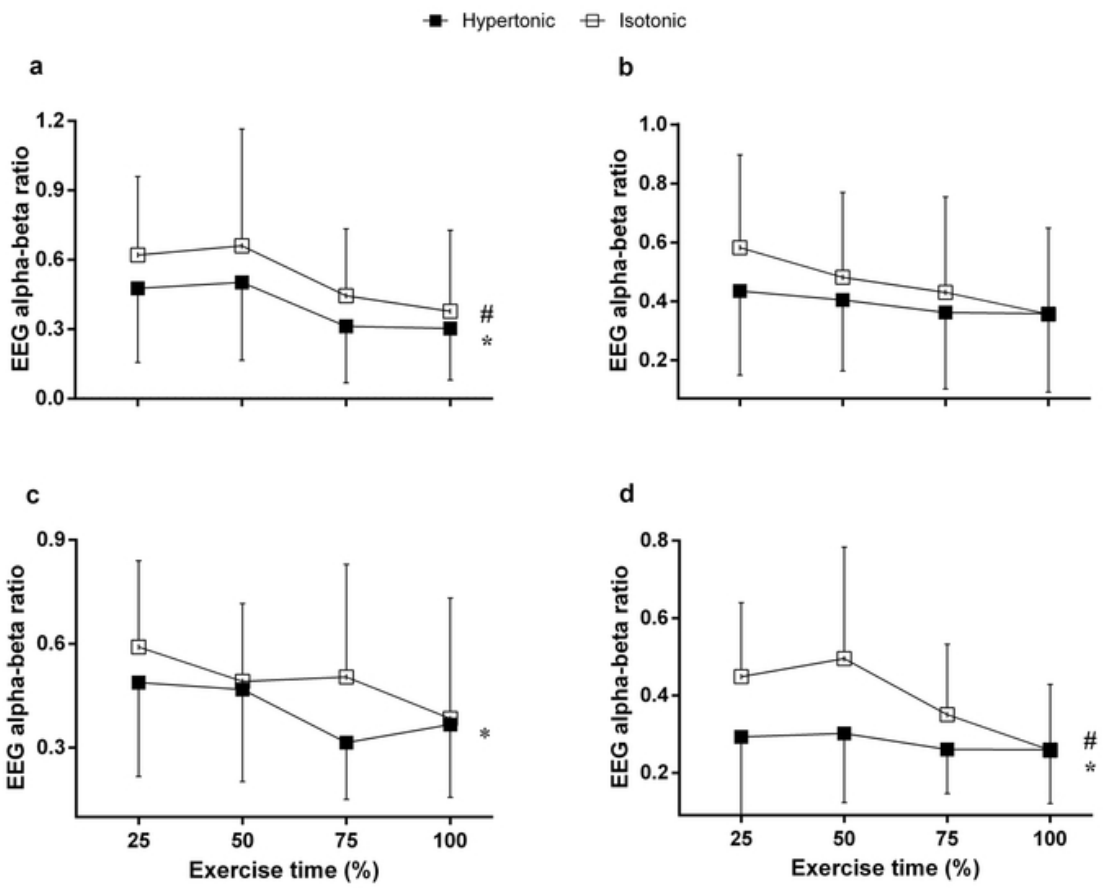
Figure 6. Pain sensation (panel a), ratings of perceived exertion (RPE; panel b) and affect (panel c) responses during the time-to-exhaustion exercise bout in hypertonic and isotonic saline solution conditions. Symbols indicate pain (#) and time (*) main effects, and pain by time interaction effects are highlighted in boxes

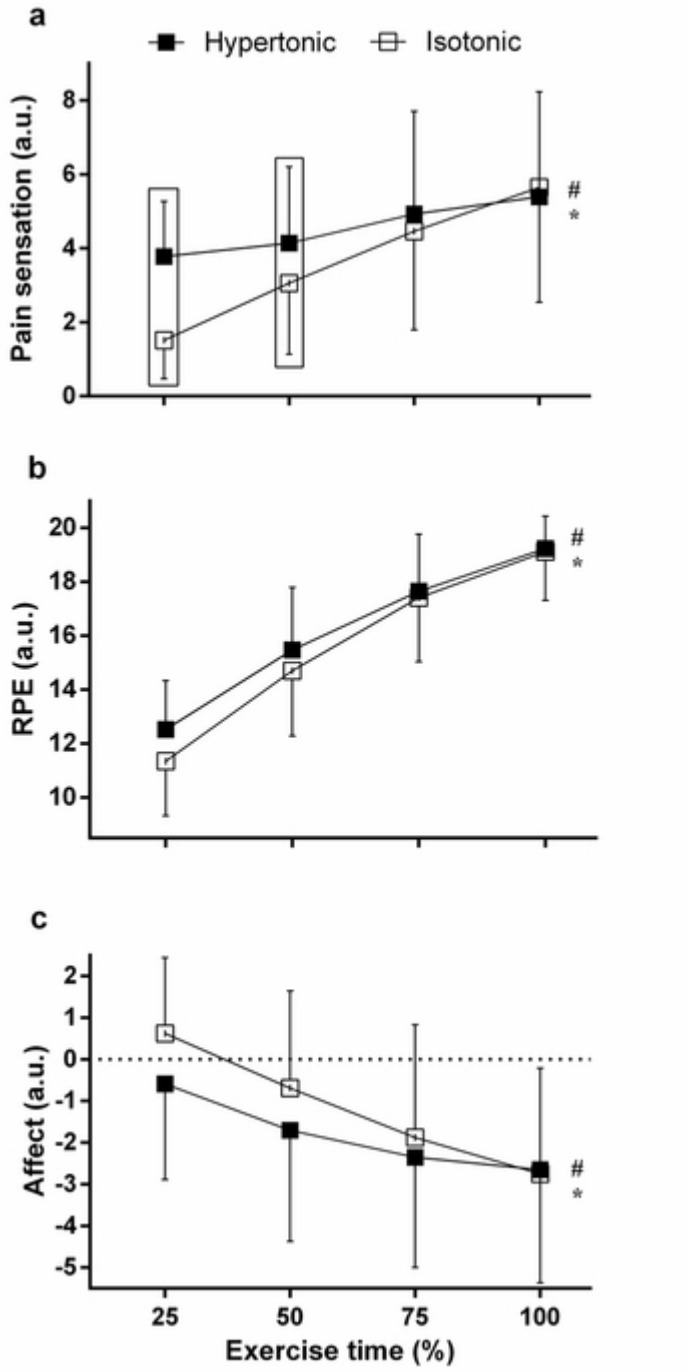

Preliminary study N = 17
Effect of different volumes
2.0 mL
2.5 mL
3.0 mL
N = 4
Time-to-exhaustion exercise reliability
N = 8
Pain time course
2.0 mL
N = 14 (pain < 7)
N = 17 (pain ≥ 7)
Proof-of-concept study N = 17
Maximal incremental + Time-to-exhaustion exercise familiarization
Time-to-exhaustion exercise
Baseline
Time-to-exhaustion exercise
2.0 mL
Counterbalanced order Hypertonic and Isotonic
2.0 mL


a
Hypertonic
Isotonic
50
45
40
35
30
VO2 (ml/kg/min)
#
*
b
150
125
100
75
50
VE (L/min)
#
*
c
190
180
170
160
150
140
HR (bpm)
#
*
25
50
75
100
Exercise time (%)


a
Hypertonic
Isotonic
VL muscle EMG
(% warm-up)
250
200
150
100
#
*
b
BF muscle EMG
(% warm-up)
250
200
150
100
#
*
c
%co-contration
80
70
60
50
40
#
25
50
75
100
Exercise time (%)


a
b
c
d
Resting EEG alpha-beta ratio
2.5
2.0
1.5
1.0
0.5
0.0
*
Hypertonic
Isotonic


Hypertonic
Isotonic
a
b
c
d
EEG alpha-beta ratio
1.2
0.9
0.6
0.3
0.0
1.0
0.8
0.4
0.2
#
*
25
50
75
100
Exercise time (%)


a
Hypertonic
Isotonic
Pain sensation (a.u.)
0
2
4
6
8
#
*
b
RPE (a.u.)
10
12
14
16
18
20
#
*
c
Affect (a.u.)
-5
-4
-3
-2
-1
0
1
2
#
*
25
50
75
100
Exercise time (%)