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Exercise-Induced Muscle Damage on Muscle and Cerebral Oxygenation and Performance

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The study aimed to investigate the effects of exercise induced muscle damage on muscle and cerebral oxygenation. Twelve healthy men performed eccentric exercise on a leg press machine at an intensity corresponding to their concentric one-repetition maximum. Muscle damage indices, muscle and cerebral oxygenation, and vastus lateralis architecture were evaluated at baseline and 48 hours post-exercise. At 48 hours post-exercise, delayed onset muscle soreness significantly increased (1.0±0.3 to 4.2±2.8; $p<0.01$), while concentric one-repetition maximum, maximal isometric force, and rate of force development decreased ($p<0.01$). The quadriceps' cross-sectional area and muscle thickness significantly increased ($p<0.05$). During a 5-second maximal isometric contraction, the tissue oxygen saturation index (TSI) of the vastus lateralis (63±3 to 61±4 %; $p>0.05$) and the prefrontal cortex (68±2 to 67±1 %; $p>0.05$) did not change significantly. Deoxyhemoglobin showed a marginally significant decrease (1.16±1.14 to 0.06±1.10 μM; $p=0.049$). No significant changes were observed in muscle and cerebral oxygenation parameters during the 30 s maximal isometric contraction. The eccentric exercise protocol induced muscle damage and altered muscle architecture. However, these changes were not sufficient to affect muscle or cerebral TSI during either short- or long-duration maximal isometric contraction. Eccentric exercise-induced muscle damage was not found to induce changes in cerebral oxygenation.

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Abstract

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Key words: Cerebral oxygenation, Cross sectional area, Delayed onset muscle soreness, Eccentric exercise, Oxyhemoglobin, Ultrasound

Introduction

Eccentric muscle contraction is defined as the active lengthening of a muscle in response to an external load. The growing popularity of eccentric exercise is based on its unique physiological (e.g., lower metabolic demands compared to concentric exercise) and mechanical characteristics (e.g., activation of the structural protein titin) [1, 2]. However, exercise with pure eccentric contractions is not common in everyday or sports activities. Due to its unaccustomed nature, eccentric exercise may significantly affect muscle function and performance, a condition known as exercise-induced muscle damage [3-5]. Exercise-induced muscle damage decreases muscle strength and pain-free range of movement, increases delayed onset muscle soreness and muscle swelling/stiffness, and increases the levels of inflammation and oxidative stress indicators in the blood [6, 7]. These indicators usually peak between 12 and 48 h post eccentric exercise and typically diminish after 5-7 days [3-5, 8].

In the days following unaccustomed exercise, resting metabolic rate is increased to meet the metabolic demands of muscle regeneration and due to repair processes [9-11]. Moreover, the days post eccentric exercise ultrasound assessments reveal significant increase in muscle thickness and fascicle length [12, 13]. Disruption of skeletal muscle structure due to exercise-induced muscle damage may impair the microvascular network [14, 15], which is accompanied with increased blood flow during exercise [16, 17]. It has been suggested that following eccentric exercise-induced muscle damage, muscle oxygenation is maintained through increased local blood flow, which likely compensates the microcirculatory dysfunction [18, 19]. Furthermore, muscle damage may impair the rate of force development – the ability to rapidly generate muscular force [20] – which can significantly affect short and high-intensity isometric contractions [21].

Eccentric exercise-induced muscle damage has been shown to affect both the peripheral and central nervous systems. Peripheral mechanisms may contribute to neuromuscular dysfunction observed after eccentric exercise, as evidenced by the immediate and prolonged loss of isometric strength [22]. This loss is primarily attributed to factors located at or beyond the neuromuscular junction [22]. Central fatigue, defined as the central nervous system's reduced ability to fully activate motor neurons, may result from decreased cerebral oxygenation, which compromises the oxidative metabolism of brain cells [23]. It is plausible that following muscle-damaging eccentric exercise, increased cortical activation may be necessary to maintain a given force output due to impaired muscle function. However, this should not be interpreted as evidence of central fatigue. This central mechanism may serve to limit force production and prevent further injury [24].

It can also be hypothesized that, since muscle damage from eccentric exercise may impair proprioceptive feedback [5, 25, 26], the central nervus system may face increased demands in organizing motor synergies. This would likely require greater involvement of higher cerebral centers to manage the additional challenges of motor control [27]. Furthermore, coordination leg movements after exercise-induced muscle damage may demand increased cortical engagement to ensure effective motor execution and heightened attentional focus [28]. To the best of our knowledge, no study has yet examined cerebral oxygenation following eccentric exercise-induced muscle damage. Based on the considerations above, it is reasonable to hypothesize that cerebral oxygenation may be compromised in the days after such muscle damage. However, the rationale for potential changes in cerebral oxygenation during exercise performed after eccentric muscle-damaging task remains unclear and insufficiently supported. While cerebral oxygenation is often used as a proxy for neural activation, the presence of muscle (motor unit) damage and the increased effort required to produce maximal force do not consistently or conclusively explain possible impairments in cerebral oxygenation.

To the best of our knowledge, no studies have measured cerebral oxygenation following eccentric exercise-induced muscle damage. Moreover, despite extensive research on exercise-induced muscle damage, there is a significant gap in understanding how this condition affects muscle and cerebral oxygenation during intense muscle actions. The aim of the present study was to investigate the effect of eccentric exercise-induced muscle damage on muscle and cerebral oxygenation, during short and long duration isometric maximal voluntary muscle contractions (lasting 5 and 30 seconds, respectively), as well as on muscle architecture during intense muscle actions following a muscle damaging bout of eccentric exercise. It was hypothesized that both muscle and cerebral oxygenation could be significantly affected by eccentric exercise-induced muscle damage, measured during exercise.

Methods

Subjects

Twelve, healthy young men [n=12, age 25.2 ± 4.8 years, body mass 79.4 ± 10.2 kg, height 180 ± 10 cm, and body mass index (BMI) 24.5 ± 2.2 kg/m^2] volunteered to participate in the present investigation. Body mass was measured to the nearest 0.05 kg (Beam Balance 710, Seca, UK), with participants lightly dressed and barefoot. Standing height was measured to the nearest 1 cm (Stadiometer 208, Seca, UK). Participants were students from the Department of Sport Science who attended practical classes as part of their academic program, averaging approximately 4 hours per week. Apart from these classes, they did not engage in any structured training and had no experience with high-intensity unaccustomed exercise for at least six months prior to enrolling in the study. Additionally, participants were not taking any anti-inflammatory, analgesic medications or nutritional supplements during the study period and one month before the initiation of the experiment. Volunteers were also instructed to abstain from any strenuous exercise during their participation in the study. All procedures were conducted in accordance with the Declaration of Helsinki and were reviewed and approved by the institutional's Ethics Committee (Protocol Record #1099/13-02-2019). All participants signed a written informed consent before entering in the study.

Study design

Volunteers visited the laboratory and completed a weekly self-reported physical activity questionnaire. Participants underwent a medical examination, anthropometric assessment and evaluation of lower extremity dominance using the revised Waterloo Footedness Questionnaire [WFQ-R, intraclass correlation coefficient (ICC) = 0.92]. Subsequently, the maximum single-leg press strength of both lower extremities was evaluated. To minimize the potential influence of limb dominance on the outcomes, we employed a counterbalanced, randomized assignment of dominant and non-dominant limbs among participants, where one limb was selected as the experimental limb, while the other served as the control limb, to exploit the methodological and statistical advantages of the single-limb exercise model [29].

During the second visit, all participants performed a unilateral leg press eccentric exercise session with the experimental limb. For both legs, a single-leg concentric and isometric maximal voluntary contraction (MVC), DOMS, rate of force development (RFD) were assessed immediately before and 48 h post eccentric exercise. During isometric MVC, muscle and cerebral oxygenation were measured via near infrared spectroscopy (NIRS) and muscle morphology was assessed through ultrasonography [1, 30].

Procedures

A warm-up session was performed at both visits and was included 5 min on a cycle ergometer at 50 Watts (Monark ergomedic 834E, Vansbro, Sweden) followed by 5 min stretching exercises.

Leg Press Maximum Strength

Maximal single-leg concentric strength (one repetition maximum; 1RM) was assessed separately for each leg before and 48 h after the eccentric exercise using a leg press machine with a 45° inclination [the same machine used for the eccentric exercise session]. Participants performed 2-3 warm-up sets of 6-8 repetitions with increasing loads. Subsequently, they determined their 1RM, defined as the last successful lifting effort with gradually increased loads with knee joint angle reaching 90°. A 3 min rest interval was maintained between efforts. In all cases, two trained experimenters were present to provide vocal encouragement during each trial. The ICC for 1RM testing was ICC = 0.96 (95% CI: lower = 0.81, upper = 0.94, n = 13).

Eccentric Exercise Protocol

Participants performed a single-leg eccentric exercise session using the same leg press machine. This leg press machine, commonly found in gyms, allows for the downward phase (eccentric muscle contraction) to occur in a diagonal trajectory. Each participant performed only the downward movement (eccentric contraction), and was instructed to lower the leg-press platform in a constant, controlled tempo, completing each eccentric phase in 2 s, and were guided by a metronome. The starting position was set at a knee angle of 175º (180º being full knee extension). To stop the downward movement of the leg press platform at the correct position, a block was placed 1 cm beneath each individual's point of full knee flexion. To avoid the concentric phase of the contraction, the ascent of the leg press platform back to the initial position was performed with an electric motor, and the next repetition was started 5 s after the completion of the previous one. Given that eccentric muscle contractions produce higher force outputs than concentric contractions and considering that the eccentric exercise was performed using a standard leg press machine, the external load was set to 100% of the participant's maximum concentric strength for safety reasons. Each participant performed 5 set of 10 repetition, with 3 min interval between sets.

Delayed onset of muscle soreness

Before and 48 h after the eccentric exercise, participants assessed their perceived level of muscle soreness using a visual analog scale ranging from 1 (normal) to 10 (very, very sore) as previously described [26]. Each participant evaluated soreness in each leg by self-palpation of the muscle belly at the distal region of the vastus lateralis, both in a seated position with relaxed muscles and in a semi-squat position.

Isometric Force and Rate of Force Development

Isometric force was measured using a force platform positioned perpendicular on a concrete laboratory wall (Applied Measurements Ltd Co. UK, WP800, 1000kg weighting platform, 80x80 cm, sampling frequency 1000 Hz). Participants were seated on a custom-made steel leg press chair, with each foot placed separately on the force platform. The knee angle was set at 120° and the hip angle was set at 100º, as previously described [31]. Two isometric tests were conducted: one consisted of two efforts lasted 5s each, and the other was consisted of a single effort lasting 30 s, with 5 min interval between efforts. Participants were instructed to apply maximum force as fast as possible and maintain the force until the end of the test. Before both tests, two 3 s efforts of increasingly intensity were performed. Participants were vocally encouraged to perform their best. Real-time visual feedback of the force applied was provided for each effort via a computer monitor placed just above the force platform.

For the 5 s effort, the variables calculated from the force-time curve were the maximum isometric force (MIF; as the highest peak on the curve) and the RFD [RFD ($N\cdot s^{-1}$) = $\Delta Force \cdot \Delta Time^{-1}$] at 80, 150 and 200 ms. During the 30 s effort, the fatigue index (FI) was calculated using of the formula: FI (%) = [(MIF30sec - LIF30sec)/ MIF30sec] x 100 (LIF: lowest isometric force) [32]. The ICC for MIF and RFD were: ICC = 0.90, (95% CI: Lower = 0.86, Upper = 0.96) and ICC = 0.92, (95% CI: Lower = 0.80, Upper = 0.98) respectively.

Evaluation of Vastus Lateralis Cross-Sectional Area and Architecture

Vastus lateralis cross sectional area, muscle thickness, pennation angle and fascicle length of both legs were assessed in a prone position using B-mode ultrasonography (Product model Z5, Shenzhen Mindray Bio-Medical Electronics Co., Ltd, Shenzhen, China) with a 10 MHz linear array probe (38mm width). The vastus lateralis was selected as it has been reported to be a good surrogate for whole quadriceps exercise-induced changes [33]. To determine the vastus lateralis cross sectional area (CSA), the point at 40% (proximal to the knee) of two locations was marked: a) the center of the patella and b) the medial aspect of the anterior superior iliac spine. This point was chosen because it represents the largest cross-sectional area along the thigh [34]. A perpendicular guideline was then draw along the thigh with an indelible marker and the probe was drawn transversely across the thigh. Full visualization of quadriceps was achieved using a panoramic picture method, specifically the extended-field-of-view mode [33, 34].

184 Two images were taken and analyzed for each participant per leg and mean values were used for statistical analysis. To determine muscle thickness, pennation angle and fascicle length a continuous single view was taken from ~40 mm before, to ~40 mm after the same marked point (40% of the thick proximal to the knee), by moving the probe along the fascicles [34]. All images were analyzed using image analysis software (ImageJ; U.S. National Institutes of Health, Bethesda, MD, USA). The ICC for vastus lateralis CSA is 0.962 (95%CI: 0.835-0.991) ($n = 36$; $P < 0.001$). The ICC for vastus lateralis muscle thickness, fascicle angle, and fascicle length was 0.970 (95% CI: lower = 0.856, upper = 0.987), 0.880 (95% CI: lower = 0.609, upper = 0.965) and 0.840 (95% CI: lower = 0.470, upper = 0.955), respectively.

Muscle and cerebral oxygenation

Muscle and cerebral oxygenation were assessed before and 48 h after eccentric exercise during 5 and 30 s of maximum isometric contraction. Muscle and cerebral oxygenation were assessed non-invasively using the continuous wave NIRS, employing two wavelengths of near-infrared light (760 and 850 nm) (Artinis Medical System, PortaMon/PortaLite, Zetten, The Netherlands) as previously described [1, 35]. The NIRS units (i.e., PortaMon and PortaLite) are equipped with multi-distance optical probes configured with one optical receiver and three optical source emitters, allowing simultaneous monitoring of three separate tissue regions. The three source emitters are aligned on the same line as the detector. The inter-optode spacing between emitters and receiver was 30, 35, and 40 mm, with a penetration depth of approximately one-half of the distance between the emitter and the receiver (i.e., 15, 17.5, and 20 mm). NIRS data were collected at a frequency of 10 Hz, and the average values of the three optical signals were used for data analysis, while, for the analyses, the average value of all parameters recorded during the 5-second maximal isometric contraction was used. While NIRS devices cannot differentiate between chromophores (such as haemoglobin and myoglobin) within the muscle, it is noted that myoglobin content tends to remain constant during exercise. Thus, changes in NIRS signals can be attributed to changes in hemoglobin levels [36].

PortaMon and Portalite NIRS units, using spatial resolved spectroscopy, provide the tissue saturation index (TSI), which reflects the balance between oxygen supply and demand, and is expressed as a percentage at absolute values [37]. Tissue saturation index is a more robust indicator of muscle oxygenation, as it is less sensitive to motion artifacts and provides absolute values that are independent of blood volume changes. Moreover, the NIRS system provided concentration changes of muscle and cerebral microvascular of deoxyhaemoglobin (Δ[HHb]), which reflect the dynamic balance between muscle oxygen delivery and extraction in the underlying tissue [36]. During exercise, there is an alteration in muscle's haemodynamic response, characterized by an increase in Δ[HHb] [38]. Finally, total haemoglobin [tHb] concentration changes may be an indirect index of microvascular blood volume changes in response to exercise [39]. The NIRS technique and the units used in the present investigation have been validated in similar research [40].

Muscle oxygenation was assessed by placing the NIRS unit (PortaMon) at the lower third of the vastus lateralis muscle (approximately 12 cm above the patella and 5 cm lateral to the midline) after shaving and cleaning the site with an alcohol swab. The vastus lateralis muscle was chosen for its accessibility and because it exhibits substantially faster deoxyhemoglobin kinetics compared to the deep and superficial rectus femoris muscle [41]. The skinfold thickness of vastus lateralis was measured using a skinfold caliper (Harpenden, John Bull, St. Albans, England) to determine adipose tissue thickness (i.e., skinfold thickness/2), as it can influence the amplitude of the NIRS signal [42]. In this study, NIRS measurements were not influenced by adipose tissue for all participants, since the average adipose tissue thickness for the left and the right vastus lateralis muscles were 11.6 ± 5.2 and 11.7 ± 5.2 mm, respectively, both below the minimum NIRS light penetration depth (i.e., 15 mm). Considering that tissue oxygenation and blood flow responses can vary between different muscles and regions of the same muscle, the probes were always positioned by the same experimenter to ensure reproducibility of NIRS placement as much as possible.

Cerebral oxygenation was assessed by attaching the NIRS unit (PortaLite) on the surface of the left prefrontal cortex. Participants were instructed to keep their heads as still as possible during exercise to minimize motion artifacts in the cerebral NIRS signal. Both muscle and cerebral NIRS units and probes were covered with a black bandage and secured with tape on the cleaned skin to minimize external light intrusion and prevent movement. Notably, no sliding of the NIRS systems was observed at the end of the exercise sessions in all participants.

Statistical Analyses

The sample size was determined to achieve 85% statistical power (effect size 0.90) using G*Power software (ver. 3.1.9.6), based on data from previous measurements of the same nature conducted by our group. Shapiro-Wilks test was used to assess the normality of our data and no violation of normality was found ($p > 0.05$). All data are presented as mean and standard deviation (mean ± SD). A two-way repeated analysis of variance [2 x 2 ANOVA; condition (experimental and control limb) x time (pre- and post-intervention) in all measures. Bonferroni corrections were applied, when necessary, in the post-hoc comparisons. Effect sizes were calculated using Pearson equation where 0.1-0.3, 0.3-0.5 and higher than 0.5 were considered to be small, moderate and high, respectively. Statistical analyses were performed with SPSS Statistics v.20 (IBM Corporation, USA) with two-tailed significance was accepted at $p \leq 0.05$.

Results

Baseline measurements revealed no significant differences between the two lower limbs across all examined parameters ($p > 0.250$ in all cases).

Muscle damage

Eccentric exercise on the experimental limb caused muscle damage, evidenced by significant alterations on the muscle damage indicators. Specifically, 48 h post eccentric exercise, there were significant changes in concentric 1RM (206 ± 38 kg vs. 175 ± 33 kg; $p = 0.001$, effect size (ES) = 0.763; Fig. 1, panel A), DOMS during squat of the muscle belly (1 ± 0 vs. 4.3 ± 2.5; $p < 0.001$, ES = 0.886; Fig. 1, panel B), DOMS during palpation the movement (1.2 ± 0.4 vs. 4 ± 2; $p < 0.001$, ES = 0.741; Fig. 1, panel C). These parameters showed significant differences between the control and experimental limb at 48 h post eccentric exercise (concentric 1RM 205 ± 47 kg vs. 175 ± 33 kg; DOMS during palpation 1 ± 0 vs. 4.3 ± 2.5; DOMS during squat movement 1.2 ± 0.4 vs. 4.0 ± 2.0; p = 0.001 – 0.032, ES = 0.490 – 0.752; Fig. 1, panels A-C).

Maximal isometric force during the 5 s effort, fatigue index and average force production during the 30 s isometric maximal contraction were significantly reduced only in the experimental limb 48 h post eccentric exercise (1,924 ± 475 N vs. 1,509 ± 439 N, 23.1 ± 6.5 % vs. 30.0 ± 6.5 % and 1,409 ± 418 N vs. 1,060 ± 277 N, respectively; p = 0.001 – 0.023, ES = 0.613 – 0.887; Fig. 2, panels A-C). The same parameters showed significant differences between the control and experimental limb at 48 h post eccentric exercise (1,703 ± 326 N vs. 1,509 ± 439 N, 25.6 ± 6.7 % vs. 30.0 ± 6.5 %, 1,353 ± 373 N vs. 1,060 ± 277 N, respectively; p = 0.001 – 0.032, ES = 0.442 – 0.984; Fig. 2, panels A-C).

Similarly, the RFD measured at 80, 150 and 250 ms were significantly reduced only in the experimental limb 48 h post eccentric exercise (7,278 ± 2,521 N/s vs. 5,199 ± 2,323 N/s, 6,902 ± 2,044 N/s vs. 4,685 ± 2,194 N/s, 6,071 ± 1,658 N/s vs. 4,240 ± 1,796 N/s, respectively; p = 0.005- 0.023, ES = 0.702 – 0.817; Fig. 3, panels A-C). These parameters also exhibited significant differences between the control and experimental limb at 48 h post eccentric exercise (6,393 ± 2,315 N/s vs. 5,199 ± 2,323 N/s, 6,044 ± 1,860 N/s vs. 4,685 ± 2,194 N/s, 5,310 ± 1,411 N/s vs. 4,240 ± 1,796 N/s, respectively; p = 0.001 – 0.005, ES = 0.756 – 0.806; Fig. 3, panels A-C).

Muscle architecture

The cross-sectional area of the vastus lateralis significantly increased 48 h after eccentric exercise only for the experimental limb (24.4 ± 6.3 cm^2 vs. 27.7 ± 6.9 cm^2; p = 0.001, ES = 0.989) while at the same time point there was a significant difference between conditions (25.6 ± 6.3 cm^2 vs. 27.7 ± 6.9 cm^2; p = 0.042, ES = 0.906) (Fig. 4, panel A). Muscle thickness of the vastus lateralis also increased 48 h post eccentric exercise only in the experimental limb (2.4 ± 0.4 cm vs. 2.5 ± 0.2 cm; $p = 0.001$, ES = 0.983), with no difference observed between the two conditions (Fig. 4, panel B). Pennation angle and fascicle length did not change significantly over time or between limbs ($p = 0.737 – 0.808$, ES = 0.444 – 0.821) (Fig. 4, panels C and D, respectively).

Muscle and cerebral oxygenation

Muscle and cerebral TSI during 5 s (Fig. 5, panels A1 and A2) and 30 s (Fig. 5, panels B1 and B2) maximal isometric contraction showed no significant differences either over time ($p = 0.247 – 0.633$, ES = 0.408 – 0.854) or between limbs ($p = 0.298 – 0.788$, ES = 0.201 – 0.921). Collectively, there was no effect of time and condition on TSI during the 5 s and 30 s maximal isometric efforts.

In experimental vastus lateralis Δ[HHb] there was a significant alteration over time during the 5 s of maximal isometric contraction (1.16 ± 1.83 μM vs. 0.06 ± 1.93 μM; $p = 0.049$, ES = 0.577; Fig. 6, panel A1). However, for the same parameter (i.e., 5s of maximal isometric contraction), there was no significant interaction in vastus lateralis Δ[tHb] ($p = 0.449$; Fig. 6, panel A2). Muscle Δ[HHb], and Δ[tHb] during 30 s of maximal isometric contraction (Fig. 6, panels B1-B2) showed no significant differences either over time or between limbs in Δ[HHb], and Δ[tHb] ($p = 0.301 – 0.854$, ES = -0.225 – 0.777).

No significant differences were found in cerebral Δ[HHb], and Δ[tHb] at 5 s (Fig. 7, panels A1-A2) or at 30 s (Fig. 7, panels B1-B2) during maximal isometric contraction either over time ($p = 0.353 – 0.917$, ES = 0.130 – 0.601) or between limbs ($p = 0.326 – 0.878$, ES = 0.347 – 0.679).

Discussion

The aim of the present investigation was to explore the potential effects of eccentric-induced muscle damage on muscle architecture, as well as muscle and cerebral oxygenation. To the best of our knowledge, this is the first study to concurrently investigate the effects of exercise-induced muscle damage on both muscle and cerebral oxygenation. Based on our findings, oxygenation of the vastus lateralis and the prefrontal cortex – as primarily assessed by the TSI – was not affected by eccentric exercise-induced muscle damage during maximal isometric contraction of either short or long duration. Unilateral eccentric exercise performed in a leg press machine at a 45° inclination induced muscle damage, as evidenced by significant alterations in several indirect indicators of muscle damage. Additionally, significant changes were observed in the cross-sectional area and muscle thickness of the vastus lateralis, as assess via ultrasonography 48 h post eccentric exercise.

One of the most valid and reliable indirect measures of muscle damage in humans is a prolonged decrease in muscle force output after exercise [43]. The eccentric exercise protocol applied in the present study caused a 16% reduction in MVC assessed in the knee extensors of the experimental limb, indicating muscle damage [44]. Furthermore, significant reductions in RFD at 80, 150 and 250 ms were observed which are in accordance with previous data [45]. The reduction in RFD observed in this study may be attributed to impaired recruitment of type II motor units following eccentric exercise [46], along with a greater reliance on type I motor units, which contributes to the slower RFD post eccentric exercise [47]. The RFD is not only considered a valid additional indirect marker of muscle damage but also a more specific and sensitive indicator of eccentric exercise-induced muscle damage than MVC [48]. Another important biomarker supporting the induction of muscle damage was the increase in CSA and muscle thickness 48 h after eccentric exercise, indicating the occurrence of edema in the affected muscle. Indeed, it has been previously observed that the days post eccentric exercise ultrasound assessments reveal significant increase in muscle thickness and fascicle length [30]. Several studies suggest that muscle swelling after eccentric exercise occurs due to fluid penetration into the interstitial space of the cells [49]. It has been proposed that unaccustomed eccentric exercise may lead to sarcomere disruption by opening the mechanosensitive stretch channels of cell membranes, resulting in the intracellular influx of Ca^{2+} and Na^{+} ions, which may cause cell damage, swelling, inflammation, and a transient reduction in force production [50]. However, in the present study we did not use techniques such as muscle needle biopsy and magnetic resonance imaging, that may provide more direct measurements muscle damage.

A slight decrease in deoxyhemoglobin was observed in the experimental limb 48 h after eccentric exercise, but only during the 5 s maximal isometric contraction – not during the 30 s effort. This reduction may be related to the concurrent decrease in the RFD. It can be hypothesized that the lower rate of force generation after eccentric muscle-damaging exercise results from increased reliance on type I muscle fibers, which are intrinsically slower at producing force. This shift may occur because type II motor units are predominantly damaged after eccentric exercise [46]. This hypotheses align with our findings, as muscle damage was associated with reduced force output 48 h post eccentric exercise – likely indicating greater recruitment of type I muscle fibers – which may, in turn, explain the unexpected reduction in muscle deoxyhaemoglobin.

During the prolonged maximal isometric contraction – which places greater demand on oxidative metabolism – no disturbances in muscle oxygenation were observed, despite the presence of exercise-induced muscle damage [17]. This preservation of oxygenation may be attributed to an increase in local muscle blood flow, potentially serving to compensate for microcirculatory dysfunction [18, 19]. The absence of changes in muscle oxygenation parameters could also be explained by the lower absolute force output following eccentric exercise-induced muscle damage, likely due to greater reliance on type I muscle fibers, which are more metabolically oxidative. These conditions may have masked potential effects of muscle damage on muscle oxygenation, as such effects are influenced by the metabolic demands of the task. Indeed, the reduced force output during the 30 s of maximal isometric contraction at 48 h post eccentric exercise could have resulted in decreased muscle compression and intramuscular pressure. This, in turn, may have helped preserve blood flow, oxygen delivery, muscle oxygenation status, and oxygen extraction.

The lower [HHb] values observed during the 5 s trial after eccentric exercise could be related to the reduction in maximal isometric force, resulting in lower intramuscular pressure and, consequently, greater blood flow and enhanced oxygen delivery. Deoxyhemoglobin has been reported to be an indirect index of oxygen extraction during exercise [41]. Previous studies have found that severe eccentric exercise muscle damage can significantly impact the microvascular response during exercise initiation [14, 18, 19]. Indeed, 48 h after eccentric exercise-induced muscle damage, slow [HHb] responses to severe-intensity exercise were observed [18]. High intensity muscle contractions (i.e., 5 - 20 s in duration, comparable duration used in the present investigation) were also found to slow microvascular reactivity, causing a mismatch between the delivery of oxygen and its utilization in the muscle tissue [14]. Similarly, in rat spinotrapezius muscle, which provides a close analog to human quadriceps muscle (e.g., muscle fibre type, oxidative capacity), eccentric exercise was found to disrupt capillary geometry by increasing the capillary luminal shape and area (i.e., luminal ellipticity), which was suggested to impair muscle microcirculatory flow and the balance between oxygen delivery and consumption at the onset of electrically stimulated contractions [19].

To the best of our knowledge, this is the first study to examine the effect of eccentric-induced muscle damage on cerebral oxygenation. Based on our results, eccentric muscle damaging exercise did not affect cerebral oxygenation, as assessed during maximal intensity exercise. In previous investigations by our group, no differences were found in cerebral oxygenation between eccentric and concentric isokinetic exercise, whether assessed during continuous submaximal exercise [1] or during high intensity interval training [35]. These similar findings on cerebral oxygenation contradict the differences in cortical activation between conditions. Eccentric exercise requires advanced cortical control and recruitment of brain areas to activate high-threshold motor units while lowering the discharge rate of activated motor units [51]. Similarly, the absence of alterations in cerebral oxygenation found in the present study contradicted the initial hypothesis, since it was expected that after eccentric muscle damaging exercise, higher brain function would be required to control muscle effort as a central protective mechanism to regulate force generation to prevent further injury [24]. Additionally, it can be hypothesized that incorporating a submaximal, long duration isokinetic assessment after exercise-induced muscle damage might have elicited a more pronounced cerebral oxygenation response, due to the increased effort required to sustain the predetermined force. Moreover, it was expected that the sensation of pain in response to eccentric exercise-induced muscle damage could have interfered with cerebral oxygenation.

Regarding cerebral oxygenation, it can be hypothesized that a higher volume of eccentric exercise (> 200-300 repetitions) may be necessary to elicit detectable changes, which could reflect increased cortical activation. In such conditions, greater cortical involvement might be required to maintain a given force output due to impaired muscle function resulting from the higher degree of muscle damage. This activation likely promotes cerebral vasodilation, thereby helping to maintain brain oxygen supply [52-55].

This study provides novel insights in both muscle and cerebral oxygenation in response to muscle damaging eccentric exercise. The results of this study suggest, for the first time, that both short and a long duration maximal isometric contractions (i.e., 5 and 30 s, respectively) performed 48 h after eccentric exercise do not alter muscle or cerebral oxygenation, as measured by TSI, nor blood volume, as indicated by changes in total hemoglobin (Δ[tHb]). However, a marginal change in muscle O_2 extraction was observed during the 5 s maximal isometric contraction, evidenced by a decrease in Δ[HHb], which was unexpected at 48 h post eccentric exercise. No alterations were observed in prefrontal cortex oxygenation during 30 s maximal isometric contraction performed 48 h post eccentric exercise. The absence of alterations in cerebral oxygenation contradicts our initial hypothesis that muscle damage would require increased brain activity to control muscle effort as a central protective mechanism to regulate force generation to prevent further injury [24]. Furthermore, the unexpected decrease in deoxyhemoglobin during the short (5 seconds) maximal isometric contraction may be linked to the reduced isometric force production observed following eccentric exercise-induced muscle damage. This reduction in force could result from a greater reliance on type I muscle fibers activation, as type II fibers are more susceptible to damage from eccentric exercise [46].

Limitations

In the present study, direct blood markers of inflammation and muscle damage (e.g., creatine kinase, myoglobin, C-reactive protein) were not measured, which could be considered a limitation, as the inclusion of such blood indicators might have strengthened the evidence that eccentric exercise induces not only local but also systemic disturbances. Furthermore, cerebral oxygenation was assessed only at a single site – the pre-frontal cortex – which may have restricted the detection of potential changes across other brain regions; the use of a multi-channel functional NIRS (fNIRS) system, specialized for assessing brain function, might have provided a more comprehensive picture of cerebral oxygenation alterations [28]. Regarding muscle and cerebral oxygenation during the 30 s protocol, it is possible that the lower absolute force output following eccentric exercise-induced muscle damage masked any potential effects. This may be due to the fact that the impact of muscle damage on oxygenation is influenced by the metabolic demands of the task. The relatively small sample size, composed of recreationally trained individuals, also limits the generalizability of our findings, as the results may not extend to broader populations or specific groups such as athletes, highly trained individuals, or clinical populations. Additionally, although the single-limb model used in this study offers methodological advantages, it could also be seen as a limitation given that previous studies have reported cross-education effects of eccentric exercise, either providing a protective effect against contralateral muscle damage [56] or causing reduced neuromuscular activity and physical capacity in the unexercised limb [57].

Practical Applications

In this study, a leg press machine was used to induce muscle damage, enhancing ecological validity since such equipment is widely available and commonly used by athletes at all levels, from recreational to elite. Many physical activities involve brief, high intensity muscle efforts, underscoring the physiological importance of the ability to rapidly increase blood flow to meet oxygen delivery demands. Our findings suggest that eccentric exercise-induced muscle damage does not appear to affect muscle oxygenation during either short (5 s) or longer (30 s) maximal isometric contractions. Similarly, no significant alterations were observed in cerebral oxygenation. Furthermore, the absence of changes in cerebral oxygenation suggests effective brain autoregulatory mechanisms that maintain oxygen supply despite the decreased muscle function, implying a potential mismatch between the athletes' perceived willingness to perform and their actual physical capacity, thus increasing the risk of unintentional overexert during the recovery.

Conclusion

Unilateral eccentric exercise performed in a leg press machine with a 45° inclination induced muscle damage and decreased muscle performance, that is, maximal strength and the rate of force development. Additionally, muscle damage induced alterations in muscle morphology, as measured via ultrasonography. Vastus lateralis CSA and muscle thickness are increased two days after the eccentric protocol, indicating the occurrence of muscle damage and the presence of edema. The structural impairments in skeletal muscle resulting from muscle damage were not sufficient to alter the muscle oxygenation levels during either short- or long- duration maximal isometric contraction tests. Similarly, cerebral oxygenation was not affected by the muscle damage as assessed two days after the eccentric protocol.

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470 *Data availability statement:* The data that supports the findings of this study are available from the corresponding author upon reasonable request.

References

1. Cherouveim ED, Margaritelis NV, Koulouvaris P et al. Skeletal muscle and cerebral oxygenation levels during and after submaximal concentric and eccentric isokinetic exercise. J Sports Sci 2022; 40: 195-202. doi:10.1080/02640414.2021.1983248
2. Hessel AL, Lindstedt SL, Nishikawa KC. Physiological Mechanisms of Eccentric Contraction and Its Applications: A Role for the Giant Titin Protein. Front Physiol 2017; 8: 70. doi:10.3389/fphys.2017.00070
3. Margaritelis NV, Theodorou AA, Chatzinikolaou PN et al. Eccentric exercise per se does not affect muscle damage biomarkers: early and late phase adaptations. Eur J Appl Physiol 2021; 121: 549-559. doi:10.1007/s00421-020-04528-w
4. Paschalis V, Koutedakis Y, Jamurtas AZ et al. Equal volumes of high and low intensity of eccentric exercise in relation to muscle damage and performance. J Strength Cond Res 2005; 19: 184-188. doi:10.1519/R-14763.1
5. Paschalis V, Nikolaidis MG, Giakas G et al. The effect of eccentric exercise on position sense and joint reaction angle of the lower limbs. Muscle Nerve 2007; 35: 496-503. doi:10.1002/mus.20723
6. Margaritelis NV, Theodorou AA, Baltzopoulos V et al. Muscle damage and inflammation after eccentric exercise: can the repeated bout effect be removed? Physiol Rep 2015; 3. doi:10.14814/phy2.12648
7. Paschalis V, Nikolaidis MG, Fatouros IG et al. Uniform and prolonged changes in blood oxidative stress after muscle-damaging exercise. In Vivo 2007; 21: 877-883
8. Peake JM, Neubauer O, Della Gatta PA et al. Muscle damage and inflammation during recovery from exercise. J Appl Physiol (1985) 2017; 122: 559-570. doi:10.1152/japplphysiol.00971.2016
9. Paschalis V, Nikolaidis MG, Giakas G et al. Beneficial changes in energy expenditure and lipid profile after eccentric exercise in overweight and lean women. Scand J Med Sci Sports 2010; 20: e103-111. doi:10.1111/j.1600-0838.2009.00920.x
10. Paschalis V, Nikolaidis MG, Theodorou AA et al. The effects of eccentric exercise on muscle function and proprioception of individuals being overweight and underweight. J Strength Cond Res 2013; 27: 2542-2551. doi:10.1519/JSC.0b013e31827fc9a6
11. Tee JC, Bosch AN, Lambert MI. Metabolic consequences of exercise-induced muscle damage. Sports Med 2007; 37: 827-836. doi:10.2165/00007256-200737100-00001
12. Brandao MCA, Matias LA, da Matta TT et al. Biceps brachii elastography in well-trained men post eccentric exercise-induced muscle damage. J Ultrasound 2024. doi:10.1007/s40477-024-00886-9. doi:10.1007/s40477-024-00886-9
13. Murayama M, Inami T, Shima N et al. Changes in biceps brachii muscle hardness assessed by a push-in meter and strain elastography after eccentric versus concentric contractions. Sci Rep 2022; 12: 9214. doi:10.1038/s41598-022-13184-3
14. Larsen RG, Hirata RP, Madzak A et al. Eccentric exercise slows in vivo microvascular reactivity during brief contractions in human skeletal muscle. J Appl Physiol (1985) 2015; 119: 1272-1281. doi:10.1152/japplphysiol.00563.2015
15. Larsen RG, Thomsen JM, Hirata RP et al. Impaired microvascular reactivity after eccentric muscle contractions is not restored by acute ingestion of antioxidants or dietary nitrate. Physiol Rep 2019; 7: e14162. doi:10.14814/phy2.14162
16. Ahmadi S, Sinclair PJ, Foroughi N et al. Monitoring muscle oxygenation after eccentric exercise-induced muscle damage using near-infrared spectroscopy. Appl Physiol Nutr Metab 2008; 33: 743-752. doi:10.1139/h08-048
17. Laaksonen MS, Kivela R, Kyrolainen H et al. Effects of exhaustive stretch-shortening cycle exercise on muscle blood flow during exercise. Acta Physiol (Oxf) 2006; 186: 261-270. doi:10.1111/j.1748-1716.2006.01532.x
18. Davies RC, Eston RG, Poole DC et al. Effect of eccentric exercise-induced muscle damage on the dynamics of muscle oxygenation and pulmonary oxygen uptake. J Appl Physiol (1985) 2008; 105: 1413-1421. doi:10.1152/japplphysiol.90743.2008

19. Kano Y, Padilla DJ, Behnke BJ et al. Effects of eccentric exercise on microcirculation and microvascular oxygen pressures in rat spinotrapezius muscle. J Appl Physiol (1985) 2005; 99: 1516-1522. doi:10.1152/japplphysiol.00069.2005
20. Mirkov DM, Nedeljkovic A, Milanovic S et al. Muscle strength testing: evaluation of tests of explosive force production. Eur J Appl Physiol 2004; 91: 147-154. doi:10.1007/s00421-003-0946-8
21. Cettolo V, Ferrari M, Biasini V et al. Vastus lateralis O2 desaturation in response to fast and short maximal contraction. Med Sci Sports Exerc 2007; 39: 1949-1959. doi:10.1249/mss.0b013e3181453476
22. Byrne C, Twist C, Eston R. Neuromuscular function after exercise-induced muscle damage: theoretical and applied implications. Sports Med 2004; 34: 49-69. doi:10.2165/00007256-200434010-00005
23. Nybo L, Rasmussen P. Inadequate cerebral oxygen delivery and central fatigue during strenuous exercise. Exerc Sport Sci Rev 2007; 35: 110-118. doi:10.1097/jes.0b013e3180a031ec
24. Proske U, Gregory JE, Morgan DL et al. Force matching errors following eccentric exercise. Hum Mov Sci 2004; 23: 365-378. doi:10.1016/j.humov.2004.08.012
25. Methenitis S, Theodorou AA, Chatzinikolaou PN et al. The effects of chronic concentric and eccentric training on position sense and joint reaction angle of the knee extensors. Eur J Sport Sci 2023; 23: 1164-1174. doi:10.1080/17461391.2023.2184726
26. Paschalis V, Nikolaidis MG, Giakas G et al. Position sense and reaction angle after eccentric exercise: the repeated bout effect. Eur J Appl Physiol 2008; 103: 9-18. doi:10.1007/s00421-007-0663-9
27. Perrey S. Brain activation associated with eccentric movement: A narrative review of the literature. Eur J Sport Sci 2018; 18: 75-82. doi:10.1080/17461391.2017.1391334
28. Borot L, Vergotte G, Perrey S. Different Hemodynamic Responses of the Primary Motor Cortex Accompanying Eccentric and Concentric Movements: A Functional NIRS Study. Brain Sci 2018; 8. doi:10.3390/brainsci8050075
29. Newton MJ, Sacco P, Chapman D et al. Do dominant and non-dominant arms respond similarly to maximal eccentric exercise of the elbow flexors? J Sci Med Sport 2013; 16: 166-171. doi:10.1016/j.jsams.2012.06.001
30. Penailillo L, Blazevich AJ, Nosaka K. Muscle fascicle behavior during eccentric cycling and its relation to muscle soreness. Med Sci Sports Exerc 2015; 47: 708-717. doi:10.1249/MSS.0000000000000473
31. Methenitis S, Karandreas N, Spengos K et al. Muscle Fiber Conduction Velocity, Muscle Fiber Composition, and Power Performance. Med Sci Sports Exerc 2016; 48: 1761-1771. doi:10.1249/MSS.0000000000000954
32. Margaritelis NV, Chatzinikolaou PN, Bousiou FV et al. Dietary Cysteine Intake is Associated with Blood Glutathione Levels and Isometric Strength. Int J Sports Med 2021; 42: 441-447. doi:10.1055/a-1255-2863
33. Spiliopoulou P, Methenitis S, Zaras N et al. Vastus Lateralis and Vastus Intermedius as Predictors of Quadriceps Femoris Muscle Hypertrophy after Strength Training. Applied Sciences 2022; 12: 9133. doi:10.3390/app12189133
34. Noorkoiv M, Nosaka K, Blazevich AJ. Assessment of quadriceps muscle cross-sectional area by ultrasound extended-field-of-view imaging. Eur J Appl Physiol 2010; 109: 631-639. doi:10.1007/s00421-010-1402-1
35. Perentis PA, Cherouveim ED, Malliou VJ et al. The Effects of High-Intensity Interval Exercise on Skeletal Muscle and Cerebral Oxygenation during Cycling and Isokinetic Concentric and Eccentric Exercise. J Funct Morphol Kinesiol 2021; 6. doi:10.3390/jfmk6030062
36. Barstow TJ. Understanding near infrared spectroscopy and its application to skeletal muscle research. J Appl Physiol (1985) 2019; 126: 1360-1376. doi:10.1152/japplphysiol.00166.2018

37. Buchheit M, Ufland P, Haydar B et al. Reproducibility and sensitivity of muscle reoxygenation and oxygen uptake recovery kinetics following running exercise in the field. Clin Physiol Funct Imaging 2011; 31: 337-346. doi:10.1111/j.1475-097X.2011.01020.x

38. Celie B, Boone J, Van Coster R et al. Reliability of near infrared spectroscopy (NIRS) for measuring forearm oxygenation during incremental handgrip exercise. Eur J Appl Physiol 2012; 112: 2369-2374. doi:10.1007/s00421-011-2183-x

39. Cardinale M, Ferrari M, Quaresima V. Gastrocnemius medialis and vastus lateralis oxygenation during whole-body vibration exercise. Med Sci Sports Exerc 2007; 39: 694-700. doi:10.1249/mss.0b013e31803084d8

40. Scott BR, Slattery KM, Sculley DV et al. Reliability of telemetric electromyography and near-infrared spectroscopy during high-intensity resistance exercise. J Electromyogr Kinesiol 2014; 24: 722-730. doi:10.1016/j.jelekin.2014.07.008

41. Koga S, Okushima D, Barstow TJ et al. Near-infrared spectroscopy of superficial and deep rectus femoris reveals markedly different exercise response to superficial vastus lateralis. Physiol Rep 2017; 5. doi:10.14814/phy2.13402

42. van Beekvelt MC, Borghuis MS, van Engelen BG et al. Adipose tissue thickness affects in vivo quantitative near-IR spectroscopy in human skeletal muscle. Clin Sci (Lond) 2001; 101: 21-28. doi:10.1042/cs20000247

43. Warren GL, Lowe DA, Armstrong RB. Measurement tools used in the study of eccentric contraction-induced injury. Sports Med 1999; 27: 43-59. doi:10.2165/00007256-199927010-00004

44. Paulsen G, Mikkelsen UR, Raastad T et al. Leucocytes, cytokines and satellite cells: what role do they play in muscle damage and regeneration following eccentric exercise? Exerc Immunol Rev 2012; 18: 42-97

45. Crameri RM, Aagaard P, Qvortrup K et al. Myofibre damage in human skeletal muscle: effects of electrical stimulation versus voluntary contraction. J Physiol 2007; 583: 365-380. doi:10.1113/jphysiol.2007.128827

46. Lieber RL, Friden J. Selective damage of fast glycolytic muscle fibres with eccentric contraction of the rabbit tibialis anterior. Acta Physiol Scand 1988; 133: 587-588. doi:10.1111/j.1748-1716.1988.tb08446.x

47. Chen YW, Hubal MJ, Hoffman EP et al. Molecular responses of human muscle to eccentric exercise. J Appl Physiol (1985) 2003; 95: 2485-2494. doi:10.1152/japplphysiol.01161.2002

48. Penailillo L, Blazevich A, Numazawa H et al. Rate of force development as a measure of muscle damage. Scand J Med Sci Sports 2015; 25: 417-427. doi:10.1111/sms.12241

49. Agten CA, Buck FM, Dyer L et al. Delayed-Onset Muscle Soreness: Temporal Assessment With Quantitative MRI and Shear-Wave Ultrasound Elastography. AJR Am J Roentgenol 2017; 208: 402-412. doi:10.2214/AJR.16.16617

50. Hughes JD, Johnson NA, Brown SJ et al. Effects of eccentric exercise-induced muscle damage on intramyocellular lipid concentration and high energy phosphates. Eur J Appl Physiol 2010; 110: 1135-1141. doi:10.1007/s00421-010-1605-5

51. Gruber M, Linnamo V, Strojnik V et al. Excitability at the motoneuron pool and motor cortex is specifically modulated in lengthening compared to isometric contractions. J Neurophysiol 2009; 101: 2030-2040. doi:10.1152/jn.91104.2008

52. Bhambhani Y, Malik R, Mookerjee S. Cerebral oxygenation declines at exercise intensities above the respiratory compensation threshold. Respir Physiol Neurobiol 2007; 156: 196-202. doi:10.1016/j.resp.2006.08.009

53. Ogoh S, Ainslie PN. Cerebral blood flow during exercise: mechanisms of regulation. J Appl Physiol (1985) 2009; 107: 1370-1380. doi:10.1152/japplphysiol.00573.2009

54. Prodel E, Balanos GM, Braz ID et al. Muscle metaboreflex and cerebral blood flow regulation in humans: implications for exercise with blood flow restriction. Am J Physiol Heart Circ Physiol 2016; 310: H1201-1209. doi:10.1152/ajpheart.00894.2015

55. Secher NH, Seifert T, Van Lieshout JJ. Cerebral blood flow and metabolism during exercise: implications for fatigue. J Appl Physiol (1985) 2008; 104: 306-314. doi:10.1152/japplphysiol.00853.2007
56. Tseng WC, Nosaka K, Tseng KW et al. Contralateral Effects by Unilateral Eccentric versus Concentric Resistance Training. Med Sci Sports Exerc 2020; 52: 474-483. doi:10.1249/MSS.0000000000002155
57. Hedayatpour N, Izanloo Z, Falla D. The effect of eccentric exercise and delayed onset muscle soreness on the homologous muscle of the contralateral limb. J Electromyogr Kinesiol 2018; 41: 154-159. doi:10.1016/j.jelekin.2018.06.003

Figure Legends

Figure 1 Concentric 1 repetition maximum (A), DOMS during squat movement (B) and DOMS during palpation (C), pre and 48-h post eccentric exercise for the control (white bars) and the experimental (black bars) limb.

* Significant difference ($p<0.05$) between pre and post exercise condition for the same condition.

significant difference ($p<0.05$) between control and experimental limb at the same time point.

Figure 2 Isometric peak force during a 5 s effort (A), Fatigue index (B) and Average force (C) during a 30 s maximal isometric effort, pre and 48-h post eccentric exercise for the control (white bars) and the experimental (black bars) limb.

* Significant difference ($p<0.05$) between pre and post exercise condition for the same condition.

significant difference ($p<0.05$) between control and experimental limb at the same time point.

Figure 3 Rate of force development at 80 ms (A) 150 ms (B) and 250 ms (C), pre and 48 h post eccentric exercise for the control (white bars) and the experimental (black bars) limb.

* Significant difference ($p<0.05$) between pre and post exercise condition for the same condition.

significant difference ($p<0.05$) between control and experimental limb at the same time point.

Figure 4 Cross sectional area (A), muscle thickness (B), pennation angle (C) and fascicle length (D) of knee extensors, pre and 48 h post eccentric exercise for the control (white bars) and the experimental (black bars) limb.

* Significant difference ($p<0.05$) between pre and post exercise condition for the same condition.

# denotes significant difference ($p<0.05$) between control and experimental limb at the same time point.

Figure 5 Tissue saturation index (TSI) for muscle and cerebral tissue measured during 5s (A1-A2) and 30s (B1-B2) isometric force, pre and 48 h post eccentric exercise.

Figure 6 Muscle oxygenation parameters [i.e., deoxyhemoglobin (HHb) and total hemoglobin (tHb)] measured during 5s (A1-A2) and 30s (B1-B2) isometric force, pre and 48 h post eccentric exercise. * Significant difference ($p<0.05$) between pre and post exercise condition for the same condition.

Figure 7 Cerebral oxygenation parameters [i.e., deoxyhemoglobin (HHb) and total hemoglobin (tHb)] measured during 5s (A1-A2) and 30s (B1-B2) isometric force, pre and 48 h post eccentric exercise.

Exercise-Induced Muscle Damage on Muscle and Cerebral Oxygenation and Performance

Abstract

The study aimed to investigate the effects of exercise-induced muscle damage on muscle and cerebral oxygenation. Twelve healthy men performed eccentric exercise on a leg press machine at an intensity corresponding to their concentric one-repetition maximum. Muscle damage indices, muscle and cerebral oxygenation, and vastus lateralis architecture were evaluated at baseline and 48 hours post-exercise. At 48 hours post-exercise, delayed onset muscle soreness significantly increased (1.0±0.3 to 4.2±2.8; $p<0.01$), while concentric one-repetition maximum, maximal isometric force, and rate of force development decreased ($p<0.01$). The quadriceps' cross-sectional area and muscle thickness significantly increased ($p<0.05$). During a 5-second maximal isometric contraction, the tissue oxygen saturation index (TSI) of the vastus lateralis (63±3 to 61±4 %; $p>0.05$) and the prefrontal cortex (68±2 to 67±1 %; $p>0.05$) did not change significantly. Deoxyhemoglobin showed a marginally significant decrease (1.16±1.14 to 0.06±1.10 µM; $p=0.049$). No significant changes were observed in muscle and cerebral oxygenation parameters during the 30 s maximal isometric contraction. The eccentric exercise protocol induced muscle damage and altered muscle architecture. However, these changes were not sufficient to affect muscle or cerebral TSI during either short- or long-duration maximal isometric contraction. Eccentric exercise-induced muscle damage was not found to induce changes in cerebral oxygenation.

Key words: Cerebral oxygenation, Cross sectional area, Delayed onset muscle soreness, Eccentric exercise, Oxyhemoglobin, Ultrasound

Introduction

Eccentric muscle contraction is defined as the active lengthening of a muscle in response to an external load. The growing popularity of eccentric exercise is based on its unique physiological (e.g., lower metabolic demands compared to concentric exercise) and mechanical characteristics (e.g., activation of the structural protein titin) [1, 2]. However, exercise with pure eccentric contractions is not common in everyday or sports activities. Due to its unaccustomed nature, eccentric exercise may significantly affect muscle function and performance, a condition known as exercise-induced muscle damage [3-5]. Exercise-induced muscle damage decreases muscle strength and pain-free range of movement, increases delayed onset muscle soreness and muscle swelling/stiffness, and increases the levels of inflammation and oxidative stress indicators in the blood [6, 7]. These indicators usually peak between 12 and 48 h post eccentric exercise and typically diminish after 5-7 days [3-5, 8].

In the days following unaccustomed exercise, resting metabolic rate is increased to meet the metabolic demands of muscle regeneration and due to repair processes [9-11]. Moreover, the days post eccentric exercise ultrasound assessments reveal significant increase in muscle thickness and fascicle length [12, 13]. Disruption of skeletal muscle structure due to exercise-induced muscle damage may impair the microvascular network [14, 15], which is accompanied with increased blood flow during exercise [16, 17]. It has been suggested that following eccentric exercise-induced muscle damage, muscle oxygenation is maintained through increased local blood flow, which likely compensates the microcirculatory dysfunction [18, 19]. Furthermore, muscle damage may impair the rate of force development – the ability to rapidly generate muscular force [20] – which can significantly affect short and high-intensity isometric contractions [21].

Eccentric exercise-induced muscle damage has been shown to affect both the peripheral and central nervous systems. Peripheral mechanisms may contribute to neuromuscular dysfunction observed after eccentric exercise, as evidenced by the immediate and prolonged loss of isometric strength [22]. This loss is primarily attributed to factors located at or beyond the neuromuscular junction [22]. Central fatigue, defined as the central nervous system's reduced ability to fully activate motor neurons, may result from decreased cerebral oxygenation, which compromises the oxidative metabolism of brain cells [23]. It is plausible that following muscle-damaging eccentric exercise, increased cortical activation may be necessary to maintain a given force output due to impaired muscle function. However, this should not be interpreted as evidence of central fatigue. This central mechanism may serve to limit force production and prevent further injury [24].

It can also be hypothesized that, since muscle damage from eccentric exercise may impair proprioceptive feedback [5, 25, 26], the central nervus system may face increased demands in organizing motor synergies. This would likely require greater involvement of higher cerebral centers to manage the additional challenges of motor control [27]. Furthermore, coordination leg movements after exercise-induced muscle damage may demand increased cortical engagement to ensure effective motor execution and heightened attentional focus [28]. To the best of our knowledge, no study has yet examined cerebral oxygenation following eccentric exercise-induced muscle damage. Based on the considerations above, it is reasonable to hypothesize that cerebral oxygenation may be compromised in the days after such muscle damage. However, the rationale for potential changes in cerebral oxygenation during exercise performed after eccentric muscle-damaging task remains unclear and insufficiently supported. While cerebral oxygenation is often used as a proxy for neural activation, the presence of muscle (motor unit) damage and the increased effort required to produce maximal force do not consistently or conclusively explain possible impairments in cerebral oxygenation.

To the best of our knowledge, no studies have measured cerebral oxygenation following eccentric exercise-induced muscle damage. Moreover, despite extensive research on exercise-induced muscle damage, there is a significant gap in understanding how this condition affects muscle and cerebral oxygenation during intense muscle actions. The aim of the present study was to investigate the effect of eccentric exercise-induced muscle damage on muscle and cerebral oxygenation, during short and long duration isometric maximal voluntary muscle contractions (lasting 5 and 30 seconds, respectively), as well as on muscle architecture during intense muscle actions following a muscle damaging bout of eccentric exercise. It was hypothesized that both muscle and cerebral oxygenation could be significantly affected by eccentric exercise-induced muscle damage, measured during exercise.

Methods

Subjects

Twelve, healthy young men [n=12, age 25.2 ± 4.8 years, body mass 79.4 ± 10.2 kg, height 180 ± 10 cm, and body mass index (BMI) 24.5 ± 2.2 kg/m^2] volunteered to participate in the present investigation. Body mass was measured to the nearest 0.05 kg (Beam Balance 710, Seca, UK), with participants lightly dressed and barefoot. Standing height was measured to the nearest 1 cm (Stadiometer 208, Seca, UK). Participants were students from the Department of Sport Science who attended practical classes as part of their academic program, averaging approximately 4 hours per week. Apart from these classes, they did not engage in any structured training and had no experience with high-intensity unaccustomed exercise for at least six months prior to enrolling in the study. Additionally, participants were not taking any anti-inflammatory, analgesic medications or nutritional supplements during the study period and one month before the initiation of the experiment. Volunteers were also instructed to abstain from any strenuous exercise during their participation in the study. All procedures were conducted in accordance with the Declaration of Helsinki and were reviewed and approved by the institutional's Ethics Committee (Protocol Record #1099/13-02-2019). All participants signed a written informed consent before entering in the study.

Study design

Volunteers visited the laboratory and completed a weekly self-reported physical activity questionnaire. Participants underwent a medical examination, anthropometric assessment and evaluation of lower extremity dominance using the revised Waterloo Footedness Questionnaire [WFQ-R, intraclass correlation coefficient (ICC) = 0.92]. Subsequently, the maximum single-leg press strength of both lower extremities was evaluated. To minimize the potential influence of limb dominance on the outcomes, we employed a counterbalanced, randomized assignment of dominant and non-dominant limbs among participants, where one limb was selected as the experimental limb, while the other served as the control limb, to exploit the methodological and statistical advantages of the single-limb exercise model [29].

During the second visit, all participants performed a unilateral leg press eccentric exercise session with the experimental limb. For both legs, a single-leg concentric and isometric maximal voluntary contraction (MVC), DOMS, rate of force development (RFD) were assessed immediately before and 48 h post eccentric exercise. During isometric MVC, muscle and cerebral oxygenation were measured via near infrared spectroscopy (NIRS) and muscle morphology was assessed through ultrasonography [1, 30].

Procedures

A warm-up session was performed at both visits and was included 5 min on a cycle ergometer at 50 Watts (Monark ergomedic 834E, Vansbro, Sweden) followed by 5 min stretching exercises.

Leg Press Maximum Strength

Maximal single-leg concentric strength (one repetition maximum; 1RM) was assessed separately for each leg before and 48 h after the eccentric exercise using a leg press machine with a 45° inclination [the same machine used for the eccentric exercise session]. Participants performed 2-3 warm-up sets of 6-8 repetitions with increasing loads. Subsequently, they determined their 1RM, defined as the last successful lifting effort with gradually increased loads with knee joint angle reaching 90°. A 3 min rest interval was maintained between efforts. In all cases, two trained experimenters were present to provide vocal encouragement during each trial. The ICC for 1RM testing was ICC = 0.96 (95% CI: lower = 0.81, upper = 0.94, n = 13).

Eccentric Exercise Protocol

Participants performed a single-leg eccentric exercise session using the same leg press machine. This leg press machine, commonly found in gyms, allows for the downward phase (eccentric muscle contraction) to occur in a diagonal trajectory. Each participant performed only the downward movement (eccentric contraction), and was instructed to lower the leg-press platform in a constant, controlled tempo, completing each eccentric phase in 2 s, and were guided by a metronome. The starting position was set at a knee angle of 175º (180º being full knee extension). To stop the downward movement of the leg press platform at the correct position, a block was placed 1 cm beneath each individual's point of full knee flexion. To avoid the concentric phase of the contraction, the ascent of the leg press platform back to the initial position was performed with an electric motor, and the next repetition was started 5 s after the completion of the previous one. Given that eccentric muscle contractions produce higher force outputs than concentric contractions and considering that the eccentric exercise was performed using a standard leg press machine, the external load was set to 100% of the participant's maximum concentric strength for safety reasons. Each participant performed 5 set of 10 repetition, with 3 min interval between sets.

Delayed onset of muscle soreness

Before and 48 h after the eccentric exercise, participants assessed their perceived level of muscle soreness using a visual analog scale ranging from 1 (normal) to 10 (very, very sore) as previously described [26]. Each participant evaluated soreness in each leg by self-palpation of the muscle belly at the distal region of the vastus lateralis, both in a seated position with relaxed muscles and in a semi-squat position.

Isometric Force and Rate of Force Development

Isometric force was measured using a force platform positioned perpendicular on a concrete laboratory wall (Applied Measurements Ltd Co. UK, WP800, 1000kg weighting platform, 80x80 cm, sampling frequency 1000 Hz). Participants were seated on a custom-made steel leg press chair, with each foot placed separately on the force platform. The knee angle was set at 120° and the hip angle was set at 100º, as previously described [31]. Two isometric tests were conducted: one consisted of two efforts lasted 5s each, and the other was consisted of a single effort lasting 30 s, with 5 min interval between efforts. Participants were instructed to apply maximum force as fast as possible and maintain the force until the end of the test. Before both tests, two 3 s efforts of increasingly intensity were performed. Participants were vocally encouraged to perform their best. Real-time visual feedback of the force applied was provided for each effort via a computer monitor placed just above the force platform.

For the 5 s effort, the variables calculated from the force-time curve were the maximum isometric force (MIF; as the highest peak on the curve) and the RFD [RFD ($N \cdot s^{-1}$) = $\Delta Force \cdot \Delta Time^{-1}$] at 80, 150 and 200 ms. During the 30 s effort, the fatigue index (FI) was calculated using of the formula: FI (%) = [(MIF30sec - LIF30sec)/ MIF30sec] x 100 (LIF: lowest isometric force) [32]. The ICC for MIF and RFD were: ICC = 0.90, (95% CI: Lower = 0.86, Upper = 0.96) and ICC = 0.92, (95% CI: Lower = 0.80, Upper = 0.98) respectively.

Evaluation of Vastus Lateralis Cross-Sectional Area and Architecture

Vastus lateralis cross sectional area, muscle thickness, pennation angle and fascicle length of both legs were assessed in a prone position using B-mode ultrasonography (Product model Z5, Shenzhen Mindray Bio-Medical Electronics Co., Ltd, Shenzhen, China) with a 10 MHz linear array probe (38mm width). The vastus lateralis was selected as it has been reported to be a good surrogate for whole quadriceps exercise-induced changes [33]. To determine the vastus lateralis cross sectional area (CSA), the point at 40% (proximal to the knee) of two locations was marked: a) the center of the patella and b) the medial aspect of the anterior superior iliac spine. This point was chosen because it represents the largest cross-sectional area along the thigh [34]. A perpendicular guideline was then draw along the thigh with an indelible marker and the probe was drawn transversely across the thigh. Full visualization of quadriceps was achieved using a panoramic picture method, specifically the extended-field-of-view mode [33, 34].

Two images were taken and analyzed for each participant per leg and mean values were used for statistical analysis. To determine muscle thickness, pennation angle and fascicle length a continuous single view was taken from ~40 mm before, to ~40 mm after the same marked point (40% of the thick proximal to the knee), by moving the probe along the fascicles [34]. All images were analyzed using image analysis software (ImageJ; U.S. National Institutes of Health, Bethesda, MD, USA). The ICC for vastus lateralis CSA is 0.962 (95%CI: 0.835-0.991) ($n = 36$; $P < 0.001$). The ICC for vastus lateralis muscle thickness, fascicle angle, and fascicle length was 0.970 (95% CI: lower = 0.856, upper = 0.987), 0.880 (95% CI: lower = 0.609, upper = 0.965) and 0.840 (95% CI: lower = 0.470, upper = 0.955), respectively.

Muscle and cerebral oxygenation

Muscle and cerebral oxygenation were assessed before and 48 h after eccentric exercise during 5 and 30 s of maximum isometric contraction. Muscle and cerebral oxygenation were assessed non-invasively using the continuous wave NIRS, employing two wavelengths of near-infrared light (760 and 850 nm) (Artinis Medical System, PortaMon/PortaLite, Zetten, The Netherlands) as previously described [1, 35]. The NIRS units (i.e., PortaMon and PortaLite) are equipped with multi-distance optical probes configured with one optical receiver and three optical source emitters, allowing simultaneous monitoring of three separate tissue regions. The three source emitters are aligned on the same line as the detector. The inter-optode spacing between emitters and receiver was 30, 35, and 40 mm, with a penetration depth of approximately one-half of the distance between the emitter and the receiver (i.e., 15, 17.5, and 20 mm). NIRS data were collected at a frequency of 10 Hz, and the average values of the three optical signals were used for data analysis, while, for the analyses, the average value of all parameters recorded during the 5-second maximal isometric contraction was used. While NIRS devices cannot differentiate between chromophores (such as haemoglobin and myoglobin) within the muscle, it is noted that myoglobin content tends to remain constant during exercise. Thus, changes in NIRS signals can be attributed to changes in hemoglobin levels [36].

PortaMon and Portalite NIRS units, using spatial resolved spectroscopy, provide the tissue saturation index (TSI), which reflects the balance between oxygen supply and demand, and is expressed as a percentage at absolute values [37]. Tissue saturation index is a more robust indicator of muscle oxygenation, as it is less sensitive to motion artifacts and provides absolute values that are independent of blood volume changes. Moreover, the NIRS system provided concentration changes of muscle and cerebral microvascular of deoxyhaemoglobin (Δ[HHb]), which reflect the dynamic balance between muscle oxygen delivery and extraction in the underlying tissue [36]. During exercise, there is an alteration in muscle's haemodynamic response, characterized by an increase in Δ[HHb] [38]. Finally, total haemoglobin [tHb] concentration changes may be an indirect index of microvascular blood volume changes in response to exercise [39]. The NIRS technique and the units used in the present investigation have been validated in similar research [40].

Muscle oxygenation was assessed by placing the NIRS unit (PortaMon) at the lower third of the vastus lateralis muscle (approximately 12 cm above the patella and 5 cm lateral to the midline) after shaving and cleaning the site with an alcohol swab. The vastus lateralis muscle was chosen for its accessibility and because it exhibits substantially faster deoxyhemoglobin kinetics compared to the deep and superficial rectus femoris muscle [41]. The skinfold thickness of vastus lateralis was measured using a skinfold caliper (Harpenden, John Bull, St. Albans, England) to determine adipose tissue thickness (i.e., skinfold thickness/2), as it can influence the amplitude of the NIRS signal [42]. In this study, NIRS measurements were not influenced by adipose tissue for all participants, since the average adipose tissue thickness for the left and the right vastus lateralis muscles were 11.6 ± 5.2 and 11.7 ± 5.2 mm, respectively, both below the minimum NIRS light penetration depth (i.e., 15 mm). Considering that tissue oxygenation and blood flow responses can vary between different muscles and regions of the same muscle, the probes were always positioned by the same experimenter to ensure reproducibility of NIRS placement as much as possible.

Cerebral oxygenation was assessed by attaching the NIRS unit (PortaLite) on the surface of the left prefrontal cortex. Participants were instructed to keep their heads as still as possible during exercise to minimize motion artifacts in the cerebral NIRS signal. Both muscle and cerebral NIRS units and probes were covered with a black bandage and secured with tape on the cleaned skin to minimize external light intrusion and prevent movement. Notably, no sliding of the NIRS systems was observed at the end of the exercise sessions in all participants.

Statistical Analyses

The sample size was determined to achieve 85% statistical power (effect size 0.90) using G*Power software (ver. 3.1.9.6), based on data from previous measurements of the same nature conducted by our group. Shapiro-Wilks test was used to assess the normality of our data and no violation of normality was found ($p > 0.05$). All data are presented as mean and standard deviation (mean ± SD). A two-way repeated analysis of variance [2 x 2 ANOVA; condition (experimental and control limb) x time (pre- and post-intervention) in all measures. Bonferroni corrections were applied, when necessary, in the post-hoc comparisons. Effect sizes were calculated using Pearson equation where 0.1-0.3, 0.3-0.5 and higher than 0.5 were considered to be small, moderate and high, respectively. Statistical analyses were performed with SPSS Statistics v.20 (IBM Corporation, USA) with two-tailed significance was accepted at $p \leq 0.05$.

Results

Baseline measurements revealed no significant differences between the two lower limbs across all examined parameters ($p > 0.250$ in all cases).

Muscle damage

Eccentric exercise on the experimental limb caused muscle damage, evidenced by significant alterations on the muscle damage indicators. Specifically, 48 h post eccentric exercise, there were significant changes in concentric 1RM (206 ± 38 kg vs. 175 ± 33 kg; $p = 0.001$, effect size (ES) = 0.763; Fig. 1, panel A), DOMS during squat of the muscle belly (1 ± 0 vs. 4.3 ± 2.5; $p < 0.001$, ES = 0.886; Fig. 1, panel B), DOMS during palpation the movement (1.2 ± 0.4 vs. 4 ± 2; $p < 0.001$, ES = 0.741; Fig. 1, panel C). These parameters showed significant differences between the control and experimental limb at 48 h post eccentric exercise (concentric 1RM 205 ± 47 kg vs. 175 ± 33 kg; DOMS during palpation 1 ± 0 vs. 4.3 ± 2.5; DOMS during squat movement 1.2 ± 0.4 vs. 4.0 ± 2.0; p = 0.001 – 0.032, ES = 0.490 – 0.752; Fig. 1, panels A-C).

Maximal isometric force during the 5 s effort, fatigue index and average force production during the 30 s isometric maximal contraction were significantly reduced only in the experimental limb 48 h post eccentric exercise (1,924 ± 475 N vs. 1,509 ± 439 N, 23.1 ± 6.5 % vs. 30.0 ± 6.5 % and 1,409 ± 418 N vs. 1,060 ± 277 N, respectively; p = 0.001 – 0.023, ES = 0.613 – 0.887; Fig. 2, panels A-C). The same parameters showed significant differences between the control and experimental limb at 48 h post eccentric exercise (1,703 ± 326 N vs. 1,509 ± 439 N, 25.6 ± 6.7 % vs. 30.0 ± 6.5 %, 1,353 ± 373 N vs. 1,060 ± 277 N, respectively; p = 0.001 – 0.032, ES = 0.442 – 0.984; Fig. 2, panels A-C).

Similarly, the RFD measured at 80, 150 and 250 ms were significantly reduced only in the experimental limb 48 h post eccentric exercise (7,278 ± 2,521 N/s vs. 5,199 ± 2,323 N/s, 6,902 ± 2,044 N/s vs. 4,685 ± 2,194 N/s, 6,071 ± 1,658 N/s vs. 4,240 ± 1,796 N/s, respectively; p = 0.005- 0.023, ES = 0.702 – 0.817; Fig. 3, panels A-C). These parameters also exhibited significant differences between the control and experimental limb at 48 h post eccentric exercise (6,393 ± 2,315 N/s vs. 5,199 ± 2,323 N/s, 6,044 ± 1,860 N/s vs. 4,685 ± 2,194 N/s, 5,310 ± 1,411 N/s vs. 4,240 ± 1,796 N/s, respectively; p = 0.001 – 0.005, ES = 0.756 – 0.806; Fig. 3, panels A-C).

Muscle architecture

The cross-sectional area of the vastus lateralis significantly increased 48 h after eccentric exercise only for the experimental limb (24.4 ± 6.3 cm^2 vs. 27.7 ± 6.9 cm^2; p = 0.001, ES = 0.989) while at the same time point there was a significant difference between conditions (25.6 ± 6.3 cm^2 vs. 27.7 ± 6.9 cm^2; p = 0.042, ES = 0.906) (Fig. 4, panel A). Muscle thickness of the vastus lateralis also increased 48 h post eccentric exercise only in the experimental limb (2.4 ± 0.4 cm vs. 2.5 ± 0.2 cm; $p = 0.001$, ES = 0.983), with no difference observed between the two conditions (Fig. 4, panel B). Pennation angle and fascicle length did not change significantly over time or between limbs ($p = 0.737 – 0.808$, ES = 0.444 – 0.821) (Fig. 4, panels C and D, respectively).

Muscle and cerebral oxygenation

Muscle and cerebral TSI during 5 s (Fig. 5, panels A1 and A2) and 30 s (Fig. 5, panels B1 and B2) maximal isometric contraction showed no significant differences either over time ($p = 0.247 – 0.633$, ES = 0.408 – 0.854) or between limbs ($p = 0.298 – 0.788$, ES = 0.201 – 0.921). Collectively, there was no effect of time and condition on TSI during the 5 s and 30 s maximal isometric efforts.

In experimental vastus lateralis Δ[HHb] there was a significant alteration over time during the 5 s of maximal isometric contraction (1.16 ± 1.83 μM vs. 0.06 ± 1.93 μM; $p = 0.049$, ES = 0.577; Fig. 6, panel A1). However, for the same parameter (i.e., 5s of maximal isometric contraction), there was no significant interaction in vastus lateralis Δ[tHb] ($p = 0.449$; Fig. 6, panel A2). Muscle Δ[HHb], and Δ[tHb] during 30 s of maximal isometric contraction (Fig. 6, panels B1-B2) showed no significant differences either over time or between limbs in Δ[HHb], and Δ[tHb] ($p = 0.301 – 0.854$, ES = -0.225 – 0.777).

No significant differences were found in cerebral Δ[HHb], and Δ[tHb] at 5 s (Fig. 7, panels A1-A2) or at 30 s (Fig. 7, panels B1-B2) during maximal isometric contraction either over time ($p = 0.353 – 0.917$, ES = 0.130 – 0.601) or between limbs ($p = 0.326 – 0.878$, ES = 0.347 – 0.679).

Discussion

The aim of the present investigation was to explore the potential effects of eccentric-induced muscle damage on muscle architecture, as well as muscle and cerebral oxygenation. To the best of our knowledge, this is the first study to concurrently investigate the effects of exercise-induced muscle damage on both muscle and cerebral oxygenation. Based on our findings, oxygenation of the vastus lateralis and the prefrontal cortex – as primarily assessed by the TSI – was not affected by eccentric exercise-induced muscle damage during maximal isometric contraction of either short or long duration. Unilateral eccentric exercise performed in a leg press machine at a 45° inclination induced muscle damage, as evidenced by significant alterations in several indirect indicators of muscle damage. Additionally, significant changes were observed in the cross-sectional area and muscle thickness of the vastus lateralis, as assess via ultrasonography 48 h post eccentric exercise.

One of the most valid and reliable indirect measures of muscle damage in humans is a prolonged decrease in muscle force output after exercise [43]. The eccentric exercise protocol applied in the present study caused a 16% reduction in MVC assessed in the knee extensors of the experimental limb, indicating muscle damage [44]. Furthermore, significant reductions in RFD at 80, 150 and 250 ms were observed which are in accordance with previous data [45]. The reduction in RFD observed in this study may be attributed to impaired recruitment of type II motor units following eccentric exercise [46], along with a greater reliance on type I motor units, which contributes to the slower RFD post eccentric exercise [47]. The RFD is not only considered a valid additional indirect marker of muscle damage but also a more specific and sensitive indicator of eccentric exercise-induced muscle damage than MVC [48]. Another important biomarker supporting the induction of muscle damage was the increase in CSA and muscle thickness 48 h after eccentric exercise, indicating the occurrence of edema in the affected muscle. Indeed, it has been previously observed that the days post eccentric exercise ultrasound assessments reveal significant increase in muscle thickness and fascicle length [30]. Several studies suggest that muscle swelling after eccentric exercise occurs due to fluid penetration into the interstitial space of the cells [49]. It has been proposed that unaccustomed eccentric exercise may lead to sarcomere disruption by opening the mechanosensitive stretch channels of cell membranes, resulting in the intracellular influx of Ca^{2+} and Na^{+} ions, which may cause cell damage, swelling, inflammation, and a transient reduction in force production [50]. However, in the present study we did not use techniques such as muscle needle biopsy and magnetic resonance imaging, that may provide more direct measurements muscle damage.

A slight decrease in deoxyhemoglobin was observed in the experimental limb 48 h after eccentric exercise, but only during the 5 s maximal isometric contraction – not during the 30 s effort. This reduction may be related to the concurrent decrease in the RFD. It can be hypothesized that the lower rate of force generation after eccentric muscle-damaging exercise results from increased reliance on type I muscle fibers, which are intrinsically slower at producing force. This shift may occur because type II motor units are predominantly damaged after eccentric exercise [46]. This hypotheses align with our findings, as muscle damage was associated with reduced force output 48 h post eccentric exercise – likely indicating greater recruitment of type I muscle fibers – which may, in turn, explain the unexpected reduction in muscle deoxyhaemoglobin.

During the prolonged maximal isometric contraction – which places greater demand on oxidative metabolism – no disturbances in muscle oxygenation were observed, despite the presence of exercise-induced muscle damage [17]. This preservation of oxygenation may be attributed to an increase in local muscle blood flow, potentially serving to compensate for microcirculatory dysfunction [18, 19]. The absence of changes in muscle oxygenation parameters could also be explained by the lower absolute force output following eccentric exercise-induced muscle damage, likely due to greater reliance on type I muscle fibers, which are more metabolically oxidative. These conditions may have masked potential effects of muscle damage on muscle oxygenation, as such effects are influenced by the metabolic demands of the task. Indeed, the reduced force output during the 30 s of maximal isometric contraction at 48 h post eccentric exercise could have resulted in decreased muscle compression and intramuscular pressure. This, in turn, may have helped preserve blood flow, oxygen delivery, muscle oxygenation status, and oxygen extraction.

The lower [HHb] values observed during the 5 s trial after eccentric exercise could be related to the reduction in maximal isometric force, resulting in lower intramuscular pressure and, consequently, greater blood flow and enhanced oxygen delivery. Deoxyhemoglobin has been reported to be an indirect index of oxygen extraction during exercise [41]. Previous studies have found that severe eccentric exercise muscle damage can significantly impact the microvascular response during exercise initiation [14, 18, 19]. Indeed, 48 h after eccentric exercise-induced muscle damage, slow [HHb] responses to severe-intensity exercise were observed [18]. High intensity muscle contractions (i.e., 5 - 20 s in duration, comparable duration used in the present investigation) were also found to slow microvascular reactivity, causing a mismatch between the delivery of oxygen and its utilization in the muscle tissue [14]. Similarly, in rat spinotrapezius muscle, which provides a close analog to human quadriceps muscle (e.g., muscle fibre type, oxidative capacity), eccentric exercise was found to disrupt capillary geometry by increasing the capillary luminal shape and area (i.e., luminal ellipticity), which was suggested to impair muscle microcirculatory flow and the balance between oxygen delivery and consumption at the onset of electrically stimulated contractions [19].

To the best of our knowledge, this is the first study to examine the effect of eccentric-induced muscle damage on cerebral oxygenation. Based on our results, eccentric muscle damaging exercise did not affect cerebral oxygenation, as assessed during maximal intensity exercise. In previous investigations by our group, no differences were found in cerebral oxygenation between eccentric and concentric isokinetic exercise, whether assessed during continuous submaximal exercise [1] or during high intensity interval training [35]. These similar findings on cerebral oxygenation contradict the differences in cortical activation between conditions. Eccentric exercise requires advanced cortical control and recruitment of brain areas to activate high-threshold motor units while lowering the discharge rate of activated motor

392 units [51]. Similarly, the absence of alterations in cerebral oxygenation found in the present study
393 contradicted the initial hypothesis, since it was expected that after eccentric muscle damaging exercise,
394 higher brain function would be required to control muscle effort as a central protective mechanism to
395 regulate force generation to prevent further injury [24]. Additionally, it can be hypothesized that
396 incorporating a submaximal, long duration isokinetic assessment after exercise-induced muscle damage
397 might have elicited a more pronounced cerebral oxygenation response, due to the increased effort required
398 to sustain the predetermined force. Moreover, it was expected that the sensation of pain in response to
399 eccentric exercise-induced muscle damage could have interfered with cerebral oxygenation.

400

401 Regarding cerebral oxygenation, it can be hypothesized that a higher volume of eccentric exercise (> 200-
402 300 repetitions) may be necessary to elicit detectable changes, which could reflect increased cortical
403 activation. In such conditions, greater cortical involvement might be required to maintain a given force
404 output due to impaired muscle function resulting from the higher degree of muscle damage. This
405 activation likely promotes cerebral vasodilation, thereby helping to maintain brain oxygen supply [52-
406 55].

407

408 This study provides novel insights in both muscle and cerebral oxygenation in response to muscle
409 damaging eccentric exercise. The results of this study suggest, for the first time, that both short and a long
410 duration maximal isometric contractions (i.e., 5 and 30 s, respectively) performed 48 h after eccentric
411 exercise do not alter muscle or cerebral oxygenation, as measured by TSI, nor blood volume, as indicated
412 by changes in total hemoglobin (Δ[tHb]). However, a marginal change in muscle O_2 extraction was
413 observed during the 5 s maximal isometric contraction, evidenced by a decrease in Δ[HHb], which was
414 unexpected at 48 h post eccentric exercise. No alterations were observed in prefrontal cortex oxygenation
415 during 30 s maximal isometric contraction performed 48 h post eccentric exercise. The absence of
416 alterations in cerebral oxygenation contradicts our initial hypothesis that muscle damage would require
417 increased brain activity to control muscle effort as a central protective mechanism to regulate force

generation to prevent further injury [24]. Furthermore, the unexpected decrease in deoxyhemoglobin during the short (5 seconds) maximal isometric contraction may be linked to the reduced isometric force production observed following eccentric exercise-induced muscle damage. This reduction in force could result from a greater reliance on type I muscle fibers activation, as type II fibers are more susceptible to damage from eccentric exercise [46].

Limitations

In the present study, direct blood markers of inflammation and muscle damage (e.g., creatine kinase, myoglobin, C-reactive protein) were not measured, which could be considered a limitation, as the inclusion of such blood indicators might have strengthened the evidence that eccentric exercise induces not only local but also systemic disturbances. Furthermore, cerebral oxygenation was assessed only at a single site – the pre-frontal cortex – which may have restricted the detection of potential changes across other brain regions; the use of a multi-channel functional NIRS (fNIRS) system, specialized for assessing brain function, might have provided a more comprehensive picture of cerebral oxygenation alterations [28]. Regarding muscle and cerebral oxygenation during the 30 s protocol, it is possible that the lower absolute force output following eccentric exercise-induced muscle damage masked any potential effects. This may be due to the fact that the impact of muscle damage on oxygenation is influenced by the metabolic demands of the task. The relatively small sample size, composed of recreationally trained individuals, also limits the generalizability of our findings, as the results may not extend to broader populations or specific groups such as athletes, highly trained individuals, or clinical populations. Additionally, although the single-limb model used in this study offers methodological advantages, it could also be seen as a limitation given that previous studies have reported cross-education effects of eccentric exercise, either providing a protective effect against contralateral muscle damage [56] or causing reduced neuromuscular activity and physical capacity in the unexercised limb [57].

Practical Applications

In this study, a leg press machine was used to induce muscle damage, enhancing ecological validity since such equipment is widely available and commonly used by athletes at all levels, from recreational to elite. Many physical activities involve brief, high intensity muscle efforts, underscoring the physiological importance of the ability to rapidly increase blood flow to meet oxygen delivery demands. Our findings suggest that eccentric exercise-induced muscle damage does not appear to affect muscle oxygenation during either short (5 s) or longer (30 s) maximal isometric contractions. Similarly, no significant alterations were observed in cerebral oxygenation. Furthermore, the absence of changes in cerebral oxygenation suggests effective brain autoregulatory mechanisms that maintain oxygen supply despite the decreased muscle function, implying a potential mismatch between the athletes' perceived willingness to perform and their actual physical capacity, thus increasing the risk of unintentional overexert during the recovery.

Conclusion

Unilateral eccentric exercise performed in a leg press machine with a 45° inclination induced muscle damage and decreased muscle performance, that is, maximal strength and the rate of force development. Additionally, muscle damage induced alterations in muscle morphology, as measured via ultrasonography. Vastus lateralis CSA and muscle thickness are increased two days after the eccentric protocol, indicating the occurrence of muscle damage and the presence of edema. The structural impairments in skeletal muscle resulting from muscle damage were not sufficient to alter the muscle oxygenation levels during either short- or long- duration maximal isometric contraction tests. Similarly, cerebral oxygenation was not affected by the muscle damage as assessed two days after the eccentric protocol.

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470 *Data availability statement:* The data that supports the findings of this study are available from the corresponding author upon reasonable request.

References

1. Cherouveim ED, Margaritelis NV, Koulouvaris P et al. Skeletal muscle and cerebral oxygenation levels during and after submaximal concentric and eccentric isokinetic exercise. J Sports Sci 2022; 40: 195-202. doi:10.1080/02640414.2021.1983248
2. Hessel AL, Lindstedt SL, Nishikawa KC. Physiological Mechanisms of Eccentric Contraction and Its Applications: A Role for the Giant Titin Protein. Front Physiol 2017; 8: 70. doi:10.3389/fphys.2017.00070
3. Margaritelis NV, Theodorou AA, Chatzinikolaou PN et al. Eccentric exercise per se does not affect muscle damage biomarkers: early and late phase adaptations. Eur J Appl Physiol 2021; 121: 549-559. doi:10.1007/s00421-020-04528-w
4. Paschalis V, Koutedakis Y, Jamurtas AZ et al. Equal volumes of high and low intensity of eccentric exercise in relation to muscle damage and performance. J Strength Cond Res 2005; 19: 184-188. doi:10.1519/R-14763.1
5. Paschalis V, Nikolaidis MG, Giakas G et al. The effect of eccentric exercise on position sense and joint reaction angle of the lower limbs. Muscle Nerve 2007; 35: 496-503. doi:10.1002/mus.20723
6. Margaritelis NV, Theodorou AA, Baltzopoulos V et al. Muscle damage and inflammation after eccentric exercise: can the repeated bout effect be removed? Physiol Rep 2015; 3. doi:10.14814/phy2.12648
7. Paschalis V, Nikolaidis MG, Fatouros IG et al. Uniform and prolonged changes in blood oxidative stress after muscle-damaging exercise. In Vivo 2007; 21: 877-883
8. Peake JM, Neubauer O, Della Gatta PA et al. Muscle damage and inflammation during recovery from exercise. J Appl Physiol (1985) 2017; 122: 559-570. doi:10.1152/japplphysiol.00971.2016
9. Paschalis V, Nikolaidis MG, Giakas G et al. Beneficial changes in energy expenditure and lipid profile after eccentric exercise in overweight and lean women. Scand J Med Sci Sports 2010; 20: e103-111. doi:10.1111/j.1600-0838.2009.00920.x
10. Paschalis V, Nikolaidis MG, Theodorou AA et al. The effects of eccentric exercise on muscle function and proprioception of individuals being overweight and underweight. J Strength Cond Res 2013; 27: 2542-2551. doi:10.1519/JSC.0b013e31827fc9a6
11. Tee JC, Bosch AN, Lambert MI. Metabolic consequences of exercise-induced muscle damage. Sports Med 2007; 37: 827-836. doi:10.2165/00007256-200737100-00001
12. Brandao MCA, Matias LA, da Matta TT et al. Biceps brachii elastography in well-trained men post eccentric exercise-induced muscle damage. J Ultrasound 2024. doi:10.1007/s40477-024-00886-9. doi:10.1007/s40477-024-00886-9
13. Murayama M, Inami T, Shima N et al. Changes in biceps brachii muscle hardness assessed by a push-in meter and strain elastography after eccentric versus concentric contractions. Sci Rep 2022; 12: 9214. doi:10.1038/s41598-022-13184-3
14. Larsen RG, Hirata RP, Madzak A et al. Eccentric exercise slows in vivo microvascular reactivity during brief contractions in human skeletal muscle. J Appl Physiol (1985) 2015; 119: 1272-1281. doi:10.1152/japplphysiol.00563.2015
15. Larsen RG, Thomsen JM, Hirata RP et al. Impaired microvascular reactivity after eccentric muscle contractions is not restored by acute ingestion of antioxidants or dietary nitrate. Physiol Rep 2019; 7: e14162. doi:10.14814/phy2.14162
16. Ahmadi S, Sinclair PJ, Foroughi N et al. Monitoring muscle oxygenation after eccentric exercise-induced muscle damage using near-infrared spectroscopy. Appl Physiol Nutr Metab 2008; 33: 743-752. doi:10.1139/h08-048
17. Laaksonen MS, Kivela R, Kyrolainen H et al. Effects of exhaustive stretch-shortening cycle exercise on muscle blood flow during exercise. Acta Physiol (Oxf) 2006; 186: 261-270. doi:10.1111/j.1748-1716.2006.01532.x
18. Davies RC, Eston RG, Poole DC et al. Effect of eccentric exercise-induced muscle damage on the dynamics of muscle oxygenation and pulmonary oxygen uptake. J Appl Physiol (1985) 2008; 105: 1413-1421. doi:10.1152/japplphysiol.90743.2008

19. Kano Y, Padilla DJ, Behnke BJ et al. Effects of eccentric exercise on microcirculation and microvascular oxygen pressures in rat spinotrapezius muscle. J Appl Physiol (1985) 2005; 99: 1516-1522. doi:10.1152/japplphysiol.00069.2005
20. Mirkov DM, Nedeljkovic A, Milanovic S et al. Muscle strength testing: evaluation of tests of explosive force production. Eur J Appl Physiol 2004; 91: 147-154. doi:10.1007/s00421-003-0946-8
21. Cettolo V, Ferrari M, Biasini V et al. Vastus lateralis O2 desaturation in response to fast and short maximal contraction. Med Sci Sports Exerc 2007; 39: 1949-1959. doi:10.1249/mss.0b013e3181453476
22. Byrne C, Twist C, Eston R. Neuromuscular function after exercise-induced muscle damage: theoretical and applied implications. Sports Med 2004; 34: 49-69. doi:10.2165/00007256-200434010-00005
23. Nybo L, Rasmussen P. Inadequate cerebral oxygen delivery and central fatigue during strenuous exercise. Exerc Sport Sci Rev 2007; 35: 110-118. doi:10.1097/jes.0b013e3180a031ec
24. Proske U, Gregory JE, Morgan DL et al. Force matching errors following eccentric exercise. Hum Mov Sci 2004; 23: 365-378. doi:10.1016/j.humov.2004.08.012
25. Methenitis S, Theodorou AA, Chatzinikolaou PN et al. The effects of chronic concentric and eccentric training on position sense and joint reaction angle of the knee extensors. Eur J Sport Sci 2023; 23: 1164-1174. doi:10.1080/17461391.2023.2184726
26. Paschalis V, Nikolaidis MG, Giakas G et al. Position sense and reaction angle after eccentric exercise: the repeated bout effect. Eur J Appl Physiol 2008; 103: 9-18. doi:10.1007/s00421-007-0663-9
27. Perrey S. Brain activation associated with eccentric movement: A narrative review of the literature. Eur J Sport Sci 2018; 18: 75-82. doi:10.1080/17461391.2017.1391334
28. Borot L, Vergotte G, Perrey S. Different Hemodynamic Responses of the Primary Motor Cortex Accompanying Eccentric and Concentric Movements: A Functional NIRS Study. Brain Sci 2018; 8. doi:10.3390/brainsci8050075
29. Newton MJ, Sacco P, Chapman D et al. Do dominant and non-dominant arms respond similarly to maximal eccentric exercise of the elbow flexors? J Sci Med Sport 2013; 16: 166-171. doi:10.1016/j.jsams.2012.06.001
30. Penailillo L, Blazevich AJ, Nosaka K. Muscle fascicle behavior during eccentric cycling and its relation to muscle soreness. Med Sci Sports Exerc 2015; 47: 708-717. doi:10.1249/MSS.0000000000000473
31. Methenitis S, Karandreas N, Spengos K et al. Muscle Fiber Conduction Velocity, Muscle Fiber Composition, and Power Performance. Med Sci Sports Exerc 2016; 48: 1761-1771. doi:10.1249/MSS.0000000000000954
32. Margaritelis NV, Chatzinikolaou PN, Bousiou FV et al. Dietary Cysteine Intake is Associated with Blood Glutathione Levels and Isometric Strength. Int J Sports Med 2021; 42: 441-447. doi:10.1055/a-1255-2863
33. Spiliopoulou P, Methenitis S, Zaras N et al. Vastus Lateralis and Vastus Intermedius as Predictors of Quadriceps Femoris Muscle Hypertrophy after Strength Training. Applied Sciences 2022; 12: 9133. doi:10.3390/app12189133
34. Noorkoiv M, Nosaka K, Blazevich AJ. Assessment of quadriceps muscle cross-sectional area by ultrasound extended-field-of-view imaging. Eur J Appl Physiol 2010; 109: 631-639. doi:10.1007/s00421-010-1402-1
35. Perentis PA, Cherouveim ED, Malliou VJ et al. The Effects of High-Intensity Interval Exercise on Skeletal Muscle and Cerebral Oxygenation during Cycling and Isokinetic Concentric and Eccentric Exercise. J Funct Morphol Kinesiol 2021; 6. doi:10.3390/jfmk6030062
36. Barstow TJ. Understanding near infrared spectroscopy and its application to skeletal muscle research. J Appl Physiol (1985) 2019; 126: 1360-1376. doi:10.1152/japplphysiol.00166.2018

37. Buchheit M, Ufland P, Haydar B et al. Reproducibility and sensitivity of muscle reoxygenation and oxygen uptake recovery kinetics following running exercise in the field. Clin Physiol Funct Imaging 2011; 31: 337-346. doi:10.1111/j.1475-097X.2011.01020.x

38. Celie B, Boone J, Van Coster R et al. Reliability of near infrared spectroscopy (NIRS) for measuring forearm oxygenation during incremental handgrip exercise. Eur J Appl Physiol 2012; 112: 2369-2374. doi:10.1007/s00421-011-2183-x

39. Cardinale M, Ferrari M, Quaresima V. Gastrocnemius medialis and vastus lateralis oxygenation during whole-body vibration exercise. Med Sci Sports Exerc 2007; 39: 694-700. doi:10.1249/mss.0b013e31803084d8

40. Scott BR, Slattery KM, Sculley DV et al. Reliability of telemetric electromyography and near-infrared spectroscopy during high-intensity resistance exercise. J Electromyogr Kinesiol 2014; 24: 722-730. doi:10.1016/j.jelekin.2014.07.008

41. Koga S, Okushima D, Barstow TJ et al. Near-infrared spectroscopy of superficial and deep rectus femoris reveals markedly different exercise response to superficial vastus lateralis. Physiol Rep 2017; 5. doi:10.14814/phy2.13402

42. van Beekvelt MC, Borghuis MS, van Engelen BG et al. Adipose tissue thickness affects in vivo quantitative near-IR spectroscopy in human skeletal muscle. Clin Sci (Lond) 2001; 101: 21-28. doi:10.1042/cs20000247

43. Warren GL, Lowe DA, Armstrong RB. Measurement tools used in the study of eccentric contraction-induced injury. Sports Med 1999; 27: 43-59. doi:10.2165/00007256-199927010-00004

44. Paulsen G, Mikkelsen UR, Raastad T et al. Leucocytes, cytokines and satellite cells: what role do they play in muscle damage and regeneration following eccentric exercise? Exerc Immunol Rev 2012; 18: 42-97

45. Crameri RM, Aagaard P, Qvortrup K et al. Myofibre damage in human skeletal muscle: effects of electrical stimulation versus voluntary contraction. J Physiol 2007; 583: 365-380. doi:10.1113/jphysiol.2007.128827

46. Lieber RL, Friden J. Selective damage of fast glycolytic muscle fibres with eccentric contraction of the rabbit tibialis anterior. Acta Physiol Scand 1988; 133: 587-588. doi:10.1111/j.1748-1716.1988.tb08446.x

47. Chen YW, Hubal MJ, Hoffman EP et al. Molecular responses of human muscle to eccentric exercise. J Appl Physiol (1985) 2003; 95: 2485-2494. doi:10.1152/japplphysiol.01161.2002

48. Penailillo L, Blazevich A, Numazawa H et al. Rate of force development as a measure of muscle damage. Scand J Med Sci Sports 2015; 25: 417-427. doi:10.1111/sms.12241

49. Agten CA, Buck FM, Dyer L et al. Delayed-Onset Muscle Soreness: Temporal Assessment With Quantitative MRI and Shear-Wave Ultrasound Elastography. AJR Am J Roentgenol 2017; 208: 402-412. doi:10.2214/AJR.16.16617

50. Hughes JD, Johnson NA, Brown SJ et al. Effects of eccentric exercise-induced muscle damage on intramyocellular lipid concentration and high energy phosphates. Eur J Appl Physiol 2010; 110: 1135-1141. doi:10.1007/s00421-010-1605-5

51. Gruber M, Linnamo V, Strojnik V et al. Excitability at the motoneuron pool and motor cortex is specifically modulated in lengthening compared to isometric contractions. J Neurophysiol 2009; 101: 2030-2040. doi:10.1152/jn.91104.2008

52. Bhambhani Y, Malik R, Mookerjee S. Cerebral oxygenation declines at exercise intensities above the respiratory compensation threshold. Respir Physiol Neurobiol 2007; 156: 196-202. doi:10.1016/j.resp.2006.08.009

53. Ogoh S, Ainslie PN. Cerebral blood flow during exercise: mechanisms of regulation. J Appl Physiol (1985) 2009; 107: 1370-1380. doi:10.1152/japplphysiol.00573.2009

54. Prodel E, Balanos GM, Braz ID et al. Muscle metaboreflex and cerebral blood flow regulation in humans: implications for exercise with blood flow restriction. Am J Physiol Heart Circ Physiol 2016; 310: H1201-1209. doi:10.1152/ajpheart.00894.2015

55. Secher NH, Seifert T, Van Lieshout JJ. Cerebral blood flow and metabolism during exercise: implications for fatigue. J Appl Physiol (1985) 2008; 104: 306-314. doi:10.1152/japplphysiol.00853.2007
56. Tseng WC, Nosaka K, Tseng KW et al. Contralateral Effects by Unilateral Eccentric versus Concentric Resistance Training. Med Sci Sports Exerc 2020; 52: 474-483. doi:10.1249/MSS.0000000000002155
57. Hedayatpour N, Izanloo Z, Falla D. The effect of eccentric exercise and delayed onset muscle soreness on the homologous muscle of the contralateral limb. J Electromyogr Kinesiol 2018; 41: 154-159. doi:10.1016/j.jelekin.2018.06.003

Figure Legends

Figure 1 Concentric 1 repetition maximum (A), DOMS during squat movement (B) and DOMS during palpation (C), pre and 48-h post eccentric exercise for the control (white bars) and the experimental (black bars) limb.

* Significant difference ($p<0.05$) between pre and post exercise condition for the same condition.

significant difference ($p<0.05$) between control and experimental limb at the same time point.

Figure 2 Isometric peak force during a 5 s effort (A), Fatigue index (B) and Average force (C) during a 30 s maximal isometric effort, pre and 48-h post eccentric exercise for the control (white bars) and the experimental (black bars) limb.

* Significant difference ($p<0.05$) between pre and post exercise condition for the same condition.

significant difference ($p<0.05$) between control and experimental limb at the same time point.

Figure 3 Rate of force development at 80 ms (A) 150 ms (B) and 250 ms (C), pre and 48 h post eccentric exercise for the control (white bars) and the experimental (black bars) limb.

* Significant difference ($p<0.05$) between pre and post exercise condition for the same condition.

significant difference ($p<0.05$) between control and experimental limb at the same time point.

Figure 4 Cross sectional area (A), muscle thickness (B), pennation angle (C) and fascicle length (D) of knee extensors, pre and 48 h post eccentric exercise for the control (white bars) and the experimental (black bars) limb.

* Significant difference ($p<0.05$) between pre and post exercise condition for the same condition.

# denotes significant difference ($p<0.05$) between control and experimental limb at the same time point.

Figure 5 Tissue saturation index (TSI) for muscle and cerebral tissue measured during 5s (A1-A2) and 30s (B1-B2) isometric force, pre and 48 h post eccentric exercise.

663 **Figure 6** Muscle oxygenation parameters [i.e., deoxyhemoglobin (HHb) and total hemoglobin (tHb)] measured during 5s (A1-A2) and 30s (B1-B2) isometric force, pre and 48 h post eccentric exercise. * Significant difference ($p<0.05$) between pre and post exercise condition for the same condition.

667 **Figure 7** Cerebral oxygenation parameters [i.e., deoxyhemoglobin (HHb) and total hemoglobin (tHb)] measured during 5s (A1-A2) and 30s (B1-B2) isometric force, pre and 48 h post eccentric exercise.

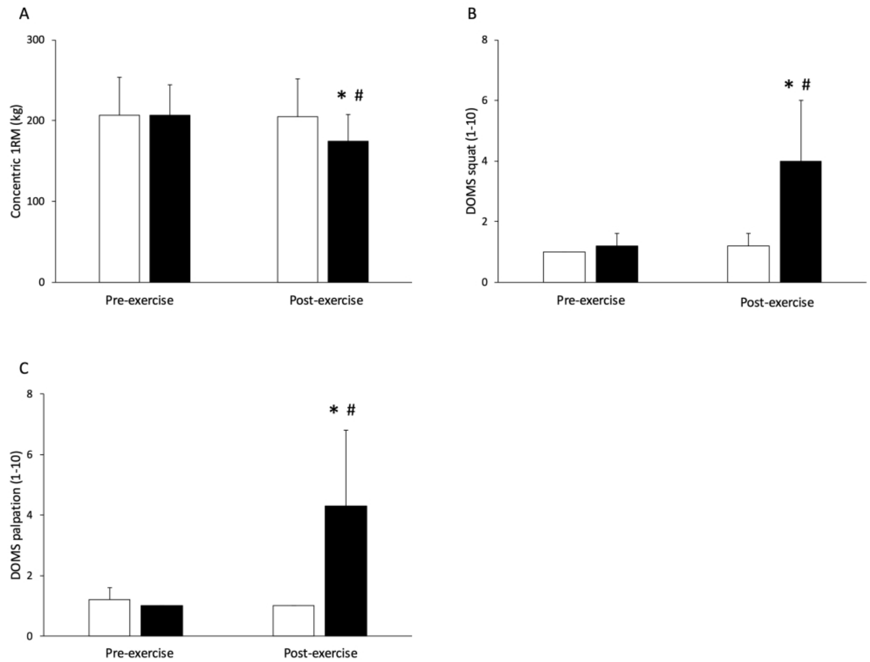

Figure 1: Concentric 1 repetition maximum (A), DOMS during squat movement (B) and DOMS during palpation (C), pre and 48-h post eccentric exercise for the control (white bars) and the experimental (black bars) limb.
* Significant difference ($p<0.05$) between pre and post exercise condition for the same condition.
# significant difference ($p<0.05$) between control and experimental limb at the same time point.

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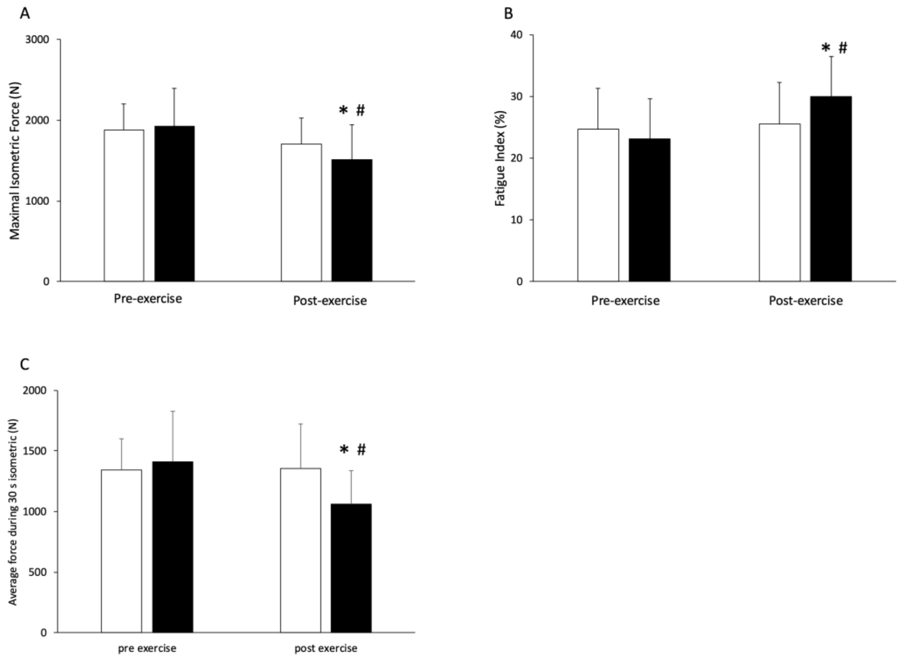

Figure 2: Isometric peak force during a 5 s effort (A), Fatigue index (B) and Average force (C) during a 30 s maximal isometric effort, pre and 48-h post eccentric exercise for the control (white bars) and the experimental (black bars) limb.
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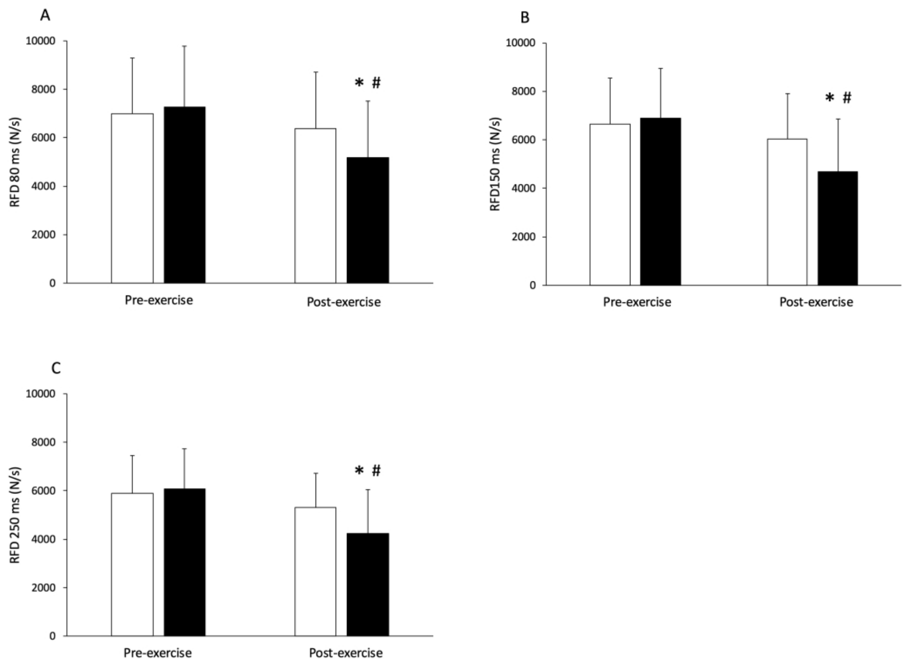

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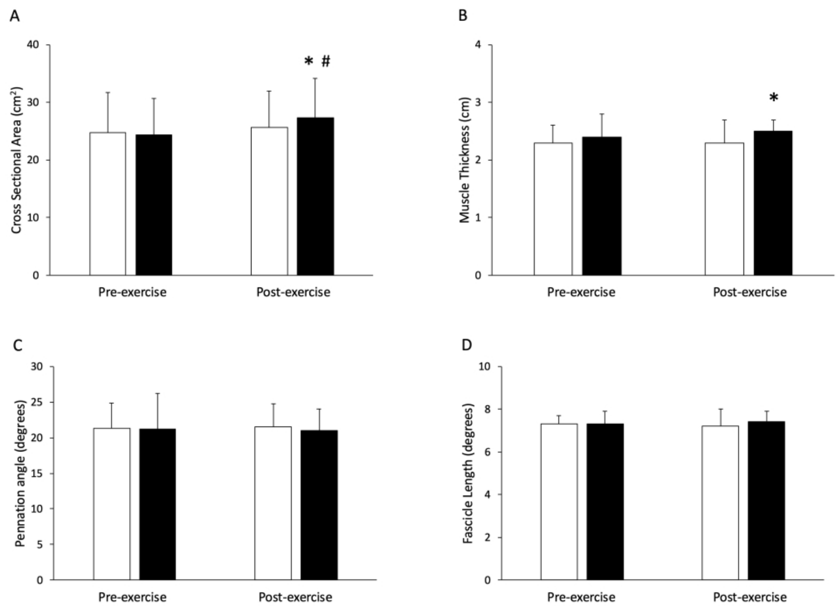

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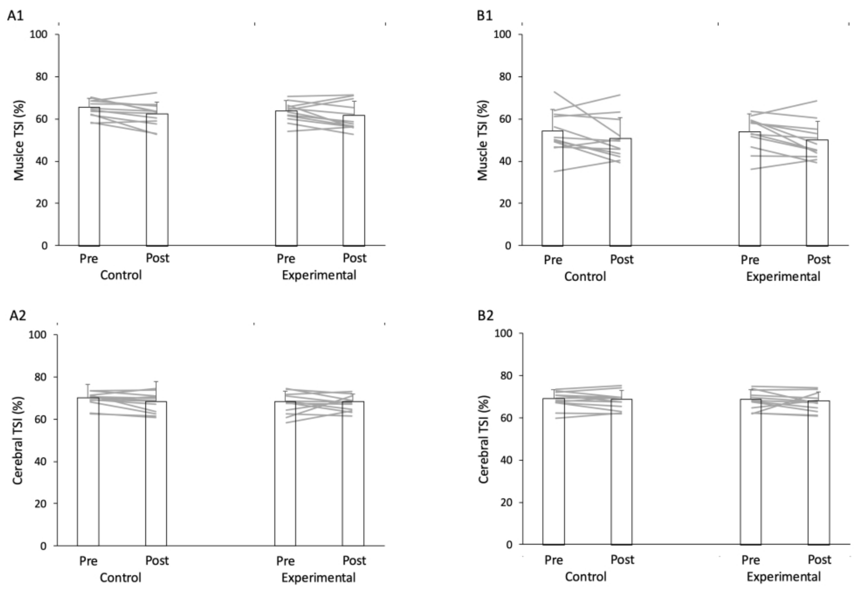

Figure 5: Tissue saturation index (TSI) for muscle and cerebral tissue measured during 5s (A1-A2) and 30s (B1-B2) isometric force, pre and 48 h post eccentric exercise.

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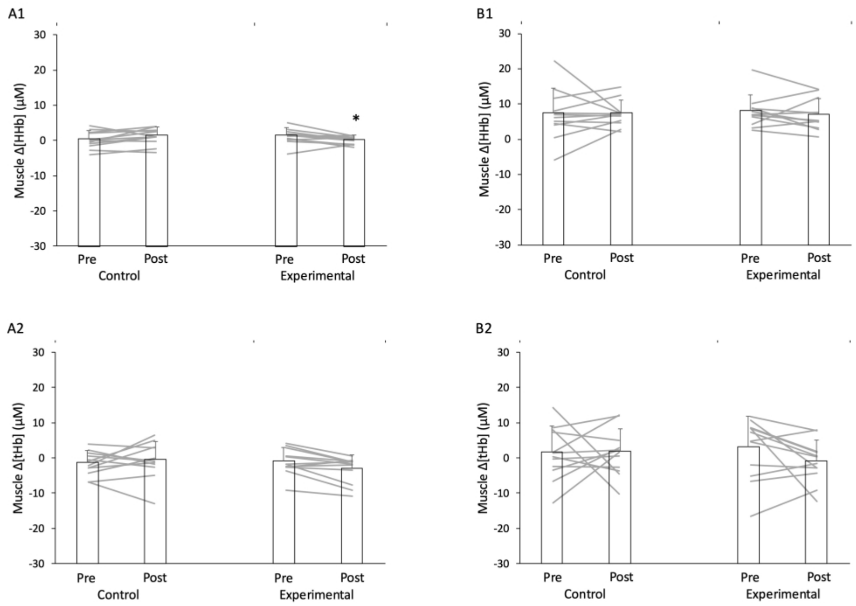

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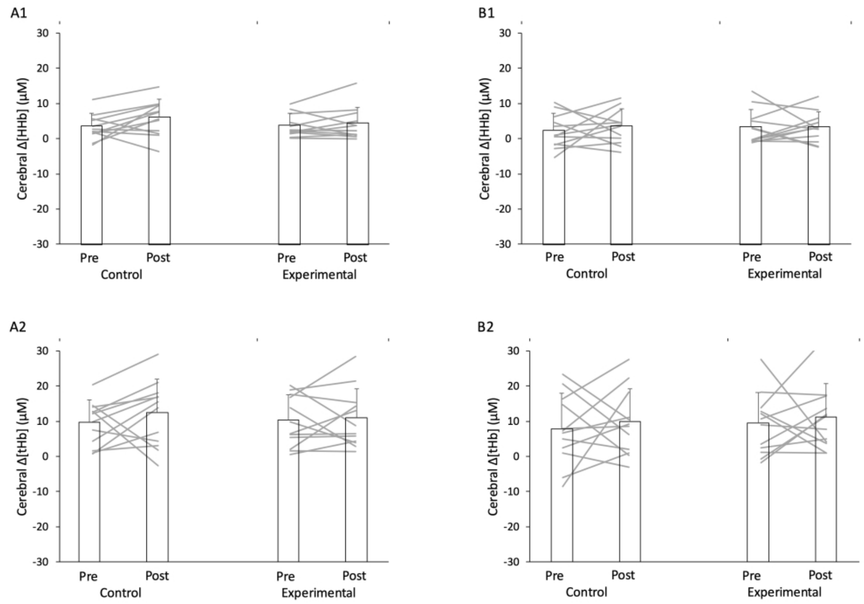

Figure 7: Cerebral oxygenation parameters [i.e., deoxyhemoglobin (HHb) and total hemoglobin (tHb)] measured during 5s (A1-A2) and 30s (B1-B2) isometric force, pre and 48 h post eccentric exercise.

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