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# Muscle damage and repeated-bout effect after a typical Nordic hamstring exercise session

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**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Abstract:**
The present study investigated muscle damage and repeated-bout effect after a typical Nordic hamstring exercise (NHE) session. Thirteen trained male adults were recruited. Muscle damage was monitored through eccentric strength, passive range of motion (ROM), muscle soreness, and muscle swelling. Assessments were performed at baseline and one (day-1), two (day-2), three (day-3), and four (day-4) days after 24 NHE repetitions. The procedures were replicated after four weeks to examine the repeated-bout effect. After the first bout, hamstring eccentric strength decreased up to day-3 [effect size (ES) range: -2.28/-1.95]. Passive ROM decreased up to day-4 (ES range: -1.62/-0.64), while muscle soreness increased on day-2 (ES=1.63) and day-3 (ES=1.53). No change in muscle swelling was observed. After the second bout, hamstring eccentric strength (ES=-1,20) decreased only on day-1. Passive ROM decreased up to day-3 (ES -0.85/-0.34). These changes were by a smaller magnitude compared to the first bout. The first NHE bout impaired hamstring strength and passive ROM, with muscle soreness also perceived, while the second bout reduced muscle damage symptoms and shortened the time to recovery. While recovery is needed when an NHE session is performed for the first time, following NHE sessions may scarcely interfere with the training routine.

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**Muscle damage and repeated-bout effect after a typical Nordic hamstring exercise session**


## ABSTRACT

The present study investigated muscle damage and repeated-bout effect after a typical Nordic hamstring exercise (NHE) session. Thirteen trained male adults were recruited. Muscle damage was monitored through eccentric strength, passive range of motion (ROM), muscle soreness, and muscle swelling. Assessments were performed at baseline and one (day-1), two (day-2), three (day-3), and four (day-4) days after 24 NHE repetitions. The procedures were replicated after four weeks to examine the repeated-bout effect. After the first bout, hamstring eccentric strength decreased up to day-3 [effect size (ES) range: -2.28/-1.95]. Passive ROM decreased up to day-4 (ES range: -1.62/-0.64), while muscle soreness increased on day-2 (ES=1.63) and day-3 (ES=1.53). No change in muscle swelling was observed. After the second bout, hamstring eccentric strength (ES=-1,20) decreased only on day-1. Passive ROM decreased up to day-3 (ES -0.85/-0.34). These changes were by a smaller magnitude compared to the first bout. The first NHE bout impaired hamstring strength and passive ROM, with muscle soreness also perceived, while the second bout reduced muscle damage symptoms and shortened the time to recovery. While recovery is needed when an NHE session is performed for the first time, following NHE sessions may scarcely interfere with the training routine.



**Keywords**: delayed onset muscle soreness; eccentric; injury prevention; recovery; resistance training ultrasound.

## INTRODUCTION

Performing a session of unaccustomed eccentric-biased exercise leads to muscle damage whatever the exercise modality performed, such as isokinetic or constant-external load [1], flywheel technology [2], downhill running [3], or cycling [4]. Indirect muscle damage markers such as strength loss, passive range of motion (ROM), muscle soreness, and muscle swelling have been commonly used to assess the recovery time course after the eccentric session [5]. However, while the initial muscle damage may discourage practitioners from the incorporation of regular eccentric exercise, the first session confers protection against muscle damage deriving from subsequent eccentric sessions, i.e., the repeated-bout effect [6]. As such, muscle damage markers will recover much faster after the second session, making eccentric-biased exercise more suitable to be included into the training routine [7].

The hamstring strain injury is one of the most problematic challenges practitioners have to cope with when conditioning running-based sports athletes, with injury incidence incrementing by 2.3% yearly [8] and constituting 24% of all injuries in men’s professional football [9]. Besides the multifactorial nature of injuries, some risk factors like low knee flexors eccentric strength [10], and short biceps femoris fascicles [11] have been acknowledged. Consequently, eccentric-biased exercise training has been proven effective in increasing eccentric strength and elongating fascicles, possibly reducing the risk of hamstring strain injury [12]. Among the eccentric-biased training modalities and exercises, Nordic hamstring exercise (NHE) gained popularity since prevalently stimulates the hamstring eccentrically [13], without the need for expensive tools and with the possibility to be easily performed in the field. Several studies have been hence conducted, which appear to confirm the effectiveness of NHE long-term training to increase hamstring strength and induce biceps femoris fascicle elongation, as recently reviewed [14,15]. This would set the stage for using NHE among practitioners.

Nevertheless, the main short-term negative side of NHE, i.e., muscle damage, represents a main barrier to systematic incorporation of NHE into the training routine [16–19]. Practitioners indeed perceive that the decrements in strength and the increments in muscle soreness would increase discomfort while sub-optimally performing the remaining routine for several days [17,19]. However, the literature to date does not help to incentivize the inclusion of NHE, since little information about muscle damage is available. While a study has reported that performing NHE after vs before the regular soccer training session might show further symptoms of fatigue [20], muscle damage after a session including but not limited to NHE was greater when performed in the middle than at the beginning of the weekly routine [21]. Given the scarcity of relevant scientific knowledge of the short-term effects of NHE and especially if these effects are attenuated when NHE is repeated in a subsequent session, the aim of the present study was to investigate muscle damage and repeated-bout effect after two separate sessions of NHE. For this purpose, a typical NHE session was used, so that the outcomes of the present study may provide practitioners with relevant knowledge about the recovery time course after the first and the following NHE session to properly incorporate it into the training routine. We hypothesized that the second NH bout would be followed by less muscle damage than the first NHE bout.

## MATERIAL AND METHODS

### Study design

The present investigation was conceived as a within-subject, repeated-measures, one-group study. To investigate muscle damage, the participants were tested at baseline and one (day-1), two (day-2), three (day-3), and four days (day-4) after the NHE session. To investigate the repeated-bout effect, the whole procedure was repeated after four weeks, in line with previous investigations [1,2]. Muscle damage markers were eccentric strength, passive ROM, muscle soreness, and muscle swelling [1,2,4,22]. Before the baseline assessments, two sessions were dedicated to familiarizing both the NHE exercise technique and the strength assessments. Figure 1 shows the study design. The sample size was calculated using statistical software (G-Power v.3.1.9.4, Stuttgart, Germany), referring to the loss in eccentric strength as the primary dependent variable [1]. Considering the study design, $\alpha$-error = 0.05, correlation among repeated measures = 0.5, sphericity correction = 1, Cohen's f effect size = 0.35, and a desired power (1-$\beta$) = 0.80, 11 subjects were needed. To prevent sub-standard decrements in power due to any possible drop-out, we recruited 13 participants.

Figure 1 here

**Participants**

Thirteen trained male adults [23] [age: 25.2(2.9) years; stature: 1.76(0.08) m; body mass: 77.5(11.2) kg] volunteered for the present investigation. For reasons unrelated to the procedures, one participant did not complete the second bout and was therefore deleted from the statistical analysis. The participants were healthy, without any orthopedical or muscular lower-limb disease or hamstring injury history in the previous year. Additionally, the systematic performance of hamstring eccentric-based training was used as exclusion criteria and the participants were required not to engage in strenuous physical activities for the entire duration of the current investigation. They were carefully instructed about the purposes and the possible risks deriving from the procedures and signed a written informed consent before being included. The procedures were in line with the Declaration of Helsinki (1964 and further updates) for studies involving human subjects [24] and were approved by the local Ethical Committee (CE84/23).

**NHE session**

The NHE session started with a standardized warm-up of 20 step-ups for each limb. The NHE session was based on the previous literature [25] to be replicable for practitioners, and 4 sets of 6 repetitions were then selected. The NHE technique was performed following optimal criteria provided in the literature [26]. The participants kneeled on a cushion that ended at the tibial tuberosity at a height > 15 cm, with hips and shoulders in a neutral position and the hands crossed in front of the chest [26], as shown in Figure 2. The ankles were fixed to an unmovable bar and the participants had to lower their body forward within a timeframe of approximately 5 s, and a metronome was used to pace the cadence. Feedback was provided to the participants to increase consistency. When the hips or shoulders were misaligned, the repetition was immediately stopped and repeated. The participants were provided with an external focus (i.e., “try not to fall forward”), with an inter-set rest of 4 min and an inter-repetition rest of 10 s [26,27]. To minimize the loading, they were instructed to push themselves back to the starting position with their arms once at the ending position [28]. The familiarization sessions occurred 10 and five days before the baseline assessment, during which the ROM was gradually increased, and the repetitions were initially assisted to let the participants experience the warranted body alignment and technique [26].

Figure 2 here

**Eccentric strength**

Eccentric strength was assessed while performing NHE using a load cell (Dynamo Plus, Vald Performance, Newstead, Australia) [29,30]with a sample frequency of 225 Hz, using the same NHE technique described above, as previously performed [28]. Briefly, the participants had their knees on a padded board with their ankles fixed by individual hooks secured immediately superior to the lateral malleolus by individual ankle braces, which were attached to the load cell with wireless data acquisition capabilities [28]. The ankle brace and load cell were secured to a pivot which allows the force generated by the knee flexors to be indirectly measured through the long axis of the load cells. Each assessment was preceded by a dynamic warm-up consisting of 20 step-ups for each limb. Two attempts were performed, and a third attempt was added when the first two differed more than 5%, and the highest value was inserted into the data analysis. The data refer to the dominant limb.

**Passive ROM**

Passive knee extension ROM was measured on the dominant limb using a manual goniometer with a sensitivity of 1° (Spinco, ON, Canada). This measurement was performed using the passive knee extension test while participants were in a supine position on a medical bed. The dominant hip was flexed to 90° and checked by a manual goniometer. The non-dominant limb was stabilized to minimize any compensatory movement during the measurement while the hip of the tested limb remained flexed at 90°. The operator ensured that the non-tested limb remained flat on the surface to prevent any pelvic tilt and maintained the hip alignment throughout the test. The participants were instructed to remain relaxed as much as possible and avoid any voluntary muscle contractions throughout the test. The operator extended the participant's knee by slowly lifting the lower leg until the participant verbally indicated the onset of discomfort. This point was defined as the moment when the participant first felt an uncomfortable stretch or tension in the hamstring muscles [31]. The examiner monitored for any signs of hip or pelvic rotation, ensuring that only the knee joint was moving. The axis of the goniometer was aligned with the lateral epicondyle of the femur, with one arm of the goniometer aligned along the midline of the thigh and the other arm aligned along the midline of the leg. The ROM was recorded as the angle formed between the long axis of the thigh and the leg. Three trials were performed for each participant, with the highest value used for analysis.

**Muscle soreness**

Muscle soreness was assessed through a visual analogue scale consisting of a 100 mm line with "no pain" at the left margin and "extremely painful" at the right margin [2]. The participants laid prone on a medical bed with lower limbs relaxed, and the operator applied a standardized pressure at 50% of the femur length and of the medial/lateral distance of the dominant limb. Particularly, one of the operators was trained to standardize the amount of pressure against a load cell (SN-1000N Interface, Crowthorne, UK) to 5.5 kg/cm$^2$, as extrapolated from previous literature [32], and practiced immediately after each pressure to the participants' muscles. After careful familiarization, the participants were asked to indicate their soreness marking the line, and then the distance from the left margin (0 mm) was measured and inserted into the data analysis.

**Muscle swelling**

Muscle swelling was identified through the overall anatomical cross-sectional area (ACSA) considering., biceps femoris, semitendinosus, and semimembranosus and assessed using a B-mode ultrasound (LOGIQ S7, GE Healthcare, Milwaukee, WI, US) with a linear transducer (mod. 9L, 3.1–10 MHz, 4-mm field-of-view) for image acquisition. The participants were positioned prone on a medical bed with lower limbs extended and relaxed to ensure consistent muscle length; their feet were placed just outside the bed frame. They were allowed to rest in this position for five minutes prior to imaging to allow body fluids to stabilize. Before imaging, femur length was measured from the greater trochanter to the lateral epicondyle of the femur identified through ultrasound. Two lines were drawn perpendicular to the long axis across the thigh at 50% and 30% of the femur length (0% = the femur lateral epicondyle) with a dermographic pencil [33]. This approach was adopted because different muscles within the hamstring group exhibit their peak cross-sectional area at different locations [34]. Then, an expert operator started to collect ultrasound imaging. A generous amount of water-based coupling gel was applied to the skin to enhance acoustic contact and reduce air interference. The probe was placed perpendicular to the long axis of the thigh at each location. The lateral boundaries of the region of interest extended from the borders of vastus lateralis, and the medial boundaries reached the borders of gracilis, following previous guidelines [35]. To this purpose, the device was set in extended-field-of-view (EFOV) mode. A continuous single scan was taken at each site along the drawn lines between the lateral and the medial hamstring boundaries. During the EFOV image acquisition, care was taken to avoid identifiable muscle compression and to keep the probe at the right angle by visual inspection. All EFOV acquisitions were repeated twice at each site. The contours of the ACSAs were digitized offline using ImageJ (Version1.46, National

Institutes of Health, Bethesda, MD, USA), as shown in Figure 3. The mean values obtained from the two scans at each location were used for subsequent analysis.

Figure 3 here

**Statistical analysis**

Statistical analysis was performed using statistical software (SPSS v. 29.0.2.0, IBM, Armonk, USA). The normality of data was checked using the Shapiro-Wilk test, and when a non-normal distribution was found, the analysis was performed using non-parametric tests. Intraclass correlation coefficient (ICC) with 95% confidence interval (95%CI) was calculated for each dependent variable and interpreted as follows: $\geq 0.9$ = excellent; $0.9 > \alpha \geq 0.8$ = good; $0.8 > \alpha \geq 0.7$ = acceptable; $0.7 > \alpha \geq 0.6$ = questionable; $0.6 > \alpha \geq 0.5$ = poor [36]. The variables examined were eccentric strength, passive ROM, muscle soreness, and ACSA. For normal distributions, the differences between the two bouts and the five days were calculated using a 2 (bout) x 5 (day) repeated-measures analysis of variance (ANOVA). For non-normal distributions, Friedman's and Wilcoxon's tests were used. Multiple pairwise comparisons were adjusted using Bonferroni's correction. Pairwise differences were presented with 95%CI and Cohen's d effect size was used to detect their magnitude and interpreted as follows: 0.00 to 0.19 = trivial; 0.20 to 0.59 = small; 0.60 to 1.19 = moderate; 1.20 to 1.99 = large; ≥ 2.00 = very large [37]. Data were presented as mean(SD) and significance was set at $\alpha<0.05$.

## RESULTS

ICC was excellent for the eccentric strength (ICC=0.905, 95%CI 0.705 to 0.972), passive ROM (ICC=0.976, 95%CI 0.915 to 0.993, muscle soreness (ICC=0.953, 95%CI 0.855 to 0.986), ACSA at 50% (ICC=0.993, 95%CI 0.976 to 0.998) and ACSA at 30% of the femur length ICC=0.998, 95%CI 0.992 to 0.999).

Figure 4 shows the time course of the eccentric strength loss as % of the baseline. The baseline value for eccentric strength was 425(38) N for the first and 423(43) N for the second bout. A bout x day interaction was found (p=0.011, $F_{4,44}$=6.712, $\eta_p^2$ = 0.770), with a main effect for factor bout (p<0.001, $F_{1,11}$=38.141, $\eta_p^2$ = 0.776) and day (p<0.001, F=20.587, $\eta_p^2$ = 0.911). After the first bout, eccentric strength was reduced at day-1 (-97 N, 95%CI -145 to -48, p<0.001, ES= -1.95), day-2 (-103, -146 to -62, p<0.001, ES= -2.28), day-3 (-93 N, -132 to -54, p<0.001, ES= 2.23) and returned to baseline at day-4 (p>0.05). After the second bout, eccentric strength showed a strong trend towards reduction at day-1 (-56 N, -112 to +1, p=0.053, ES= -1.20), while no difference compared to baseline was observed at day-2 (p>0.05), day-3 (p>0.05) and day-4 (p>0.05). Comparing the two bouts, eccentric strength was greater during the second vs the first bout on day-1 (40 N, 2 to 77, p=0.041, ES= 0.69), day-2 (76 N, 39 to 112, p<0.001, ES= 1.32), day-3 (96 N, 60 to 132, p<0.001, ES= 1.95) and day-4 (75 N, 38 to 112, p<0.001, ES= 1.28).

Figure 4 here

Figure 4 shows the time course of passive ROM. The baseline value for passive ROM was 70.4(8.6)° for the first and 70.6(8.3)° for the second bout. A bout x day interaction was found (p=0.015, $F_{4,44}$=6.112, $\eta_p^2$ = 0.754), as well as a main effect for factor bout (p=0.002, $F_{1,11}$=16.869, $\eta_p^2$ = 0.605) and day (p<0.001, F=17.949, $\eta_p^2$ = 0.900). After the first bout, passive ROM was reduced at day-1 (-8.0°, -11.1 to -4.9, p<0.001, ES= -0.86), day-2 (-12.5°, -15.6 to -9.4, p<0.001, ES= -1.62), day-3 (-11.3°, -14.5 to 8.0, p<0.001, ES= -1.47) and day-4 (-5.1°, -7.8 to -2.4, p=0.002, ES= -0.64). After the second bout, passive ROM was reduced at day-1 (-6.8°, -9.5 to 4.0, p<0.001, ES= -0.77), day-2 (8.0°, 11.6 to 4.6, p<0.001, ES= -0.85), day-3 (-3.0°, -5.4 to -0.6, p= 0.019, ES= -0.34) and returned to baseline at day-4 (p>0.05). Comparing the two bouts, passive ROM was greater during the second vs the first bout on day-2 (4.6°, 1.0 to 8.1, p=0.016, ES= 0.53), day-3 (8.4°, 4.2 to 12.6, p=0.001, ES= 1.01) and day-4 (5.2°, 0.1 to 10.2, p=0.047, ES= 0.47).

Figure 4 shows the time course of muscle soreness. The distribution of the muscle soreness values was not normal in most of the cases, so the data were analyzed using non-parametric tests. The baseline value for muscle soreness was 3.0(2.5) A.U. for the first and 2.9(2.0) A.U. for the second bout. Friedman's test revealed a main effect of factor day for the first bout ($p<0.001$, $F_{4,13}=24.694$). After the first bout, muscle soreness was greater than baseline on day-2 ($p=0.000$, ES= 1.63) and day-3 ($p=0.010$, ES= 1.53). Friedman's test revealed a main effect of factor day for the second bout ($p<0.001$, $F_{4,12}=19.264$). However, Bonferroni's correction did not detect any differences between the baseline and the following days ($p>0.05$). Wilcoxon's test revealed that muscle soreness was greater comparing the first vs the second bout at day-2 ($p=0.006$, ES= 0.87), day-3 ($p=0.009$, ES=1.16) and day-4 ($p=0.005$, ES= 1.37).

Figure 5 shows the time course of ACSA at 50% and 30% of the femur length. The baseline value for ACSA at 50% of the thigh length was 39.0(3.8) $cm^2$ for the hamstrings combined at the first bout and 38.5(4.3) $cm^2$ at the second bout. No bout x day interaction was found ($p=0.544$, $F_{4,44}=0.835$, $\eta_p^2$ = 0.323), and no main effect for factor bout ($p=0.734$, $F_{4,44}=0.123$, $\eta_p^2 = 0.012$) and day ($p=0.51$, $F_{1,11}=4.127$, $\eta_p^2 = 0.702$) was observed. Pairwise comparisons showed no difference compared to baseline after each bout and between the bouts on the same relative day ($p>0.05$). The baseline value for ACSA at 30% of the thigh length was 33.3(5.7) $cm^2$ for the hamstrings combined at the first bout and 33.9(4.9) $cm^2$ at the second bout. No bout x day interaction was found ($p=0.216$, $F_{4,44}=2.120$, $\eta_p^2$ = 0.629), and no main effect for factor bout ($p=0.908$, $F_{4,44}=0.014$, $\eta_p^2 = 0.002$) and day ($p=0.217$, $F_{1,11}=2.108$, $\eta_p^2 = 0.628$) was observed. Pairwise comparisons showed no difference compared to baseline after each bout and between the bouts on the same relative day ($p>0.05$).

Figure 5 here

**DISCUSSION**

We investigated for the first time the effects of a typical NHE session on muscle damage markers and the following repeated-bout effect. After the first bout, hamstring eccentric strength decreased up to day-3 and returned to baseline after four days. Concomitantly, passive ROM decreased and did not recover within four days, while muscle soreness was higher than baseline on day-2 and day-3. In contrast, overall hamstring ACSA remained unchanged. After the second bout, hamstring eccentric strength recovered after two days and passive ROM after three days, while muscle soreness did not increase at all. Noteworthy, the changes in hamstring strength and passive ROM were by a smaller magnitude compared to the first bout. The current outcomes highlighted that NHE may initially impair hamstring strength and passive with concomitant increased sensation of pain, with no visible change in hamstring swelling. However, the second bout comes with a much faster recovery, giving the possibility to include NHE in the training routine and reducing its interference with other training drills.

Some preliminary considerations would be helpful to contextualize the results and better understand the derived practical implications. The literature has well established that the first eccentric-biased session results in muscle damage, showing that the recovery from the first bout usually takes some days [5]. It should be also recalled that the muscle damage markers do not show identical time course and sensitivity [5], thus interpreting muscle damage through different markers allows a more comprehensive description of the phenomenon. Furthermore, many independent variables accompany any resistance training exercise [27], and the magnitude of the muscle damage is known to be dependent on some of them, such as the total number of repetitions [38], the external load or force exerted [39], the movement velocity [40] or the muscle length at which the exercise is performed [41]. Additionally, the magnitude of muscle damage depends also on characteristics of the population involved such as the resistance training experience [42]. Consequently, the present results must be interpreted considering that changing one or more factors may result in different muscle damage expressions, in terms of overall or specific marker extent and time course.

Hamstring eccentric strength was impaired up to day-3 and was recovered on day-4. Strength loss may depend on both neural, e.g., decreased neural drive and/or motoneuron firing rate, and peripheral mechanisms, e.g., disruption in the excitation-contraction coupling, altered intra-cellular setting and/or damaged sarcolemma and contractile proteins [43]. In contrast, passive ROM did not recover within the four days, implying that the hamstring elongation capacity should not be further stressed within such a time course. The reduction in passive ROM is likely due to edema localized in the myotendinous junction that may take more days to be fully absorbed, as well as to the damage induced in the connective tissue and the extra-cellular matrix [44]. In line, studies on different muscle groups observed that passive ROM is still reduced after four days [40], corroborating that caution is needed when including resistance exercises or specific drills that require strong hamstring elongations within this time interval. Muscle soreness was greater two and three days after the first NHE bout. The exact insurgence of muscle soreness after an eccentric-biased session is not fully understood, albeit different neurochemical mechanisms have been proposed, among which an increased nociceptors and mechanoceptors activity, or an augmented excitation of the type-III and -IV afferent fibers [45]. Although muscle soreness is easy to assess and can be effortlessly included in non-laboratory contexts, it may not be strictly associated with the magnitude of muscle damage [46]. Practitioners should be reminded that the information retrieved from the soreness perceived might not match with the actual muscle damage after NHE bouts and further markers are warranted. Should not this be feasible, caution is needed when interpreting the recovery status also considering the possible variability in perceived soreness. Alternatively, practitioners should be educated that muscle soreness is part of the adaptation process and should not be considered as a barrier. No symptom of systemic muscle swelling was retrieved in the hamstring since no change in hamstring ACSA was observed. Interestingly, NHE has a knee-based motor pattern and was seen to recruit the medial more than the lateral hamstring, in contrast to hip-based exercises that in turn tend to stimulate hamstring more laterally [47,48]. Consequently, one may expect that semimembranosus and semitendinosus would be more sensitive to the NHE bout than biceps femoris, with possible repercussions on the hamstring ACSA. The lack of change may be accounted for by the NHE technical characteristics, the number of repetitions performed, the population involved, or even a combination of them. One further possibility is that the assessment was made centrally and distally, so we cannot exclude that different scanning sites would have shown changes in ACSA.

Another novel point of the present manuscript is the investigation of the repeated-bout effect following NHE. The second NHE bout elicited muscle damage markers by a smaller magnitude with much faster recovery, showing that muscle damage after the second NHE bout comes with the protection offered by the first bout. The mechanisms associated with the repeated-bout effect encompass neural and muscle-tendon adaptations, pro-inflammatory sensitivity, and a late remodeling of the extra-cellular matrix [6]. Particularly, neural adaptations include a possible shift in motor unit recruitment towards slow-twitch fibers, an increased $\alpha$-motoneuron excitability and inhibitory feedback; muscle-tendon adaptations include a possible reduced fascicle elongation and increased tendon compliance, together with a smaller displacement of the myotendinous junction; the inflammatory mechanism an augmented expression of the pro- vs anti-inflammatory cytokines to accelerate the inflammation process; the extra-cellular matrix remodeling starts with an initial deadhesion and with a late adhesion together with an increased collagen expression which seems to take some weeks to complete the process, while few days did not appear sufficient [6]. In addition, a possible restoration of the connective may help to reduce the mechanical stress, although not clearly proven [44]. The present design does not allow any mechanistic inference, thus speculations in this regard should be avoided. However, when referring to the two main barriers that discourage the incorporation of NHE into practice, i.e., strength loss and muscle soreness, this suggests that the interference with the remaining routine following the second NHE bout lasts much less after the second bout. The only major caution might regard the hamstring elongation capacity, and exercises and drills requiring full elongation should be considered carefully before passive ROM recovers.

Interestingly, the duration of the protection offered by the first eccentric-biased session spans from few days up to six months [49], and the present bout interval (four weeks) is in line with this temporal range. Furthermore, the repeated-bout effect may be present also after light muscle damage [38,50]. In this view, practitioners may consider using fewer repetitions during and/or smaller ROM to reduce the muscle damage time course after the first bout but still accelerate the recovery process after the second NHE bout. As such, the first NHE bout might be performed pre-season, while the subsequent bouts can also be scheduled in-season, with athletes who are supposed to progressively be accustomed to the short-term effects until their possible disappearance. Lastly, while NHE stimulates hamstrings overall, it places a focus on the medial muscles so that hip-based movements like razor-curl, Romanian or stiff-leg deadlift, or hip extensions should be also considered and possibly included into the training routine [47,48].

The present investigation comes with some acknowledged limitations. First, the current outcomes derive from the protocol used and the population involved, so different combinations may result otherwise. Second and strictly related, the NHE exercise technique used here provides distinct mechanical stimuli that may vary should the technique be different. This may be relevant especially in non-professional or amateur contexts, where the strength and conditioning staff might be absent or non-sufficiently numerous to check for individual NHE techniques. Last, we did not assess the optimum angle and we did not measure any blood protein activity, and this would have deepened the discussion. However, the markers used here provide enough description of the muscle damage and the repeated-bout effect. As a perspective, muscle damage and repeated-bout effect should be examined following different eccentric-biased hamstring exercises often used in the practice, e.g., razor-curl, Romanian or stiff-leg deadlift, or hip extensions, or even using accentuated eccentric external load such as flywheel technology in hip-dominant exercises [51,52]. This may be further helpful for practitioners.

In conclusion, the present study examined the muscle damage and repeated-bout effect following an NHE typical session for the first time. The first session is followed by strength loss, reduction in passive ROM, and muscle soreness, while no change in hamstring ACSA occurred. Following the second bout, all muscle damage markers showed lesser magnitude and faster recovery. It is concluded that a first NHE bout is followed by muscle damage but confers protection for future NHE bouts.

## REFERENCES


[1] Coratella G, Bertinato L. Isoload vs isokinetic eccentric exercise: a direct comparison of exercise-induced muscle damage and repeated bout effect. Sport Sci Health 2015; 11: 87–96. doi:10.1007/s11332-014-0213-x

[2] Coratella G, Chemello A, Schena F. Muscle damage and repeated bout effect induced by enhanced eccentric squats. Journal of Sports Medicine and Physical Fitness 2016; 56: 1540–1546

[3] Coratella G, Varesco G, Rozand V, et al. Downhill running increases markers of muscle damage and impairs the maximal voluntary force production as well as the late phase of the rate of voluntary force development. Eur J Appl Physiol 2024; doi:10.1007/s00421-023-05412-z

[4] Mavropalias G, Koeda T, Barley OR, et al. Comparison between high- and low-intensity eccentric cycling of equal mechanical work for muscle damage and the repeated bout effect. Eur J Appl Physiol 2020; 120: 1015–1025. doi:10.1007/s00421-020-04341-5

[5] Clarkson PM, Hubal MJ. Exercise-induced muscle damage in humans. American journal of physical medicine & rehabilitation / Association of Academic Physiatrists 2002; 81: S52-69. doi:10.1097/01.PHM.0000029772.45258.43

[6] Hyldahl RD, Chen TC, Nosaka K. Mechanisms and Mediators of the Skeletal Muscle Repeated Bout Effect. Exerc Sport Sci Rev 2017; 45: 24–33. doi:10.1249/JES.0000000000000095

[7] Isner-Horobeti M-E, Dufour SP, Vautravers P, et al. Eccentric exercise training: modalities, applications and perspectives. Sports Med 2013; 43: 483–512. doi:10.1007/s40279-013-0052-y

[8] Ekstrand J, Waldén M, Hägglund M. Hamstring injuries have increased by 4% annually in men's professional football, since 2001: a 13-year longitudinal analysis of the UEFA Elite Club injury study. Br J Sports Med 2016; 50: 731–737. doi:10.1136/bjsports-2015-095359

[9] Ekstrand J, Bengtsson H, Waldén M, et al. Hamstring injury rates have increased during recent seasons and now constitute 24% of all injuries in men's professional football: the UEFA Elite Club Injury Study from 2001/02 to 2021/22. Br J Sports Med 2023; 57: 292–298. doi:10.1136/bjsports-2021-105407

[10] Green B, Bourne MN, Pizzari T. Isokinetic strength assessment offers limited predictive validity for detecting risk of future hamstring strain in sport: a systematic review and meta-analysis. Br J Sports Med 2018; 52: 329–336. doi:10.1136/bjsports-2017-098101

[11] Timmins RG, Bourne MN, Shield AJ, et al. Short biceps femoris fascicles and eccentric knee flexor weakness increase the risk of hamstring injury in elite football (soccer): A prospective cohort study. Br J Sports Med 2016; 50: 1524–1535. doi:10.1136/bjsports-2015-095362

[12] Brughelli M, Cronin J. Altering the length-tension relationship with eccentric exercise : implications for performance and injury. Sports Med 2007; 37: 807–826

[13] Raiteri BJ, Beller R, Hahn D. Biceps Femoris Long Head Muscle Fascicles Actively Lengthen During the Nordic Hamstring Exercise. Front Sports Act Living 2021; 3: 1–10. doi:10.3389/fspor.2021.669813

[14] Muniz Medeiros D, Marchiori C, Manfredini Baroni B. Effect of Nordic Hamstring Exercise Training on Knee Flexors Eccentric Strength and Fascicle Length: A Systematic Review and Meta-Analysis. J Sport Rehabil 2021; 30: 482–491. doi:10.1123/JSR.2019-0388

[15] Gérard R, Gojon L, Decleve P, et al. The effects of eccentric training on biceps femoris architecture and strength: A systematic review with meta-analysis. J Athl Train 2020; 55: 501–514. doi:10.4085/1062-6050-194-19

[16] Van Der Horst N, Van De Hoef S, Van Otterloo P, et al. Effective But Not Adhered to: How Can We Improve Adherence to Evidence-Based Hamstring Injury Prevention in Amateur Football? Clinical Journal of Sport Medicine 2021; 31: 42–48. doi:10.1097/JSM.0000000000000710

[17] Chesterton P, Draper G, Portas M, et al. The Uptake of Nordic Hamstring Exercise Program for Injury Prevention in Major League Soccer and Its Barriers to Implementation in Practice. J Sport Rehabil 2022; 31: 576–581. doi:10.1123/jsr.2021-0262

[18] Bahr R, Thorborg K, Ekstrand J. Evidence-based hamstring injury prevention is not adopted by the majority of Champions League or Norwegian Premier League football teams: The Nordic Hamstring survey. Br J Sports Med 2015; 49: 1466–1471. doi:10.1136/bjsports-2015-094826

[19] Ekstrand J, Bengtsson H, Walden M, et al. Still poorly adopted in male professional football: but teams that used the Nordic Hamstring Exercise in team training had fewer hamstring injuries-a retrospective survey of 17 teams of the UEFA Elite Club Injury Study during the 2020-2021 season. BMJ Open Sport Exerc Med 2022; 8: 5–8. doi:10.1136/bmjsem-2022-001368

[20] Lovell R, Siegler JC, Knox M, et al. Acute neuromuscular and performance responses to Nordic hamstring exercises completed before or after football training. J Sports Sci 2016; 34: 2286–2294. doi:10.1080/02640414.2016.1191661

[21] Lovell R, Whalan M, Marshall PWM, et al. Scheduling of eccentric lower limb injury prevention exercises during the soccer micro-cycle: Which day of the week? Scand J Med Sci Sports 2018; 28: 2216–2225. doi:10.1111/sms.13226

[22] Chapman DW, Newton M, McGuigan M, et al. Effect of lengthening contraction velocity on muscle damage of the elbow flexors. Med Sci Sports Exerc 2008; 40: 926–933. doi:10.1249/MSS.0b013e318168c82d

[23] McKay AKA, Stellingwerff T, Smith ES, et al. Defining Training and Performance Caliber: A Participant Classification Framework. Int J Sports Physiol Perform 2022; 17: 317–331. doi:10.1123/ijspp.2021-0451

[24] Harriss DJ, Jones C, MacSween A. Ethical Standards in Sport and Exercise Science Research: 2022 Update. Int J Sports Med 2022; 43: 1065–1070. doi:10.1055/a-1957-2356

[25] Alt T, Severin J, Schmidt M. Quo Vadis Nordic Hamstring Exercise-Related Research?—A Scoping Review Revealing the Need for Improved Methodology and Reporting. Int J Environ Res Public Health 2022; 19: 11225. doi:10.3390/ijerph191811225

[26] Alt T, Schmidt M. The ANHEQ Evaluation Criteria: Introducing Reliable Rating Scales for Assessing Nordic Hamstring Exercise Quality. Sports Med Open 2021; 7: 91. doi:10.1186/s40798-021-00383-x

[27] Coratella G. Appropriate reporting of exercise variables in resistance training protocols: much more than load and number of repetitions. Sports Med Open 2022; 8: 99. doi:10.1186/s40798-022-00492-1

[28] Pincheira PA, Boswell MA, Franchi M V., et al. Biceps femoris long head sarcomere and fascicle length adaptations after 3 weeks of eccentric exercise training. J Sport Health Sci 2021; doi:10.1016/j.jshs.2021.09.002

[29] González-Rosalén J, Benítez-Martínez JC, Medina-Mirapeix F, et al. Intra- and Inter-Rater Reliability of Strength Measurements Using a Pull Hand-Held Dynamometer Fixed to the Examiner's Body and Comparison with Push Dynamometry. Diagnostics 2021; 11: 1230. doi:10.3390/diagnostics11071230
[30] Thotawatta I, Azevedo A, Mayes S, et al. Reliability testing of foot and ankle strength specific to Ballet using the ForceFrame and DynaMo. J Sci Med Sport 2023; 26: S134. doi:10.1016/j.jsams.2023.08.009
[31] Brusco CM, Blazevich AJ, Radaelli R, et al. The effects of flexibility training on exercise-induced muscle damage in young men with limited hamstrings flexibility. Scand J Med Sci Sports 2018; 28: 1671–1680. doi:10.1111/sms.13068
[32] Fischer AA. Pressure algometry over normal muscles. Standard values, validity and reproducibility of pressure threshold. Pain 1987; 30: 115–126. doi:10.1016/0304-3959(87)90089-3
[33] Franchi M V., Fitze DP, Hanimann J, et al. Panoramic ultrasound vs. MRI for the assessment of hamstrings cross-sectional area and volume in a large athletic cohort. Sci Rep 2020; 10: 1–10. doi:10.1038/s41598-020-71123-6
[34] Franchi M V., Fitze DP, Hanimann J, et al. Panoramic ultrasound vs. MRI for the assessment of hamstrings cross-sectional area and volume in a large athletic cohort. Sci Rep 2020; 10: 1–10. doi:10.1038/s41598-020-71123-6
[35] Balius R, Pedret C, Iriarte I, et al. Sonographic landmarks in hamstring muscles. Skeletal Radiol 2019; 48: 1675–1683. doi:10.1007/s00256-019-03208-x
[36] Tavakol M, Dennick R. Making sense of Cronbach's alpha. Int J Med Educ 2011; 2: 53–55. doi:10.5116/ijme.4dfb.8dfd
[37] Hopkins WG, Marshall SW, Batterham AM, et al. Progressive Statistics for Studies in Sports Medicine and Exercise Science. Med Sci Sports Exerc 2009; 41: 3–13. doi:10.1249/MSS.0b013e31818cb278
[38] Chen TC, Nosaka K. Effects of number of eccentric muscle actions on first and second bouts of eccentric exercise of the elbow flexors. J Sci Med Sport 2006; 9: 57–66. doi:10.1016/j.jsams.2006.03.012
[39] Chen TC, Nosaka K, Sacco P. Intensity of eccentric exercise, shift of optimum angle, and the magnitude of repeated-bout effect. J Appl Physiol (1985) 2007; 102: 992–999. doi:10.1152/japplphysiol.00425.2006
[40] Chapman D, Newton M, Sacco P, et al. Greater muscle damage induced by fast versus slow velocity eccentric exercise. Int J Sports Med 2006; 27: 591–598. doi:10.1055/s-2005-865920
[41] Child RB, Saxton JM, Donnelly a E. Comparison of eccentric knee extensor muscle actions at two muscle lengths on indices of damage and angle-specific force production in humans. J Sports Sci 1998; 16: 301–308. doi:10.1080/02640419808559358
[42] Newton MJ, Morgan GT, Sacco P, et al. Comparison of Responses to Strenous Eccentric Exercise of the Elbow Flexors between Resistance Trained and Untrained men. J Strength Cond Res 2008; 22: 597–607
[43] Hubal MJ, Rubinstein SR, Clarkson PM. Mechanisms of variability in strength loss after muscle-lengthening actions. Med Sci Sports Exerc 2007; 39: 461–468. doi:10.1249/01.mss.0000247007.19127.da
[44] Brusco CM, Blazevich AJ, Radaelli R, et al. The effects of flexibility training on exercise-induced muscle damage in young men with limited hamstrings flexibility. Scand J Med Sci Sports 2018; 28: 1671–1680. doi:10.1111/sms.13068
[45] Mizumura K, Taguchi T. Neurochemical mechanism of muscular pain: Insight from the study on delayed onset muscle soreness. J Physiol Sci 2024; 74: 4. doi:10.1186/s12576-023-00896-y

[46] Nosaka K, Newton M, Sacco P. Delayed-onset muscle soreness does not reflect the magnitude of eccentric exercise-induced muscle damage. Scand J Med Sci Sports 2002; 12: 337–346
[47] Bourne MN, Williams MD, Opar DA, et al. Impact of exercise selection on hamstring muscle activation. Br J Sports Med 2017; 51: 1021–1028. doi:10.1136/bjsports-2015-095739
[48] Hegyi A, Péter A, Finni T, et al. Region-dependent hamstrings activity in Nordic hamstring exercise and stiff-leg deadlift defined with high-density electromyography. Scand J Med Sci Sports 2018; 28: 992–1000. doi:10.1111/sms.13016
[49] Nosaka K, Sakamoto KEI, Newton M, et al. How long does the protective effect on eccentric exercise induced muscle damage last? Med Sci Sports Exerc 2001; 33: 1490–1495
[50] Lavender AP, Nosaka K. A light load eccentric exercise confers protection against a subsequent bout of more demanding eccentric exercise. Journal of science and medicine in sport / Sports Medicine Australia 2008; 11: 291–298. doi:10.1016/j.jsams.2007.03.005
[51] Cosio PL, Moreno-Simonet L, Mechó S, et al. Individual and region-specific hamstring muscles use during a novel Flywheel Russian belt Deadlift exercise. Apunts Sports Medicine 2024; 59: 100454. doi:10.1016/j.apunsm.2024.100454
[52] Beato M, Keijzer KL De, Fleming A, et al. Post flywheel squat vs . flywheel deadlift potentiation of lower limb isokinetic peak torques in male athletes. Sports Biomech 2020; 00: 1–15. doi:10.1080/14763141.2020.1810750

## FIGURE CAPTION

**Figure 1:** The study design is depicted

**Figure 2:** The Nordic hamstring technique is shown

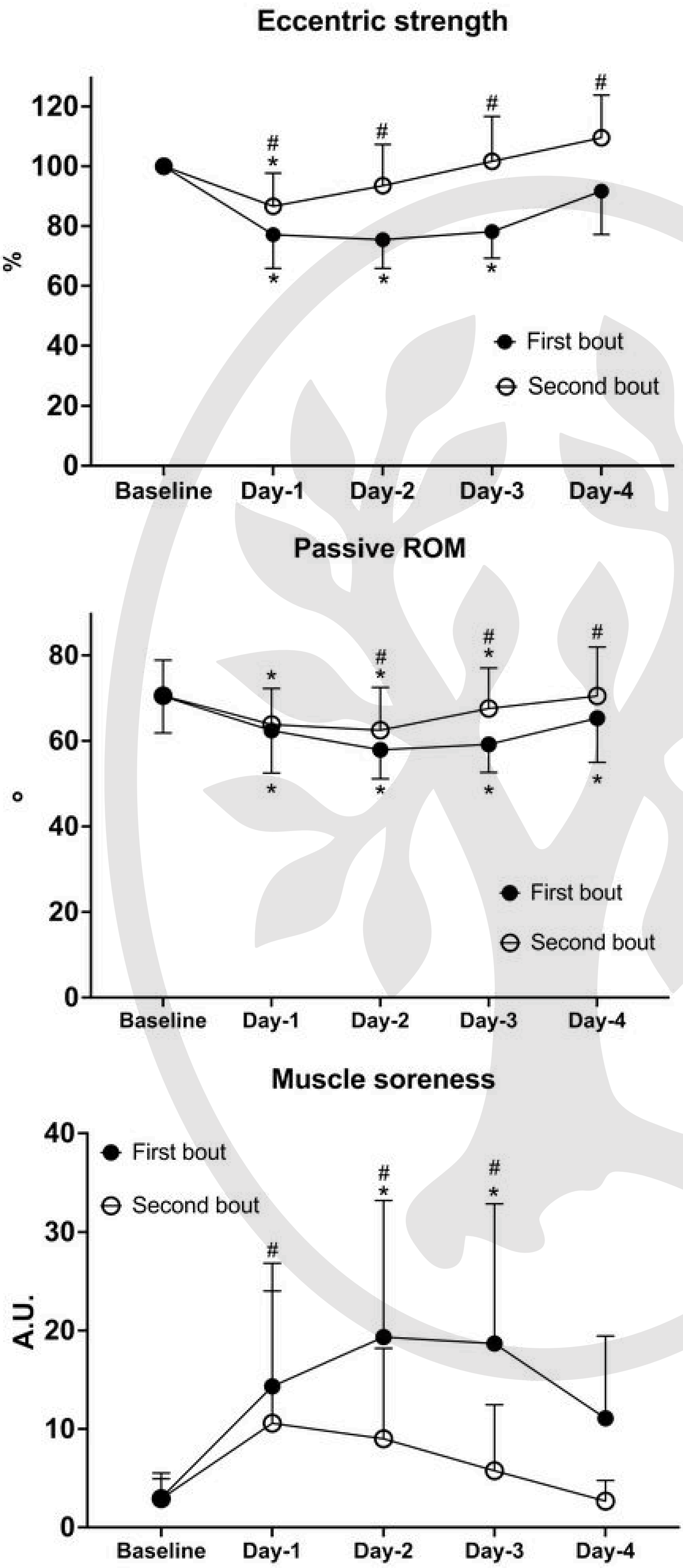
**Figure 3:** The ultrasound imaging to contour the overall hamstring anatomical cross-sectional area. The image is from a representative participant at 50% femur length.

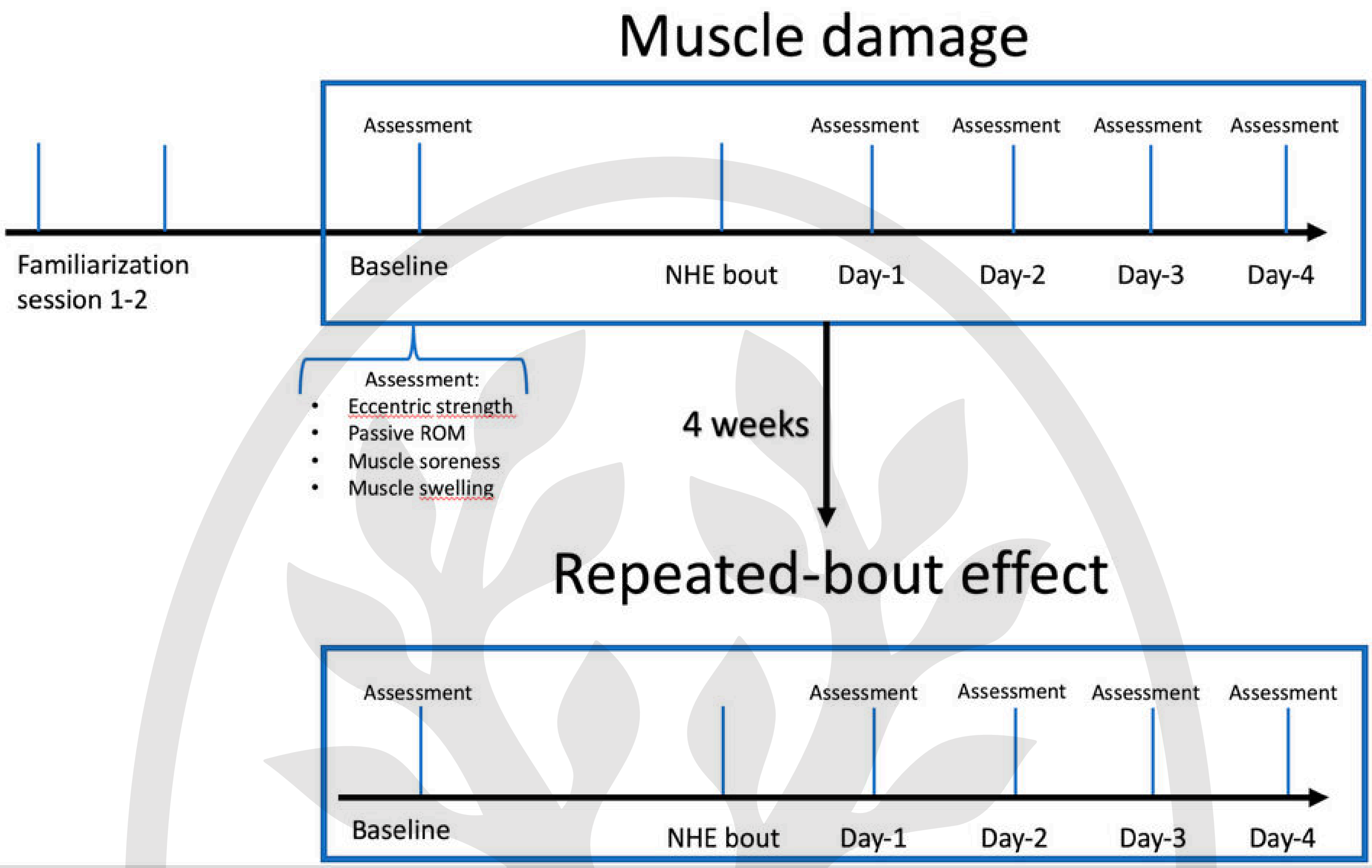
**Figure 4**: The time course of the hamstring eccentric strength, passive range of motion (ROM) and muscle soreness is shown. A.U.: arbitrary unit.

*p <0.05 different from baseline

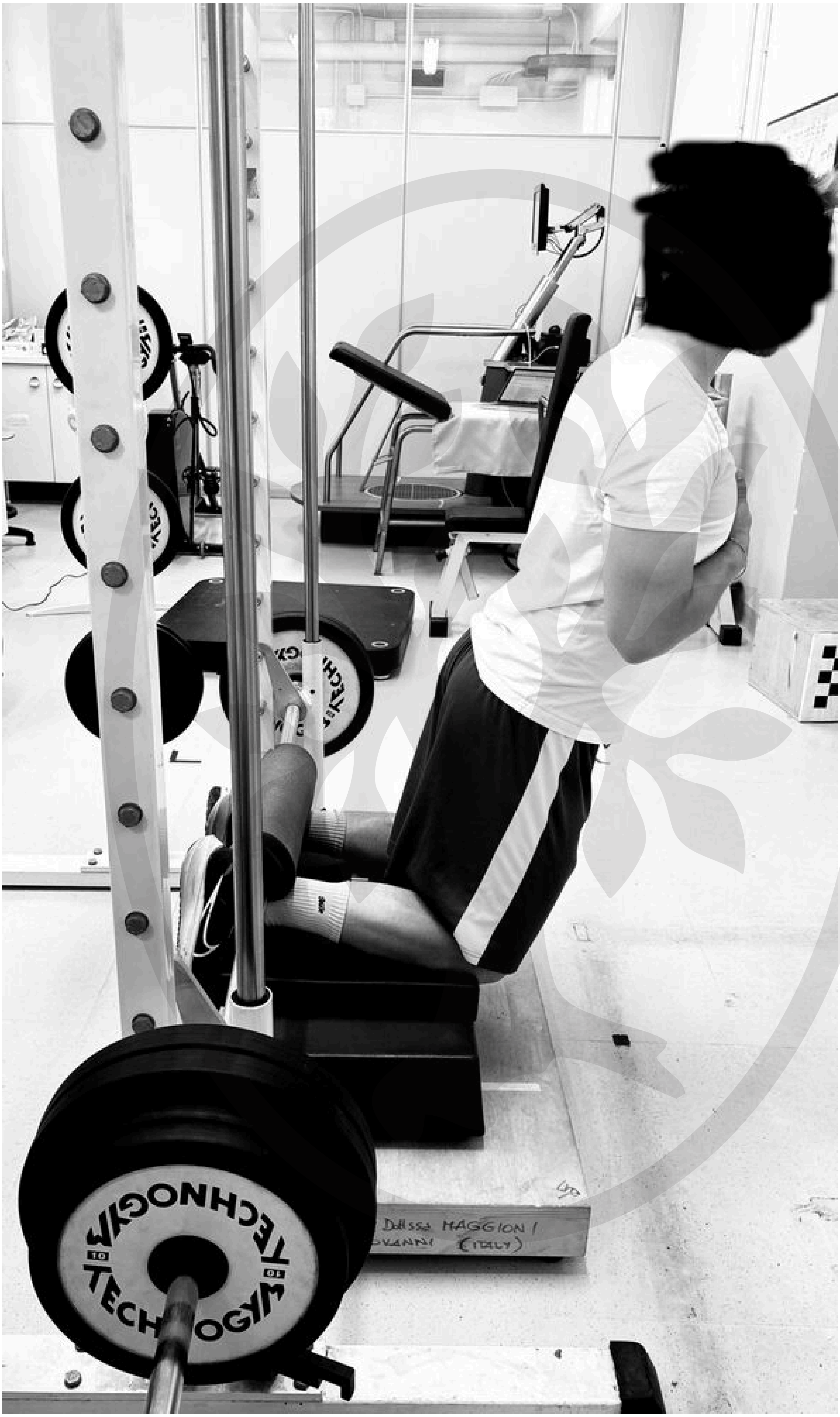
# p<0.05 between-bout difference

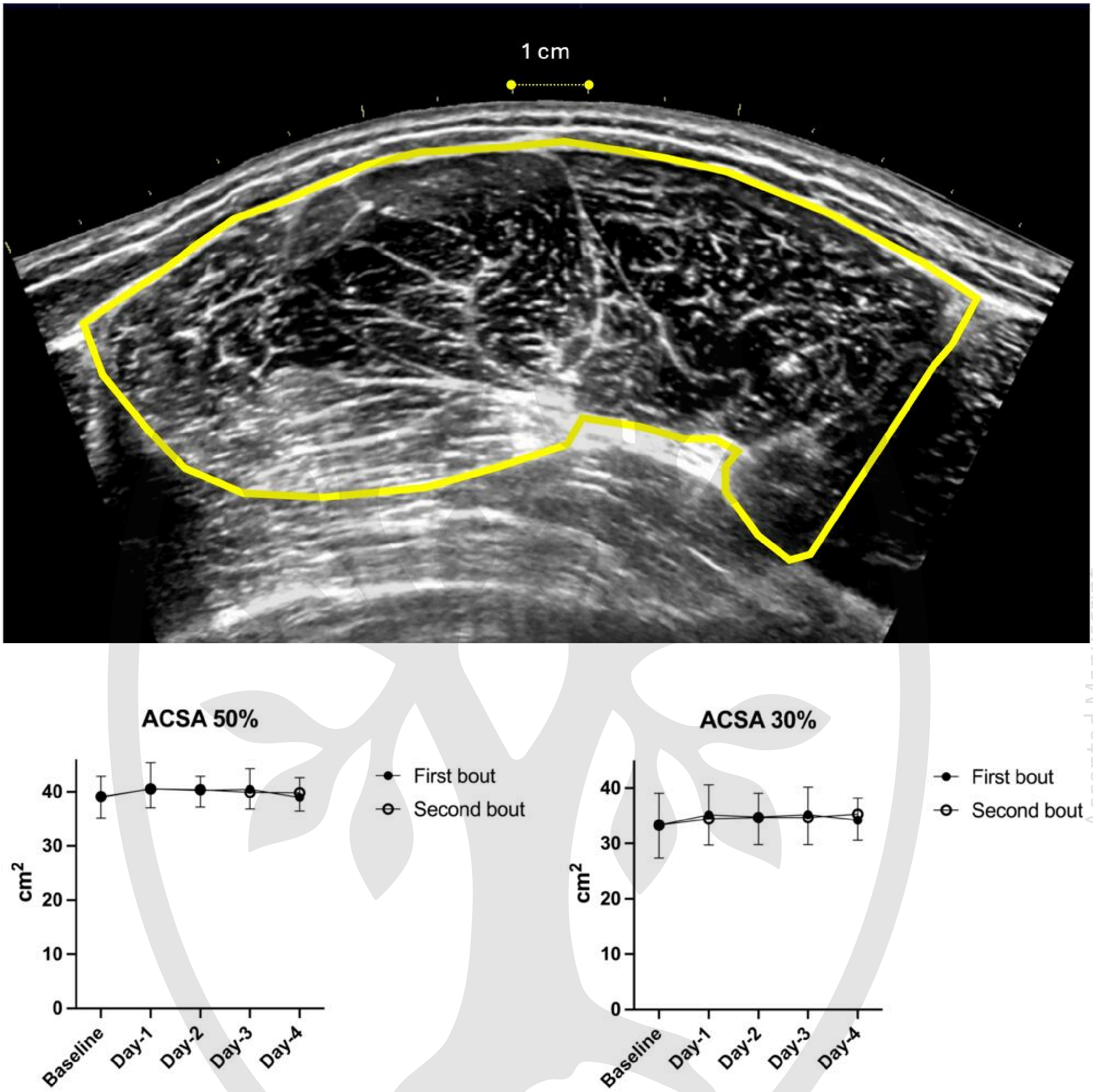
**Figure 5**: The time course of the hamstring anatomic cross-sectional area assessed at 50% (ACSA 50%) and 30% of the participants’ femur length (ACSA 30%) is shown.


Eccentric strength
%
120
100
80
60
40
20
0
Baseline
Day-1
Day-2
Day-3
Day-4
First bout
Second bout
Passive ROM
°
80
60
40
20
0
Baseline
Day-1
Day-2
Day-3
Day-4
First bout
Second bout
Muscle soreness
A.U.
40
30
20
10
0
Baseline
Day-1
Day-2
Day-3
Day-4
First bout
Second bout



Muscle damage
Assessment
Assessment
Assessment
Assessment
Assessment
Familiarization session 1-2
Baseline
NHE bout
Day-1
Day-2
Day-3
Day-4
Assessment:
• Eccentric strength
• Passive ROM
• Muscle soreness
• Muscle swelling
4 weeks
Repeated-bout effect
Assessment
Assessment
Assessment
Assessment
Assessment
Baseline
NHE bout
Day-1
Day-2
Day-3
Day-4


TECHNOGYM


1 cm
ACSA 50%
First bout
Second bout
cm2
0
10
20
30
40
Baseline
Day-1
Day-2
Day-3
Day-4
ACSA 30%
First bout
Second bout
cm2
0
10
20
30
40
Baseline
Day-1
Day-2
Day-3
Day-4