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EFFECTS OF INTER-SET STRETCHING ON ACUTE FATIGUE-RELATED CHANGES IN VASTUS LATERALIS MUSCLE ARCHITECTURE AND KNEE EXTENSOR TORQUE

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ABSTRACT

Background: Muscle fatigue, characterized by a reduction in force or torque production during resistance training, is often associated with changes in muscle architecture, such as fascicle length and pennation angle, which influence torque-generating capacity. Inter-set static stretching has been proposed as a time-efficient method to modulate these parameters, but its acute effects on muscle fatigue and architecture remain unclear.

Objective: This study aimed to investigate the impact of inter-set stretching on fatigue-induced changes in fascicle length, pennation angle, and knee extension torque.

Methods: Fifteen men performed 4 × 10 maximal isokinetic knee extensions. One limb underwent 30-second inter-set stretching, while the other limb recovered passively. Vastus lateralis muscle architecture (pennation angle and fascicle length; proximal and distal regions) and knee extensor torque (peak torque and peak torque angle; concentric and eccentric contractions) were measured and compared between protocols.

Results: Inferential tests revealed no significant differences between protocols for torque or muscle architecture. However, effect size analysis indicated that the inter-set stretching protocol increased fascicle length in the distal [1.64 (0.8; 2.2)] and proximal [0.52 (-0.0; 1.0)] regions and reduced concentric peak torque fatigue [0.86 (0.2; 1.3)]. Conversely, the passive rest protocol showed a greater proximal pennation angle [-0.81 (-1.4; -0.1)].

Conclusion: Short-duration inter-set stretching can attenuate fatigue induced by resistance exercise, with muscle architecture changes associated with these responses. Clinically, incorporating a few sets of inter-set stretching lasting 30 seconds may provide practical benefits.

KEYWORDS

Ultrasound, knee joint, resistance exercise, static stretching, fatigue

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INTRODUCTION

Muscle fatigue, defined as a decline in maximal force or power output due to sustained contractions[1], is a common consequence of resistance exercise and results from central and peripheral mechanisms[2,3]. For instance, different knee extension exercise modalities can elicit fatigue, leading to functional impairments and structural changes in the quadriceps, particularly in the vastus lateralis muscle[4]. These changes include adaptations in muscle architecture, such as fascicle length (FL) and pennation angle (PA), which are key determinants of force transmission and torque-generating capacity[5,6]. Shortened fascicles and increased PAs, typically observed with fatigue, contribute to reduced torque output by shifting the force-length curve to suboptimal muscle lengths[7]. Conversely, longer and less pennate fascicles decrease relative shortening velocity during joint rotation, enhancing fascicle force output[8].

Longer static stretching has been associated with increased FL and decreased PA[9,10]. However, these structural changes are often accompanied by reductions in maximal voluntary contraction due to decreased motoneuron excitability[11]. Additionally, time constraints are a significant barrier to regular exercise participation[12], and incorporating static stretching into resistance training sessions can further extend their duration. Acute static stretching has also been shown to induce a rightward shift in the torque-angle curve, resulting in strength gains on the descending limb after stretching exercises[13]. However, the mechanisms underlying the effects of short-duration stretching on muscle performance remain unclear[11].

Inter-set stretching is a time-efficient alternative that involves dividing longer stretches into shorter stretches between resistance exercise sets. This method has shown potential to improve muscle adaptations and function[14], including increases in strength[15,16], hypertrophy[16,17], and flexibility[15,18], or at least no impairments in these parameters[17-19]. Despite its potential, the effects of inter-set stretching on muscle fatigue during resistance training remain unclear, with conflicting findings regarding its influence on torque production and muscle function[20-25]. Importantly, no studies have examined the acute effects of inter-set stretching on muscle architecture.

Research on inter-set stretching has primarily focused on its effects on flexibility or chronic adaptations, with limited attention to its immediate impact on fatigue and muscle architecture. Understanding changes in FL and PA during inter-set stretching could provide valuable insights into the mechanisms of fatigue and its functional implications. Additionally, investigating these architectural responses may help clarify how stretching influences torque generation during subsequent sets, particularly the angle at which peak torque is produced[13,26].

This study aims to determine whether inter-set static stretching can modify (i) FL and PA in the vastus lateralis, and (ii) maximal knee extension torque and the angle at which it occurs. Using panoramic ultrasound and an isokinetic dynamometer, we assessed these variables across proximal and distal regions of the muscle, acknowledging that fascicles in different areas may respond differently[27]. We hypothesize that short-duration inter-set stretching will increase FL, decrease PA, and mitigate the decline in peak torque during exercise, potentially shifting the peak torque angle toward longer muscle lengths.

MATERIALS AND METHODS

Experimental approach to the problem

Participants attended three sessions separated by 48-hour intervals. In the first session, they were familiarized with the knee extension protocol using an isokinetic dynamometer. During the second and third sessions, each leg was randomly assigned to the inter-set stretching or passive rest protocol, counterbalancing leg dominance. Participants performed maximal knee extensions with the assigned protocol during inter-set rest periods, and ultrasound images of the vastus lateralis were captured before (preUS) and after (postUS) each protocol.

Subjects

Fifteen physically active men (age: 30.8 ± 5.0 years; height: 177.4 ± 7.9 cm; body mass: 80.5 ± 11.2 kg) engaged in lower-limb exercises at least three times per week for over a year and reported no injuries or pain. They abstained from exercise, stimulants, and alcohol 48 hours before testing. Informed consent was obtained, and the study was approved by the Ethics and Research Council of the Institution (registration number: 92424818.6.0000.5257) following the latest Declaration of Helsinki.

Isokinetic exercise

Maximal knee extensions were performed using a Biodex 4 System Pro isokinetic dynamometer (Biodex Medical Systems Inc., USA). After a warm-up (five repetitions at 180°/s), participants completed four sets of 10 maximal concentric/eccentric knee extensions at 60°/s, with a 90-second rest period between sets and range of motion set from 20° to 100° of knee flexion (adapted from similar studies; 23,25,28). The passive rest protocol involved participants seated with thighs relaxed and knees flexed at 80°. In the inter-set stretching protocol, participants were placed prone, and an evaluator performed a 30-second quadriceps stretch to the point of maximum possible knee flexion. The first and last 30 seconds of each interval were used for repositioning. Participants were encouraged to exert maximum effort and complete the full range of motion during exercises. Maximum concentric (PTconc) and eccentric (PTecc) peak torque and the angles at which they occurred (PTAconc, PTAecc) were recorded. Normalized differences (% variation) between sets (S2, S3, S4) were computed for analysis.

Ultrasound image acquisition

The vastus lateralis was assessed using an ultrasound

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Logic-e GE (40 mm transducer; 10 MHz frequency) in extended field-of-view (EFOV) mode, based on a previous study[29]. Proximal and distal regions were imaged immediately before and after the protocols.

Anatomical landmarks were marked to define regions of interest *(Fig. 1)*. Participants lay on a stretcher with their knee and hip fully extended and the hip slightly internally rotated. A reference line (line a) was drawn from the midpoint between the patella and the femur's lateral epicondyle to the femur's greater trochanter. Using this reference line, the intersections with the proximal and distal myotendinous junctions of the vastus lateralis muscle were marked on the skin (muscle length; crosses b). The distance between these junctions was measured, and tape markers were placed at 20%, 50%, and 80% of this length (dotted lines c, d, and e). The ultrasound probe was aligned longitudinally with the orientation of the fascicle (angles f). Panoramic images were acquired from 80% to 50%, and 50% to 20% of the muscle length.

The probe was moved longitudinally from the distal to the proximal region of the muscle with minimal pressure applied to the skin, and transmission gel was used to enhance acoustic coupling (Ultrex gel, Farmativa Industry and Trade Ltd., Brazil). Two high-quality preUS images from each muscle region were selected for analysis. The FL was measured as the length of the entire visible fascicle, and the PA was determined as the angle between the deep aponeurosis and the fascicle. Averages were computed from two fascicles and two images for each region, and all analyses were conducted blindly using ImageJ software (National Institutes of Health, USA, v.1.52).

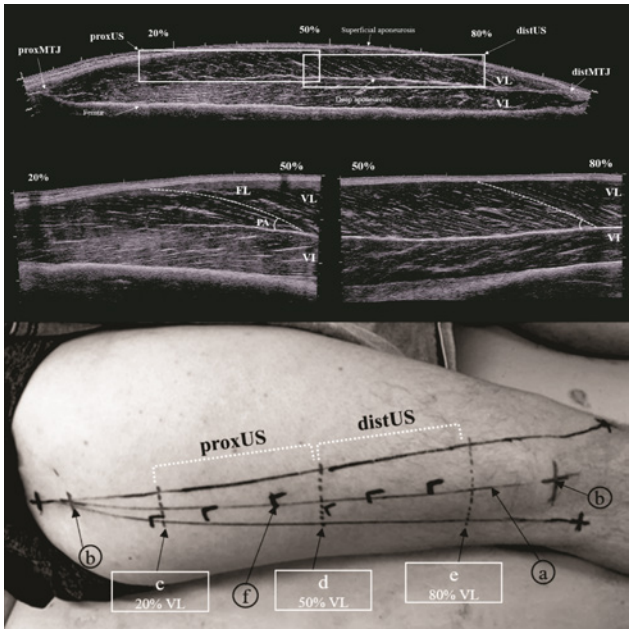


Figure 1. *Acquisition of ultrasound images. Top. Extended field-of-view images of the vastus lateralis. In top, EFOV image showing the entire muscle, used to identify and delimit the proximal (proxUS) and distal (distUS) regions; ProxUS (bottom left) and distUS (bottom right): images with their respective distances acquired to measure fascicle length (FL) and pennation angle (PA). 20, 50, and 80%: muscle lengths delimiting the regions, prox and dist, MTJ: proximal and distal myotendinous junctions, VL: vastus lateralis, VI: vastus intermedius. Bottom. Region of interest on participant's thigh. A) reference line; B) myotendinous junctions of the vastus lateralis; C-E) muscle lengths identified with measuring tape (white dotted lines); f: perpendicular angles identifying fascicle orientation with the ultrasound probe; prox and distUS: regions marked for proximal and distal ultrasound imaging.*

Statistical analysis

Parametric tests were conducted after confirming data normality. Two-way ANOVA evaluated PT and PTA between protocols for concentric and eccentric contractions, with Tukey's HSD test for post hoc comparisons. Paired t-tests compared muscle architecture and torque variations between sets 1 and 4 for each protocol. Statistical analyses were performed using StatSoft Statistica v10 software, with significance at $p \leq 0.05$. Effect sizes were calculated following Cohen's[30] and interpreted based on Sawilowsky's[31] guidelines: d = 0.01 (very small), d = 0.2 (small), d = 0.5 (medium), d = 0.8 (large), d = 1.2 (very large), and d = 2.0 (huge). This approach complements p-value-based inference, particularly in studies where practical relevance is a key consideration.

The reliability of FL and PA measurements for the proximal and distal vastus lateralis regions was evaluated using the intraclass correlation coefficient (ICC) and coefficient of variation (CoV), and interpreted based on Cicchetti's[32]. Two images from the same day were analyzed. The same experienced evaluator acquired all ultrasound images.

RESULTS

Reliability results

Muscle architecture variables indicated excellent reliability. The ICCs were 0.923 ($p < 0.001$) for the proximal PA, 0.896 ($p < 0.001$) for the distal PA, 0.963 ($p < 0.001$) for the proximal FL, and 0.945 ($p < 0.001$) for the distal FL. The average CoV values were 0.04 (± 0.03) for the proximal PA, 0.04 (± 0.02) for the distal PA, 0.04 (± 0.04) for the proximal FL, and 0.03 (± 0.02) for the distal FL.

Torque

No significant differences between protocols were observed for PT or PTA *(Fig. 2)*. For PTconc, variations between S2-S3 and S2-S4 were significant in both protocols ($p \leq 0.001$). For PTecc, significant differences were found between S2-S3 and S2-S4 in the passive rest protocol, and between S2-S4 in the inter-set stretching protocol ($p \leq 0.001$ for all). Absolute values of PT and PTA differed between sets 1 and 4 for both protocols *(Table 1)*.

Muscle architecture

No significant differences in muscle architecture were found between protocols ($p > 0.06$ for all; *Fig. 3*). Mean FL variations were: proximal passive rest (2.14% ± 10.03), proximal inter-set stretching (8.39% ± 13.62), distal passive

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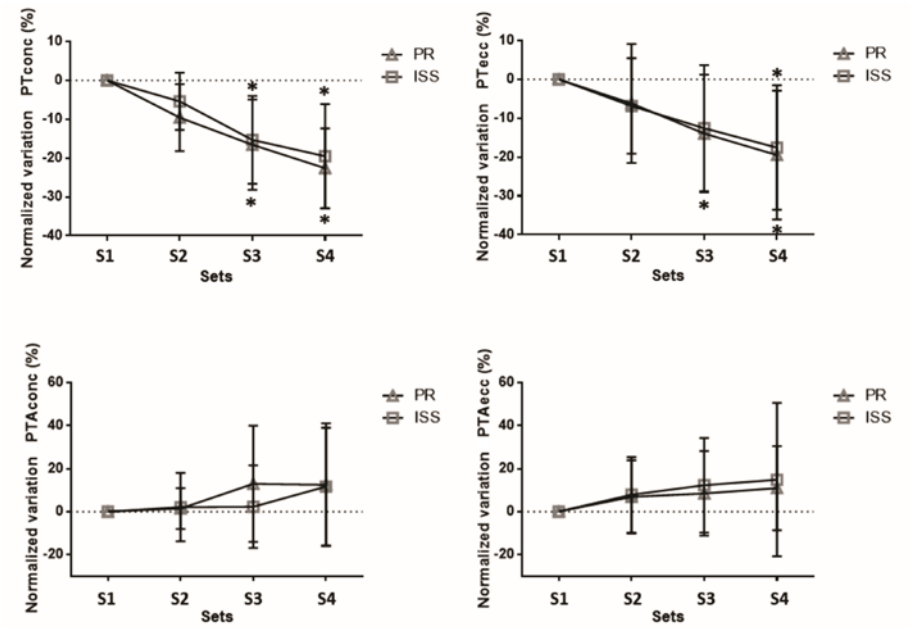


*Figure 2. Normalized variation (%) in concentric and eccentric peak torque and peak torque angle between the passive rest and inter-set stretching protocols. PT, peak torque; PTA, peak torque angle; PR, passive rest protocol; ISS, inter-set stretching protocol; conc, concentric contraction; ecc, eccentric contraction; S1, S2, S3 and S4, sets 1 to 4, respectively. *Significant difference for S2, p <0.05.*

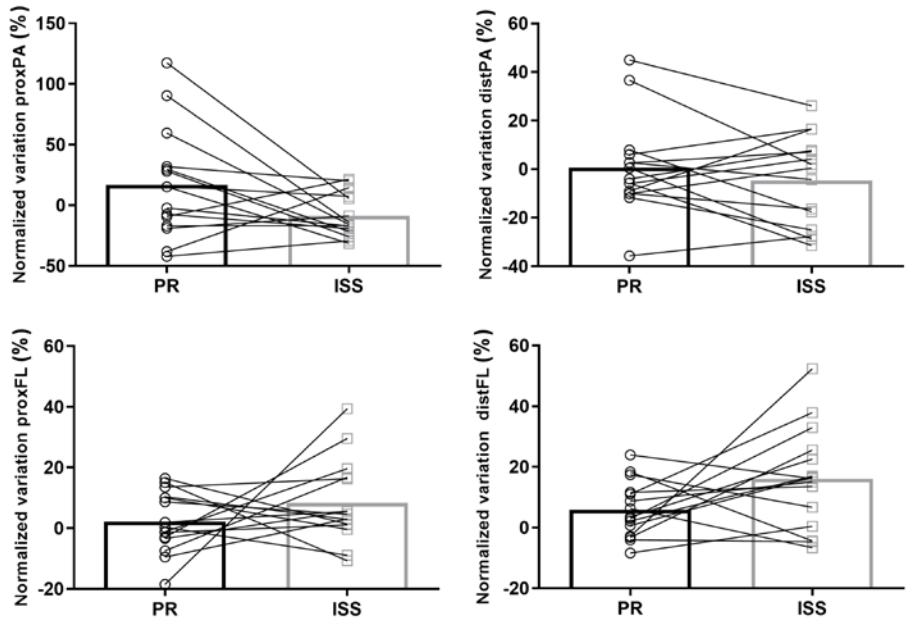


Figure 3. Normalized variation (%) in pennation angle and fascicle length for proximal and distal regions of the vastus lateralis between the passive rest (PR) and inter-set stretching (ISS) protocols. PR: passive rest protocol; ISS: inter-set stretching protocol; prox: proximal region of vastus lateralis; dist: distal region of vastus lateralis.

rest (5.88% ± 9.25), and distal inter-set stretching (16.09% ± 16.71). Mean PA variations were: proximal passive rest (16.89% ± 45.05), proximal inter-set stretching (8.71% ± 17.84), distal passive rest (0.58% ± 19.37), and distal inter-set stretching (-4.71% ± 18.58). *Table 1* summarizes the effect sizes, which favored inter-set stretching for proximal and distal FL and passive rest for proximal PA.

DISCUSSION

Our results indicate that the isokinetic exercise reduced maximal concentric and eccentric knee extension peak torque across both protocols from the first to the fourth set, confirming the expected presence of fatigue. Although inferential tests show no statistically significant differences between protocols, the effect size favored the inter-set stretching protocol for increased FL in the vastus lateralis and attenuation of concentric peak torque fatigue in the knee extensors. Conversely, the passive rest protocol showed a greater increase in PA compared to inter-set stretching.

Meta-analyses suggest that static stretches longer than 60 seconds exacerbate fatigue before resistance exercises, while stretches shorter than 30 seconds do not impair performance[33,34]. In this study, inter-set stretching did not negatively impact performance. Previous research on inter-set stretching varies in methodology and reports mixed findings[20-25]. Aydin et al.[22] observed increased fatigue, likely influenced by confounding factors such as compensatory movements and inaccurate load estimation. In contrast, Padilha et al.[25] and Cometti et al.[20], using isokinetic dynamometry, providing precise torque measurements, found different outcomes: Padilha et al.[25] reported fatigue with 45-second stretches, whereas Cometti et al.[20] found no significant effects with 20-second stretches, similar duration to our protocol. These findings suggest that dividing stretches into shorter

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Variable	Passive rest protocol			Inter-set stretching protocol			Effect size d (CI95%)
Fascicle	pre	post	p-value	pre	post	p-value	PR/ISS
PAprox (°)	19.69 (± 4.3)	21.61 (± 5.2)	0.317	20.73 (± 3.4)	18.78 (± 4.2)	0.069	-0.81 (-1.4; -0.1)
PAdist (°)	21.93 (± 4.3)	21.80 (± 4.8)	0.914	21.30 (± 2.6)	19.94 (± 2.6)	0.224	-0.28 (-0.8; 0.3)
FLprox (cm)	8.91 (± 0.9)	9.12 (± 1.4)	0.385	9.06 (± 1.2)	9.73 (± 1.0)	0.035	0.52 (-0.0; 1.0)
FLdist (cm)	6.90 (± 0.9)	7.23 (± 0.8)	0.036	6.66 (± 1.0)	7.67 (± 1.0)	0.002	1.64 (0.8; 2.2)
Torque	set 1	set 4	p-value	set 1	set 4	p-value	PR/ISS
PTconc (N.m)	220.5 (± 35.8)	175.7 (± 32.3)	<0.001	211.7 (± 31.5)	163.4 (± 29.1)	<0.0001	0.86 (0.2; 1.3)
PTecc (N.m)	271.8 (± 57.4)	221.2 (± 67.0)	0.001	264.3 (± 57.5)	209.5 (± 56.5)	<0.001	-0.50 (-1.0; 0.1)
PTAconc (°)	61.5 (± 5.1)	66.7 (± 10.6)	0.040	61.6 (± 7.4)	67.1 (± 9.5)	<0.001	0.03 (-0.4; 0.5)
PTAecc (°)	63.9 (± 11.0)	70.2 (± 14.4)	0.022	64.2 (± 9.5)	73.9 (± 13.6)	<0.001	0.26 (-0.2; 0.7)

Table 1. *Mean absolute values and standard deviations of torque and muscle architecture parameters.*
Abbreviation: PA, pennation angle; FL fascicle length; prox, proximal region; dist, distal region; PT, peak torque; PTA, peak torque angle; conc, concentric contraction; ecc, eccentric contraction; IC, confidence interval; P-value, P-values from Paired Student's t-tests comparisons.

durations (≤30 seconds) may prevent additional fatigue, potentially by minimizing the motoneuron excitability reduction seen in longer stretches[11].

The effect size analysis supported reduced fatigue with inter-set stretching for concentric peak torque, while passive rest showed benefits for eccentric peak torque. Since eccentric contractions involve both elastic and contractile components, whereas concentric ones rely mainly on contractile elements[35], stretching may reduce elastic tension, decreasing eccentric force. This hypothesis is supported by studies reporting reduced connective tissue stiffness following stretching[36,37].

Muscle architecture variables showed no significant differences between the protocols; however, the effect size indicated "medium" and "very large" effects favoring the inter-set stretching protocol for proximal and distal FL, respectively. *Table 1* highlights increased FL in both regions for the inter-set stretching protocol and the distal region for the passive rest protocol. Fascicle elongation, often observed after eccentric contractions[38] and stretching[9], aligns with our findings and prior research[27,39,40], which suggest regional differences in vastus lateralis architecture. Proximal regions typically exhibit lower PA, while distal regions have shorter FL, explaining the larger distal effect size. Similarly, PA results favor passive rest, with "large" and "small" effect sizes for proximal and distal regions, respectively. This behavior aligns with studies on conventional resistance exercises[4] and concentric contractions[38].

Although t-tests showed no significant protocol differences between protocols, concentric and eccentric contractions exhibited a shift in the optimal torque angle toward longer muscle length post-exercise. This shift likely reflects the greater tension-generating capacity of eccentric contractions[41], causing rightward adjustment in the muscle's length-tension curve. Eccentric-induced sarcomere overstretching may also explain greater force loss at shorter muscle lengths[42]. Effect size analysis revealed a "small" effect favoring inter-set stretching for the eccentric peak torque angle (PTAecc), likely due to a larger shift in PTAecc compared to PTAconc. Notably, PTAecc shifted 7° in the passive rest protocol and 10° with inter-set stretching, while PTAconc shifted 5° and 6°, respectively. These shifts were likely influenced by FL changes during the stretching, as sarcomere elongation during stretching likely influenced torque generation[13,33,43].

Some limitations warrant consideration. First, measuring muscle architecture between sets could have better-linked torque data with fascicle behavior, but the time required for panoramic ultrasound imaging made this unreasonable. Second, the intensity of stretching was not objectively quantified, although "maximum range of motion possible" is widely used[44], it is subjective and limits the generalizability of findings. Additionally, although our study focused on torque and muscle architecture under controlled isokinetic conditions, fatigue has also been shown to affect functional movements such as jumping biomechanics[45]. This underscores the importance of future studies investigating whether acute architectural changes influence dynamic, real-world tasks. Finally, the sample consisted solely of physically active men, which limits extrapolation to women or clinical populations. Future research should explore whether these findings extend to different populations, contraction types (e.g., isometric or plyometric), and

functional outcomes. Longitudinal studies are also needed to determine whether acute muscle architecture adaptations translate into long-term training benefits.

CONCLUSIONS

This study is the first to demonstrate that short-duration inter-set stretching can attenuate concentric fatigue in the quadriceps muscles of physically active men during resistance exercise. These effects appear to be associated with increased FL and reduced PA. While the underlying neuromechanical mechanisms remain uncertain, the observed influence of distal FL and proximal PA on torque modulation underscores their importance and warrants further investigation. Clinically, inter-set stretching lasting up to 30 seconds may serve as a practical and time-efficient strategy to mitigate torque loss and enhance performance in resistance training. The "large" effect favoring concentric peak torque further supports the hypothesis of improved neuromechanical efficiency, reinforcing the practical relevance of inter-set stretching even in the absence of statistically significant differences.

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