

The effects of a 10-minute triceps surae stretching session persist after 60 min: a randomized clinical trial

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HIGHLIGHTS

- Passive static stretching increases ankle range of motion.
- Stretching effects last up to one hour.
- Increased range of motion is associated with changes in myotendinous properties.
- Stretching decreases muscle stiffness and increases myotendinous junction shift.

ABBREVIATIONS

CG	Control group
<i>d</i>	Effect sizes
EG	Experimental group
EMG	Surface electromyograph
GEE	Generalized Estimation Equations
IPAQ	International Physical Activity Questionnaire
MTJ	Myotendinous junction
MTU	Muscle-tendon unit
PRE	Pre-intervention evaluations
POST-0	Immediately after the stretching intervention
POST-15	15 min after intervention
POST-30	30 min after intervention
POST-45	45 min after intervention
POST-60	60 min after intervention
ROM	Range of motion
RMS	Root mean square

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BACKGROUND: Stretching exercises increase the joint range of motion (ROM) and depend on the skeletal tissues' exposition-time to stretch. However, it is unclear how a long stretching time affects the muscle-tendon unit's passive mechanical properties.

AIM: This study aimed to analyze changes in the triceps surae muscle-tendon unit's passive mechanical properties before and after a 10-minute passive stretching protocol.

METHOD: Thirty healthy participants (26.57 ± 3.82 years old) were allocated into a control group ($n=15$), who did not perform any intervention, and to an experimental group ($n=15$), who performed one bout of a 10-minute ankle plantar flexor passive static stretching. Ankle ROM, plantar flexor passive torque, and myotendinous junction displacement were evaluated pre-intervention, immediately after, and 15, 30, 45, and 60 minutes after the end of the intervention. The stiffnesses of the muscle-tendon unit, muscle, and tendon were calculated for all moments. A generalized estimating equation test was performed to compare groups and moments.

RESULTS: The experimental group increased the ROM ($p<0.001$) from pre- to post-intervention and remained augmented up to 60 minutes. The myotendinous junction displacement decreased at post-30 and post-45 moments compared to pre-intervention. Muscular stiffness increased immediately after stretching and post-45 and post-60 minutes. Passive torque and musculotendinous unit stiffness decreased over time, with trivial, small, and moderate effect sizes, respectively.

CONCLUSION: Passive static stretching (10 min) generates an acute ROM increase associated with muscle-tendon unit passive mechanical properties reduction, which lasts up to one-hour post-intervention.

KEYWORDS: Muscle Stretching Exercises | Achilles tendon | Ultrasonography | Triceps Surae | Passive Torque | Range of Motion

INTRODUCTION

Flexibility is determined by the range of motion (ROM) in a joint or a group of joints. It is directly influenced by the tissues surrounding the joint (muscles, joint capsule, tendon, and ligaments)^{1,2}.

Evidences support that static stretching might specifically provide a small-to-moderate protective effect for muscle–tendon injury risk, especially in running-based sports

³. As static stretching has been reported to reduce the incidence of musculotendinous injuries, especially in sports with a large number of sprints and change-of-direction movements, it may be an important addition to a pre-exercise or pre-sports warm-up ⁴. Although this exercise type acutely increases ankle ROM, it does not seem to affect muscle extensibility and muscle stiffness ^{5,6}.

However, understanding the mechanisms responsible for this ROM increase is essential, as stretching exercises may affect ROM, tendon, muscle and the muscle-tendon unit (MTU) behavior ⁷. ROM is related to the soft tissues (e.g., fascia, muscle's connective tissue sheets, joint capsule, ligaments, and tendon) mechanical properties (e.g., passive tension, deformation, stiffness, stress, strain, elastic modulus), and the MTU's stretching reflex neural activity ⁷. Secondly, it is affected by a decrease in joint resistance to stretch, which could be due to a change in MTU mechanical properties ⁸. Longer periods of static stretching may induce greater decrements in muscle force output, which may be due to neurological impairments such as decrements in spinal excitability ⁹.

Understanding the stretching acute effects' duration after a single static stretching session is relevant, as it may affect physically and non-physically active individuals and help clinical decision making when using stretching exercises in clinical practice ^{3,4}. One of the essential aspects of stretching is how long its acute effects last. The effects of the duration time of a single static stretching session and how long they can last is a useful information, as it allows us to understand its real effect on daily-life and athletic tasks and determining the use or not of stretching as part of the warm-up routine for exercise ⁶. However, the existent evidence does not allow for a clear conclusion and, therefore, for the best decision in professional practice. Despite this inconclusiveness, a more compliant tissue allows exercise performance in a higher joint ROM, favoring greater joint mobility, greater MTU excursion, and strength training at higher ranges not previously achieved ^{10,11}.

Changes in the tendon's mechanical properties (e.g., stiffness, elastic modulus) may affect the production and transfer of muscle strength to bones. The tendon acts as the main transmitter of the contractile force produced by the muscle to the bones. Therefore, changes in tendinous properties can influence the magnitude of force transmission, which can produce a significant effect on muscle mechanics. Increased stiffness of elastic components, for example, can improve the connective tissues' ability to transmit muscle force ²⁶, contributing to an increase in muscle strength production ²⁷. However, a more compliant myotendinous unit allows for faster shortening of contractile components ²⁷, favoring the performance in stretching-shortening cycle tasks ¹⁰.

A more compliant MTU seems to imply a higher joint ROM. In the ankle joint, for example, a greater dorsiflexion ROM could increase the torque production of the plantar flexor muscles ²⁸. This torque increase could occur due to the displacement of the plantar flexors' torque-angle relationship for greater muscle lengths, in which plantar flexor's torque increases as ankle dorsiflexion increases ²⁸. At the same time, a more compliant MTU also allows for faster shortening of the contractile components ²⁷. Thus, this greater complacency can contribute to reducing force levels due to the force-velocity relationship ²⁶, shifting this relationship's curve to the right ²⁷. The longer lengths of more compliant tendons increase total joint ROM thereby determining a lower predisposition for muscle strain injuries.

The triceps surae's passive static stretching was evaluated in different protocols, with stretching duration varying from 2 to 8 min (with fractionated times of 4x30 sec or 5x1

min, for example), and the follow-up time reaching a maximal of 20 min post-stretching¹². Passive torque decrease was observed only immediately after the stretching intervention^{5,13}, without significant results in the follow-up for this variable. MTU stiffness decreased immediately after stretching, persisting up to 20 min post-stretching^{12,13,14}, with no studies looking at longer follow-up times. Muscle stiffness reduced immediately after stretching, lasting up to 10 min post-stretching^{5,14}. Tendon stiffness significantly increased immediately after stretching, returning to baseline values within 10 min after the stretching intervention¹⁴.

Follow-up studies on the duration of the acute effect after a single passive static stretching session have used brief stretching periods (30 sec to 1 min), with total stretching time not exceeding 8 min¹². Such protocols have shown changes in MTU's passive mechanical variables only immediately after stretching, with changes lasting no more than 10 min¹².

A decrease in tendon stiffness was observed after both 5-minute duration protocols of continuous static stretching^{10,11} and a 10-minute static stretching protocol¹⁵. However, these studies^{10,11,15} evaluated the tendon mechanical properties only immediately after stretching and did not follow up on these mechanical changes' duration.

Thus, there is a gap in the knowledge regarding the MTU mechanical adaptations to acute effects of static stretching. Therefore, to understand the mechanisms determining the increased joint ROM post-stretching, we looked at the changes in the triceps surae's MTU passive mechanical properties before and immediately after a 10-minute passive stretching protocol. We also checked what happened with these mechanical properties after 15, 30, 45, and 60 minutes of the stretching intervention. We hypothesize that the 10-minute stretching will increase ankle ROM and myotendinous junction displacement. Moreover, the intervention should reduce passive torque, MTU stiffness, muscle stiffness, and tendon stiffness, which will last up to 60 min.

METHODS

According to the Helsinki Declaration, this experimental study was approved by the Research Ethics Committee of the Federal University of Rio Grande do Sul (number 2.139.313). All participants signed a written informed consent form. This randomized clinical trial was registered in the Brazilian Registry of Clinical Trials (RBR-7fnsww).

Participants

Healthy young adults (men and women aged between 18 and 40 years old) participated in the study. The sample size was defined by the calculation for infinite populations¹⁶, with plantar flexors' passive torque as the primary outcome. A 95% confidence interval and a maximum admitted error of 5% was used. For the calculation, passive torque values (37.5 ± 2.4 Nm) from Nakamura, Ikezoe¹⁴ study indicated 7 participants per group. Due to possible losses, we recruited 16 participants per group.

After disclosing the invitation to participate in the study through social networks and on the university campus, 32 healthy male and female participants agreed to participate. After signing the informed consent form, participants completed a questionnaire for sample characterization and the International Physical Activity Questionnaire (IPAQ)¹⁷. We included only irregularly active or sedentary people in the sample. Participants should not be engaged

in strength and flexibility training. They should not have any history of musculoskeletal injury in the lower limbs. Participants with difficulty in performing dorsiflexion ROM and that had triceps surae muscle's activation in any tests were also excluded.

Experimental design

The data collection happened in a single session. After completing the previously mentioned questionnaires, participant randomization was performed by a computer program (randomization.com) that determined their group allocation (control – CG or experimental – EG) and which leg would be evaluated, preferred/non-preferred. To determine this lateral preference, participants were asked which leg they used to kick a ball. The assessment and intervention were carried out unilaterally. Participants were randomized into the experimental and control groups using the randomly exchanged block method. There was allocation confidentiality, and the evaluators of the outcomes were blinded to the participants' allocation. This computer program generated an entry order for the first 16 participants that entered the study. The next 16 participants were paired to the first group participants according to their leg length (distance between lateral malleolus and lateral femur condyle) so that homogeneous groups were formed concerning leg and tendon length. Leg length, height, and body mass were part of the anthropometric measurements conducted at the beginning of the study.

Subsequently, pre-intervention evaluations were made (PRE), which consisted of ankle ROM measurements, triceps surae's myotendinous junction displacement (MTJD), and passive torque. After PRE assessments, the EG's intervention was conducted with 10-minute plantar flexors' passive static stretching. CG participants remained seated for the same 10-minute period with no intervention. Both groups performed the same post-intervention evaluations: immediately after (POST-0), POST-15, POST-30, POST-45, and POST-60 min of the stretching intervention.

Procedures

Participants performed the assessment and the intervention sitting on a Biodex System 3 (Biodex Medical System, USA) isokinetic dynamometer, with the hip flexed at 85° (0° = hip fully extended) and knee fully extended (0°). Velcro straps stabilized their body to avoid compensatory movements. The foot was attached to the dynamometer's footplate with the ankle at 0° (90° between the foot and the leg). The lateral malleolus was aligned with the dynamometer axis¹⁸.

ROM assessment

Maximal ankle joint ROM was evaluated through the isokinetic dynamometer. The evaluator passively moved the ankle towards maximal dorsiflexion until the participant reported a discomfort sensation. Three measures were performed, and the mean of those three values was used for analysis.

Passive torque assessment

The plantar flexor passive torque was evaluated in the same position described above. The ankle was passively moved by the dynamometer at a constant angular velocity of 5°·s⁻¹ for five cycles, from 0° to the previously determined maximum dorsiflexion for each

participant at the PRE moment. The dynamometer measured passive torque for all joint angles up to the maximal ankle dorsiflexor ROM. Torque values for the three central cycles, which occurred in maximum PRE moment ROM evaluations, were used to calculate the mean passive torque value. For post-intervention evaluations (POST-0, POST-15, POST-30, POST-45, and POST-60), the same maximum dorsiflexor ROM angle of the PRE evaluation was used to calculate the average passive torque of the three cycles in each moment.

MTJ displacement assessment

The medial gastrocnemius muscle's MTJD was evaluated during the passive torque assessment. The ultrasound (US - Aloka Inc., Japan) transducer (7.5 MHz) was attached perpendicularly to the participant's leg with Velcro straps at the distal region of the medial gastrocnemius muscle, where the myotendinous junction was identified ¹⁴. In addition, an anechoic marker was fixed on the skin to control for possible probe slides during image collection. Finally, a water-soluble gel was applied to establish the probe-skin contact.

The MTJD was determined as the distance between the skin marker and the MTJ point at 0° (i.e., neutral position) and at maximum dorsiflexion ^{14,19,20}. The data were recorded in video format, and after conversion to AVI format, data analysis was performed in the Kinovea-0.8.15 software (Copyright © 2006–2011, Joan Charmant and Contributors; www.kinovea.org/). The analysis was performed in the three central cycles of the five passive torque cycles. A chronometer was used to record when the participant's ankle was at the initial (0°) and final (maximum ROM) positions (Figure 1B). During the intervention, eleven measures were performed from minute zero to minute 10. MTJD was determined as the distance difference of the MTJ from the anechoic marker in each minute of the stretching protocol with respect to the resting condition.

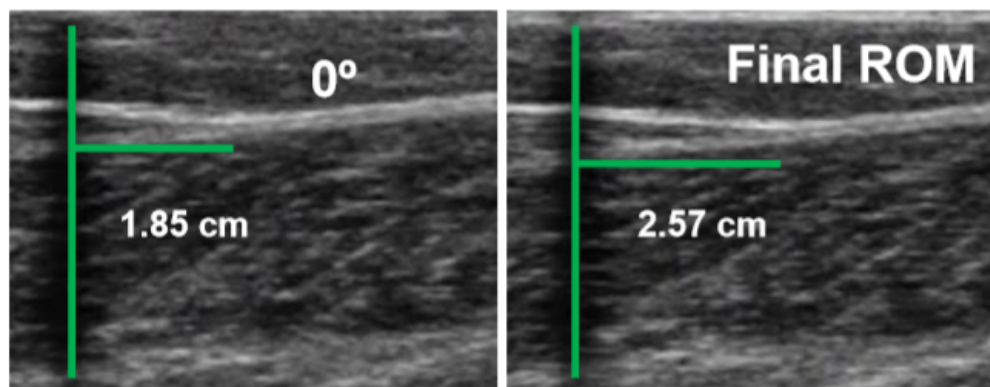


Figure 1. Ultrasound images from a representative subject of the experimental group at the initial dorsiflexion position at 0° and at the final dorsiflexion range of motion (ROM) during the pre-intervention evaluation. The intersection point between the two white lines in the figure shows the location of the myotendinous junction (MTJ). The vertical green line shows the acoustic anechoic marker, and the horizontal green line shows the distance between the marker and the MTJ. MTJ displacement was calculated by the difference between the two horizontal green lines (Source: Neuromuscular Plasticity Laboratory, LAPEX, ESEFID-UFRGS).

Measurements of MTU, muscle, and tendon stiffness

The third passive torque-angle curve's slope in the last 10 degrees of the dorsiflexion ROM of the PRE moment was considered the MTU stiffness. The slope at the same ankle joint angles (last 10 degrees of pre-evaluation ROM) was used to determine post-intervention stiffness ²⁰.

Muscle and tendon stiffness values were calculated from MTJD and passive torque measurements. Muscle stiffness was defined as the ratio between the passive torques from 0° to the maximum dorsiflexion ROM by MTJD in this ROM ⁵. Tendon stiffness was defined as the ratio between the passive torque change from 0° to the maximum ankle dorsiflexion ROM and the tendon displacement at the maximum dorsiflexion angle. Tendon displacement at maximum ankle joint angle was determined by subtracting the MTU displacement by MTJD. For the definition of the MTU displacement, the value of 0.78mm/° [estimated value of the length change from 0° to 30° of the gastrocnemius muscle by equation from Grieve, Cavanagh ²¹] was multiplied by the maximum dorsiflexion ankle angle reached by the participant ²⁰.

Intervention protocol: passive static stretching

Participants from the EG remained seated in the dynamometer in the same position in which the evaluations were performed. The dynamometer moved the participant's foot at 5°·s⁻¹ until the maximum dorsiflexion ROM measured at the PRE moment was reached. That position was maintained for 10 min in the dynamometer's passive mode.

Electromyographic signal

Surface electromyography (EMG) monitored the triceps surae muscle (medial and lateral gastrocnemius heads and soleus). After skin preparation, surface electrodes (Meditrace 100, Kendall, USA) were positioned on the medial and lateral gastrocnemius heads and soleus. A reference electrode was fixed on the medial surface of the tibia ¹⁸. To be considered at rest, the plantar flexors' EMG activity during the intervention should not oscillate more than two standard deviations above the resting value ²⁰. When above this value, the participant was excluded from the study.

The triceps surae muscles' EMG data analysis was performed using the Miotec Suite system (*Miotec Equipamentos Biomédicos, Porto Alegre, RS, Brazil*) and was used to digitize the EMG signals with a sampling frequency of 2000 Hz. Initially, a 5th order Butterworth bandpass filter was used, with 20 and 500 Hz cutoff frequencies, to eliminate possible ambient and electrical device noises. The root mean square (RMS) value at rest was used to determine possible plantar flexor muscle activation level during the passive torque test in the three central repetitions. The resting value was checked for five seconds prior to testing.

Statistical analysis

Data were presented as mean and standard deviation, and the Shapiro Wilk test was used to evaluate data normality. An independent t-test was used to evaluate between-groups homogeneity regarding the sample characterization variables. For intra-group comparison (PRE, POST-0, POST-15, POST-30, POST-45, and POST-60) and between-

group comparison (experimental and control), a Generalized Estimation Equations (GEE) test was used, with a Bonferroni post-hoc test when differences were identified. Tests were performed using the Statistical Package for the Social Sciences for Windows (SPSS, software version 20.0), adopting $\alpha=0.05$ for the significance level. In addition, effect size (Cohen's D) was calculated to express the magnitude of the differences between the post-intervention moments and the pre-intervention moment for the dependent variables from each group. Effect sizes (d) were categorized as trivial (<0.20), small (0.20-0.49), moderate (0.50-0.79), large (0.80-1.29), and very large (>1.30) ²².

RESULTS

Thirty-two participants were selected for the study. However, two participants were excluded for excessive shortening of the triceps surae muscles, which prevented their correct positioning for the tests. Of the initial 30 participants, five were excluded after EMG data analysis, which indicated that these participants were unable to keep their triceps surae muscles relaxed during the test. Therefore, EG had 12 participating and CG 13 participants (Figure 2). The data describing the sample's characterization are presented in Table 1. There were no between-group differences ($p>0.05$), showing that the groups were homogeneous at the PRE moment.

Table 1. Characterization of the participants. Values are presented as mean (standard deviation) or absolute frequency obtained at the baseline period.

Characteristics	EG (n=12)	CG (n=13)	t-value	p-values
Sex			0.75	0.45
Female	11	9		
Male	4	6		
Age (years)	26.67 (3.22)	26.47 (4.45)	-0.14	0.88
Body mass (kg)	65.88 (15.21)	71.20 (20.37)	0.810	0.42
Stature (cm)	164.00 (11.00)	169.00 (12.00)	1.09	0.28
BMI (kg/cm ²)	24.21 (3.85)	24.70 (5.72)	0.27	0.78
Leg length (cm)	39.27 (3.61)	41.00 (4.19)	1.21	0.23
Tendon length (cm)	19.70 (2.29)	20.83 (2.36)	1.33	0.19
IPAQ			0.00	1.00
IA	11	12		
Sed	1	1		

BMI: body mass index, EG: Experimental Group, CG: Control Group, IPAQ: international physical activity questionnaire, IA: irregularly active, Sed: sedentary

ROM

There was a ROM increase in both groups at all POST moments compared to the PRE moment ($p<0.001$). In addition, there was a ROM increase at POST-45 and POST-60 moments ($p=0.009$ for both) compared to the POST-0 moment. There was no between-moments difference ($p=0.938$) (Table 2). Moderate effect sizes were found for POST-0 (CG), and POST-0, POST-15, and POST-30 (EG) relative to the PRE moment, while large effect size was observed for POST-15, POST-30, POST-45, and POST-60 moments in the CG and POST-45 and POST-60 moments in EG compared to the PRE moment.

Passive torque

There was no between-groups passive torque difference in any of the moments ($p>0.05$ for all comparisons) (Table 2). However, there was a group-by-moment interaction ($p=0.021$), demonstrating that the groups' passive torque behavior was different over time. While there was no between-moments differences for CG, in EG, passive torque at the PRE moment was greater than all POST moments ($p<0.001$ for all comparisons), and the POST-30 moment was greater than the POST-60 ($p=0.009$). The passive torque's effect size was trivial for POST-0, POST-30, and POST-45 moments and small in POST-15 and POST-60 moments compared to the PRE moment in CG. The effect size was small at POST-30 and POST-45 and moderated for the POST-0, POST-15, and POST-60 moments compared to the PRE moment for EG.

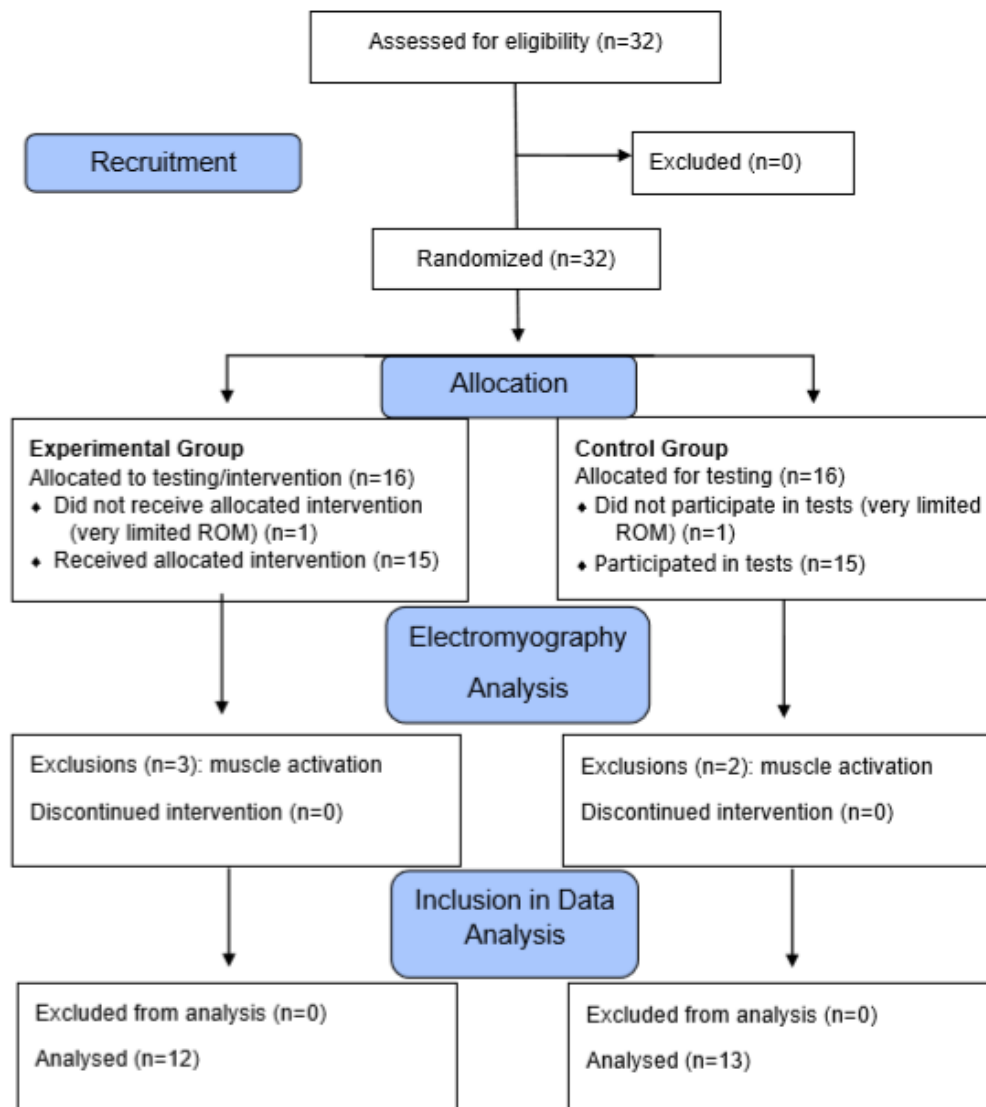


Figure 2. Flowchart showing the recruitment, allocation, and exclusion of participants.

MTJ Displacement

In EG and CG, MTJD at the PRE moment was smaller than all POST moments. There was an increase in MTJD from the PRE moment to POST-30 ($p=0.019$) and POST-45 ($p=0.036$) moments both in EG and CG, with no between-groups difference ($p=0.20$) (Table 2). The MTJD effect size was trivial for the POST-0 moment (EG), small for the POST-0, POST-15 and POST-60 moments (CG), and POST-15, POST-45 and POST-60 moments (EG), and moderate for POST-30 and POST-45 moments (CG) and POST-30 moment (EG).

MTU, muscle and tendon stiffness

In EG and CG, MTU stiffness at the PRE moment was smaller than all POST moments. MTU stiffness presented a significant group-by-moment interaction ($p=0.006$). When comparing MTU stiffness between groups at the specific moments, stiffness was greater in CG compared to EG at POST-0 ($p=0.025$). In the between-moments comparison within each group, there was no between-moments difference for CG ($p>0.05$) (Table 2), while EG showed a reduction in this variable at all POST times compared to the PRE moment ($p<0.001$ for POST-0, POST-15 and POST-30 moments' comparisons; $p=0.027$ in the POST-45 moment), except for the POST-60 ($p=0.205$) that was not different from PRE. The effect size was trivial for POST-0 moment in CG, small for POST-15, POST-30 and POST-60 moments in CG and POST-45 and POST-60 in EG, and moderate for POST-45 moment in CG and POST-0, POST-15 and POST-30 moments in EG.

In EG and CG, muscle stiffness at the PRE moment was smaller than all POST moments. Muscle stiffness was smaller in both groups at PRE compared to POST-0 ($p=0.018$), POST-45 ($p=0.004$) and POST-60 ($p=0.001$) moments, with no between-groups difference ($p=0.698$) (Table 2). The effect size was small for both groups' POST-0, POST-15 and POST-30 moments and moderate for POST-45 and POST-60 moments.

In CG, tendon stiffness values at the PRE moment were smaller than all POST moments. In EG, tendon stiffness values at the PRE moment were smaller than all POST moments. On the other hand, there were no moment ($p=0.172$) or group ($p=0.133$) differences in the tendon stiffness (Table 2). The effect sizes were trivial for POST-0 and POST-15 moments in CG and POST-15, POST-30, POST-45, and POST-60 moments in EG. Effect sizes were small in the POST-30, POST-45, and POST-60 moments in CG and POST-0 moment in EG.

Table 2. Mean, standard deviation (SD), effect size (Cohen's d) and 95% confidence interval (CI95%) of ROM, passive torque, MTJ displacement, MTU stiffness, muscle stiffness, and tendon stiffness from moments PRE, POST-0, POST-15, POST-30, POST-45, and POST-60. Post moment comparisons were made with the PRE moment.

		PRE	POST-0	POST-15	POST-30	POST-45	POST-60
ROM (°)							
CG	mean (SD)	27.23 (6.72)	32.77 (7.54) *	34.15 (8.62) *	35.62 (7.50) *	36.23 (8.04) * [#]	35.77 (8.44) * [#]
	d (CI95%)	-	0.69 (-0.10;1.49)	0.83 (0.03;1.63)	1.09 (0.26;1.91)	1.12 (0.29;1.95)	1.03 (0.20;1.85)
EG	mean (SD)	27.42 (8.66)	33.25 (9.77) *	33.75 (10.05) *	34.92 (10.18) *	36.25 (10.39) * [#]	37.75 (9.18) * [#]
	d (CI95%)	-	0.63 (-0.19;1.45)	0.62 (-0.20;1.44)	0.73 (-0.09;1.56)	0.85 (0.02;1.69)	1.07 (0.21;1.92)
PT (Nm)							
CG	mean (SD)	32.54 (10.10)	31.17 (9.27)	29.49 (12.66)	31.00 (11.07)	31.41 (12.19)	29.96 (9.88)
	d (CI95%)	-	0.13 (-0.64;0.90)	0.25 (-0.53;1.02)	0.13 (-0.64;0.90)	0.09 (-0.68;0.86)	0.24 (-0.53;1.01)
EG	mean (SD)	30.22 (6.86)	25.55 (6.13) *	26.49 (6.68) *	27.57 (7.79) *	26.81 (7.86) *	26.25 (7.06) * ^{\$}
	d (CI95%)	-	0.69 (-0.14;1.51)	0.52 (-0.29;1.34)	0.35 (-0.46;1.16)	0.41 (-0.37;1.25)	0.55 (-0.27;1.36)
MTJD (cm)							
CG	mean (SD)	0.82 (0.25)	0.90 (0.22)	0.94 (0.22)	1.03 (0.40) *	0.99 (0.29) *	0.94 (0.29)
	d (CI95%)	-	0.31 (-0.46;1.09)	0.47 (-0.31;1.25)	0.58 (-0.20;1.30)	0.58 (-0.21;1.36)	0.41 (-0.37;1.90)
EG	mean (SD)	0.71 (0.28)	0.77 (0.31)	0.85 (0.31)	0.90 (0.24) *	0.82 (0.24) *	0.83 (0.24)
	d (CI95%)	-	0.19 (-0.61;0.99)	0.44 (-0.37;1.25)	0.67 (-0.15;1.49)	0.39 (-0.42;1.20)	0.42 (-0.38;1.23)
MTUSTIF (Nm/°)							
CG	mean (SD)	1.24 (0.47)	1.20 (0.40) [†]	1.10 (0.61)	1.12 (0.47)	1.00 (0.36)	1.12 (0.36)
	d (CI95%)	-	0.09 (-0.68;0.86)	0.24 (-0.53;1.01)	0.24 (-0.53;1.01)	0.54 (-0.24;1.33)	0.27 (-0.50;1.04)
EG	mean (SD)	1.12 (0.31)	0.88 (0.28) * [†]	0.93 (0.28) *	0.94 (0.31) *	0.95 (0.35) *	0.94 (0.38)
	d (CI95%)	-	0.75 (-0.08;1.58)	0.59 (-0.22;1.41)	0.54 (-0.28;1.35)	0.47 (-0.34;1.29)	0.48 (-0.50;1.04)
MUSSTIF (Nm/cm)							
CG	mean (SD)	28.76 (12.87)	36.01 (15.14) *	34.02 (16.41)	35.97 (17.09)	36.88 (14.31) *	39.63 (19.07) *
	d (CI95%)	-	0.48 (-0.30;1.26)	0.33 (-0.44;1.10)	0.44 (-0.34;1.22)	0.55 (-0.23;1.33)	0.62 (-0.17;1.40)
EG	mean (SD)	28.82 (9.35)	33.31 (12.61) *	31.41 (10.36)	33.16 (11.85)	36.30 (12.47) *	37.41 (12.78) *
	d (CI95%)	-	0.36 (-0.44;1.17)	0.24 (-0.56;1.05)	0.38 (-0.43;1.18)	0.63 (-0.19;1.45)	0.71 (-0.12;1.53)
TENSTIF (Nm/cm)							
CG	mean (SD)	18.04 (7.18)	19.08 (6.65)	19.54 (10.13)	22.40 (14.10)	21.28 (12.19)	20.20 (9.99)
	d (CI95%)	-	0.14 (-0.63;0.91)	0.16 (-0.61;0.93)	0.36 (-0.42;1.13)	0.30 (-0.48;1.07)	0.23 (-0.54;1.00)
EG	mean (SD)	15.84 (8.54)	13.52 (4.50)	15.60 (5.75)	16.58 (6.51)	15.88 (6.79)	14.79 (5.98)
	d (CI95%)	-	0.31 (-0.49;1.12)	0.03 (-0.77;0.83)	0.09 (-0.71;0.89)	0.00 (-0.80;0.80)	0.13 (-0.67;0.94)

ROM: range of motion; PT: passive torque; MTJD: myotendinous junction displacement, MTUSTIF: muscle-tendon unit stiffness, MUSSTIF: muscle stiffness, TENSTIF: tendon stiffness. * Significant difference from pre to post-intervention moment; [†] Difference between control and experimental groups for POST-0; * Different from PRE moment; [#] Different from POST-0 moment; ^{\$} Different from POST-30 moment.

DISCUSSION

We looked at changes in the triceps surae MTU passive mechanical properties before and after a 10-minute passive stretching protocol. We also checked for possible changes in these mechanical properties after 15, 30, 45, and 60 minutes of the stretching intervention. To the best of our knowledge, our study had the longest passive stretching duration and the longest follow up time while evaluating the acute effects' duration on the mechanical properties of healthy women and men. Our main findings are that both groups increased ROM that persisted up to 60 minutes after the intervention for EG and after the testing session followed by a 10-minute rest for CG. Moreover, EG showed decreased passive torque and MTU stiffness in all post-intervention moments (except for the MTU stiffness at POST-60) compared to PRE, while CG showed no changes in the follow up, but showed a higher MTU stiffness at POST-0 compared to EG. MTJD and muscle stiffness increased in some moments, but there was no pattern for these changes. The only variable that did not change with intervention and was similar between groups was tendon stiffness, with an effect size from low to trivial for both groups at all moments.

As CG did not undergo any stretching intervention, we would expect no changes to be observed for any of the outcomes in this group. However, the fact that changes occurred over time in this group in some of the outcome variables leads to the hypothesis that the assessment type used to measure ankle maximal dorsiflexion ROM, passive torque, and MTJD may have had an unexpected effect on CG. Therefore, it appears that the three maximal ankle dorsiflexion repetitions used for maximal ROM determination, followed by five passive torque measurements of the plantar flexor muscles, were sufficient to produce an increase in ankle ROM 10 min after the tests, and this ROM increase remained about constant in the 60 min of follow up. Therefore, cyclic or dynamic stretching happened as a form of intervention for CG. For each moment of our protocol, all outcome variables were accessed, and they consisted of eight cycles where the ankle joint was passively taken up to its maximum dorsal flexion ROM. Thus, unpredictably, it seems that cyclical or dynamic stretches ended up as a form of intervention for CG, leading to changes in ROM^{23,24,25}. However, we do not know if the PRE testing session was the only factor to determine these ROM changes or if the subsequent tests (POST-0, POST-15, POST-30, and POST-60 min of the resting period) also played a role in this ROM increase in CG.

Back to the secondary objective regarding the time duration changes after the stretching protocol in EG, the study by Mizuno and Umemura²⁴ partially corroborates our results. They used a 2-minute dynamic stretching protocol in contrast to our 10-minute static stretching protocol. Mizuno and Umemura²⁴ also followed up the duration of the changes due to stretching up to 30 min after the stretching intervention. They attributed the joint ROM improvement to a stretching tolerance increase since the passive mechanical variables did not change at any time.

As in Mizuno and Umemura's²⁴ study, MTJD, muscle stiffness, and tendon stiffness did not show significant changes after our 10-minute stretching intervention. However, passive torque and MTU stiffness decreased after the stretching protocol. Passive torque is a mechanical variable representing the resistance that the MTU offers to the passive movement¹⁴. Triceps surae's MTU stiffness calculation considers the passive torque behavior according to the angular change during maximal dorsiflexion. As the triceps surae

muscle was relaxed during the tests and intervention (as determined by the triceps surae's electrical activity controlled through surface electromyography), it appears that the mechanism responsible for the ankle's ROM increase was the stretching tolerance increase, as already suggested by Mizuno and Umemura²⁴. This increased stretching tolerance may be directly related to changes in passive components (e.g., collagen alignment and other changes in the extracellular matrix of the MTU connective tissue), as well as to neural adaptations, and not to structural changes.

Stretching tests' tolerance is often used in studies that look for increased flexibility after stretch training. According to Freitas, Mendes⁸, the explanation for the joint ROM increase involves a sensory theory, which indicates that MTU may tolerate greater passive tension after the intervention. According to Blazeovich, Cannavan¹⁹, ROM-related neural changes may be associated with a change in the response of peripheral afferent receptors or to variations in the individual's perception of soft tissues' elongation during the passive stretching.

When we look at the outcome changes observed in EG after stretching, in addition to the ankle ROM increase and to the MTU stiffness decrease, an increase in MTJD and in muscle stiffness were also observed in the follow up (although MTJD's increase was only observed at the POST-30 and POST-45 moments and muscle stiffness did not increase at POST-15 and POST-30). Altogether, these results seem to support the idea mentioned above of a higher stretching tolerance of the MTU in EG.

Passive torque presented the same behavior as ROM, which differs from the study by Mizuno, Matsumoto¹³, in which passive torque changed only immediately after the five series of 1-minute stretching, returning to baseline levels 5 min after the end of the stretching.

MTU stiffness remained altered up to 45 minutes after stretching in our study. In Ryan, Beck's¹² study, which evaluated the effect of an 8-minute stretching test, this variable decreased immediately after stretching, returning to baseline level 20 min after the intervention. Nakamura, Ikezoe¹⁴ evaluated only up to 10 min after a 5-minute stretching protocol and found a decrease in MTU stiffness up to this evaluation time. In the study by Mizuno, Matsumoto¹³, MTU stiffness decreased immediately after, returning to baseline levels in 10 and 15 min after the end of the stretching intervention. The difference between our and their results may be due to our long stretching duration as opposed to their series of short stretches, and the evaluations may also have interfered with these variables' behavior.

Limitations

The study's main limitation was the fact that the participant allocation process may have generated dubious results. In the allocation process, we did not keep the randomizations' list confidential for the researchers. In addition, it was not possible to blind the evaluators of the outcomes. Another limitation is the fact that the intervention was performed with healthy physically active and sedentary participants, and, therefore, the study application is limited to this population. Moreover, we did not control the effects of the different phases of the menstrual cycle or the use of hormonal contraceptives, which may have an impact in joint laxity.

The stretching protocol's limitation was to manipulate the ROM until its "maximum" was achieved, which may have overstretched most structures crossing the ankle joint.

Therefore, the conclusions should be viewed with caution. Moreover, CG also increased ROM and MTJD even without a stretching intervention. However, the fact that there were no systematic changes in the other outcomes in CG suggests that the testing session only affected ROM. As none of the other outcome variables can explain this ROM increase, it appears that the observed changes were related to changes in the ankle joint complex, but not in the MTU system.

Implications for practice

Passive static stretching may be considered a good alternative in physical exercise for increasing flexibility due to the increased ankle joint ROM, which was associated with a decrease in the MTU passive components' resistance. Our results contribute to clinical practice of therapists and trainers who aim to gain ankle joint flexibility and find difficulties with the most used protocols. The knowledge that long-term static stretching, in addition to increasing ROM, as in shorter duration protocols, also reduces resistance in the passive and mechanical components of the joint. Thus, may contribute more effectively to cases in which the decrease in ROM is not restricted to muscle shortening.

CONCLUSION

The triceps surae's passive static stretching generates an increase in ankle ROM associated with changes in the MTU passive mechanical properties, such as decreased passive torque and muscle stiffness and increased MTJD. In addition, these changes in the passive mechanical properties' variables last up to one hour after the end of the intervention. The results should be observed with caution due to the ROM protocol limitations.

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