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There is no difference between two and five minutes of static stretching training and detraining on gastrocnemius medialis muscle thickness, pennation angle and fascicle length

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HIGHLIGHTS
- 6-weeks of passive stretching did not generate muscle architectural adaptations.
- Ultrasound analyses for muscle thickness have an excellent inter-analyzer reliability.
- The inter-analyzers reliability for fascicle length is good.
- The inter-analyzers reliability for pennation angle varies from moderated to good.

ABBREVIATIONS

CI	Confidence Interval
CV	Coefficient of variance
ES	Effect size
FL	Fascicle length
GC	Control group
GM	Gastrocnemius Medialis
G2	2-minutes of PSS
G5	5-minutes of PSS
ICC	Intraclass correlation coefficients
IPAQ	International physical activity questionnaire
MDC	Minimum detectable change
MT	Muscle thickness
PA	Pennation angle
PSS	Passive static stretching
ROM	Range of motion
SD	Standard deviation
SEM	Standard error of measurement
TTUS	Total time under stretching

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BACKGROUND: Skeletal muscle's architecture can undergo temporary or permanent adaptations when subjected to chronic passive loading, such as during passive static stretching (PSS).
AIM: We evaluated the effects of a 6-week PSS program, with two and five minutes of duration, on the architecture of the Gastrocnemius Medialis (GM) muscle. In addition, we determined the inter-analyzer reliability of the GM's muscle architecture images analysis process.
METHOD: 30 healthy adults participated in this study. Participants were divided into three groups: Control Group (CG), 2-minutes of PSS (G2) and 5-minutes of PSS (G5). Plantar flexors' PSS was applied three times a week for 6 weeks. Participants were assessed before intervention, after intervention, and two weeks post detraining. GM's muscle thickness (MT), pennation angle (PA) and fascicle length (FL) were measured with an ultrasound system by an experienced evaluator. All images were analyzed by two independent analyzers, using the Image-J software.
RESULTS: No significant effects were identified ($p>0.05$) of the PSS program on muscle architecture parameters. No architectural changes were observed following the detraining period. GM's MT results presented excellent reliability, while good reliability was found for the FL measures. For PA, good reliability was only observed for the post-intervention moment. On the pre-intervention and follow-up moments, the intraclass correlation coefficients values were moderate.
CONCLUSION: A 6-week PSS program did not generate adaptations on GM's muscle architecture parameters in healthy subjects, independent of the stretching duration. Muscle architecture parameters are reliable when analyzed by different analyzers following clinical interventions.

KEYWORDS: Ultrasound | Fascicle length | Pennation angle | Muscle thickness | Muscle Stretching Exercises

INTRODUCTION

Muscle stretching is widely used in rehabilitation programs and sports training with the aim of increasing flexibility and improving functional performance [1,2]. One of the most popular types of muscle stretching used in clinical practice is the passive static stretching (PSS). This technique consists in placing the muscles in a maximal position of stretch and holding it there for a determined time period [3].

It is clear in the literature that, in addition to the fast and easy implementation in training programs, PSS is considered one of the safest and most reliable way to obtain range of motion (ROM) gains [4-6]. These flexibility gains are explained based on theories involving neural and mechanical plastic adaptations [7]. The neural plastic adaptations are mainly related to an increase in stretch tolerance, while the mechanical plastic aspects are related to microstructural level responses, such as serial sarcomere number increase (sarcomerogenesis) and tendon or muscle mechanical properties changes [7-9].

Conflicting results have been observed regarding the chronic adaptations of the gastrocnemius medialis muscle (GM's) architecture, such as muscle thickness (MT), pennation angle (PA) and fascicle length (FL), after PSS interventions [9-18]. Chronic PSS has been reported to induce adaptations in GM's MT (increase or decrease), PA (increase or decrease), and FL (increase) [10-12], and the total time under stretching [(TTUS) = (time of each stretching set × number of sets of each session × number of sessions)] in these studies ranged from 48 to 945 minutes [10-12]. In the studies that did not find changes on GM's muscle architecture parameters, TTUS ranged from 36 to 672 minutes [9,14-18]. Furthermore, in addition to their differences in TTUS, the studies differed in terms of population, PSS duration and intensity.

We found three reviews (literature review) about PSS training effects. The first, a systematic review with meta-analysis, did not identify significant changes on muscle architecture parameters of the biceps femoris and triceps surae muscles after PSS protocols with durations between 3 and 8 weeks [19]. The second, concluded that stretching does not appear to confer beneficial changes in muscle size and architecture [20]. The third (also a systematic review with meta-analysis), identified that stretching training induces trivial increases in FL at rest and small increases in FL during stretching, but no increases were observed in either fascicle PA or MT [21]. However, due to the heterogeneity of the reviewed studies in relation to the stretching protocols and the different methodologies used, it is not possible to determine which are the chronic effects of PSS on muscle architecture parameters and how these changes can affect long-term flexibility.

In addition, PSS training effects may be affected by the methodology used for the ultrasound image data collection and data analysis. B-mode ultrasound is the most popular technique used for measuring the architectonic parameters of skeletal muscles [22,23]. Nevertheless, this technique depends of the evaluator's experience and, therefore, without due training, it can be susceptible to measurement errors [24,25]. In general, after the image acquisition process, images are exported to a specific image-analyzer software, in which muscle architecture parameters are measured manually [22,26]. On this step, factors such as different analyzers or analyzers with different time experience, different evaluation moments and the extrapolation method to quantify the FL may compromise the values of the analyzed variables [22,27].

Studies have reported excellent results for intra-analyzer and inter-analyzer reliability for muscle architecture parameters, with high values of intraclass correlation coefficients (ICC>0.82) [24,28]. These outcomes are important because they allow to determine if the method of image analyses, repeated on multiple occasions or by different analyzers, are reliable and sensitive enough to track adaptations [28]. Despite that, apparently only two studies investigated if the magnitude of standard error of measurement (SEM) of ultrasound image analysis surpasses the possible alterations on the variables of interest (MT, PA, and FL), but none of these studies determined this during and after clinical interventions [24,28]. Furthermore, only one study calculated the minimum detectable change (MDC) of these parameters [28], and, despite being reliable, due to their relatively large MDC, they suggest that clinically derived ultrasound measurements of muscle architecture in GM are more likely to be useful to detect differences between populations than to detect changes in muscle architecture following interventions. However, until the present moment, no studies were found in the literature evaluating all three muscle architecture parameters following interventions and performing a reliability analysis of the architectural parameters. Therefore, it is not clear how reliable obtained architectural values are, when determining muscular adaptations after clinical intervention (e.g., chronic stretching), and the assessment of inter-analyzer reliability helps determining strategies to minimize measurement errors [27].

Therefore, the main objective of this study was to evaluate the effects of a six-week PSS program, with different periods of execution (two and five minutes) and after two weeks of detraining, on GM's muscle architecture parameters in healthy subjects. The detraining was evaluated with the aim of verifying if changes in the musculature that may occur with PSS training are maintained when the stretching stimulus is ceased, characterizing a probable long-term structural adaptation of the musculature. The second aim of this study was to determine the inter-analyzer reliability of GM's muscle architecture image analyses process performed by two analyzers with different time-experience with the image-analysis methodology during an exercise intervention. Based on the available evidence in the literature, we hypothesize that muscle architecture parameters will remain unchanged after 6 weeks of PSS training with no changes in the two weeks of follow-up. Moreover, we expect that well-trained analyzers with different experience-time in ultrasound image-analysis can obtain excellent reliability results, independent of the intervention time and image analysis experience time.

METHODS

Study design

A randomized clinical trial study was conducted to assess the effects of PSS on muscle architecture parameters. Prior to its execution, this study was registered in Brazilian Clinical Trials Registry RBR-5j3h3c ((http://www.ensaiosclinicos.gov.br/).

Initially, the participants were randomly divided into three different groups: control group (CG), with no PSS intervention, 2-minutes group (G2) that performed PSS for two minutes and 5-minutes group (G5), that performed PSS for five minutes. For the evaluations, the participants visited the laboratory three times. In the first evaluation (pre-stretching), consent was obtained from all participants, physical activity was evaluated by the international physical activity questionnaire (IPAQ-short form), and the group and limb randomizations were performed. The participants' randomization into the groups was made through an online system (randomization.com) using the randomly exchanged blocks. Limb randomization was made through drawing between dominant and non-dominant limb. Next, the assessment of GM's muscle architecture parameters (MT, PA, and FL) was accomplished with an ultrasound system by an experienced rater. After six weeks of PSS, on the post-intervention evaluation, the same GM's muscle architecture variables were reassessed with a minimum of three days interval from the last PSS session (Figure 1). Two weeks later, the subjects returned to perform the detraining testing session (follow-up). On all occasions, participants were instructed not to perform any vigorous physical activity 48 hours before the tests [29].

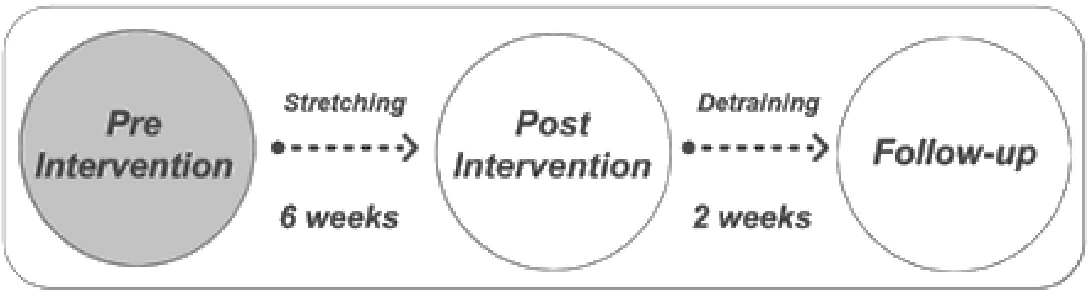

Figure 1. Study design timeline.

Participants

The G∗Power software (Kiel University, Germany) was used to calculate the sample size. A repeated measures ANOVA was used, with within-between interactions (F-test family) and, with a priori power analysis, were used to calculate the sample size with $\alpha = 0.05$, power = 0.80, and effect size f = 0.18. This calculation was performed using values from GM's PA effect size (ES) value (ES: 0.36) from a previous study [17]. A 95% Confidence Interval (CI) and a maximum admitted error of 5% were used. The sample size calculation totaled 30 subjects. To accommodate possible dropouts, 33 subjects were recruited among recreationally active university students.

Participants aged between 18 and 40 years, who were physically active but not engaged in strength and flexibility training based on the IPAQ short form, were included. The exclusion criteria were: (1) having any previous history of musculoskeletal injuries or surgery on the lower limbs; (2) presenting continuous pain on the lower limbs; (3) using analgesics, anti-inflammatory, or muscle relaxants; (4) presenting hypermobility syndrome, according to the Beighton Score; and (5) having any metabolic diseases, such as diabetes mellitus.

All participants signed an informed consent form containing all the information pertinent to this study, approved by the University's Ethics Committee for Human Research (project number: 2.139.313) according to the Declaration of Helsinki.

Procedures

Static Stretching Intervention

PSS training for the plantar flexor muscles was applied with a frequency of three times per week, during six weeks, using a step with at least 15 cm of height. Participants stood erect, with the lower limb being stretched with the forefoot supported on the step, and both arms against a wall in front of the body to provide balance (Figure 2). They were instructed to stretch the ankle with the highest intensity tolerated as possible, until reaching the greatest ankle dorsiflexion angle. The stretching protocol was made in the laboratory with researcher's supervision. G2 remained in this stretching position for 2 minutes while G5 remained for 5 minutes. Although only one lower limb was evaluated, both limbs were stretched during intervention, one at a time.

Measurements of Gastrocnemius Medialis Muscle Architecture

An ultrasound system (SSD-4000; Aloka Inc., Tokyo, Japan) with a 60mm linear array probe (7.5 MHz) was used to determine GM's MT, PA, and FL. Three ultrasound images were obtained at each time point (i.e., before, after, and two weeks post-intervention) by an experienced evaluator (3 years of experience with the technique) that was blinded to the participants group and time-point of intervention. During image acquisition, the subjects remained lying down on a stretcher in a prone position, with the feet positioned out of the stretcher and the ankle joint at 0° (neutral position). The ankle joint position was maintained with the aid of a goniometer. The ultrasound probe was positioned longitudinally to the muscle fibers and perpendicular to the skin at 30% of the distance between the popliteal crease and the lateral malleolus [30]. A layer of water-soluble transmission gel was used to provide acoustic contact between the skin and the probe.

For the muscle architecture analysis, MT was considered the distance between the deep and the superficial aponeuroses and was calculated through the mean value of five parallel lines drawn between these reference points along each ultrasonography image

(Figure 3a). FL was considered as the length of the fascicular path between the two aponeuroses, while PA was determined as the angle between the fascicle and the deep aponeurosis (Figure 3b) [30]. The probe position is shown in Figure 3c. When the final part of the fascicle length was out of the probe's field of view, FL was estimated as recommended in previous studies [31-33]. Ultrasonography images were analyzed through ImageJ 1.42q software (National Institute of Health, Bethesda, Maryland, USA). Mean values were obtained from three ultrasound images of GM in each moment to determine GM's morphological adaptations.

Figure 2. Passive static stretching exercise.

Inter-analyzer reliability of ultrasound image-analysis

For determining the inter-analyzer reliability, two independent investigators, with different time-experience in ultrasonography image analysis (analyzer A = one year, analyzer B = 6 months), manually analyzed all ultrasonographic images obtained by the experience rater during the pre-intervention, post-intervention, and follow-up assessments for each group. These analyzers were blinded to the identity of the participants and time-point at which each ultrasonography image was obtained. Each analyzer measured the muscle architecture parameters (MT, PA, and FL) once for each of the three images obtained in each time-point. The mean value of investigator A's results from each moment of evaluation was compared against the mean value of the investigator B's results from each evaluation session to determine inter-rater-test.

Statistical Analysis

Data are presented as mean and standard deviation (SD). Data were subjected to normality and homogeneity tests, by the Shapiro Wilk and Levene tests, respectively. A two-way ANOVA (group, moment) for repeated measures, followed by Bonferroni's post-hoc test, was used to verify differences between moments (pre, post and follow-up) and groups (G2, G5 and CG).

The ES was obtained by the Cohen Equation (Cohen's d), by determining the mean difference along the time point of evaluations between the groups, and then dividing the result by the pooled standard deviation (within-group's effect size). The calculated ES was 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) [34].

To verify the outcome measures' reliability, the ICC (2,1 - Agreement), CI, SEM, MDC and coefficient of variance (CV) were calculated. The ICC was classified as excellent ($r > 0.90$); good ($r = 0.75 - 0.90$); moderate ($r = 0.50 - 0.75$) or poor ($r < 0.50$) [35]. SEM was estimated using equation: $SEM = SD * \sqrt{(1\text{-}ICC)}$ [36]. The MDC was estimated based on a 95% CI (95%CI), where $MDC = 1.96 * SEM$ [37].

The level of significance adopted for all analyses was set at 5%. All statistical procedures were performed using the statistical package SPSS 20.0 (IBM, Chicago, USA) for Windows.

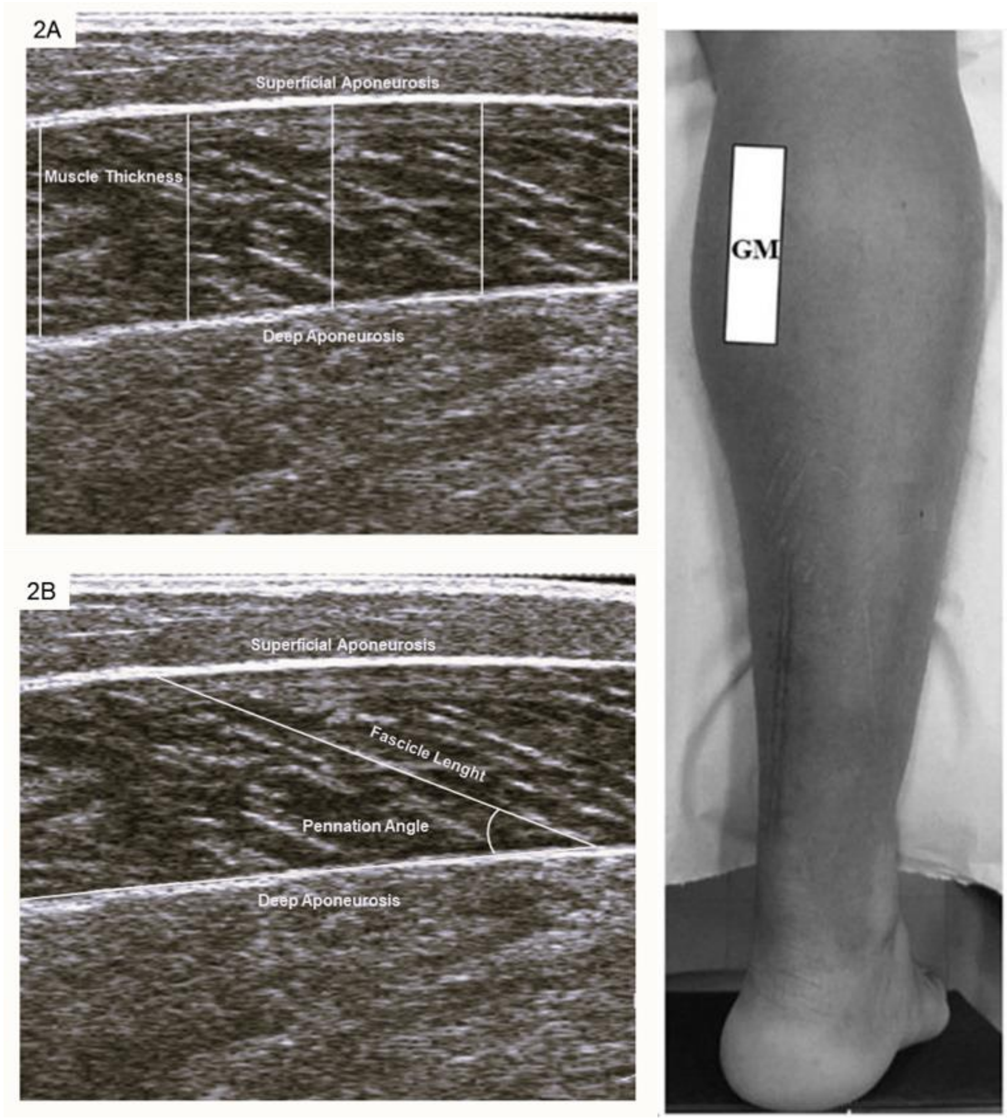

Figure 3. Ultrasound image from GM's, showing an example of the analyzed muscle architecture parameters. A: Muscle Thickness B: Fascicle Length and Pennation Angle. C: positioning of the ultrasound probe.

RESULTS

In total, thirty adults (24 females and 6 males) participated in this study - three individuals did not meet the eligibility criteria and were excluded before randomization - with 10 participants in each group. No significant differences were found between the three groups ($p>0.05$) for the baseline characteristics (Table 1). Between the beginning and the end of the intervention, no sample losses were recorded. However, in the follow-up assessment, four subjects in CG, four in G2 and three subjects in G5 did not participate in this evaluation (Figure 4). Intention to treat analysis was performed in the follow-up evaluation.

Effects of static stretching on muscle architecture parameters

The mean and standard deviation values of the PSS training in each moment for MT, PA and FL are reported on Table 2. The two-way ANOVA indicated no significant differences between moments and groups for any muscle architecture variable. All calculated effect sizes were classified as trivial (ranged from 0.003 to 0.08; Table 2).

Table 1. Characteristics of the participants in the three groups.

		CG (10)	G2(10)	G5(10)	*p-value*
Age (years)		23.80 ± 3.73	22.60 ± 3.02	24.90 ± 6.44	0.541
Body Mass (kg)		70.92 ± 18.91	62.97 ± 11.47	69.19 ± 14.02	0.471
Height (cm)		167.50 ± 7.94	163.15 ± 7.51	163.81 ± 10.22	0.491
Sex	F	7 (70%)	9 (90%)	8 (80%)	0.550
	M	3 (30%)	1 (10%)	2 (20%)	
Leg length (cm)		38.95 ± 2.24	38.65 ± 2.47	37.90 ± 3.12	0.655

CG: Control Group; G2: 2 Minutes Group; G5: 5 Minutes Group; kg = kilograms; cm = centimeters; F: Female; M: Male.

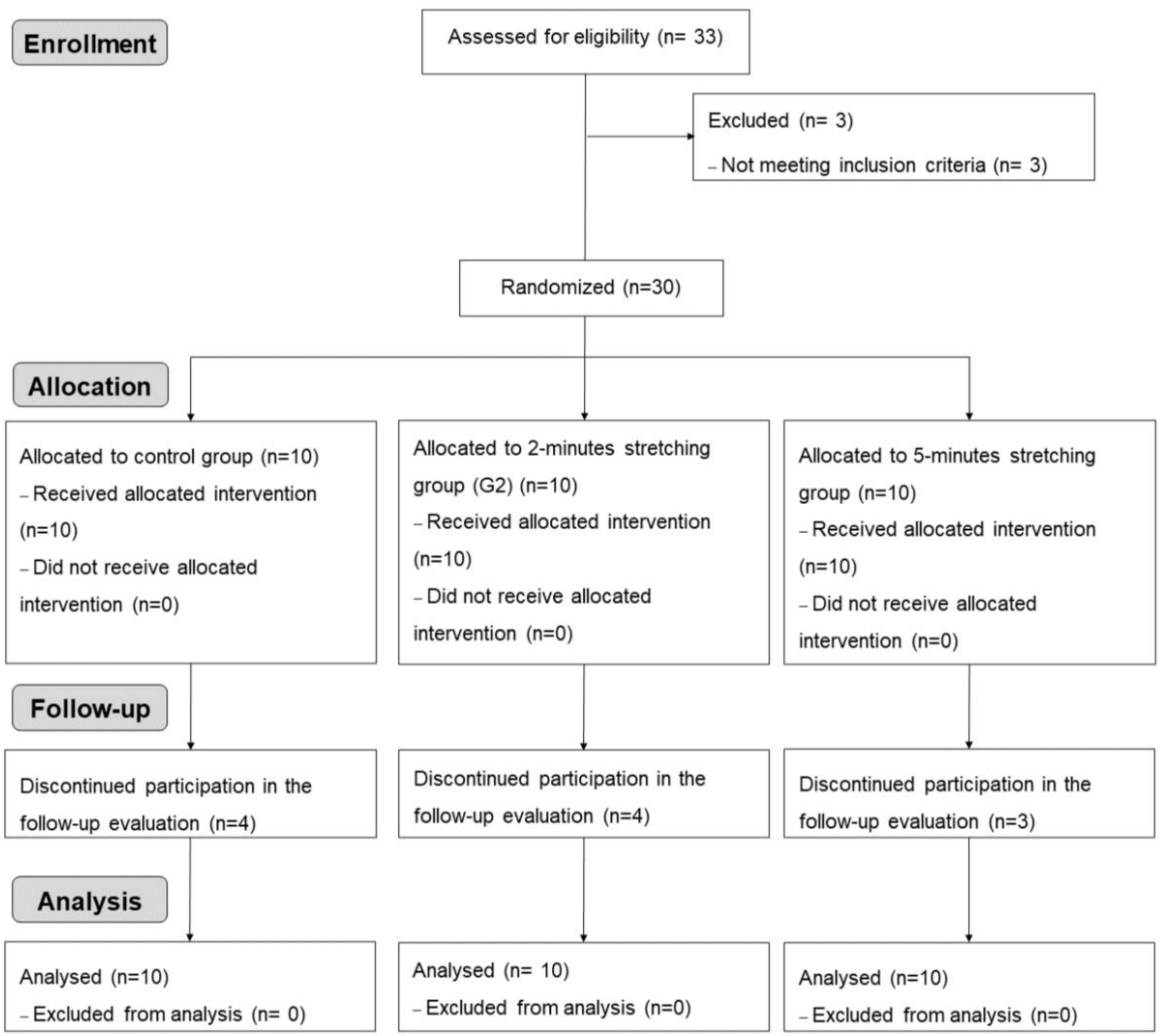

Figure 4. Flowchart of the participants during each stage of the protocol.

Inter-analyzer reliability of the image analysis process

In total, each investigator analyzed 237 images, 90 for each of the first two assessments (1 muscle x 3 images x 30 subjects = 90 images per evaluation) and 57 for the last one (follow-up = 1 muscle x 3 imagens x 19 subjects). The ICC results of GM's MT presented an excellent inter-analyzer reliability for all three time-points: pre-intervention, post-intervention, and follow-up (Table 3). In addition, in all evaluation moments, good inter-analyzer reliability was found for the FL measures. Furthermore, good inter-analyzer reliability was also observed for the PA post-intervention moment, while on the pre-intervention and follow-up moments the ICC values were considered moderated. When the three reliability parameters were compared, MT presented the highest values, followed by FL and PA. In all moments, MT presented the highest reliability results.

Table 2. Gastrocnemius medialis fascicle length, pennation angle and muscle thickness, at pre-intervention, post-intervention, and follow-up.

	Pre-intervention (Mean ± SD)	Post-Intervention (Mean ± SD)	Follow-up (Mean ± SD)	ES Cohen's d Pre-intervention to Post-intervention	ES Cohen's d Post-intervention to Follow-up
Fascicle Length (cm)					
CG	4.04 ± 0.53	4.09 ± 0.48	4.42 ± 0.30	0.010	0.081
G2	4.35 ± 0.84	4.74 ± 0.77	5.02 ± 0.55	0.048	0.042
G5	4.39 ± 0.82	4.75 ± 1.16	4.71 ± 0.95	0.040	0.005
Pennation Angle (◦)					
CG	26.77 ± 3.52	26.44 ± 2.87	26.54 ± 3.05	0.010	0.003
G2	27.40 ± 4.29	25.23 ± 3.94	26.52 ± 2.36	0.053	0.040
G5	27.64 ± 6.70	26.46 ± 5.46	26.57 ± 2.80	0.019	0.003
Muscle Thickness (cm)					
CG	1.71 ± 0.28	1.73 ± 0.33	1.88 ± 0.18	0.007	0.058
G2	1.87 ± 0.42	1.93 ± 0.41	2.10 ± 0.41	0.013	0.043
G5	1.89 ± 0.25	1.95 ± 0.30	2.00 ± 0.28	0.020	0.017

p = p-value; CG = Control group; G2 = 2-minutes group; G5 = 5-minutes group; ES = Effect size; ns = not significant; ° = degrees; cm = centimeters.

DISCUSSION

The main goal of the present study was to evaluate the effects of a six-week PSS program, with different periods of execution (two and five minutes), on GM's muscle architecture parameters. After six training weeks, we did not find significant effects of two and five minutes of PSS on muscle architecture parameters (MT, FL, PA) of GM's during all evaluation times (pre, post and follow-up).

Our lack of statistical differences on GM's muscle architecture parameters (MT, PA, and FL) after six weeks of a stretch-training protocol between all groups (CG, G2, and G5) corroborate the findings from previous studies that utilized 4-6 weeks of PSS for the GM. Nakamura et al. [16] performed two series of 60s for four weeks daily (TTUS = 56 minutes). Sato et al. [18] performed six weeks of PSS with two different frequencies (TTUS = 36 minutes): once a week (360s) and three times a week (120s per session). Konrad and Tilp [38] performed four series of 30s for six weeks, five times per week (TTUS = 60 minutes). Similar studies that evaluated GM's architecture with greater intervention time than ours also found no relevant differences on muscle architecture parameters (TTUS = 672; 450; 300 minutes, respectively) [14,15,17]. Therefore, it seems improbable that PSS in humans induces similar effects in the muscle structure to those seen in the stretching interventions performed in animal studies (i.e., FL increase) [39]. Furthermore, a review on chronic stretching changes reported that stretch-training durations of 3-8 weeks do not alter muscle or tendon properties, although it can increase the extensibility and tolerance of the muscle to a greater tensile force [19]. In addition, it is important to highlight that there are no longitudinal studies examining morphological changes over years of static stretching [7]. Therefore, a probable explanation on the literature for the ROM increase following stretching is due to an altered perception of stretch, and pain or stretch tolerance [37]. Despite both sensory aspects and muscle architecture playing an important role in determining joint ROM, joint ROM is also directly related to the structural (e.g., length, thickness, cross-sectional area) and mechanical (e.g., stress, strain, elastic modulus) of joint capsule, ligaments, fascia and tendons, in addition to possible adhesions between different connective tissue structures, and chemical substances responsible for tissue lubrication and nutrition [40]. However, it is not clear what is the contribution of each of these variables to the ROM increase following PSS training.

Table 3. Inter-analyzer reliability results for the Gastrocnemius Medialis (GM's) muscle architecture parameters at pre-intervention, post-intervention, and follow-up evaluations. Values of mean ± standard deviation (SD) of two analyzers, intraclass correlation coefficient (ICC), 95% confidence interval (95%CI), p-value, standard error of the measurement (SEM), minimum detectable change (MDC) and coefficient of variation (CV).

	Mean ± SD	ICC	95%CI	*p-value*	SEM	MDC	CV (%)
Pre-Intervention							
FL - (cm)	4.29 ± 0.74	0.755	0.410-0.892	<0.001	0.37	0.72	17.3
PA - (◦)	27.20 ± 4.80	0.518	-0.005-0.770	<0.001	3.33	6.53	17.6
MT - (cm)	1.82 ± 0.32	0.961	0.919-0.981	<0.001	0.06	0.12	17.8
Post-Intervention							
FL - (cm)	4.51 ± 0.87	0.754	0.402-0.890	<0.001	0.43	0.85	19.4
PA - (◦)	25.99 ± 4.06	0.796	0.276-0.923	<0.001	1.83	3.59	15.6
MT - (cm)	1.86 ± 0.35	0.955	0.908-0.978	<0.001	0.07	0.15	19.0
Follow-up							
FL - (cm)	4.72 ± 0.87	0.761	0.308-0.914	<0.001	0.42	0.83	18.4
PA - (◦)	26.54 ± 3.47	0.619	-0.173-0.871	<0.001	2.14	4.20	13.1
MT - (cm)	2.00 ± 0.39	0.928	0.806-0.973	<0.001	0.11	0.21	19.6

FL: Fascicle Length; PA: Pennation Angle; MT: Muscle Thickness; ˚ = degrees; cm = centimeters.

The second aim of our study was to determine the reliability of manual measurement of muscle architecture between two analyzers with different experience times on different time points of the intervention protocol. We identified excellent reliability for MT, good for FL and moderate-to-good for PA. However, measurements need to be done with caution, mainly for PA analysis.

Previous cross-sectional reliability studies reported excellent ICC values for inter-rater analysis of GM's architecture. May et al. [28] observed ICC (r) of 0.95, 0.95, and 1.00 and Konig et al. [24] observed ICC (r) of 0.93, 0.76 and 0.96 for FL, PA, and MT, respectively. Our ICC results of muscle architecture parameters demonstrated similarly high inter-analyzer reliability for MT (pre-intervention: 0.961; post-intervention: 0.955; follow-up: 0.928). However, for the PA (pre-intervention: 0.518; post-intervention: 0.796; follow-up: 0.619) and FL (pre-intervention: 0.755; post-intervention: 0.754; follow-up: 0.761) analysis, the ICC reliability values were lower than those of the previous studies, except for the ICC of PA on the post-intervention time [24,28].

Greater variability of the PA's ICC values may be associated with the novice experience of two analyzers (6 months and 1 year) [27]. Moreover, these parameters are highly sensitive to image interpretation, so more analyzers involved in the analysis process could decrease the reliability [24]. Therefore, a longer training period in the analysis of muscle architectural parameters is suggested for obtaining a greater PA's reliability [27].

The SEM is a measure of absolute reliability and is expressed in the actual units of measurement, making it easy to interpret. In other words, the smaller the SEM, the greater the reliability. The MDC is considered the minimal amount of change that is unlikely to be due to chance variation in measurement. Additionally, MDC values indicate that the magnitude of measurement error when using this method can exceed the potential changes in muscle architecture following a clinical intervention [28]. Therefore, clinicians should be aware of these MDC values when interpreting results obtained using similar techniques and consider implementing more rigorous test-retest procedures if the intention is to monitor changes in muscle architecture over time.

We observed lower values of SEM and MDC compared to May et al. [28], except for the MDC of PA, which was similar. Our SEM values corroborate with those reported by Konig et al. [24] for all muscle architecture parameters. We believe that this variation between the studies occurred due to the probe size and the different positions of the knee utilized during data collection [24,28].

Our study has some limitations. One limitation was the fact that the proportion of males was smaller than females. Therefore, is not possible to extrapolate our results for the male population. Moreover, we did not control the effects of the different phases of the menstrual cycle or the use of hormonal contraceptives in our female participants. The contribution of non-muscle structures (joint capsules, ligaments, tendons, and skin) was neglected. In addition, when the probe length is short (between 4-6cm) or the extended-field-of-view is not accessible, it is necessary to use the extrapolation method for quantifying FL [22]. This method allows to measure fascicles that extend beyond the ultrasound's field of view on 2B-mode. Linear extrapolation assumes that a fascicle follows a linear path and does not account for fascicle or aponeurosis curvature, which has important implications for the accurate calculation of muscle architecture [24]. We also did not evaluate the chronic effects of PSS on the muscle architecture of the other two ankle plantar flexors (i.e., gastrocnemius lateralis and soleus), and therefore we cannot ascertain if our stretching protocol was able to change these muscles architecture. Finally, we used only PSS, and muscle architecture might be more adaptable to dynamic stretching protocols.

Practical Application

PSS may be considered a viable alternative in physical exercise for increasing flexibility, despite that muscle architecture does not seem to be a factor to explain the gains in joint ROM. Future studies should assess neural aspects related to pain/discomfort tolerance during stretching. In addition, for morphological changes to occur, it is necessary to provide a variety of stressors to the motion (i.e., neuromuscular, and skeletal) system. Therefore, physical therapists and trainers should incorporate a range of stretching styles or modalities, including static, dynamic, and proprioceptive neuromuscular facilitation, to optimize muscle architectural adaptations [7].

The good to excellent inter-analyzer reliability is evidence that MT and FL are reliable muscle architecture parameters when obtained by different analyzers. The inter-analyzers PA's moderate reliability results suggest that a higher training period should be observed when evaluating this parameter.

CONCLUSION

In summary, the results of the present study demonstrated that a 6-week passive stretching program with different durations of stretching execution for the plantar flexors did not generate adaptations on GM's muscle architecture in young healthy subjects. In addition, inter-analyzer reliability for MT is excellent, for FL is good and for PA is moderate-to-good, and therefore these variables may be used to evaluate muscle architecture in intervention studies.

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