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EFFECTS OF DIFFERENT FOAM ROLLING PROTOCOLS ON MUSCULOTENDINOUS PROPERTIES AND FUNCTIONAL PERFORMANCE: A COMPREHENSIVE STUDY

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ABSTRACT

EFFECTS OF DIFFERENT FOAM ROLLING PROTOCOLS ON MUSCULOTENDINOUS PROPERTIES AND FUNCTIONAL PERFORMANCE: A COMPREHENSIVE STUDY

Foam rolling (FR) is a self-massage technique widely used by professionals in the field of rehabilitation and physical training. The technique's popularity stems from the functional effects reported following its application, such as increased flexibility, and decreased muscle pain. Similar to other massage and self-massage techniques, it is speculated that these functional effects occur due to changes in the myotendinous structures' stiffness. However, the current studies present conflicting results regarding changes in soft tissue stiffness after FR or other massage techniques, as well as their most recommended protocols. Regarding FR protocols, different application times per sets have been investigated, but results are still conflicting. Furthermore, the relationship between application time and its effects remains unclear. Thus, the objective of this master's degree dissertation is to identify the effects of different massage techniques described in the literature on soft tissue stiffness and to verify the effects of different FR protocols on the musculotendinous properties of plantar flexors and functional performance. The dissertation was divided into two chapters. In Chapter I, a systematic review with meta-analysis was developed to verify the effects of different massage and self-massage techniques (including FR) on the stiffness of soft tissues (myotendinous unit [MTU], muscle, and tendon tissues). The searches were done in the following databases: PubMed, Embase, Web of Science, PEDro, Preprint.org, and MedRxiv. 2167 studies were identified and 27 clinical trials were included. The studies data were extracted and the risk of bias was analyzed using the PEDro scale. Our analysis showed that massage techniques decreased muscle stiffness (SMD: -0.392; CI95%: -0.545 to -0.239; p<0.001; I²: 49%), mainly in rectus femoris (SMD: -0.413; CI95%: -0.642 to -0.184; p<0.001; I²: 12%;) and gastrocnemius medialis (SMD: -0.353; CI95%: -0.553 to-0.152, p: 0.001; I²: 0%); but did not change tendon stiffness and MTU (SMD: 0.146; CI95%: -0.046 to 0.339; p: 0.136; I²: 34%). Chapter II presents the results of an experimental study that investigated the effects of different FR protocols (FR90: three sets of 30s, FR180: three sets of 60s) and a control condition (CTRL) on the musculotendinous properties of plantar flexors, as well as functional performance. The Achilles tendon (AT) evaluations were: a) morphological properties (cross-sectional area and tendon length) assessed at rest by ultrasonography; b) mechanical (force, elongation, stiffness), and; c) material properties (stress, strain, Young's modulus) assessed during ramp contractions in an isokinetic dynamometer. The passive stiffness of the gastrocnemius medialis and the MTU were also evaluated. Functional performance was assessed by the unilateral countermovement jump height by two-dimensional kinematics. The skin temperature (plantar flexors and AT) was assessed by thermography. Reported pain in calf and/or other body region was verified using a visual analogue scale after both interventions with FR. No changes were found in AT morphological properties. However, the AT stiffness (CTRL: -8.0%, FR90: -4.3%, FR180: -30.5%), and Young's modulus (CTRL: -8.8%, FR90: -4.4%, FR180: -31.3%) decreased after all conditions. Despite no difference between the conditions, the relative reduction in AT stiffness and Young's modulus was greater in the FR180. In addition, the muscle (CTRL: -33.2%, FR90: -43.2%, FR180: -41.5%) and MTU passive stiffness (CTRL: -30.0%, FR90: -32.9%, FR180: -39.3%), and jump height (CTRL: -6.3%, FR90: -1.4%, FR180: -4.5%) decreased after all conditions; whereas skin temperature increased for all conditions in plantar flexors (mean - CTRL: 1.8%, FR90: 1.7%, FR180: 2.6%) and AT region (mean - CTRL: 2.5%, FR90: 1.5%, FR180: 2.5%). Reported pain in the upper limbs was higher in FR180, with no differences between conditions for calf region. The reduction in stiffness that occurred in all conditions may have been due to the evaluation stimuli and the creep effect. Considering both studies, it is concluded that massage techniques can reduce muscle and tendon stiffness, especially in the rectus femoris, gastrocnemius medialis, and AT, possibly due to the thixotropic property of tissues and/or neurophysiological mechanisms (i.e., cross-bridge breaks or inhibitory effect in nociceptors). However, as the FR effects on AT depends on a longer application time, this may not bring benefits due to discomfort during rolling. In addition, the control group is essential in studies that involve massage techniques in order to identify effects inherent to interventions.

Keywords: Self-massage; massage; Achilles tendon; plantar flexors; stiffness.

RESUMO

EFEITOS DE DIFERENTES PROTOCOLOS DE *FOAM ROLLING* NAS PROPRIEDADES MUSCULOTENDÍNEAS E DESEMPENHO FUNCIONAL: UM ESTUDO ABRANGENTE

O *foam rolling* (FR) é uma técnica de automassagem muito utilizada por profissionais da área de reabilitação e treinamento físico. A popularidade da técnica decorre dos efeitos funcionais relatados após sua aplicação, como aumento da flexibilidade e diminuição da dor muscular. Semelhante a outras técnicas de massagem e automassagem, especula-se que estes efeitos funcionais ocorram devido a alterações na rigidez das estruturas miotendíneas. Porém, os estudos atuais apresentam resultados conflitantes em relação às alterações na rigidez dos tecidos moles após FR ou outras técnicas de massagem, bem como seus protocolos mais recomendados. Em relação aos protocolos FR, foram investigados diferentes tempos de aplicação por série, mas os resultados ainda são conflitantes. Além disso, a relação entre o tempo de aplicação e os seus efeitos ainda não é clara. Assim, o objetivo desta dissertação de mestrado é identificar os efeitos de diferentes técnicas de massagem descritas na literatura na rigidez de estruturas miotendíneas e verificar os efeitos de diferentes protocolos de FR nas propriedades musculotendíneas e no desempenho funcional dos flexores plantares. A dissertação foi dividida em dois capítulos. No Capítulo I, foi desenvolvida uma revisão sistemática com meta-análise para verificar os efeitos de diferentes técnicas de massagem e automassagem (incluindo FR) na rigidez dos tecidos moles (unidade miotendína [UMT], tecidos muscular e tendíneo). As buscas foram feitas nas seguintes bases de dados: PubMed, Embase, Web of Science, PEDro, Preprint.org e MedRxiv. Foram identificados 2.167 estudos e incluídos 27 ensaios clínicos. Os dados dos estudos foram extraídos e o risco de viés dos artigos foi analisado por meio da escala PEDro. Nossa análise mostrou que as técnicas de massagem diminuíram a rigidez muscular (SMD: -0,392; IC95%: -0,545 a -0,239; p<0,001; I²: 49%), principalmente no reto femoral (SMD: -0,413; IC95%: -0,642 a -0,184; p<0,001; I²: 12%) e gastrocnêmio medial (SMD: -0,353; IC95%: -0,553 a -0,152, p: 0,001; I²: 0%); mas não alterou a rigidez do tendão e a UMT (SMD: 0,146; IC95%: -0,046 a 0,339; p: 0,136; I²: 34%). O Capítulo II apresenta os resultados de um estudo experimental que investigou os efeitos de diferentes protocolos de FR (FR90: três séries de 30s, FR180: três séries de 60s) e uma condição controle (CTRL) nas propriedades musculotendíneas dos flexores plantares, bem como desempenho funcional. As avaliações do tendão de Aquiles (TA) foram: a) propriedades morfológicas (área de secção transversa e comprimento do tendão) avaliadas em repouso por ultrassonografia; b) propriedades mecânicas (força, deformação, rigidez) e; c) materiais (*stress*, *strain* módulo de Young) avaliadas durante contrações em rampa em um dinamômetro isocinético. A rigidez passiva do gastrocnêmio medial e da UMT também foram avaliados. O desempenho funcional foi avaliado pela altura do salto *countermovement* unilateral por meio de cinemática bidimensional. A temperatura da pele (flexores plantares e TA) foi avaliada por termografia. A dor relatada na panturrilha e/ou outras regiões corporais foi verificada por meio de escala visual analógica após ambas as intervenções com FR. Não foram encontradas alterações nas propriedades morfológicas do TA. No entanto, a rigidez (CTRL: -8,0%, FR90: -4,3%, FR180: -30,5%) e o módulo de Young do TA (CTRL: -8,8%, FR90: -4,4%, FR180: -31,3%) diminuíram após todas as condições. Apesar de não haver diferença entre as condições, a redução relativa na rigidez do TA e no módulo de Young foi maior no FR180. Além disso, a rigidez passiva muscular (CTRL: -33,2%, FR90: -43,2%, FR180: -41,5%) e da UMT (CTRL: -30,0%, FR90: -32,9%, FR180: -39,3%) e altura de salto (CTRL: -6,3%, FR90: -1,4%, FR180: -4,5%) diminuíram após todas as condições; enquanto a temperatura da pele aumentou para todas as condições nas regiões de flexores plantares (média - CTRL: 1,8%, FR90: 1,7%, FR180: 2,6%) e TA (média - CTRL: 2,5%, FR90: 1,5%, FR180: 2,5%). A dor referida nos membros superiores foi maior no FR180, sem diferenças entre as condições para região da panturrilha. A redução da rigidez em todas as condições pode ter ocorrido devido aos estímulos de avaliação e ao efeito de *creep*. Considerando ambos os estudos, conclui-se que as técnicas de massagem podem reduzir a rigidez muscular e tendínea, especialmente no reto femoral, gastrocnêmio medial e TA, possivelmente devido à propriedade tixotrópica dos tecidos e/ou mecanismos neurofisiológicos (i.e., quebras de pontes cruzadas ou efeito inibitório sobre os nociceptores). Porém, como os efeitos da FR sobre o TA dependem de um tempo de aplicação maior, isso pode não trazer benefícios devido ao desconforto durante o rolamento. Além disso, o grupo controle é essencial em estudos que envolvem técnicas de massagem para identificar os efeitos inerentes às intervenções.

Palavras-chave: Automassagem; massagem; tendão de Aquiles; flexores plantares; rigidez.

LIST OF ABBREVIATION

AT	Achilles tendon
BW	Body weight
CMJ	Countermovement jump
CSA	Cross-sectional area
CTRL	Control condition
EMG	Electromyography
ES	Effect size
FR	Foam rolling
FR180	Foam rolling for three sets of 60s
FR90	Foam rolling for three sets of 30s
GM	Gastrocnemius medialis
GRF	Ground reaction force
ICC	Intraclass correlation coefficient
IPAQ	International Physical Activity Questionnaire
MTJ	Myotendinous junction
MTU	Myotendinous unit
MVIC	Maximal voluntary isometric contraction
PA	Pennation angle
PF	Plantar flexors
POST	Post-intervention evaluations
PRE	Pre-intervention evaluations
ROI	Region of interest
ROM	Range of motion
TE	Typical error
TL	Tendon length
US	Ultrasonography
VISA-A	Victorian Institute of Sports Assessment self-administered Achilles questionnaire

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SUMMARY

PREFACE

Massage includes different techniques aiming to mobilize soft tissues through the application of mechanical pressure (Weerapong *et al.*, 2005). It is frequently used in the sporting environment to enhance performance and/or aid muscle recovery (Crommert *et al.*, 2015; Mine; Lei; Nakayama, 2018). Among these techniques, there is the self-massage, wherein the individual uses their body weight to apply pressure to soft tissues (Cheatham *et al.*, 2015). The application can involve the use of balls, sticks, plastic tubes, or foam rollers (Capobianco; Mazzo; Enoka, 2019; Cheatham *et al.*, 2015). The foam rolling (FR) as a self-massage technique became popular among the general population in recent years (Cheatham, 2019). This intervention is performed using a dense and rigid foam roller, mobilizing and applying pressure on tissues such as fascia, muscles, and tendons, which are stretched and massaged upon contact with the device. Despite its popularity, its effects and mechanisms are still not understood.

The FR and other massage techniques are often associated with the term “myofascial release”, suggesting a biomechanical mechanism for reducing soft tissue stiffness (Behm, Wilke, 2019). Therefore, this “release” would provide the functional changes, such as increased flexibility (Beardsley; Škarabot, 2015) or jump performance (Hendricks *et al.*, 2020). This possible stiffness reduction could occur due to viscoelastic properties in these tissues (Meyer *et al.*, 2011), which are susceptible to the thixotropic effects (i.e., when a viscous material becomes less dense and more fluid due to heat, shaking or pressure exposure, that is, what happens during massage) (Beardsley; Škarabot, 2015; Reiner *et al.*, 2021; Schleip, 2003). However, this does not seem to be a consensus in the literature, since some studies found no changes in soft tissues stiffness after massage (Chang *et al.*, 2021; Ikeda *et al.*, 2019; Thomson *et al.*, 2015). A systematic review investigated the effects of FR on fascial and muscle stiffness (Glänzel *et al.*, 2023) and found no effects of FR. However, it did not investigate tendon or myotendinous unit (MTU) stiffness. Since then, new studies have been published (Nakamura *et al.*, 2022; Klich *et al.*, 2022; Chang *et al.*, 2021), but conflicts persist regarding the effects on: a) muscle stiffness (Nakamura *et al.*, 2022; Chang *et al.*, 2021); b) tendon stiffness (Klich *et al.*, 2022; Chang *et al.*, 2021), and; c) the most recommended FR protocol (regarding application time) for structural or functional effects. Therefore, this dissertation aims to identify the effects of different massage techniques described in the literature on soft tissue stiffness and to verify the effects of different FR protocols on the musculotendinous properties of plantar flexors, functional performance. Thus, the dissertation is composed of two chapters organized in the form of original papers.

In Chapter I, we conducted a systematic review investigating the effects of different massage techniques on MTU, muscle, and tendon stiffness. The literature search was performed in four online databases (PubMed, Embase, Web of Science, and PEDro) and in two preprint databases (Preprint.org and MedRxiv). The syntheses of included studies' data and the studies' quality appraisal assessment were performed by two independent investigators. Assessed outcomes were myotendinous structures stiffness, and the available data were pooled through meta-analyses.

In Chapter II, we aimed to investigate the effects of two different FR protocols (FR90: three sets of 30s; FR180: three sets of 60s) on musculotendinous properties, and functional performance of Achilles tendon and plantar flexors. We conducted a randomized crossover study involving 20 participants. Weight-bearing during the FR protocols was controlled by a force platform, in order to investigate the effects of application time. Assessments of Achilles tendon morphological (cross-sectional area, tendon length), mechanical (force, elongation, stiffness), and material (stress, strain, Young's modulus) properties were conducted before and after each condition. Additionally, the muscle and MTU passive stiffness, unilateral countermovement jump performance, and skin temperature were investigated before and after the intervention.

CHAPTER I: MASSAGE TECHNIQUES ACUTE EFFECTS ON THE MYOTENDINOUS STRUCTURES' STIFFNESS: A SYSTEMATIC REVIEW AND META-ANALYSIS

ABSTRACT

Introduction: Massage techniques are widely used in rehabilitation/physical training and sports. The mechanical stimulus during massage interventions could change the stiffness of the myotendinous structures. However, the massages' effects on the muscle, tendon and myotendinous unit (MTU) stiffness are unclear. This systematic review with meta-analysis aimed to investigate clinical trials that tested the massage techniques' acute effects on the myotendinous structure's stiffness in healthy adults or athletes. Methods: This review (CRD42022307586) was performed following the Cochrane the Preferred Reporting Items for Systematic Reviews and Meta-Analyzes Recommendations. Syntheses of included studies' data were performed, and the PEDro scale was used to assess the studies' quality appraisal. Assessed outcomes were myotendinous structures stiffness. Available data were pooled through meta-analyses. Results: 2167 studies were identified and 27 clinical trials were included. The most evaluated technique (n=17) was the self-massage applied through foam roller, whereas few studies evaluated manual techniques (n=5) and instrumental massage applied by a therapist (n=5). The massage techniques showed a small effect for decreased muscle stiffness (SMD: -0.392; CI95%: -0.545 to -0.239; $p<0.001$; I²: 49%), mainly in rectus femoris (SMD: -0.413; CI95%: -0.642 to -0.184; $p<0.001$; I²: 12%;) and gastrocnemius medialis (SMD: -0.353; CI95%: -0.553 to-0.152, p: 0.001; I²: 0%); but did not change MTU (SMD: 0.146; CI95%: -0.046 to 0.339; p: 0.136; I²: 34%) and tendon stiffness. These results may arise from the thixotropic effects on muscle tissue, i.e., through compression and/or heat during massage, muscle tissue becomes less viscous, thereby reducing its stiffness. The same effects may not happen in tendon and MTU due, probably, the different composition of the connective tissue. Conclusion: Massage techniques do not change the tendon and MTU, but can induces small decreases with small effects on the muscle stiffness, especially in rectus femoris and gastrocnemius medialis.

Keywords: Self-massage. Myofascial Release. Manual Treatment. Muscle Stiffness. Tendon Stiffness.

INTRODUCTION

Massage techniques are widely used in rehabilitation and sports (Best *et al.*, 2008; Pérez-Bellmunt *et al.*, 2021). These techniques have been shown to induce relaxation (Weerapong *et al.*, 2005), reduce muscle soreness (Davis *et al.*, 2020; Dupuy *et al.*, 2018; Guo *et al.*, 2017), enhance flexibility (i.e., increased joint range of motion [ROM]) (Beardsley & Škarabot, 2015; Davis *et al.*, 2020; Skinner *et al.*, 2020), and alleviate non-specific musculoskeletal pain (Furlan *et al.*, 2015; van den Dolder *et al.*, 2014). Through the years, these effects were explained by different speculative mechanisms that can occur simultaneously by the mechanical pressure during intervention, such as psychological (relaxation and wellness) (Weerapong *et al.*, 2005), physiological (increased muscle and skin temperature, and increased blood flow and supply) (Behm; Wilke, 2019; Weerapong *et al.*, 2005), neural (as decreased neuromuscular excitability) (Aboodarda *et al.*, 2018; Behm; Wilke, 2019; Keraútret *et al.*, 2021; Weerapong *et al.*, 2005), and biomechanical mechanisms (as decreased tissues stiffness) (Beardsley; Škarabot, 2015; Weerapong *et al.*, 2005).

The biomechanical factors became recognized as a potential main mechanism of massage when manual and instrumental techniques began to be described as “myofascial release”. In theory, the mechanical stimuli would eliminate "restrictions" in myofascial tissue (Barnes, 1997) and enable the reported effects (i.e., increased flexibility and relaxation) in both sports and rehabilitation contexts. However, the current evidence indicates that the term "myofascial release" suggests a misleading mechanism, as these practices would not break up or remove myofascial adhesions (Behm; Wilke, 2019; Schleip, 2003). Thus, all these methods (manual or instrumental) can be considered as different massage techniques, as they have in common the soft tissues mobilization (APTA, 2014; Sheldon; Karas, 2022).

Although massage techniques may not directly “release” myofascial adhesions, they do involve mechanical stimulus, which could lead to other biomechanical effects, as decreased myotendinous structures stiffness (Behm; Wilke, 2019, Weerapong *et al.*, 2005). Considering that the stiffness is the capacity of a tissue to resist elongation by an applied force (Baumgart *et al.*, 2019), a decreased muscle, tendon or muscle-tendon unit (MTU) stiffness (which represents both muscle and tendon tissue mechanics) (Morse *et al.*, 2008) would allow greater tissue elongation, which could lead to increase or decrease flexibility of these structures (Behm; Wilke, 2019; Weerapong *et al.*, 2005). Therefore, the mechanical stimuli of massage techniques could reduce the stiffness of these tissues, due to the thixotropic effects (i.e., when a viscous material becomes less dense and more fluid due to heat, shaking or pressure exposure) (Beardsley; Škarabot, 2015; Reiner *et al.*, 2021; Schleip, 2003), or neurophysiological effects (inhibitory effect in pain receptors or muscle relaxation after a break in cross-bridges) (Bavencoffe *et al.*, 2014; Crommert *et al.*, 2014; Proske, Morgan, 1999).

In the current evidence about massage effects on soft tissues stiffness, some studies found decreases in muscle (Baumgart *et al.*, 2019; Chang *et al.*, 2021; Crommert *et al.*, 2015; Pérez-Bellmunt *et al.*, 2021; Reiner *et al.*, 2021), tendon (Klich *et al.*, 2022), and fascial tissue stiffness (Griefahn *et al.*, 2017), and increase in MTU passive stiffness (Nakamura *et al.*, 2021a). However, other studies found no changes in muscle (Ikeda *et al.*, 2019; Mayer *et al.*, 2020; Nakamura *et al.*, 2022), tendon (Chang *et al.*, 2021), and MTU stiffness (Nakamura *et al.*,2021b,2021c; Thomson *et al.*, 2015), demonstrating that there is no consensus in these results. Therefore, it is still unclear about the tissues' stiffness individual responses after massage techniques. In addition, understanding the mechanisms of massage techniques, as well as their effects on tissues, is important to know when to prescribe it (e.g., before or after physical practice), as tissue changes can also result in functional changes and/or tissues recovery (Weerapong *et al.*, 2005). Thus, this systematic review with meta-analysis aimed to investigate clinical trials that tested the massage techniques' acute effects on the myotendinous structure's stiffness in healthy adults or athletes.

METHODS

This systematic review with meta-analysis (CRD42022307586) was performed following the recommendations from the Cochrane Collaboration (Higgins *et al.*, 2023), and the Preferred Reporting Items for Systematic Reviews and Meta-Analyzes (PRISMA) reporting guidelines (Page *et al.*, 2021) (Supplementary Table S1 – appendix A).

The study selection, data extraction, the included studies' quality appraisal, and the certainty of evidence were conducted by two independent investigators (R.L. and I.M.P.). In case of disagreements, a third reviewer (M.H.G.) was consulted.

Search strategies

The literature search was performed in four online databases (PubMed, Embase, Web of Science, and PEDro) and in two preprint databases (Preprint.org and MedRxiv), according to Cochrane recommendations, until September 29, 2023. No time or language restrictions have been adopted. The search strategy was adapted for each database, including keywords about intervention and interest outcomes. A full detailed search strategy is presented in the Supplementary Table S2 (Appendix B). Besides that, the references list of all the included studies and previous reviews about massage techniques (Beardsley; Škarabot, 2015; Davis *et al.*, 2020; Glänzel *et al.*, 2022; Hendricks *et al.*, 2020; Skinner *et al.*, 2020; Weerapong *et al.*, 2005; Wiewelhove *et al.*, 2019; Wilke *et al.*, 2020) also were checked to identify other potentially eligible studies.

Selection criteria

The inclusion criteria were related to the PICOS question: a) healthy adults or athletes; b) presenting intervention with massage or self-massage techniques alone (before or without any exercise or activity); c) at least data of the pre-post treatment for the massage condition (crossover studies) or comparison of post-treatment measurements between intervention and control groups (parallel groups); d) access massage acute (i.e., described as immediately after, that is, as soon as possible after the massage treatment with a limit up to 5-min) (Wilke *et al.*, 2020) and local effects on myotendinous structures stiffness (MTU; muscle or tendon stiffness); and e) studies with clinical trial design. In studies measuring acute and chronic effects (long-term), only acute effects were considered. Exclusion criteria were as follows: interventions that did not use only pressure techniques (e.g., interventions that used vibrating devices); use of cream/oil with anti-inflammatory substance or similar; studies that combined massage techniques with other intervention types (e.g., stretch, cycling, or other exercise); studies that evaluated non-local effect (remote effect, except for the tendon, as we considered the effect of massage on the tendon of the muscle that received the treatment); and studies that used massage as recovery (e.g., after exercise-induced muscle damage).

Data extraction

Data (mean and standard deviation [SD] values) related to study design, subjects' characteristics (sex, age, weight, height, and physical activity level), intervention protocols (technique, applied pressure and volume), methodological items (sample calculation, experience level of therapist or practitioner, familiarization procedures with the intervention, and the assessment methods and techniques), and results (post-intervention changes or between-conditions comparisons after massage application and its effects on the MTU, muscle, and tendon stiffness) were extracted individually and exported to a spreadsheet.

Data synthesis and statistics

When the studies provide incomplete data, the study's corresponding author was contacted and requested to obtain access to the study database. When information or access to the database was provided, raw data were used for quantitative analysis. When authors did not respond or could not provide the required data, the mean and SD values were obtained manually from the plots using the ImageJ tool (version 1.48v, National Institutes of Health, Bethesda, MA). In addition, when access to the data was not possible, the study was not included in the quantitative analysis.

Common outcomes among five or more studies were pooled through meta-analyses using the standardized mean difference (SMD), standard error (SE), and confidence interval (CI) as measures of effect and dispersion, respectively. The effect size was classified according to Cohen's d-values (Higgins *et al.*, 2023), where SMD: <0.40 = small effect; 0.40–0.70 = moderate effect; >0.70 = large effect (Cohen, 1988). When a study presented more than one intervention protocol, we chose the protocol with the lowest application volume to estimate the pooled effect, based on a conservative decision (i.e., if the lowest volume represents significant effects, it is more possible that the larger volume also presents it, since larger volume can produce greater changes in some functional outcomes [Phillips *et al.*, 2021]). When a study used more than one assessment method or evaluated more than one site in the same structure, the methods and sites most similar to the other meta-analyses' studies were chosen. Studies were considered more than once when they reported values for different muscles. Regarding the plantar flexor muscles' stiffness, it was standardized the assessment performed with the ankle in a neutral position (0º), due to the possible influence associated with the length-tension relationship (Lima *et al.*, 2019). Therefore, assessment made with the plantar flexor muscles in shortened or stretched positions were not pooled through meta-analysis.

In the crossover trials, when a study did not present the correlation coefficient (r) values between the preintervention and postintervention, we adopted a conservative estimate of $r = 0.7$, as recommended by Rosenthal (1991) and used in previous studies (Glänzel *et al.*, 2022; Kishita *et al.*, 2017). The random-effects model was used in all meta-analyses, as a result of the heterogeneity in the types of intervention and measures of the results, and confirmed by the I^2 test interpreted according to Higgins *et al.* (2003), where values above 25 and 50% were classified as moderate and high heterogeneity, respectively. When moderate or high heterogeneity was found (values > 25%), sensitivity analysis was performed, and the heterogeneity was explored. All statistical analyses were performed using the Comprehensive Meta-Analysis (version 2; Biostat, Englewood, NJ). The level of statistical significance was determined as $\alpha \leq 0.05$.

Studies' quality appraisal

The included studies' quality appraisal was assessed by the PEDro scale. This scale consists of 11 items. Each item (except item 1) contributes one point to the total score when present. The total score was made by the sum of all responses, ranging from 0 to 10. Studies' total scores equal to or greater than 6 were considered to have high methodological quality (Maher *et al.*, 2003).

Risk of bias and confidence in evidence

The grading strength of recommendations was assessed using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach (Guyatt *et al.*, 2008). The GRADE is based on 5 aspects: study limitations (risk of bias), inconsistency of results (heterogeneity), indirectness of evidence, imprecision of results (95% confidence intervals), and publication bias. The criteria used to determine the meta-analyses' level of recommendation were as follows: a) methodological quality: classified according to PEDro mean score of the meta-analyses included, that was ≥ 6 points "no serious", 4–5 "serious", and 0–3 "very serious"; b) inconsistency: I^2 values < 25% "no serious", 25-50% "serious", and > 50% "very serious"; c) indirectness (the evidence was considered to be direct when it answered the PICOS question, so the criterion "not serious" was adopted for all meta-analyses); d) imprecision: CI amplitude < SMD values was considered "no serious", CI amplitude up to half SMD value as "serious", CI amplitude greater than half SMD value as "very serious", and CI amplitude greater than twice the SMD value as "extremely serious"; and e) publication bias: detectable if there was a tendency to publish studies only with positive results.

RESULTS

Study selection

The study selection flowchart is illustrated in Figure 1. Initially, 2167 studies were identified in the databases. After the study selection steps, 23 studies (Baumgart *et al.*, 2019; Chang *et al.*, 2021; Crommert *et al.*, 2015; Ikeda *et al.*, 2019; Klich *et al.*, 2022; Krause *et al.*, 2019; Kuruma *et al.*, 2013; Mayer *et al.*, 2020; Morales-Artacho *et al.*, 2017; Nakamura *et al.*, 2021a, 2021b, 2021c, 2022; Reiner *et al.*, 2021, 2023; Satkunskiene *et al.*, 2021; Schroeder *et al.*, 2021; Thomson *et al.*, 2015; Vardiman *et al.*, 2015; Wilke *et al.*, 2019; Weber *et al.*, 2023; Ichikawa *et al.*, 2015; Macgregor *et al.*, 2018) were included in this review. No preprint was included as it did not meet the inclusion criteria. Also, three additional studies (Martínez-Cabrera & Núñez-Sánchez, 2016; Pérez-Bellmunt *et al.*, 2021; Schroeder *et al.*, 2017) were identified in the articles' reference list and one (Kasahara *et al.*, 2023) was identified in other sources (i.e., manual search) and were also included, totaling 27 articles.

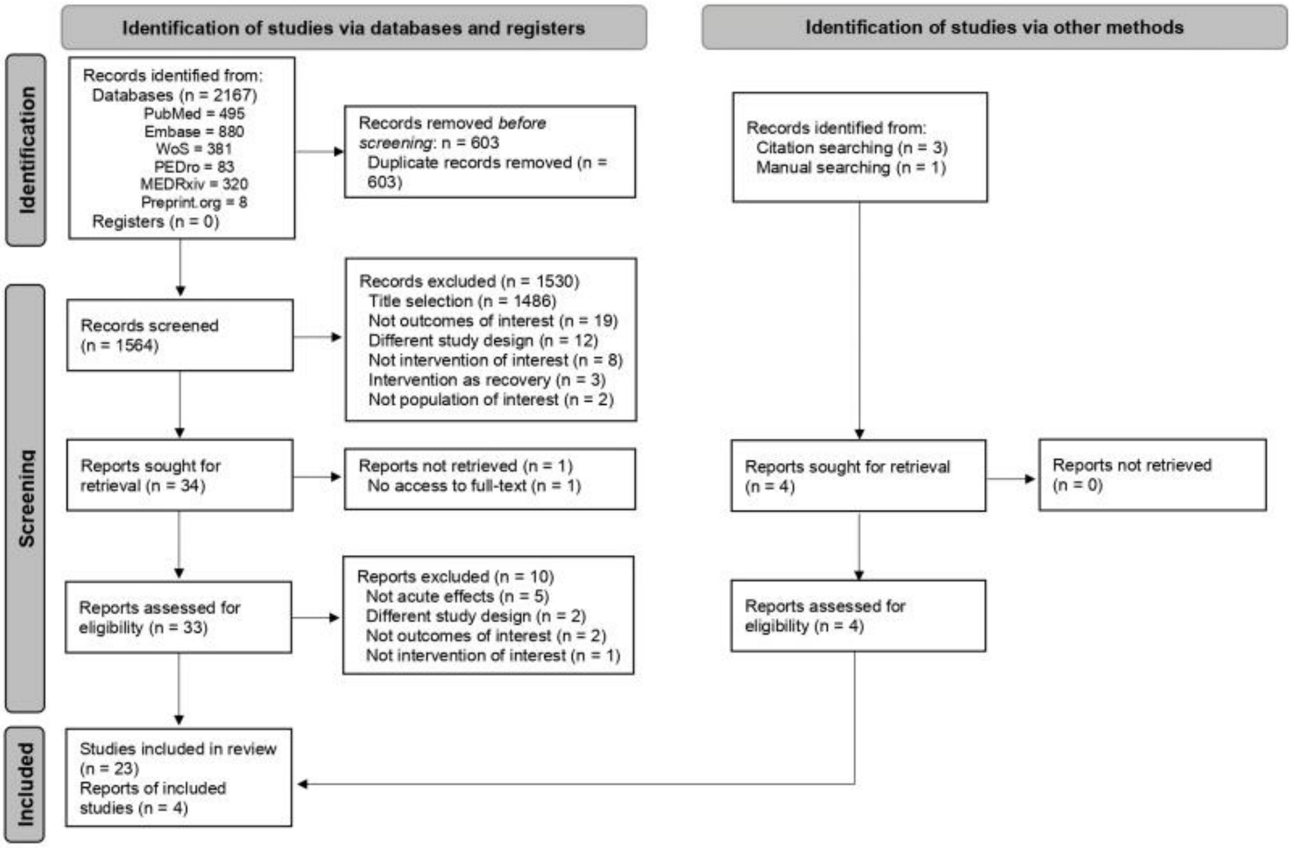


Figure 1 - PRISMA flowchart of the included studies.

Studies characteristics

The table 1 summarizes the included studies' main information. The randomized design was adopted by most studies (n=25) (Baumgart *et al.*, 2019; Chang *et al.*, 2021; Ichikawa *et al.*, 2015; Ikeda *et al.*, 2019; Klich *et al.*, 2022; Krause *et al.*, 2019; Kuruma *et al.*, 2013; Macgregor *et al.*, 2018; Martínez-Cabrera & Núñez-Sánchez, 2016; Morales-Artacho *et al.*, 2017; Nakamura *et al.*, 2021a; Nakamura *et al.*, 2021b; Nakamura *et al.*, 2021c; Pérez-Bellmunt *et al.*, 2021; Reiner *et al.*, 2021; Satkunskiene *et al.*, 2021; Schroeder *et al.*, 2017, 2021; Thomson *et al.*, 2015; Vardiman *et al.*, 2015; Wilke *et al.*, 2019; Weber *et al.*, 2023; Nakamura *et al.*, 2022; Reiner *et al.*, 2023; Kasahara *et al.*, 2023), whereas few studies (n=2) did not randomized the intervention conditions (Crommert *et al.*, 2015; Mayer *et al.*, 2020).

Ten studies compared the pre-post changes after massage techniques (Ichikawa *et al.*, 2015; Klich *et al.*, 2022; Mayer *et al.*, 2020; Nakamura *et al.*, 2021a; Nakamura *et al.*, 2021b; Reiner *et al.*, 2021; Satkunskiene *et al.*, 2021; Schroeder *et al.*, 2017; Weber *et al.*, 2023; Kasahara *et al.*, 2023), whereas another 17 studies compared the massage acute effects with control condition (i.e., without any treatment). Within the 17 studies, some of these (n=7) used the contralateral lower limb as control (Crommert *et al.*, 2015; Martínez-Cabrera & Núñez-Sánchez, 2016; Nakamura *et al.*, 2021c; Pérez-Bellmunt *et al.*, 2021; Schroeder *et al.*, 2021; Thomson *et al.*, 2015; Vardiman *et al.*, 2015), while in the others (n=10) there was a control group (Baumgart *et al.*, 2019; Chang *et al.*, 2021; Ikeda *et al.*, 2019; Krause *et al.*, 2019; Kuruma *et al.*, 2013; Macgregor *et al.*, 2018; Morales-Artacho *et al.*, 2017; Wilke *et al.*, 2019; Nakamura *et al.*, 2022; Reiner *et al.*, 2023). Furthermore, of the 27 included studies, 11 also compared massage techniques with other interventions (e.g., hot pack therapy, tissue flossing, static stretching) (Baumgart *et al.*, 2019; Ichikawa *et al.*, 2015; Klich *et al.*, 2022; Krause *et al.*, 2019; Kuruma *et al.*, 2013; Martínez-Cabrera & Núñez-Sánchez, 2016; Morales-Artacho *et al.*, 2017; Reiner *et al.*, 2021; Satkunskiene *et al.*, 2021; Schroeder *et al.*, 2017; Thomson *et al.*, 2015; Weber *et al.*, 2023; Kasahara *et al.*, 2023), while the other studies did not investigate different interventions.

Table 1 - Summary of studies on the massage techniques effects on myotendinous structures.

Study	Subjects	Technique	Protocol	Main outcomes and assessment tools	Results
Baumgart *et al.* (2019)	Recreational athletes n=20 (M = 20) 26.6 ± 2.7 y	FR	2x 30reps QD and PF	Muscle stiffness; Myotonometry	RF: ↓2.6% GM: ns
Chang *et al.* (2021)	Healthy individuals n=50 (M=25) IG 22.5 ± 2.4 y	FR	2x 60s [30s rest between sets]; pressure 7/10 discomfort scale PF	Muscle and tendon stiffness; Myotonometry	GM: ↓13.3% GL: ↓13.3% AT: ns
Crommert *et al.* (2014)	Healthy individuals n=18 (M = 12) 28 ± 6.4 y	Manual massage	7min PF	Muscle stiffness; SWE	GM: ↓6.4%
Ichikawa *et al.* (2015)	Healthy individuals n=12 (M=12) 27 y	Myofascial release	4min VL	Muscle stiffness; RTE and durometer	VL: ↓69-78.4% by RTE ↓32.9% by durometer
Ikeda *et al.* (2019)	Healthy individuals n=14 (M=11) 24 ± 4 y	IASTM	5min; pressure < 4/5 pain scale PF and AT	Muscle and MTU passive stiffness; SWE and passive torque	GM: ns SO: ns MTU: ns
Kasahara *et al.* (2023)	Recreationally active individuals n=15 (M = 15) 22 ± 1.3 y	FR	1x 30s or 2x30s [30s rest between sets]; 15 cycles/set; maximal pressure QD	Muscle stiffness; Tissue hardness meter	RF: ↓11.9%
Klich *et al.* (2022)	College athletes n=32 (M = 32) 22 ± 1.5 y	FR	3x 30s [15s rest between sets]; 50 bpm; maximum pressure; PF	Tendon stiffness; Myotonometry	AT: ns (distal insertion); ↓7.9% (joint center); ↓5% (intermuscular septum)
Krause *et al.* (2019)	Healthy individuals n=16 (M = 10) 32.1 ± 5 y	FR	2x 60s [30s rest between sets]; 15 rolls/min; pressure 7/10 discomfort scale QD	MTU passive stiffness; Passive torque	MTU: ns
Kuruma *et al.* (2013)	Healthy individuals n=40 (M = 20) 21 y	Myofascial release	8min QD	Muscle stiffness; Durometer	VL: ns

Table 1 - Summary of studies on the massage techniques effects on myotendinous structures. *(continued)*

Study	Subjects	Technique	Protocol	Main outcomes and assessment tools	Results
Macgregor *et al.* (2018)	Physically active individuals n=16 (M = 16) 24.5 ± 4.4 y	FR	2min; cadence 2s; self-selected pressure QD	Muscle stiffness; Tensiomyography	RF: ns VL: ns
Martínez-Cabrera, Nuñez-Sanchez (2016)	Professional soccer players n=17 (M = 17) 21.4 ± 3.8 y	FR	2x 15s [2min rest between sets]; 30rpm QD	Muscle displacement; Tensiomyography	RF: ns
Mayer *et al.* (2020)	College athletes n=40 (M = 20) exp 24.7 ± 2.3, nexp 25.3 ± 3.6 y	FR	5x 45s [20s rest between sets]; 1 roll/2s lateral thigh	Muscle stiffness; SWE	VL: ns VI: ns
Morales-Artacho *et al.* (2016)	Physically active individuals n=14 (M = 14) 26.6 ± 4.5 y	FR	1x 60s with both lower limbs + 1x 60s with each lower limb [30s]; 27rpm HA	Muscle stiffness and MTU passive stiffness; SWE and passive torque	HA: ns MTU knee extension: ns
Nakamura *et al.* (2021a)	Healthy individuals n=29 (M=15) 21.9 ± 2.3 y	Roller massage	3x 60s [30s rest between sets]; 60bpm; pressure 7/10 discomfort scale PF	Muscle stiffness and MTU passive stiffness; SWE and passive torque	GM: ns MTU: ↑6.8% (M) ↑27.8% (F)
Nakamura *et al.* (2021b)	Healthy and sedentary individuals n=45 (M=23) 21 ± 1.5 y	FR	1x – 10x 30s [30s rest between sets]; 30rpm; pressure 7/10 discomfort scale PF	Muscle stiffness and MTU passive stiffness; SWE and passive torque	GM: ns MTU: ns
Nakamura *et al.* (2021c)	Healthy individuals n=15 (M=7) 22.8 ± 3 y	Roller massage	3x 60s [30s rest between sets]; 60bpm; pressure 7/10 discomfort scale PF	Muscle stiffness and MTU passive stiffness; SWE and passive torque	GM: ns MTU: ns
Nakamura *et al.* (2022)	Untrained university students n=16 (M=16) 21.7 ± 1.3 y	FR	3x 60s [30s rest between sets]; 15 cycles/min; maximum pressure PF	Muscle stiffness and MTU passive stiffness; SWE and passive torque	GM: ns MTU: ↑18.3%

Table 1 - Summary of studies on the massage techniques effects on myotendinous structures. *(continued)*

Study	Subjects	Technique	Protocol	Main outcomes and assessment tools	Results
Perez-Bellmunt *et al.* (2021)	Healthy college athletes n=30 (M=20) 23.8 ± 6.1 y	Manual massage	5min PF	Muscle stiffness; Myotonometry	GM: ↓8.33%
Reiner *et al.* (2021)	Physically active individuals n=21 (M=21) 25.2 ± 3.8 y	FR	3x 60s [30s rest between sets]; 30rpm; maximum pressure QD	Muscle stiffness and MTU passive stiffness; SWE and passive torque	VM: ns VL: ns RF: ↓15% MTU: ns
Reiner *et al.* (2023)	Physically active individuals n=38 (M=24) M: 26.6 ± 5.3; F: 27.5 ± 4.1 y	Ball massage	2min, pressure until the point of discomfort PMa	Muscle stiffness; SWE	PMa: ns
Satkunskiene *et al.* (2022)	Soccer players n=15 (M=15) 18 ± 1.4 y	FR	6x 45s [15s rest between sets]; 15 rolls/set; pressure 5/10 discomfort scale HA	MTU passive stiffness; Passive torque	MTU: ns
Schroeder *et al.* (2017)	Healthy individuals n=12 (M=6) 28.8 ± 5.7 y	FR	3x 60s [60s rest between sets]; 2 sec/roll; HA	Muscle stiffness; Tensiomyography	BF: ns
Schroeder *et al.* (2021)	Physically active individuals n=20 (M=9) 25 ± 3.5 y	FR	2x 60s or 2x 3min [60s rest between sets]; 30rpm QD	Muscle stiffness; Myotonometry	↓1.6% (2 sets of 60s); ↓1.9% (2 sets of 3min)
Thomson *et al.* (2015)	Healthy individuals n=29 (M=17) 22.1 ± 5.5 y	Manual massage	10min; intensity (reported) strong but tolerable PF	MTU passive stiffness; Passive torque	MTU: ns
Vardiman *et al.* (2014)	Healthy individuals n=11 (M=11) 23 ± 3 y	IASTM	7~8min; 3x 7 strokes/area; pressure was standardized by device PF	MTU passive stiffness; Passive torque	MTU: ns
Weber *et al.* (2023)	Senior-level soccer players n=67 (M=0) 20.9 ± 3.9 y	IASTM	8min, maximum pressure ESM	Muscle stiffness; Cross-correlation software	ESM: ns

Table 1 - Summary of studies on the massage techniques effects on myotendinous structures. *(ended)*

Study	Subjects	Technique	Protocol	Main outcomes and assessment tools	Results
Wilke *et al.* (2019)	Physically active individuals n=17 (M=7) 25 ± 2 y	FR	4x 45s [30s rest between sets]; 60 or 6bpm; pressure 6~7/10 discomfort scale QD	Muscle stiffness; Indentometer	QD: ns

AT: Achilles tendon; BF: biceps femoris; ESM: erector spinae muscle; GM: gastrocnemius medialis; GL: gastrocnemius lateralis; HA: hamstrings; MTU: myotendinous unit; PF: plantar flexors; PMa: Pectoralis major; QD: quadriceps; RF: rectus femoris; SO: soleus; VI: vastus intermedius; VL: vastus lateralis; VM: vastus medialis; FR: foam rolling; IASTM: instrument assisted soft tissue mobilization; IG: intervention group; F: female; M: male; RTE: real time elastography; SWE: shear wave elastography; bpm: beats per minute; rpm: repetitions per minute; rep: repetitions; exp: experienced with intervention group; nexp: inexperienced with intervention group; min: minutes; ns: non-significant; s: seconds; y: years; [interval time].

Twenty-one studies evaluated healthy adults (Baumgart *et al.*, 2019; Chang *et al.*, 2021; Crommert *et al.*, 2015; Ikeda *et al.*, 2019; Kasahara *et al.*, 2023 Krause *et al.*, 2019; Kuruma *et al.*, 2013; Morales-Artacho *et al.*, 2017; Nakamura *et al.*, 2021a, 2021b, 2021c, 2022; Reiner *et al.*, 2021, 2023; Schroeder *et al.*, 2017, 2021; Thomson *et al.*, 2015; Vardiman *et al.*, 2015; Wilke *et al.*, 2019; Ichikawa *et al.*, 2015; Macgregor *et al.*, 2018) and six assessed athletes (Klich *et al.*, 2022; Pérez-Bellmunt *et al.*, 2021; Martínez-Cabrera & Núñez-Sánchez, 2016; Mayer *et al.*, 2020; Satkunskiene *et al.*, 2021; Weber *et al.*, 2023). The total sample size varied from 11 (Vardiman *et al.*, 2015) to 67 subjects (Weber *et al.*, 2023). Sixteen studies (Ikeda *et al.*, 2019; Klich *et al.*, 2022; Krause *et al.*, 2019; Macgregor *et al.*, 2018; Morales-Artacho *et al.*, 2017; Nakamura *et al.*, 2021c; Nakamura *et al.*, 2021a; Nakamura *et al.*, 2021b; Pérez-Bellmunt *et al.*, 2021; Reiner *et al.*, 2021; Satkunskiene *et al.*, 2021; Schroeder *et al.*, 2021; Wilke *et al.*, 2019; Nakamura *et al.*, 2022; Reiner *et al.*, 2023; Kasahara *et al.*, 2023) determined the sample size, while the remaining studies did not describe this information. A total of 669 subjects (414 men and 255 women), with the mean age ranging between 18 (Satkunskiene *et al.*, 2021) and 32 years (Krause *et al.*, 2019), were evaluated across all studies.

Eleven studies described the therapist's or practitioner's level of experience related to the applied technique (Crommert *et al.*, 2015; Ichikawa *et al.*, 2015; Ikeda *et al.*, 2019; Pérez-Bellmunt *et al.*, 2021; Thomson *et al.*, 2015; Vardiman *et al.*, 2015; Baumgart *et al.*, 2019; Mayer *et al.*, 2020; Nakamura *et al.*, 2021a; Schroeder *et al.*, 2017, 2021). Six of them mentioned the therapist’s years of experience (Crommert *et al.*, 2015; Ichikawa *et al.*, 2015; Ikeda *et al.*, 2019; Pérez-Bellmunt *et al.*, 2021; Thomson *et al.*, 2015; Vardiman *et al.*, 2015), that ranged between three (Crommert *et al.*, 2015) and 25 years (Ichikawa *et al.*, 2015). Two studies applied self-massage techniques in no prior experienced subjects (Baumgart *et al.*, 2019; Mayer *et al.*, 2020), while three studies applied self-massage in prior experienced subjects (Mayer *et al.*, 2020; Schroeder *et al.*, 2017, 2021) and 13 did not mention the subjects' experience (Chang *et al.*, 2021; Klich *et al.*, 2022; Krause *et al.*, 2019; Macgregor *et al.*, 2018; Martínez-Cabrera & Núñez-Sánchez, 2016; Morales-Artacho *et al.*, 2017; Nakamura *et al.*, 2021b, 2022; Reiner *et al.*, 2021; 2023; Satkunskiene *et al.*, 2021; Wilke *et al.*, 2019; Kasahara *et al.*, 2023). The subjects' experience level ranged between "just familiarity" with the technique (Schroeder *et al.*, 2017, 2021) and a minimum practice of 15min per week for more than six months (Mayer *et al.*, 2020). Regarding familiarization procedures, 13 studies (Baumgart *et al.*, 2019; Chang *et al.*, 2021; Krause *et al.*, 2019; Macgregor *et al.*, 2018; Mayer *et al.*, 2020; Nakamura *et al.*, 2021c; Reiner *et al.*, 2021; Satkunskiene *et al.*, 2021; Vardiman *et al.*, 2015; Wilke *et al.*, 2019; Nakamura *et al.*, 2022; Reiner *et al.*, 2023; Kasahara *et al.*, 2023) described having performed some type of procedure to familiarize the subjects with self-applied techniques.

The interval time between intervention and the post-intervention assessment was reported from seven studies (Klich *et al.*, 2022; Krause *et al.*, 2019; Mayer *et al.*, 2020; Morales-Artacho *et al.*, 2017; Nakamura *et al.*, 2021c; Nakamura *et al.*, 2021a; Nakamura *et al.*, 2021b), which ranged between less than 30s (Krause *et al.*, 2019) and 5min (Klich *et al.*, 2022; Morales-Artacho *et al.*, 2017), while some studies defined it as "immediately after" (Crommert *et al.*, 2015; Macgregor *et al.*, 2018; Satkunskiene *et al.*, 2021; Thomson *et al.*, 2015; Vardiman *et al.*, 2015; Wilke *et al.*, 2019; Nakamura *et al.*, 2022; Reiner *et al.*, 2023; Kasahara *et al.*, 2023) or described it only as post-intervention assessment (Baumgart *et al.*, 2019; Chang *et al.*, 2021; Ichikawa *et al.*, 2015; Ikeda *et al.*, 2019; Kuruma *et al.*, 2013; Martínez-Cabrera & Núñez-Sánchez, 2016; Pérez-Bellmunt *et al.*, 2021; Reiner *et al.*, 2021; Schroeder *et al.*, 2017, 2021; Weber *et al.*, 2023).

The most evaluated technique (n=17) was the self-massage applied through foam roller (FR) (Baumgart *et al.*, 2019; Chang *et al.*, 2021; Klich *et al.*, 2022; Krause *et al.*, 2019; Macgregor *et al.*, 2018; Martínez-Cabrera & Núñez-Sánchez, 2016; Mayer *et al.*, 2020; Morales-Artacho *et al.*, 2017; Nakamura *et al.*, 2021b, 2022; Reiner *et al.*, 2021; Satkunskiene *et al.*, 2021; Schroeder *et al.*, 2017, 2021; Wilke *et al.*, 2019; Kasahara *et al.*, 2023) or ball massage (Reiner *et al.*, 2023), whereas few studies (n=6) evaluated manual techniques (massage [Crommert *et al.*, 2015; Pérez-Bellmunt *et al.*, 2021; Thomson *et al.*, 2015]; "myofascial release" [Ichikawa *et al.*, 2015; Kuruma *et al.*, 2013]); and instrumental massage applied by a therapist (n=4) (instrument assisted soft tissue mobilization [Ikeda *et al.*, 2019; Vardiman *et al.*, 2015; Weber *et al.*, 2023]; and roller massager [Nakamura *et al.*, 2021c; Nakamura *et al.*, 2021a]). The massage application time ranged between 30s (Nakamura *et al.*, 2021b; Kasahara *et al.*, 2023) and 300s (Nakamura *et al.*, 2021b) for the self-massage, and between 90s (Nakamura *et al.*, 2021c; Nakamura *et al.*, 2021a) and 10min (Thomson *et al.*, 2015) for the other techniques (manual or instrumental).

Massage effects on muscle stiffness

The studies investigated the massage effects on muscle stiffness, which assessed upper limbs and/or trunk (n=2), quadriceps (n=10), hamstrings (n=2), and plantar flexors (n=9). Regarding the upper limbs and trunk, Reiner *et al.* (2023) found no differences for pectoralis major stiffness after self-massage and Weber *et al.* (2023) also found no differences for erector spinae muscles after instrument soft tissue mobilization. The assessments methods for muscle stiffness were shear wave elastography (SWE) (n=10), myotonometer (n=4), tensiomiography (n=3), durometer (n=2), real-time elastography (n=1) tissue hardness meter (n=1), indentometer (n=1), and or cross-correlation method (n=1).

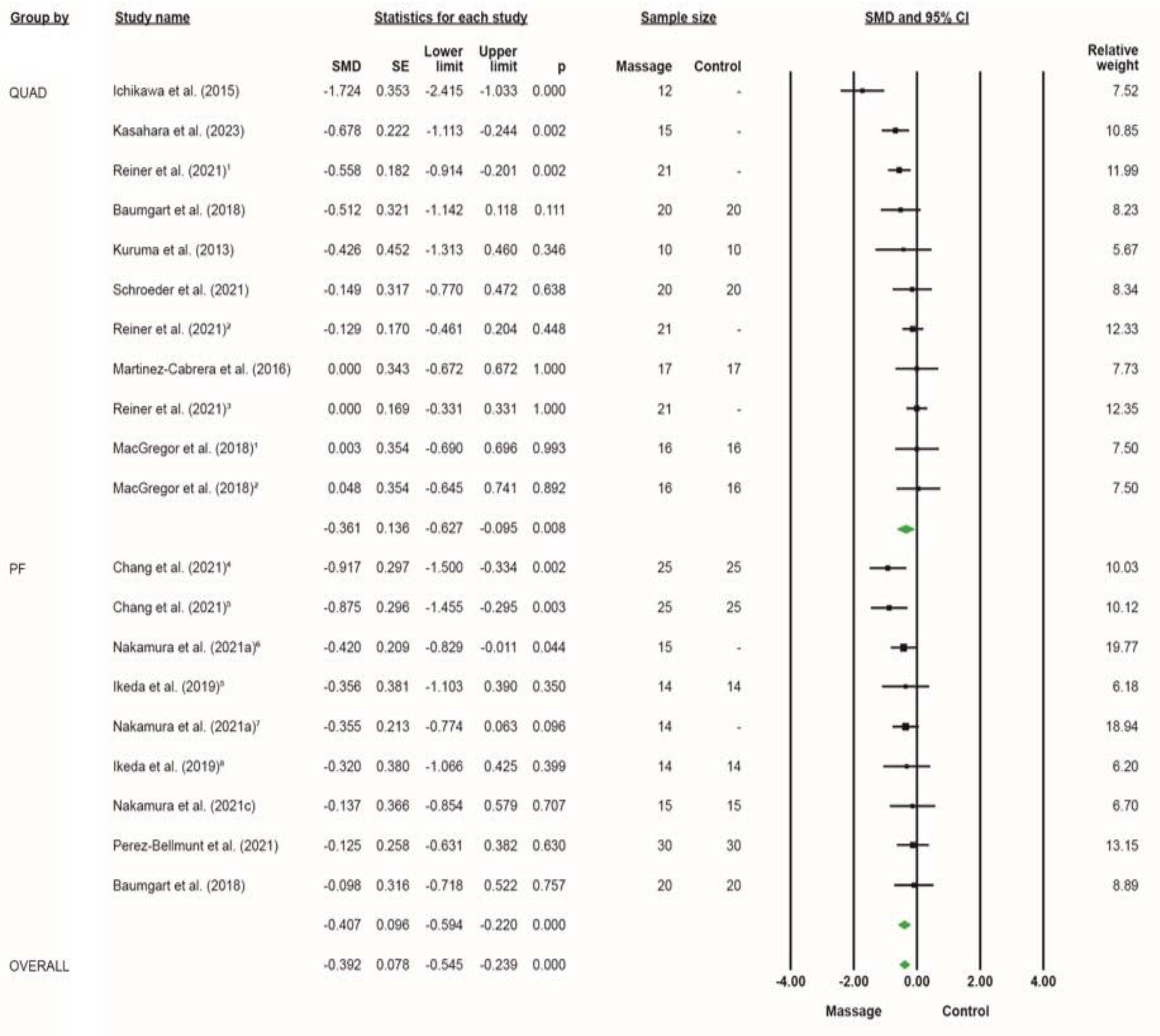
The studies that evaluated the quadriceps muscles assessed rectus femoris (n=7) (Baumgart *et al.*, 2019; Macgregor *et al.*, 2018; Martínez-Cabrera & Núñez-Sánchez, 2016; Reiner *et al.*, 2021; Schroeder *et al.*, 2021; Wilke *et al.*, 2019; Kasahara *et al.*, 2023), vastus lateralis (n=5) (Ichikawa *et al.*, 2015; Kuruma *et al.*, 2013; Macgregor *et al.*, 2018; Mayer *et al.*, 2020; Reiner *et al.*, 2021), vastus medialis (n=1) (Reiner *et al.*, 2021), and vastus intermedius (n=1) (Mayer *et al.*, 2020) stiffness. Regarding the rectus femoris (n=7), four studies (Baumgart *et al.*, 2019; Reiner *et al.*, 2021; Schroeder *et al.*, 2021; Kasahara *et al.*, 2023) found decreased stiffness, and three studies found no changes (Macgregor *et al.*, 2018; Martínez-Cabrera & Núñez-Sánchez, 2016; Wilke *et al.*, 2019). The studies that found decreased stiffness used protocols between 30s (Kasahara *et al.*, 2023) to 360s (Schroeder *et al.*, 2021), whereas the others had applied the FR between 30s (Martínez-Cabrera & Núñez-Sánchez, 2016) and 180s (Wilke *et al.*, 2019). All studies used self-massage with FR. For the other quadriceps muscles, four studies found no changes on muscle stiffness after FR (Macgregor *et al.*, 2018; Mayer *et al.*, 2020; Reiner *et al.*, 2021) or myofascial release (Kuruma *et al.*, 2013). Only Ichikawa (2015) reported decreases in vastus lateralis stiffness after 4min of myofascial release. Additionally, regarding the thigh muscles, two studies investigated the hamstrings stiffness (one with available data). Both studies investigated self-massage with FR after 120s (Morales-Artacho *et al.*, 2017) or 180s (Schroeder *et al.*, 2017).

The studies found no changes for biceps femoris (Morales-Artacho *et al.*, 2017; Schroeder *et al.*, 2017), semimembranosus, and semitendinosus (Morales-Artacho *et al.*, 2017) stiffness.

Nine studies investigated the plantar flexors stiffness after massage techniques. Most studies (n=6) found no changes in gastrocnemius medialis (Baumgart *et al.*, 2019; Ikeda *et al.*, 2019; Nakamura *et al.*, 2021c; Nakamura *et al.*, 2021a; Nakamura *et al.*, 2021b; Nakamura *et al.*, 2022) and soleus (n=1) (Ikeda *et al.*, 2019) stiffness, while three studies found decreased stiffnes for gastrocnemius medialis (Crommert *et al.*, 2015; Pérez-Bellmunt *et al.*, 2021; Chang *et al.*, 2021) or lateralis (Chang *et al.*, 2021). Regarding the massage techniques, FR showed contradictory results (reduction and maintenance of stiffness), and instrumental massage showed no changes, whereas both studies with manual massage resulted in decreased gastrocnemius medialis stiffness. The protocols varied between 30s and 300s for FR, 180s to 5min for instrumental massage, and 5-7min of manual massage.

A meta-analysis was performed to estimate the overall effect of massage on muscle stiffness (n=13) (Baumgart *et al.*, 2019; Chang *et al.*, 2021; Ichikawa *et al.*, 2015; Ikeda *et al.*, 2019; Kuruma *et al.*, 2013; Macgregor *et al.*, 2018; Martínez-Cabrera & Núñez-Sánchez, 2016; Nakamura *et al.*, 2021c; Nakamura *et al.*, 2021a; Pérez-Bellmunt *et al.*, 2021; Reiner *et al.*, 2021; Schroeder *et al.*, 2021; Kasahara *et al.*, 2023). Studies were pooled according to the criteria for meta-analysis and available data. One study presented two different groups with different intervention experience levels that received the same intervention (Mayer *et al.*, 2020) and were not included in the meta-analysis because it was not possible to choose between the subjects' level of experience, since this information was not reported in most studies.

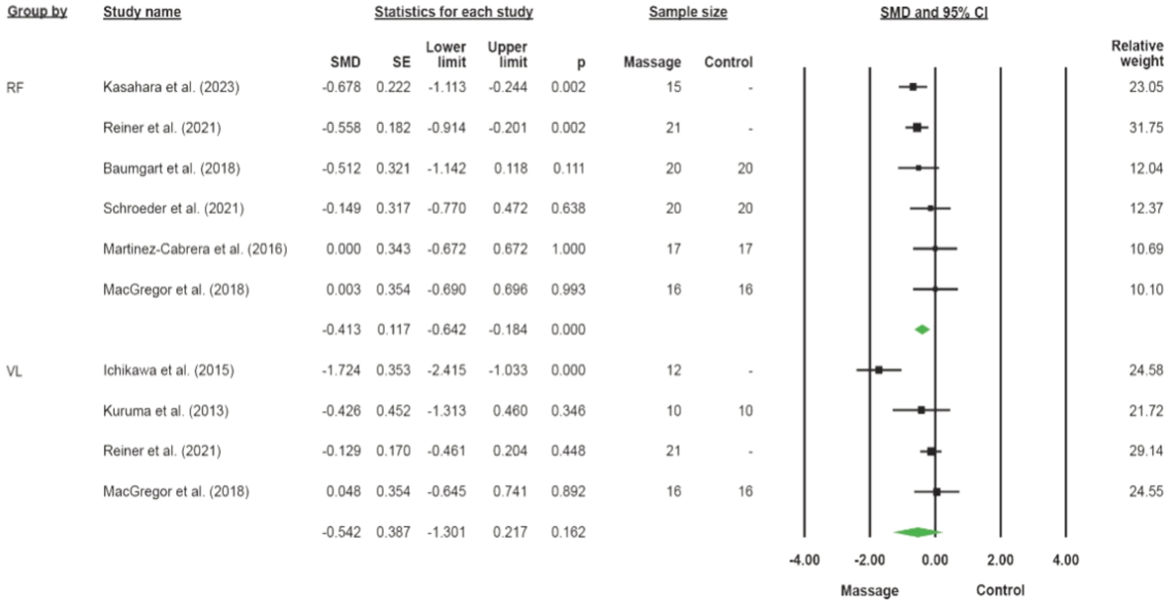
A small effect was observed for decreased stiffness after massage (SMD: -0.392; CI95%: -0.545 to -0.239; p<0.001; I^2: 49%). Due to the moderate heterogeneity levels, subgroup analysis were performed according to muscle group, allowing to separately analysis for quadriceps (n=8) (Baumgart *et al.*, 2019; Ichikawa *et al.*, 2015; Kuruma *et al.*, 2013; Macgregor *et al.*, 2018; Martínez-Cabrera & Núñez-Sánchez, 2016; Reiner *et al.*, 2021; Schroeder *et al.*, 2021; Kasahara *et al.*, 2023) and plantar flexors (n=6) (Baumgart *et al.*, 2019; Chang *et al.*, 2021; Ikeda *et al.*, 2019; Nakamura *et al.*, 2021c; Nakamura *et al.*, 2021a; Pérez-Bellmunt *et al.*, 2021) muscles stiffness. The subgroup analysis showed a small effect for quadriceps (SMD: -0.361; CI95%: -0.627 to -0.095; p: 0.008; I^2: 65%), and a moderate effect for plantar flexors (SMD: -0.407; CI95%: -0.594 to -0.220; p<0.001; I^2: 3%), suggesting that massage can decrease muscle stiffness, mainly in the quadriceps and plantar flexor muscles (Figure 2). The quadriceps stiffness showed high heterogeneity levels, and therefore, it was explored. It was identified that Ichikawa *et al.* (2015) was the principal source of this high heterogeneity, however, a sensitivity analysis showed that its exclusion would not affect the direction and magnitude of the effect.



Group by	Study name	SMD	SE	Lower limit	Upper limit	p	Massage	Control	Relative weight
QUAD	Ichikawa et al. (2015)	-1.724	0.353	-2.415	-1.033	0.000	12	-	7.52
	Kasahara et al. (2023)	-0.678	0.222	-1.113	-0.244	0.002	15	-	10.85
	Reiner et al. (2021)[1]	-0.558	0.182	-0.914	-0.201	0.002	21	-	11.99
	Baumgart et al. (2018)	-0.512	0.321	-1.142	0.118	0.111	20	20	8.23
	Kuruma et al. (2013)	-0.426	0.452	-1.313	0.460	0.346	10	10	5.67
	Schroeder et al. (2021)	-0.149	0.317	-0.770	0.472	0.638	20	20	8.34
	Reiner et al. (2021)[2]	-0.129	0.170	-0.461	0.204	0.448	21	-	12.33
	Martinez-Cabrera et al. (2016)	0.000	0.343	-0.672	0.672	1.000	17	17	7.73
	Reiner et al. (2021)[3]	0.000	0.169	-0.331	0.331	1.000	21	-	12.35
	MacGregor et al. (2018)[1]	0.003	0.354	-0.690	0.696	0.993	16	16	7.50
	MacGregor et al. (2018)[2]	0.048	0.354	-0.645	0.741	0.892	16	16	7.50
		-0.361	0.136	-0.627	-0.095	0.008			
PF	Chang et al. (2021)[4]	-0.917	0.297	-1.500	-0.334	0.002	25	25	10.03
	Chang et al. (2021)[5]	-0.875	0.296	-1.455	-0.295	0.003	25	25	10.12
	Nakamura et al. (2021a)[6]	-0.420	0.209	-0.829	-0.011	0.044	15	-	19.77
	Ikeda et al. (2019)[5]	-0.356	0.381	-1.103	0.390	0.350	14	14	6.18
	Nakamura et al. (2021a)[7]	-0.355	0.213	-0.774	0.063	0.096	14	-	18.94
	Ikeda et al. (2019)[8]	-0.320	0.380	-1.066	0.425	0.399	14	14	6.20
	Nakamura et al. (2021c)	-0.137	0.366	-0.854	0.579	0.707	15	15	6.70
	Perez-Bellmunt et al. (2021)	-0.125	0.258	-0.631	0.382	0.630	30	30	13.15
	Baumgart et al. (2018)	-0.098	0.316	-0.718	0.522	0.757	20	20	8.89
		-0.407	0.096	-0.594	-0.220	0.000			
OVERALL		-0.392	0.078	-0.545	-0.239	0.000			

Figure 2 - Effects of massage techniques vs. non-exercise control on muscle stiffness.Forest charts with grouped standardized mean differences (SMD), standard errors (SE), and 95% confidence intervals (CI) are shown: subgroup analysis of acute effects separated by muscle group (QUAD = quadriceps muscle [SMD: -0.361; CI95%: -0.627 to -0.095; *p*: 0.008; I²: 65%; effects from eight studies]; PF = plantar flexors muscles [SMD: -0.407; CI95%: -0.594 to -0.220; *p*<0.001; I²: 3%; effects from six studies]; and overall [SMD: -0.392; CI95%: -0.545 to -0.239; *p*<0.001; I²: 49%; effects from 13 studies]). [1]Rectus femoris; [2]Vastus lateralis; [3]Vastus medialis; [4]Gastrocnemius lateralis; [5]Gastrocnemius medialis; [6]Male group; [7]Female group; [8]Soleus.

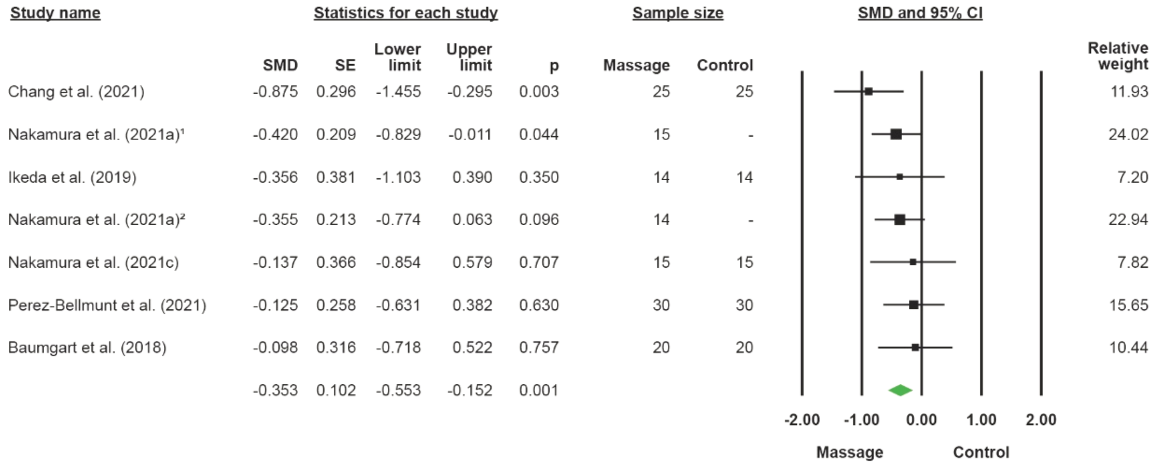
Due to the high heterogeneity observed in estimating the pooled effect of the quadriceps, the eight studies (Baumgart *et al.*, 2019; Ichikawa *et al.*, 2015; Kuruma *et al.*, 2013; Macgregor *et al.*, 2018; Martínez-Cabrera & Núñez-Sánchez, 2016; Reiner *et al.*, 2021; Schroeder *et al.*, 2021; Kasahara *et al.*, 2023) were pooled in another subgroup analysis to estimate the massage effects on separated quadriceps muscles. The analysis showed moderate effect for decreased stiffness on rectus femoris (SMD: -0.413; CI95%: -0.642 to -0.184; *p*<0.001; I²: 12%; effects from six studies), and moderate but not statistically significant effect for vastus lateralis (SMD: -0.542; CI95%: -1.301 to 0.217; *p*: 0.162; I²: 84%; effects from four studies) (Figure 3). In the same way as in the quadriceps analysis, this heterogeneity was explored. Ichikawa *et al.* (2015) was also the main source of the high heterogeneity; however, its exclusion would not affect the direction and magnitude of the effect, therefore, the study was maintained in the meta-analysis.



Group by	Study name	Statistics for each study					Sample size		Relative weight
		SMD	SE	Lower limit	Upper limit	p	Massage	Control	
RF	Kasahara et al. (2023)	-0.678	0.222	-1.113	-0.244	0.002	15	-	23.05
	Reiner et al. (2021)	-0.558	0.182	-0.914	-0.201	0.002	21	-	31.75
	Baumgart et al. (2018)	-0.512	0.321	-1.142	0.118	0.111	20	20	12.04
	Schroeder et al. (2021)	-0.149	0.317	-0.770	0.472	0.638	20	20	12.37
	Martinez-Cabrera et al. (2016)	0.000	0.343	-0.672	0.672	1.000	17	17	10.69
	MacGregor et al. (2018)	0.003	0.354	-0.690	0.696	0.993	16	16	10.10
		-0.413	0.117	-0.642	-0.184	0.000			
VL	Ichikawa et al. (2015)	-1.724	0.353	-2.415	-1.033	0.000	12	-	24.58
	Kuruma et al. (2013)	-0.426	0.452	-1.313	0.460	0.346	10	10	21.72
	Reiner et al. (2021)	-0.129	0.170	-0.461	0.204	0.448	21	-	29.14
	MacGregor et al. (2018)	0.048	0.354	-0.645	0.741	0.892	16	16	24.55
		-0.542	0.387	-1.301	0.217	0.162			

Figure 3 - Effects of massage techniques vs. non-exercise control on quadriceps muscles stiffness. Forest charts with grouped standardized mean differences (SMD), standard errors (SE), and 95% confidence intervals (CI) are shown: subgroup analysis of acute effects separated by muscle (RF = rectus femoris [SMD: -0.413; CI95%: -0.642 to -0.184; *p* < 0.001; I²: 12%; effects from six studies]; and VL = vastus lateralis [SMD: -0.542; CI95%: -1.301 to 0.217; *p*: 0.162; I²: 84%; effects from four studies]).

Six studies that assessed gastrocnemius medialis also were pooled through meta-analysis (Baumgart *et al.*, 2019; Chang *et al.*, 2021; Ikeda *et al.*, 2019; Nakamura *et al.*, 2021c; Nakamura *et al.*, 2021a; Pérez-Bellmunt *et al.*, 2021) and showed a small effect for decreased muscle stiffness after massage application (SMD: -0.353; CI95%: -0.553 to-0.152, *p*: 0.001; I²: 0%; effects from six studies) (Figure 4).



Study name	SMD	SE	Lower limit	Upper limit	p	Massage	Control	Relative weight
Chang et al. (2021)	-0.875	0.296	-1.455	-0.295	0.003	25	25	11.93
Nakamura et al. (2021a)¹	-0.420	0.209	-0.829	-0.011	0.044	15	-	24.02
Ikeda et al. (2019)	-0.356	0.381	-1.103	0.390	0.350	14	14	7.20
Nakamura et al. (2021a)²	-0.355	0.213	-0.774	0.063	0.096	14	-	22.94
Nakamura et al. (2021c)	-0.137	0.366	-0.854	0.579	0.707	15	15	7.82
Perez-Bellmunt et al. (2021)	-0.125	0.258	-0.631	0.382	0.630	30	30	15.65
Baumgart et al. (2018)	-0.098	0.316	-0.718	0.522	0.757	20	20	10.44
	-0.353	0.102	-0.553	-0.152	0.001			

Figure 4 - Effects of massage techniques vs. non-exercise control on medial gastrocnemius stiffness. Forest charts with grouped standardized mean differences (SMD), standard errors (SE), and 95% confidence intervals (CI) are shown (SMD: -0.353; CI95%: -0.553 to-0.152, *p*: 0.001; I²: 0%; effects from six studies). ^{1}Male group; ^{2}Female group.

Massage effects on tendon stiffness

Only two studies investigated the stiffness responses to self-massage application through FR (Chang *et al.*, 2021; Klich *et al.*, 2022), and measured these effects on the Achilles tendon through myotonometer. Concerning the intervention protocol, Klich *et al.* (2022) applied the technique for less time (three sets of 30s, each set emphasizing each evaluated region [insertion at calcaneus, joint center, and intermuscular septum site]) than Chang *et al.* (2021) (three sets of 60s in plantar flexors). Klich *et al.* (2022) found decreased tendon stiffness in the joint center site and in the intermuscular septum, but no changes in the insertion site (at calcaneus bone). However, Chang *et al.* (2021) evaluated the tendon stiffness 4cm proximal to the tendon insertion and found no differences after self-massage application.

Massage effects on muscle-tendon unit stiffness

Eleven studies investigated the massage techniques effects on the MTU passive stiffness for knee flexors (n=2) (Krause *et al.*, 2019; Reiner *et al.*, 2021), knee extensors (n=2) (Morales-Artacho *et al.*, 2017; Satkunskiene *et al.*, 2021), and plantar flexors muscles (n=7) (Ikeda *et al.*, 2019; Nakamura *et al.*, 2021c; Nakamura *et al.*, 2021a; Nakamura *et al.*, 2021b; Thomson *et al.*, 2015; Vardiman *et al.*, 2015; Nakamura *et al.*, 2022). Althoug Nakamura *et al.* (2021b, 2022) found increases in plantar flexors MTU stiffness, most studies (n=9) found no changes on plantar flexors, knee flexors and extensors.

Regarding the techniques, six studies investigated self-massage with FR (Krause *et al.*, 2019; Morales-Artacho *et al.*, 2017; Nakamura *et al.*, 2021b; Reiner *et al.*, 2021; Satkunskiene *et al.*, 2021; Nakamura *et al.*, 2022). Two studies analyzed the same roller massage protocol (Nakamura *et al.*, 2021a,c), two studies used instrumental soft tissue mobilization (Ikeda *et al.*, 2019; Vardiman *et al.*, 2015), and Thomson *et al.* (2015) investigated the manual massage. The application time varied between 30s (Nakamura *et al.*, 2021b) and 10min (Thomson *et al.* 2015). All the studies assessed the MTU passive stiffness through passive torque assessment.

A pooled effect (n=9, studies with available data) (Ikeda *et al.*, 2019; Nakamura *et al.*, 2021c; Nakamura *et al.*, 2021a; Nakamura *et al.*, 2021b; Reiner *et al.*, 2021; Satkunskiene *et al.*, 2021; Thomson *et al.*, 2015; Vardiman *et al.*, 2015; Nakamura *et al.*, 2022). In addition, studies were considered more than once when they reported values for different groups, i.e., male and female groups (Nakamura *et al.*, 2021a) (as in the meta-analyses for muscle stiffness). The pooled effect showed no statistically significant changes on the MTU passive stiffness for plantar flexors, knee flexors and extensors (SMD: 0.146; CI95%: -0.046 to 0.339; *p*: 0.136; I^2: 34%) (Figure 5A). 44% (n=4) of the studies included in meta-analysis evaluated self-massage FR, 44% (n=4) used instrumental massage and 11% (n=1) investigated manual massage. 89% (n=8) of the studies evaluated healthy adults and 11% (n=1) athletes. Considering that most studies evaluated the plantar flexors MTU, a specific analysis was made with seven studies. We found no effects of massage techniques on plantar flexors MTU passive stiffness (SMD: 0.218; CI95%: -0.016 to 0.451; *p*: 0.0.067, I^2: 32%) (Figure 5B).

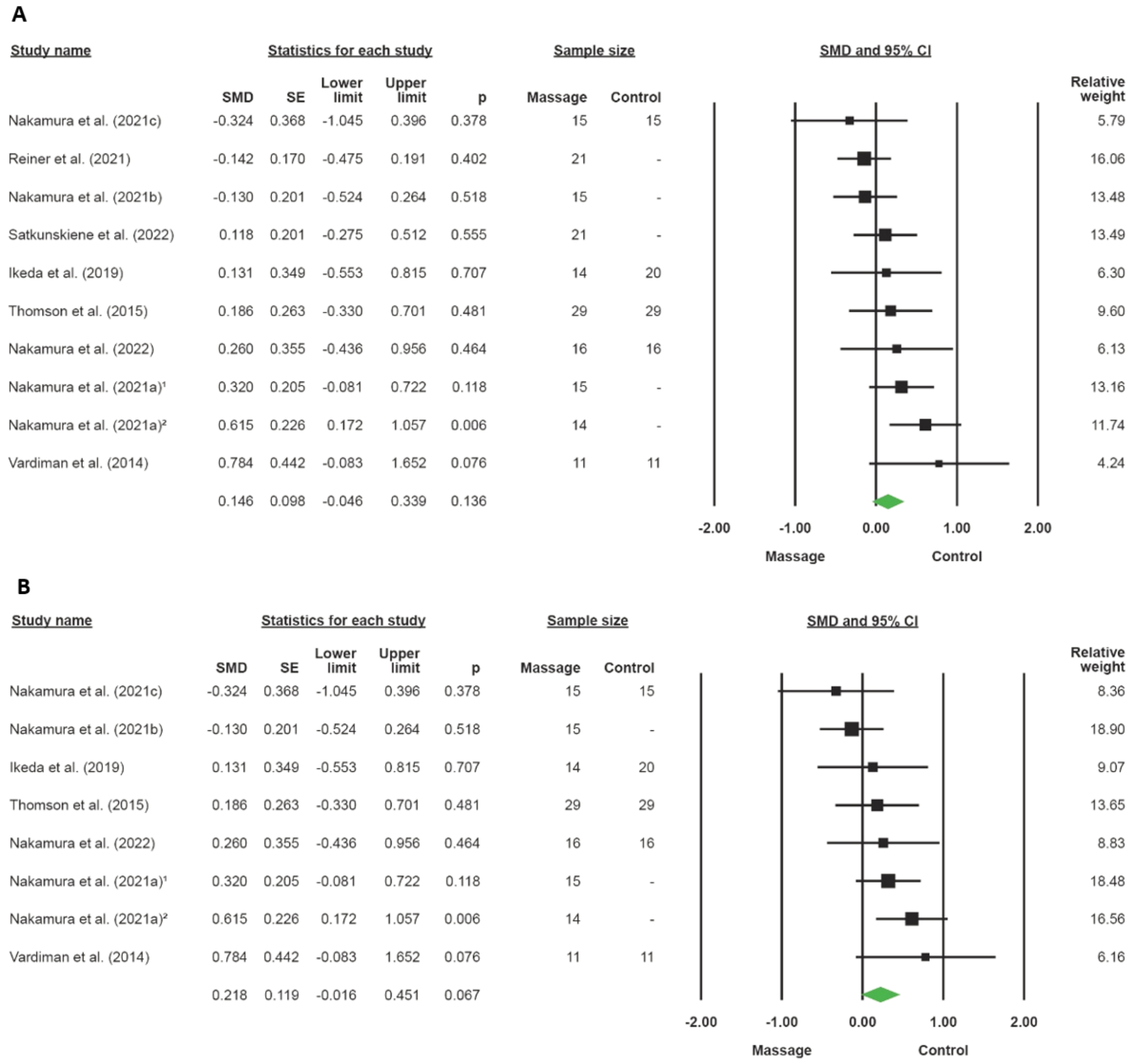


Figure 5 - Effects of non-exercise control vs. massage techniques on MTU stiffness overall (A) and on plantar flexors (B). Forest charts with grouped standardized mean differences (SMD), standard errors (SE), and 95% confidence intervals (CI) are shown: analysis of massage acute effects on passive torque overall (A [SMD: 0.146; CI95%: -0.046 to 0.339; *p*: 0.136; I^2: 34%; effects from nine studies]) and on dorsiflexion (B [SMD: 0.218; CI95%: -0.016 to 0.451; *p*: 0.0.067, I^2: 32%; effects from seven studies]. [1]Male group; [2]Female group.

Studies' quality appraisal

The included studies' scores ranged between 4 and 7 points out of 10 (mean 5.7±0.8), indicating a low methodological quality (Table 2). The main methodological issues found were allocation concealed (0.0% attended), blinding of subjects (0.0% attended), therapists (0.0% attended), and assessors (18.5% attended).

Table 2 - Methodological quality of the included studies (ratings on the PEDro scale).

Study	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Total (points)
Baumgart *et al.* (2019)	+	+	-	+	-	-	-	+	+	-	+	5
Chang *et al.* (2021)	+	+	-	+	-	-	-	+	+	+	+	6
Crommert *et al.* (2014)	+	-	-	+	-	-	+	+	+	+	+	6
Ichikawa *et al.* (2015)	+	+	-	+	-	-	-	+	+	+	+	6
Ikeda *et al.* (2019)	+	+	-	+	-	-	-	+	+	-	+	5
Kasahara *et al.* (2023)	-	+	-	+	-	-	-	+	+	+	+	6
Klich *et al.* (2022)	+	+	-	+	-	-	+	+	+	+	+	7
Krause *et al.* (2019)	+	+	-	+	-	-	-	+	+	+	+	6
Kuruma *et al.* (2013)	-	+	-	+	-	-	-	+	+	+	+	6
Macgregor *et al.* (2018)	+	+	-	+	-	-	-	+	+	+	-	5
Martínez-Cabrera, Nuñez-Sanchez (2016)	+	-	-	+	-	-	-	+	+	+	+	5
Mayer *et al.* (2020)	+	-	-	+	-	-	-	+	+	+	+	5
Morales-Artacho *et al.* (2016)	-	+	-	+	-	-	-	+	+	-	+	5
Nakamura *et al.* (2021a)	+	-	-	-	-	-	-	+	+	+	+	4
Nakamura *et al.* (2021b)	+	+	-	+	-	-	-	+	+	+	+	6
Nakamura *et al.* (2021c)	+	+	-	+	-	-	-	+	+	+	+	6
Nakamura *et al.* (2022)	+	+	-	+	-	-	-	+	+	+	+	6
Perez-Bellmunt *et al.* (2021)	+	+	-	+	-	-	+	+	+	+	+	7
Reiner *et al.* (2021)	+	+	-	+	-	-	-	+	+	+	+	6
Reiner *et al.* (2023)	-	+	-	+	-	-	-	+	+	+	+	6
Satkunskiene *et al.* (2022)	+	+	-	+	-	-	-	+	+	-	+	5
Schroeder *et al.* (2017)	+	+	-	+	-	-	-	+	+	+	+	6
Schroeder *et al.* (2021)	+	+	-	+	-	-	-	+	+	+	+	6
Thomson *et al.* (2015)	+	+	-	+	-	-	+	+	+	+	+	7
Vardiman *et al.* (2014)	+	+	-	+	-	-	-	+	+	+	-	5
Weber *et al.* (2023)	+	+	-	+	-	-	+	+	+	+	+	7
Wilke *et al.* (2019)	+	+	-	+	-	-	-	+	+	+	-	5

Q1: inclusion criteria; Q2: random allocation; Q3: concealed allocation; Q4: similarity at baseline; Q5: subject blinding; Q6: therapist blinding; Q7: assessor blinding; Q8: >85% follow-up; Q9: intention-to-treat analysis; Q10: between-group comparisons; Q11: point estimates and variability; + point awarded; - no point awarded.

Table 3 - GRADE assessment for the certainty of evidence.

Outcomes	**Study design (n)**	**Risk of bias in individual studies**	**Publication bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Confidence in evidence**	**Recommendation**
Muscle stiffness overall	CTs (n = 15)	Serious[a]	Undetected	Serious[b]	No serious	No serious	Very Low	No recommendation can be provided
QUAD muscle stiffness	CTs (n = 8)	Serious[a]	Undetected	Very serious[c]	No serious	Very serious[d]	Very Low	No recommendation can be provided
PF muscle stiffness	CTs (n = 7)	Serious[a]	Undetected	No serious	No serious	No serious	Very Low	No recommendation can be provided
RF muscle stiffness	CTs (n = 6)	Serious[a]	Undetected	No serious	No serious	Serious[e]	Very Low	No recommendation can be provided
VL muscle stiffness	CTs (n = 4)	Serious[a]	Undetected	Very serious[c]	No serious	Extremely serious[f]	Very Low	No recommendation can be provided
GM muscle stiffness	CTs (n = 7)	Serious[a]	Undetected	Very Serious[c]	No serious	Serious[e]	Very Low	No recommendation can be provided
MTU stiffness overall	CTs (n =11)	Serious[a]	Undetected	Serious[b]	No serious	Extremely serious[f]	Very Low	No recommendation can be provided
Dorsiflexion MTU stiffness	CTs (n = 9)	Serious[a]	Undetected	Serious[b]	No serious	Very serious[d]	Very Low	No recommendation can be provided

CT: clinical trial; RCT: randomized controlled trial; MTU: myotendinous unit; QUAD: quadriceps; PF: plantar flexors; RF: rectus femuris; VL: vastus lateralis; GM: gastrocnemius medialis; a. PEDro mean scores between 4-6; b. Heterogeneity values are classified as moderate (I^2 values between 25-50%); c. Heterogeneity values are classified as high (I^2 values >50%); d. The confidence interval is greater than half the mean; e. The confidence interval is greater than the mean (but less than half the mean); f. the confidence interval is equal or more than twice the mean.

Confidence in evidence

The GRADE assessments are presented below (Table 3). We found very low certainty for all the analyzed outcomes (MTU, and muscle stiffness) indicating that the included controlled trials have important limitations and that any effect estimate is very uncertain (Guyatt *et al.*, 2008). The main confidence issues found were risk of bias (0% attended), inconsistency 25% attended), and imprecision (25% attended).

DISCUSSION

To the best of our knowledge, this study presents the first synthesis of available evidence about the acute effects of different massage techniques on the myotendinous structure's stiffness. Although we observed no effects on MTU stiffness, we found decreased muscle stiffness after massage techniques, mainly on the thigh (rectus femoris) and calf (gastrocnemius medialis) muscles. However, our findings require careful interpretation due to the low studies’ quality appraisal and the very low certainty evidence level.

The possible changes in stiffness muscle after massage can occur due to thixotropy effects. When a material becomes more fluid, it resists less strain by an applied force, decreasing the stiffness (Lakie; Campbell, 2019; Dunn; Silver, 1983). The thixotropic response occurs when weak bonds between the materials’ constituent molecules are destroyed temporarily by stimuli (i.e., agitation, heat, pressure) and then reform progressively (Lakie; Campbell, 2019; Dunn; Silver, 1983). Previous studies in animal (Bosboom *et al.*, 2001) and virtual models (Van Loocke *et al.*, 2008) have already shown how the behavior of the muscle’s mechanical properties is influenced by compressive loading (Crawford *et al.*, 2014). Thus, mechanical compression could rearrange the fluid present in muscle fibers’ citoplasm rendering it less viscous (Van Loocke *et al.*, 2008). This would occur especially with compression across the fibers direction, as otherwise the fluid could be constrained by the endomysium and perimysium layers (Van Loocke *et al.*, 2008). Moreover, other studies showed an increased temperature in muscle tissue after massage techniques (Crommert *et al.*, 2015; Drust *et al.*, 2003). The thixotropic effect can also occur through heat (Beardsley & Škarabot, 2015), therefore, this increase in muscle temperature could also make the intramuscular fluid content less viscous after massage.

In addition, the muscle tissue extensibility could change due to neurophysiological factors. Manual massage techniques already showed to increase the skin blood flow (Hinds *et al.*, 2004) and self-massage techniques also showed to increase the intramuscular blood flow immediately after the intervention (Hotfiel *et al.*, 2017). This changes in tissue perfusion are explain through a nitric oxide release on endothelial cells after mechanical stress, which contributes to the reduction of arterial and blood vessel stiffness, increasing the perfusion (Hotfiel *et al.*, 2017; Okamoto *et al.*, 2014). The nitric oxide also seems to play an inhibitory effect in nociceptors (i.e., pain receptors), reducing the spinal transmission (Bavencoffe *et al.*, 2014). If a stimulus modifies autoregulation of the autonomic nervous system and increases the threshold for activation of these nociceptors (Maio *et al.*, 2023), greater pain tolerance could allow a greater muscle extensibility. Furthermore, after this increased tissue perfusion, a break in cross-bridges that remains in the muscle during resting also could cause a muscle relaxation (Crommert *et al.*, 2014; Proske, Morgan, 1999), which would also contribute to increasing muscle extensibility. However, it should be noted that these relaxation mechanisms (fluid rearrange, greater pain tolerance, cross-bridges break) have been discussed hypothetically after massage (Crommert *et al.*, 2014; Proske, Morgan, 1999), and was not proven by experimental studies.

Several studies observed decreased stiffness in quadriceps after massage techniques (Baumgart *et al.*, 2019; Ichikawa *et al.*, 2015; Reiner *et al.*, 2021; Schroeder *et al.*, 2021; Kasahara *et al.*, 2023), but we demonstrated that this effect occurs in the rectus femoris muscle, but not in vastus lateralis. Rectus femoris it is a superficial muscle and is the most centralized of the quadriceps group, which allows the rectus femoris greater emphasis during application (e.g, greater pressure and/or application to a larger part of the muscle) (Reiner *et al.*, 2021). Corroborating with this relationship, Ichikawa *et al.* (2015) emphasized the lateral portion of the quadriceps during the manual technique (described as myofascial release), and was the only study to find a decreased stiffness in vastus lateralis. In addition, our meta-analysis for plantar flexors found decreased muscle stiffness, with a moderate effect. Considering the possible relationship between muscles emphasized during massage and effects on stiffness reduction (Reiner *et al.*, 2021), it is possible that massage techniques can emphasize the gastrocnemius during the application and result in decreased stiffness. In addition, it is possible that superficial muscles also receive more emphasis from the application compared to deep muscles. Although subgroup analysis was not feasible for the other plantar flexor muscles, Chang *et al.* (2021) was the only study to evaluate the gastrocnemius lateralis and found a reduction in muscle stiffness, whereas the only study that assessed soleus (deepest muscle) did not find changes (Ikeda *et al.*, 2019).

Although our meta-analyses showed a reduction in muscle stiffness, this is not a consensus in the included studies. This disagreements results may be due to differences in application protocols. It is possible that pressure applied influences these results, as the muscle thixotropic response may depend on the interaction with more superficial structures, such as adipose tissue (Crawford *et al.*, 2014), which would dissipate part of the mechanical stimulus that reaches the muscle. In addition, the studies that found an increase in skin temperature and intramuscular temperature after massage (an effect that could explain thixotropy due to heat) used techniques with high pressure/weight bearing during intervention (Hotfiel *et al.*, 2017; Hinds *et al.*, 2004). Most studies did not provide detailed information about the pressure applied, so it is not possible to determine with certainty its influence on the results. Part of the studies that applied maximum pressure found a reduction in stiffness (Reiner *et al.*, 2021; Kasahara *et al.*, 2023), whereas the others investigated deeper muscles (erector spinae) (Weber *et al*, 2023) or the limited familiarization with the technique could influenced (Nakamura *et al.*, 2023). In addition, studies using submaximal pressure (assessed by a discomfort scale), no changes in stiffness were observed (Ikeda *et al.*, 2019; Nakamura *et al.*, 2021a, 2021b, 2021c; Wilke *et al.*, 2018). Regarding methodological issues, the majority of the studies did not include a control group and/or perform sample calculations. The absence of a control condition does not allow asserting that the results can be attributed to the technique itself or due to some other factor (e.g., assessment protocol or test learning). Whereas the sample size may have been insufficient to identify changes through intervention. This further limits conclusions about the protocols used.

Although we found decreased stiffness for muscle tissue after massage, few studies have investigated tendon tissue. In our review, only two studies (Chang *et al.*, 2021; Klich *et al.*, 2022) investigated the massage acute effects on tendon tissue stiffness. Both studies used self-massage through foam roller over the plantar flexors and evaluated the Achilles tendon’s stiffness (Chang *et al.*, 2021; Klich *et al.*, 2022). Only Klich *et al.* (2022) found decreased tendon stiffness. Regarding the massage protocols, while Chang *et al.* (2021) used the highest application time with submaximal discomfort level (i.e., 3min; 7/10 discomfort scale during intervention), Klich *et al.* (2022) applied a lesser time massage (i.e., 90s) associated with maximum tolerable pressure. Therefore, a longer application time appears to have no effects on the tendon tissue, but greater pressure may have influenced the stiffness reduction seen by Klich et al (2022). However, the absence of a control condition in this study leads to uncertain conclusions, since the results may not be attributed to the technique itself. In addition, the studies that identified thixotropic effects on muscle tissue after compressive loading (Bosboom *et al.*, 2001; Van Loocke *et al.*, 2008) did not investigate the tendon tissue. Although connective tissue also has thixotropic properties (Meyer *et al.*, 2011), the tendon is a different tissue from the muscle in terms of composition, function, and adaptive responses, presenting more type I collagen (stiffer than other types) and less fluid content (Maganaris *et al.*, 2008). Therefore, the reduction in muscle tissue stiffness attributed to the thixotropic effects of compression and/or heat on its fluid content (Van Loocke *et al.*, 2008) may not happen in tendon due the different composition (Maganaris *et al.*, 2008). Furthermore, the tendon presents more tension receptors (Proske *et al.*, 2012), considering that the stimulus during intervention is mostly compression, the tendon receptors may not respond directly to the inhibition of nociceptors that can occur in the muscle after massage. Consequently, it can be expected that adaptations to massage found in muscle tissue may not occur in tendon.

Similar to tendon results, our meta-analyses indicated no changes in MTU stiffness. In agreement with our findings, most studies found no changes in MTU passive stiffness (Morales-Artacho *et al.*, 2017; Satkunskiene *et al.*, 2021, Krause *et al.*, 2019; Reiner *et al.*, 2021, Ikeda *et al.*, 2019; Nakamura *et al.*, 2021b, 2021c; Thomson *et al.*, 2015; Vardiman *et al.*, 2015). According to previous studies that investigated the MTU, it is possible that the same mechanical stimulus causes greater deformations in muscle tissue than in tendon due to the inherent stiffness of tendons compared to relaxed muscle tissue (Kay; Husbands-Beasley; Blazevich, 2015; Morse *et al.*, 2008). In addition, considering that MTU assessments were passive evaluations, it is possible that MTU was more influenced by the tendon tissue, and the involved passive joint movements can be limited by tendon tissue stiffness. Furthermore, our findings are in line with Kay *et al.* (2015) results, which showed that the stiffness response is tissue-dependent, *i.e.*, an assessment of MTU alone may not represent the response of both structures.

Our study has some limitations. First, we focused on investigating the effects massage techniques on myotendinous structures in healthy adults or athletes. Thus, our findings can’t be applied to other populations, such as unhealthy subjects (e.g., musculoskeletal injuries). In addition, some analyzes showed high heterogeneity, but, when possible, sensitivity analysis was performed to identify the heterogeneity sources. The conclusions about our results also require caution due the different evaluation methods employed, different protocols (even with the same technique), poor methodological quality (observed from PEDro’s average scores; scores below 6 points out of 10), and low strength of evidence recommendation (observed from GRADE’s analysis). However, our study has considerable strengths. We gather different massage and self-massage techniques, allowing a discussion of the mechanical stimulus by the soft tissue mobilization rather than a separate discussion of each technique. Our review was also conducted across various databases with a broad search strategy, and the review steps (selection, data extraction, risk of bias analysis) were carried out by two independent evaluators.

Practical applications

Massage techniques can be used to complement the functional effects expected from a reduction in superficial muscle stiffness. Different massage and self-massage techniques have been used to increase relaxation in muscles and ROM (Weerapong *et al.*, 2005). Our findings demonstrate that the mechanism of these effects is, at least in part, biomechanical, since decreased muscle stiffness allows a sensation of relaxation and can increase the ROM. This occurs because, with decreased stiffness, the muscle tissue is less resistant to movement, enabling greater elongation. Therefore, it may be an option to assist increases in knee flexion and dorsiflexion ROM, and in quadriceps and plantar flexors relaxation in healthy adults and athletes. It can be employed in specific scenarios, such as situations where an acute increase in flexibility is required, and/or to induce superficial muscle relaxation (e.g., after sports practices or in cases of muscle tension reported).

CONCLUSION

Massage techniques can induce decreases in rectus femoris and gastrocnemius medialis stiffness, but do not change the tendon and MTU stiffness. However, due to the small effect sizes, high heterogeneity, and low evidence certainty levels, these findings need to be interpreted with caution.

CHAPTER II: EFFECTS OF DIFFERENT FOAM ROLLING PROTOCOLS ON PLANTAR FLEXORS’ MUSCULOTENDINOUS PROPERTIES AND FUNCTIONAL PERFORMANCE: RANDOMISED CROSSOVER TRIAL

ABSTRACT

Introduction: Foam rolling (FR) is a self-massage technique widely used in sports and rehabilitation. Reductions in soft tissue stiffness may occur after FR, but there are conflicting results in the literature. Furthermore, the relationship between application time and FR effects remains unclear. Aim: To investigate the effects of different FR protocols on the Achilles tendon (AT), gastrocnemius medialis (GM), and plantar flexors myotendinous unit (MTU) stiffness in healthy participants. Materials and methods: Twenty participants (10 male/10 female; 26.7±5.2 years old) were included in this crossover study and exposed to three conditions: FR90 (FR application by three sets of 30s), FR180 (FR application by three sets of 60s), and a control condition (CTRL, without any intervention). Weight bearing during the FR application was measured by a force plate. AT morphological (length and cross-sectional area), mechanical (force, elongation, and stiffness), and material (stress, strain, and Young's modulus) properties, passive GM and MTU stiffness were assessed using ultrasound and isokinetic dynamometry. Unilateral countermovement jump performance was evaluated by two-dimensional kinematics. Skin temperature was measured by thermography. Results: The weight distribution during FR application was similar between the FR90 and FR180 conditions. No changes were found in morphological AT properties, tendon force, or stress. AT elongation (CTRL: 18.7%, FR90: 9.8%, FR180: 11.9%) and strain (CTRL: 22.8%, FR90: 22.2%, FR180: 13.9%) increased for all conditions, while stiffness (CTRL: -8.0%, FR90: -4.3%, FR180: -30.5%) and Young's modulus decreased (CTRL: -8.8%, FR90: -4.4%, FR180: -31.3%). The FR180 presented greater stiffness and Young’s modulus reduction than other conditions (FR90 and CTRL). The GM (CTRL: -33.2%, FR90: -43.2%, FR180: -41.5%) and MTU passive stiffness (CTRL: -30.0%, FR90: -32.9%, FR180: -39.3%) and jump height (CTRL: -6.3%, FR90: -1.4%, FR180: -4.5%) decreased in all three conditions. Skin temperature increased for all conditions in plantar flexors (mean - CTRL: 1.8%, FR90: 1.7%, FR180: 2.6%) and AT region (mean - CTRL: 2.5%, FR90: 1.5%, FR180: 2.5%). Conclusions: Applying FR for a longer time generated greater relative reductions in AT stiffness when compared to shorter time of FR or CTRL condition, with no differences in the changes for GM and UMT stiffness and jumping height.

Keywords: Self-massage. Achilles tendon. Plantar flexors. Stiffness. Thermography.

INTRODUCTION

Foam rolling (FR) is a self-massage technique performed using body weight on a dense, rigid foam roller (Smith *et al.*, 2019), being widely utilized by professionals in the fields of rehabilitation, physical training, and sports (Cheatham, 2019). During its application, direct pressure is exerted on soft tissues such as fascia, muscles, and tendons, which are stretched and massaged upon contact with the device (Behm; Wilke, 2019; Wiewelhove *et al.*, 2019). The popularity of this technique can be attributed to its effects as described in various studies, primarily its role in increasing range of motion (ROM) (Connolly *et al.*, 2020; Skinner; Moss; Hammond, 2020; Wilke *et al.*, 2020), as well as in reducing post-exercise muscle pain (Hendricks *et al.*, 2020; Macdonald *et al.*, 2014), and possible increased jump performance (Konrad; Tilp; Nakamura, 2021; Tsai; Chen, 2021).

Despite these known functional effects of FR, its mechanisms are unclear. The technique is often associated with the term “myofascial release”, suggesting a biomechanical mechanism for reducing soft tissue stiffness (Behm; Wilke, 2019). This reduction in stiffness supposedly occurs due to the viscoelastic property of myofascial tissues (Meyer; McCulloch; Lieber, 2011) and the thixotropic effects (i.e., when a viscous material becomes less dense and more fluid) that can occur with the imposition of compressive loads, making the tissue less resistant to elongation (Beardsley; Škarabot, 2015; Crawford *et al.*, 2014; Reiner *et al.*, 2021; Schleip, 2003). A systematic review, with meta-analysis, investigated the effects of FR on fascial and muscle stiffness (Glänzel *et al.*, 2023). Although the systematic review showed reductions in stiffness, the meta-analysis did not confirm these results. However, few studies were included. Furthermore, the thixotropic effects also may occur as a result of elevated muscle tissue temperature (Schleip, 2003). The FR has shown an increase (Kerautret *et al.*, 2021; Adamczyk *et al.*, 2020), and maintenance (Murray *et al.*, 2016) of skin temperature, which can be related to muscle temperature (Drust *et al.*, 2003).

Regarding the FR acute effects on both muscle and tendon properties, only plantar flexors (PF) stiffness was investigated. Recent studies investigated the FR effects on the PF (Nakamura *et al.*, 2022, 2021; Chang *et al.*, 2021) due their fundamental role in activities of daily living (Olmos *et al.*, 2019), sports performance (Kay; Blazevich, 2009), and activities involving jumping (Farris *et al.*, 2016). The jump performance may be influenced by both pennation angle of the PF (Earp *et al.*, 2010; Secomb *et al.*, 2015), and the tendon stiffness (Kubo *et al.* 2005). A highly pennated PF muscles could increase jump performance due to a greater production of maximal force and ability to store elastic energy (Earp *et al.*, 2010; Secomb *et al.*, 2015). Regarding the FR protocols, different application times are used in practice and a supposed dose-response relationship has been discussed (Nakamura *et al.*, 2021). In the Achilles tendon (AT) tissue, Chang *et al.* (2021) found no differences in stiffness after three sets of 60s, whereas Klich *et al.* (2022) showed a decreased tendon stiffness after three sets of 30s. Concerning the PF muscles' stiffness, different sets (1, 3, and 10) of 30s of application did not alter the gastrocnemius medialis (GM) stiffness (Nakamura *et al.*, 2021), while three sets of 60s reduced (Chang *et al.*, 2021) or did not change stiffness in this muscle (Nakamura *et al.* 2022). These results indicate that application time per set is relevant to tendon and muscle stiffness changes. However, extensive application protocols (e.g., 10 sets of 30s) (Nakamura *et al.*, 2021) could generate high levels of fatigue, making it difficult to properly apply the FR protocol. Thus, the relationship between FR volumes and changes in soft tissue stiffness is not very clear yet, particularly in the PF.

Therefore, given the conflicting results on the mechanisms of FR application as well as differences in the application protocols, this study aims to investigate the effects of different FR protocols on the AT, GM, and myotendinous unit (MTU) stiffness in healthy participants.

MATERIAL AND METHODS

Study design

This study is characterized by a crossover randomized single-blind controlled trial, registered under the Clinical Trials (NCT05801302) and followed the Consolidated Standards of Reporting Trials - CONSORT recommendations for crossover studies (Dwan *et al.*, 2019). All participants included in the study signed a consent form approved by the local research ethics committee (39064720.5.0000.5347). The participants underwent three different conditions in a randomized order: self-massage with FR for three sets of 30s (FR90), three sets of 60s (FR180), and a control condition with no intervention (CTRL).

The participants visited the laboratory four times with a one-week interval wash-out. On the first visit, after obtaining informed consent, the participants were familiarized with the intervention (FR), the conditions were randomly assigned (by closed envelopes) without disclosing the chosen order, and the following questionnaires were answered: personal information, physical activity level by the International Physical Activity Questionnaire (IPAQ) (Craig *et al.*, 2003), and AT function by the Victorian Institute of Sports Assessment self-administered Achilles questionnaire (VISA-A) (de Mesquita *et al.*, 2018) In the remaining three visits, assessments and interventions were conducted (Figure 1). Before the conditions, participants underwent pre-intervention (PRE) assessments: (1) AT morphological properties (at rest); (2) AT mechanical properties (pre-conditioning and active ramp contractions); (3) passive MTU properties; (4) unilateral jump height (after ≈10-minute); and (5) skin temperature. After the intervention, reassessments (POST) began with the skin temperature and, after that, we followed the same order as the PRE assessments. All assessments and analyses were conducted by an evaluator who was blind to the interventions.

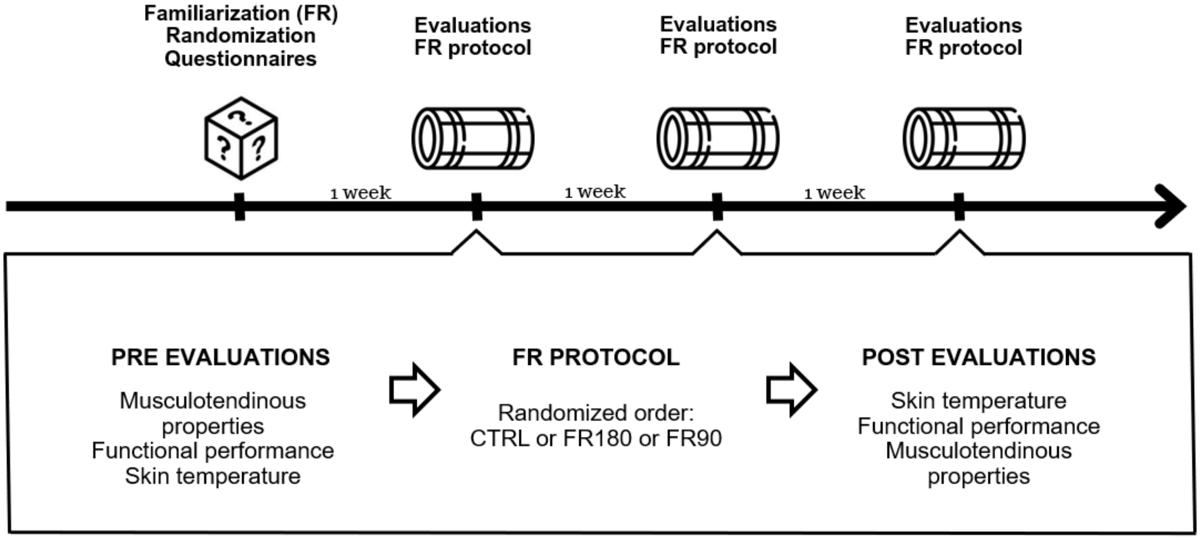


Figure 1 - Experimental study design. FR: foam rolling; PRE: evaluations before intervention; POST: evaluations after interventions; CTRL: control condition; FR90: foam rolling during 90s (three sets of 30s); FR180: foam rolling during 180s (three sets of 60s).

Participants

The sample size calculation was performed in the software G*Power (Kiel University, Germany) and resulted in a minimum of 18 participants (effect size = 0.94; significance level = 0.05; power = 0.90) from GM muscle stiffness values (Chang *et al.*, 2021). Considering a possible loss of 10% of sample size, 20 participants were stipulated (Krause *et al.*, 2019). Healthy and physically active participants (18–35 years of age) were solicited for the study. Participants were included if they: (1) were not engaged in lower limb strength and/or resistance training program in the 4 months preceding the study start; (2) had no history of musculoskeletal injuries or disorders in the evaluated lower limb within the past year; and (3) had not been regularly using FR in the last month (twice a week, at least 15 minutes) (Mayer *et al.*, 2020). Additionally, to be included, female participants were required to use a hormonal contraceptive method (combined synthetic estrogen and progestin pill) and the evaluations were during the pill consumption phase (day 1 to day 21 or 24 of active pill). Throughout the study, participants were instructed to refrain from any systematic physical activity involving the lower limbs.

Participants would be excluded if they: (1) exhibited symptoms of inflammation, acute pain, or delayed muscle pain on the day of assessment; (2) engaged in high-intensity physical activity within 72 hours prior to the assessments; (3) consumed analgesic medication, alcoholic beverages, or caffeine within the last 24 hours before the assessments; (4) had any contraindications for performing maximal exercises; (5) used anabolic steroids. The participants flow diagram is shown in Figure 2.

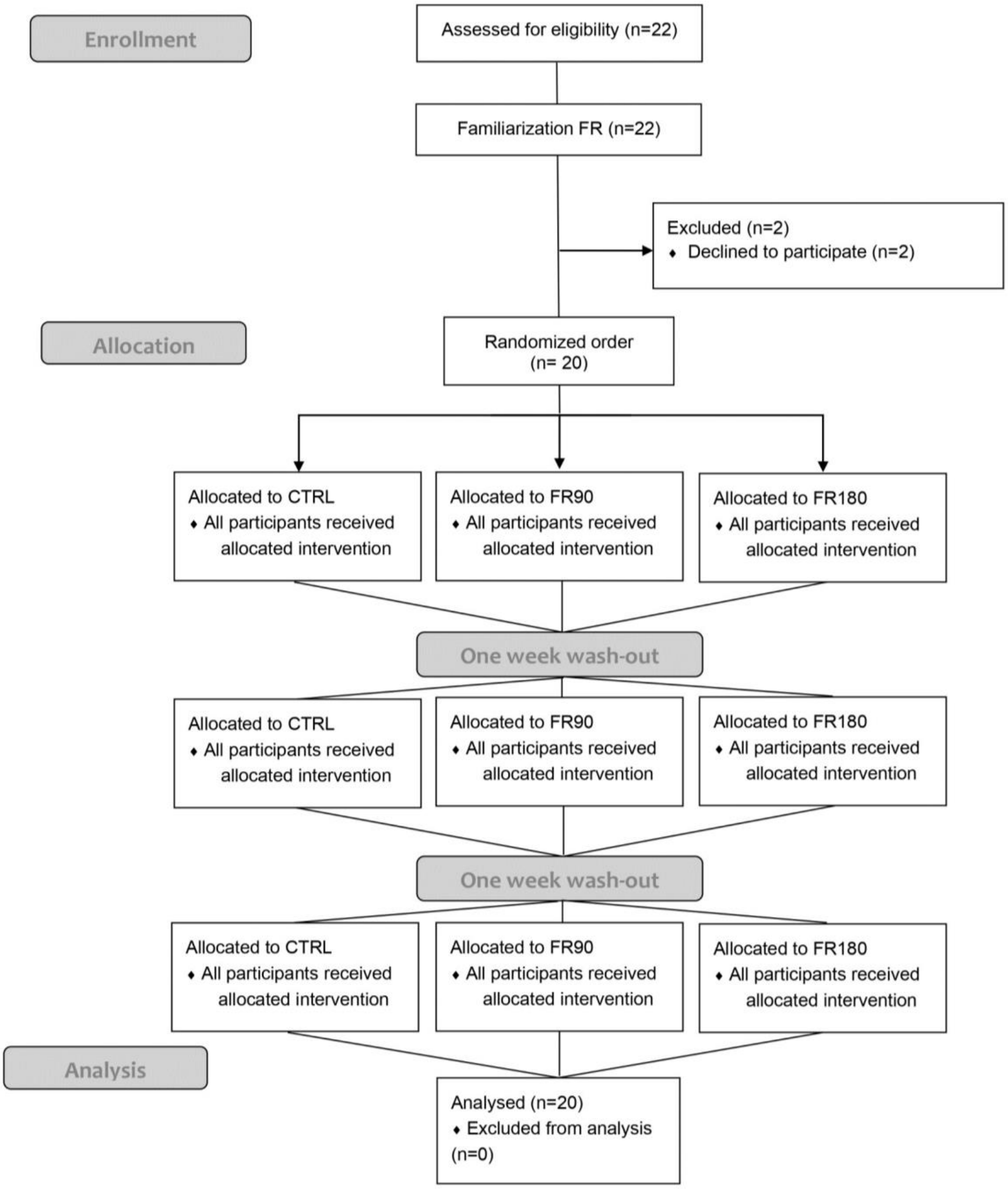


Figure 2 - Participants flow diagram. CTRL: control condition; FR90: foam rolling during 90s (three sets of 30s); FR180: foam rolling during 180s (three sets of 60s).

Intervention

Participants completed two FR protocols and a control assessment on distinct days. In the lower volume protocol (FR90), participants performed three sets of 30s (Nakamura *et al.*, 2021). In the higher volume protocol (FR180), three sets of 60s were performed (Chang *et al.*, 2021). The interval between series was 30s (Chang *et al.*, 2021; Nakamura *et al.*, 2021), and a one-week wash-out was used between each condition (Baumgart *et al.*, 2019; Macgregor *et al.*, 2018).

In the FR90 and FR180 conditions, the application of FR was over the PF and AT of the dominant lower limb, defined as the preferred limb for kicking a ball (Geremia *et al.*, 2015). The participants were positioned in a sitting position, with hands supported to the ground, keeping the body off the ground, and the non-assessed leg crossed above the leg of interest (Chang *et al.*, 2021; Nakamura *et al.*, 2021) (Figure 3). Familiarization procedures were carried out one week before the start of the assessments, where participants performed the movement for the desired time and asked questions about the execution.

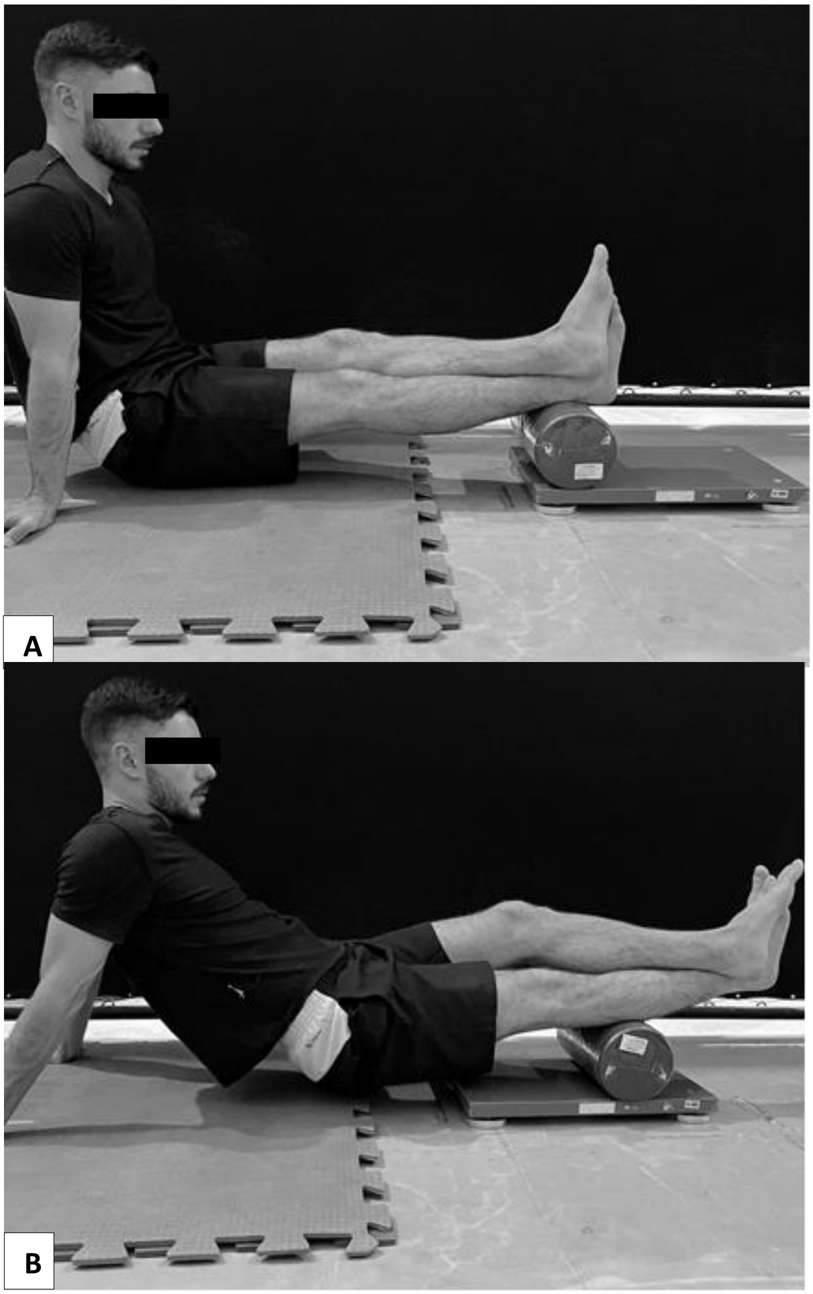


Figure 3 - Initial (A) and final position (B) during foam rolling application.

The FR application was carried out using a device with dimensions of 30cm x 15cm (Foam Roller Brasil, Brazil), positioned on a force platform to monitor weight bearing during FR (Baumgart *et al.*, 2019; Macgregor *et al.*, 2018). The same device was used in all interventions. To standardize weight bearing, participants were instructed to apply the greatest weight bearing possible during rolling (Klich *et al.*, 2022), while the execution speed of the rolling was controlled using a metronome set at a frequency of 30bpm (Nakamura *et al.*, 2021). The FR movements were performed between the popliteal fossa and the insertion of the AT into the calcaneus bone. Weight distribution during FR application was determined through the measurement of ground reaction forces. A force platform (Type 2812A, Kistler, Switzerland) was utilized allowing measurement of the Fz along the Z axle (Baumgart *et al.*, 2019; Macgregor *et al.*, 2018). Ground reaction forces were recorded for the same duration as each FR condition (90s and 180s) at a sampling rate of 200 Hz. Weight bearing was analyzed based on data from the center of pressure recorded by the contact area on the Fz axle. The means of the force curves were calculated relative to the participants' body mass (Baumgart *et al.*, 2019; Curran; Fiore; Crisco, 2008).

In order to assess perceived pain levels, a visual analogue scale was administered to participants during the FR execution. Perceived pain was recorded at the conclusion of each protocol, with specific inquiries made regarding calf pain as well as any discomfort experienced elsewhere on the body. This scale comprises a horizontal line measuring 10cm, where 0 denotes the absence of pain and 10 represents the most severe pain the participant could be experiencing (Haefeli; Elfering, 2006).

In the CTRL condition, participants were not subjected to any treatment, remaining seated at rest for 180s, in a position similar to that of the intervention (sitting position, non-assessed leg crossed above the leg of interest, with hands supported to the ground).

Achilles tendon morphological properties

To obtain data regarding the cross-sectional area (CSA) and AT length (TL), participants were positioned in a prone position with the ankle in neutral position (i.e., foot surface perpendicular to the shank). The ultrasonography (US) probe (ALOKA SSD 4000, Japan), was placed perpendicular to the tendon. Images were captured transversely at distances of 2cm, 4cm, and 6cm from the distal insertion of the AT into the calcaneal bone (Geremia *et al.*, 2015, 2018) (Figure 4). Anatomical reference points, skin marks, and the ultrasonography scanning sites were mapped on a malleable plastic sheet to ensure that repeated scans were taken from the same site.

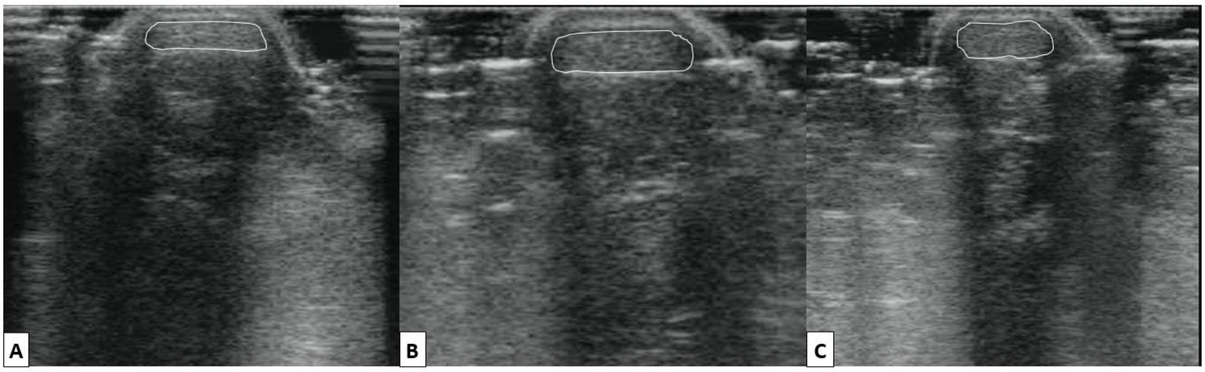


Figure 4 - Cross-sectional area at 2 (A), 4 (B), and 6cm (C) from the distal insertion of the Achilles tendon.

The CSA at each of the three positions was measured five times, and the average was calculated for each position. The mean value from the three positions was taken as the Achilles tendon CSA. The TL was obtained with the US probe positioned longitudinally along the AT. The most distal portion of the AT on the calcaneus (in a sagittal view) was identified by US and marked on the skin. Subsequently, the myotendinous junction (MTJ) of the GM muscle was located, and this point was also marked on the skin. The distance between the two marked points was then measured using a tape measure, and this distance was considered representative of the TL (Geremia *et al.*, 2018).

Tendon elongation during plantar flexor ramp contractions

Tendon elongation was obtained with the US probe positioned at the MTJ, strapped to the shank. A skin marker was used to detect undesired movements of the US probe with respect to the skin during passive motion and during the maximal voluntary isometric contractions (MVICs) (Figure 5). Whenever movement of the probe relative to the skin was detected in the horizontal axis of a movie frame, all markers were corrected by the magnitude of the observed movement. The participants were positioned on the isokinetic dynamometer (Biodex System 3 Pro, USA) and performed five-ramp plantar flexor MVICs for familiarization and tendon pre-conditioning (Seynnes *et al.*, 2015). Next, participants performed three-ramp plantar flexor MVICs lasting 5s each (Jerger *et al.*, 2022; Seynnes *et al.*, 2015), with a 120s rest interval between contractions. During the ramp MVICs, torque, US images, tibialis anterior electromyography (EMG) signal and ankle kinematics were obtained with a synchronization system (HORITA, Video Stop Watch VS-50; HORITA

Company Inc., USA). The US images were recorded by an external DVD recording unit (R130/XAZ, 32 Hz, Samsung Seoul Inc., South Korea; sampling frequency 30 frames per second).

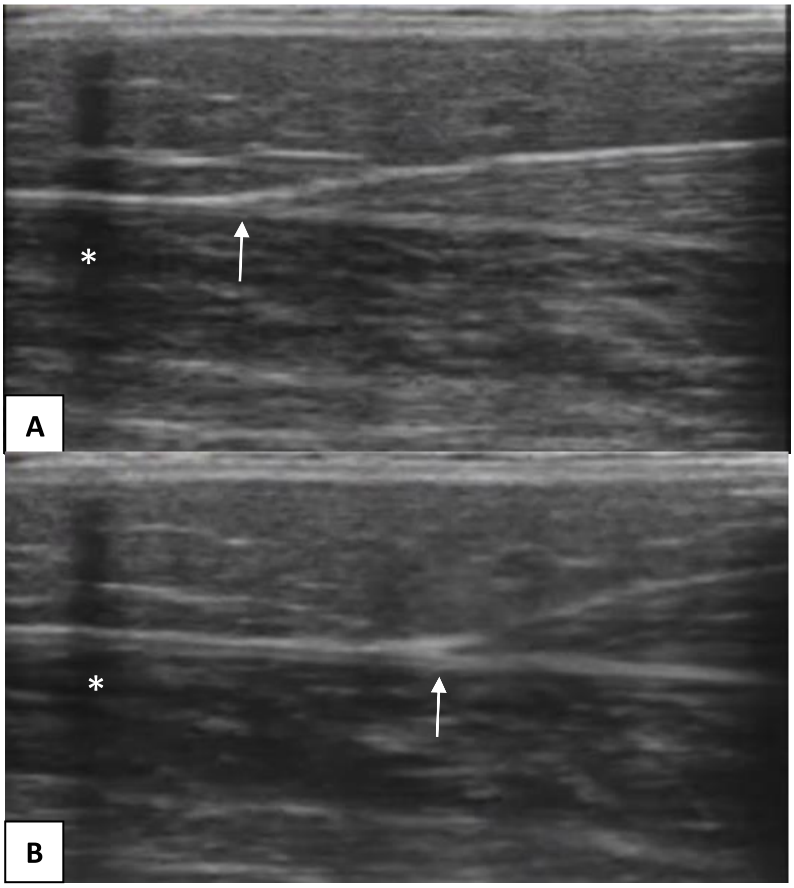


Figure 5 - Displacement of the myotendinous junction (arrow) between the initial (A) and the final (B) of the ramp contraction. *Skin marker.

Plantar flexor torque correction through muscle electrical activity evaluation

The torque recorded by the dynamometer corresponds to the final plantar flexion torque, which differs from the torque produced by the PF if antagonist activation occurs (Geremia *et al.*, 2015; Magnusson *et al.*, 2001). The estimate of the actual torque generated by the plantar flexors was improved using a relation established between tibialis anterior EMG signal and the corresponding dorsiflexor torque (Arya; Kulig, 2010; Geremia *et al.*, 2015, 2018). Tibialis anterior activation was obtained using an EMG system (Delsys Trigno Wireless, USA) in three different conditions (Geremia *et al.*, 2015, 2018; Mademli *et al.*, 2004): (a) at rest, (b) at a lower and (c) at a higher activation than that produced during the ramp plantar flexor MVIC. The EMG signals were digitized at 2000 Hz simultaneously with the dynamometer's torque signal by a data acquisition system (Labchart, ADInstruments, Australia). EMG signals were band-pass filtered (Butterworth, 20-500Hz), rectified, smoothed with a low-pass filter (Butterworth, 4 Hz). A linear relation was established between the mean rectified and smoothed EMG signals and the obtained dorsiflexor torque at the three different activation levels. From this relation, the dorsiflexor torque was estimated and used to improve our estimate of the actual torque produced by the PF from the measured net ankle torque.

Tendon displacement correction through evaluation of ankle joint rotation

During plantar flexor MVICs, joint rotation may occur independent of external limb fixations, causing undesired ankle joint rotation and overestimation of tendon elongation (Magnusson *et al.*, 2001). To reduce this source of error, retroreflective markers were placed at (1) the middle third of the leg, (2) the malleolus, (3) the first metatarsal head. The position of the markers during the ramp MVICs was monitored with a video camera (HDR-CX 220, 60 Hz, Sony, Japan) (Muramatsu *et al.*, 2001) and synchronized to the isokinetic dynamometer using a single-pulse generator connected to a small lamp. The Tracker software (Physlets, USA) was used to calculate the plantar flexion angle during the MVICs.

AT elongation was corrected after tracking the MTJ during ankle passive motion (Magnusson *et al.*, 2001) at a constant angular velocity of 5° s^{-1} from 0° (tibia perpendicular to the foot line with knee fully extended) to 35° (plantar flexion). Three passive plantar flexion cycles were executed, and cycles were repeated if there was any EMG activity of the soleus or tibialis anterior muscles (Geremia *et al.*, 2018).

MTJ tracking during passive motion was performed using Tracker software. MTJ displacement was obtained for each joint angle at the three executed motion cycles, and a mean MTJ displacement value was calculated for further analysis. Finally, MTJ displacement was corrected by subtracting the undesired displacement obtained during the ramp MVICs (Geremia *et al.*, 2018; Magnusson *et al.*, 2001; Muramatsu *et al.*, 2001).

Achilles tendon mechanical and material properties

AT elongation was obtained from the MTJ displacement on the US video files during the ramp MVICs. Using ShotCut software (Meltytech, USA), the video file was screened frame by frame to select the desired images (ramp contraction). MTJ displacement was then determined by Tracker software. To calculate AT force, first it was determined the AT moment arm from the video and US images (Geremia *et al.*, 2018; Manal; Cowder; Buchanan, 2010; Zhao *et al.*, 2009). A retroreflective marker was positioned on the medial malleolus, and the US probe was longitudinally positioned over the AT, with the probe centre aligned with the marker. In this position, two images were obtained: (1) one video image of the medial malleolus; and (2) one US image of the AT. The distances between the marker and the probe (*d1*), and between the AT line of action and the skin (*d2*) were obtained with ImageJ software (National Institutes of Health, Maryland, USA). The difference between *d1* and *d2* was used as measure of the AT moment arm. Therefore, AT force was obtained by dividing the corrected plantar flexor torque by the AT moment arm (Geremia *et al.*, 2018; Manal; Cowder; Buchanan, 2010; Zhao *et al.*, 2009).

AT force and elongation were obtained at intervals of 10% of the MVIC force (from 0 to 100%) (Geremia *et al.*, 2018). The slope of the force–elongation curve, obtained from 80 to 100% MVIC was considered as the tendon stiffness for each participant (Seynnes *et al.*, 2015). Stress was obtained by taking the ratio between AT force and the tendon's CSA, and strain by taking the ratio between tendon elongation and TL at rest. Stresses and strains were obtained at 10% torque steps throughout the ramp MVIC. The Young's modulus was determined as the slope of the stress–strain curve, obtained from 80 to 100% MVIC. The slopes of the force-elongation and stress-strain curves were obtained by linear regressions. All calculations necessary to obtain AT mechanical and material properties were performed in MATLAB software (MATrix LABoratory, MathWorks Inc., Natick, USA).

Medial gastrocnemius and muscle tendon unit passive stiffness

To assess passive GM and MTU stiffness, the passive torque was initially measured during passive dorsiflexion at 5°/s using the isokinetic dynamometer in a reference position (Kay; Husbands-Beasley; Blazevich, 2015). Three movement cycles were performed until the maximum range of dorsiflexion for each subject (by reported discomfort), And the last cycle was chosen for analysis (Kay; Husbands-Beasley; Blazevich, 2015). The slope in the passive torque curve in the final 10° of dorsiflexion was considered as the passive stiffness of the MTU. During the post-evaluation of each intervention, the slope of the curve was calculated at the same 10° end of pre-assessment dorsiflexion (Kay; Husbands-Beasley; Blazevich, 2015). EMG signs of the gastrocnemius lateralis, soleus, and anterior tibialis were monitored to ensure that the movements were performed exclusively passively (Kay; Husbands-Beasley; Blazevich, 2015).

During the passive torque performance, the GM MTJ displacement was monitored by US. The stiffness of the GM muscle was calculated as the passive torque variation in the final 10° of dorsiflexion divided by the change in GM MTJ (Kay; Husbands-Beasley; Blazevich, 2015). All calculations necessary to obtain GM and UMT passive torque were performed in MATLAB software (MATrix LABoratory, MathWorks Inc., Natick, USA).

Plantar flexors' pennation angle

In order to identify possible relationships between plantar flexor pennation angle and jumping performance (Earp *et al.*, 2010; Secomb *et al.*, 2015), the pennation angle for each plantar flexor muscle was assessed by US images. Three US images were obtained for each plantar flexors muscle with the subject at rest (prone position, ankle in neutral position - 0°). The US probe was covered with water-soluble transmission gel and positioned longitudinally to the muscle fibers and perpendicular to the skin at 50% (soleus) and 30% (GM and lateral gastrocnemius) of the distance between the popliteal crease and the lateral malleolus (Geremia *et al.*, 2019; Kawakami, 2012) (Figure 6). Anatomical reference points, skin marks, and the ultrasonography scanning sites were mapped on a malleable plastic sheet to ensure that repeated scans were taken from the same site.

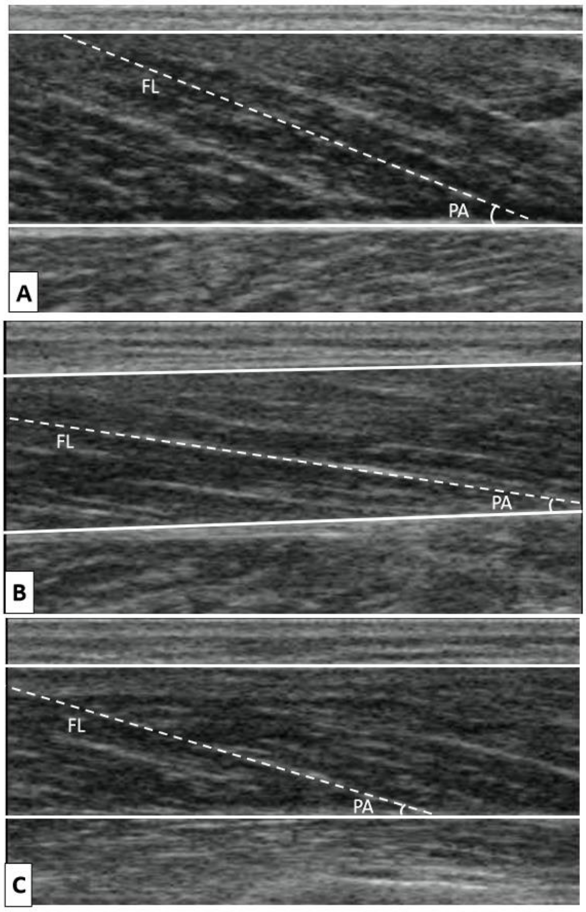


Figure 6 – Pennation angle for gastrocnemius medialis (A), gastrocnemius lateralis (B), and soleus (C). PA: pennation angle; FL: fascicle length.

The images were analyzed by ImageJ software. The best fascicle (i.e., the fascicle that best could be seen in its entirety from its insertion on the deep aponeurosis into the superficial aponeurosis, or to the US probe field-of-view end) in each image was used for pennation angle analysis (Geremia *et al.*, 2019). Pennation angle was calculated as the angle between the muscle fascicle and the deep aponeurosis (Geremia *et al.*, 2019). Mean values were obtained from three US images for each muscle.

Unilateral countermovement jump

The unilateral countermovement jump (CMJ) was performed using the dominant limb (Panidi *et al.*, 2021). Kinematic two-dimensional data were captured using a video camera (HDR-CX, 60Hz, Sony, Japan) positioned in the sagittal plane. A reflective marker was placed on the trochanter to monitor jump height (Aizawa *et al.*, 2016). The analysis of images for the initial moment and the highest point of the jump was conducted using Tracker software.

Participants began the assessment in a single-leg balance position, with their hands positioned on their waist. They were then instructed to perform a descending movement (knee and hip flexion) and immediately follow it with a unilateral jump involving knee and hip extension, landing unilaterally (Panidi *et al.*, 2021). Five maximum tests were conducted with a 2-minute interval between each test. The highest jump height achieved was used for the analysis (Panidi *et al.*, 2021).

Skin temperature assessment

All thermal images were captured by the same evaluator using a thermal camera FLIR-T450sc with a resolution of 320 x 240 pixels (Flir Systems Inc., Wilsonville, Oregon, USA) in an air-conditioned environment (room temperature: 25°c) (Quesada, 2017). An anti-reflective panel was placed behind the participant to avoid interferences from radiation emitted by a non-neutral background (Quesada, 2017). All images were captured at 1.5 m from the region of interest (ROI), with the camera lens perpendicular to the ROI, skin emissivity of 0.98, and the participants were exposed to the room temperature for at least 5 min before the assessment (Quesada, 2017). In the post-intervention, the skin temperature was the first evaluation to minimize the influence of other stimuli on skin temperature (e.g., ultrasound gel, EMG electrodes, MVICs).

Thermacam Researcher Pro 2.10 software (FLIR, Wilsonville, OR, USA) was used to process all the images. The ROI corresponding to PF was determined as the area between the largest region of the calf muscles and the AT (i.e., change in color between the muscle and the tendon and judged by the assessor) (Da Silva *et al.*, 2022) (Figure 7A). For the ROI corresponding to AT, the assessor defined a standardized rectangle area for all images. This rectangle tool was placed over the length and width of the AT with the lower border of the box in line with the superior border of the calcaneus as defined by the change in color between the bone and the tendon and judged by the assessor (Figure 7B) (Tumilty *et al.*, 2019). The mean, maximum, and minimum temperature values were obtained for each ROI.

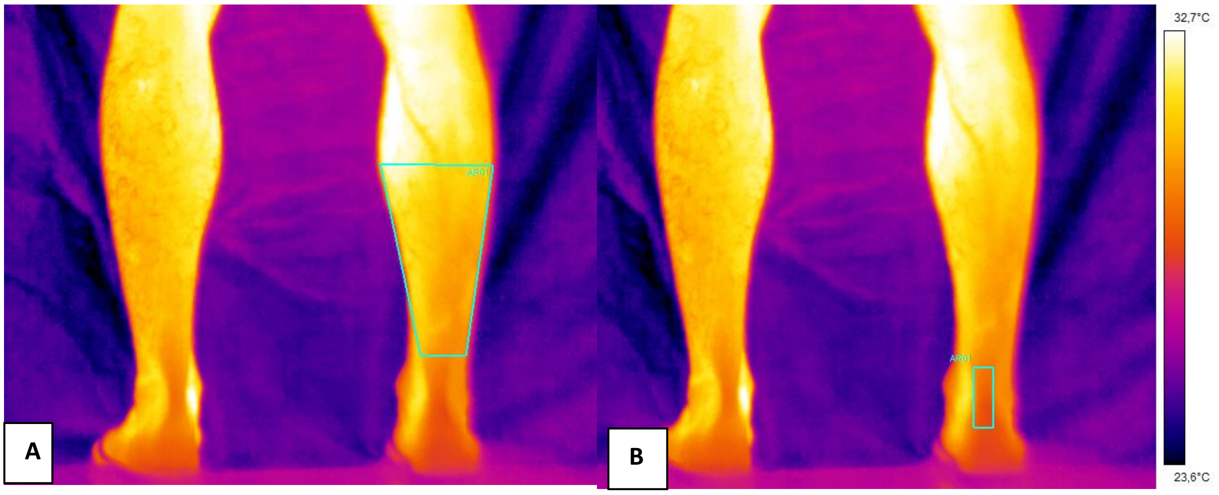


Figure 7. Region of interest for plantar flexors (A) and Achilles tendon (B) skin temperature.

Statistical analysis

All statistical procedures were carried out using SPSS software (version 20.0; IBM Corporation, Armonk, New York, USA). An intraclass correlation coefficient (ICC) was applied to determine the test–retest reliability between two PRE conditions with an interval of at least 7 days, for all measurements. The reliability was interpreted as follows: null (0.0), weak (0.1-0.3), moderate (0.4-0.6), strong (0.7-0.9), and perfect (1.0) (Dancey; Reidy, 2013).

Normality of the data were assessed using the Shapiro-Wilk test. To compare the weight bearing and reported pain during the two FR protocols, the t-test for paired samples or Wilcoxon test was used. A two-way repeated measures ANOVA was utilized to compare the conditions (CTRL, FR90, and FR180) and time points (pre and post). The sphericity was tested by Mauchly test. The effect size (ES) (Cohen's d) was calculated for each variable. Values <0.20 indicated a trivial effect size, values between 0.20 and 0.50 indicated a small effect size, values between 0.51 and 0.80 indicated a moderate effect size, and values >0.80 indicated a large effect size (Cohen, 1988). Typical error (TE) also was calculated (Hopkins, 2000). Relative changes to the PRE evaluation and comparison of time intervals from the intervention to the reassessment were determined for each outcome variable using a one-way repeated measures ANOVA, followed by LSD *post-hoc* test.

Responsiveness to the conditions (percent change from pre- to post-intervention) was determined by TE criteria (Bonafiglia *et al.*, 2016; Cadore *et al.*, 2018; Geremia *et al.*, 2019). The TE was calculated by the equation $TE = \Delta SD/\sqrt{2}$, where ΔSD is difference between the standard deviations of two pretests. Non-responsive participants were defined as those that did not achieve an expected change that was two times higher than the TE whereas those participants who presented unexpected changes two times higher than TE were considered adverse responders (Bonafiglia *et al.*, 2016). All statistical analyses used a significance level of $\alpha \leq 0.05$.

RESULTS

Twenty volunteers (10 male/10 female; 26.7 ± 5.2 years old; 1.7 ± 0.1m of height; 72.2 ± 16.0kg of body mass; 25.0 ± 4.3 of body mass index; 58.3 ± 50.3 min of physical activity/week; 20 right legs) participated in the study. The VISA-A score was 96.3 ± 5.3 points, indicating healthy ATs (> 70 points) and there were no reports of current pain.

High test–retest reliability between two PRE conditions was observed from the ICC values for the follow variables: AT force (r = 0.991), AT elongation (r = 0.872), AT stiffness (r = 0.894), stress (r = 0.998), strain (r = 0.963), Young's modulus (r = 0.892), CSA (r = 0.997), TL (r = 0.932), GM elongation (r = 0.834), GM passive stiffness (r = 0.819), UMT passive stiffness (r = 0.833), CMJ height (r = 0.952), plantar flexors' pennation angle (GM: r = 0.845; GL: r = 0.901, SO: r = 0.943), PF skin temperature (mean: r = .972; maximum: r = 0.976, minimum: r = 0.921), and, and TA skin temperature (mean: r = 0.900; maximum: r = 0.943, minimum: r = 0.899).

Foam rolling application

The mean of relative weight distribution during FR application was similar between the FR90 and FR180 conditions (Table 1).

Table 1. Relative weight distribution during foam rolling conditions (mean ± standard deviation).

	FR90	FR180	*P*
Mean vertical GRF (%BW)	39.2 ± 3.3	38.9 ± 4	0.573
Maximum vertical GRF (%BW)	43.9 ± 3.9	44.2 ± 4.7	0.545
Minimum vertical GRF (%BW)	16.1 ± 3.5	10.0 ± 6.2	0.121

FR90: foam rolling during 3 sets of 30s; FR180: foam rolling during 3 sets of 60s GRF: ground reaction force; BW: body weight; s: seconds.

During the FR90, 60% (n=12) reported pain in calf region, and 70% (n=14) declared pain in upper limbs region (3.6 ± 2.1cm). For the FR180 protocol, 75% (n=15) reported calf pain (2.0 ± 2.3cm) and 95% (n=19) presented pain in upper limbs (4.8 ± 2.7cm). The reported pain in the upper limbs was higher in FR180 (p = 0.001), with no difference between the groups for the calf region ($p > 0.05$). Furthermore, in the intra-group comparison, pain in the upper limbs was greater than pain in the calf for both conditions ($p < 0.05$).

The mean time between the intervention and the post-evaluation for the main outcomes (soft tissue stiffness) was 42.5 ± 6min for CTRL condition, 42.7 ± 9.4min for FR90, and 40.1 ± 7.4min for FR180. There were no differences in interval time between the groups (p = 0.248).

Achilles tendon properties

Table 2 shows the AT morphological, mechanical and material properties observed in pre and post moments between the conditions. No changes ($p > 0.05$) were found in TL, CSA, force, and stress. The elongation increased in the post-evaluation for CTRL (ES = 0.51), FR90 (ES = 0.48), and FR180 (ES = 0.42), whereas the stiffness decreased for CTRL (ES = 0.39), FR90 (ES = 0.33), and FR180 (ES = 1.26). No differences ($p > 0.05$) among the conditions were found for AT elongation and stiffness.

In addition, the strain increased (CTRL: ES = 0.52; FR90: ES = 0.68; FR180: ES = 0.44) and Young’s modulus decreased (CTRL: ES = 0.45; FR90: ES = 0.40; FR180: ES = 1.28) between the pre-post moments, with no differences between conditions ($p > 0.05$). FR180 presented greater relative reductions for stiffness and Young’s modulus ($p < 0.05$). The force–elongation (Figure 8A) and stress–strain (Figure 8B) relationships changed between pre and post evaluations in all conditions.

Table 2. Achilles tendon morphological, mechanical and material properties (mean ± standard deviation).

	CTRL		FR90		FR180	
	PRE	POST	PRE	POST	PRE	POST
Morphological properties						
Tendon length (mm)	205.8 ± 26.2	206.6 ± 24.7	204.7 ± 23.6	205.9 ± 21.5	205.2 ± 22.8	204.9 ± 24.3
Δ%	0.3 ± 1.2		0.3 ± 1.4		-0.1 ± 2.7	
CSA (mm^2)	45.1 ± 12.2	45.0 ± 12.0	45.2 ± 11.6	45.2 ± 10.3	45.2 ± 12.1	45.5 ± 11.9
Δ%	-0.2 ± 1.1		1.0 ± 5.0		-0.2 ± 1.6	
Mechanical properties						
Force (N)	2062.8 ± 392.8	2026.5 ± 371.1	2084.8 ± 447.8	1990.5 ± 470.2	2083.3 ± 421.0	2017.9 ± 384.6
Δ%	-1.2 ± 11.2		-4.1 ± 14.0		-2.3 ± 12.0	
Elongation (mm)	15.9 ± 3.4	17.6 ± 3.2*	15.9 ± 2.3	17.3 ± 3.4*	15.7 ± 3.2	17.1 ± 3.5*
Δ%	12.7 ± 20.6		9.8 ± 18.7		11.9 ± 30.4	
Stiffness (N/mm)	211.1 ± 72.7	182.7 ± 72.3*	207.2 ± 67.8	185.8 ± 60.6*	217.0 ± 50.0	148.7 ± 58.0*
Δ%	-8.0 ± 34.9		-4.3 ± 38.6		-30.5 ± 25.1$^\#$	
Material properties						
Stress (MPa)	44.6 ± 15.1	44.6 ± 11.5	45.0 ± 15.7	44.6 ± 8.2	47.7 ± 14.3	45.9 ± 10.0
Δ%	5.2 ± 21.9		7.4 ± 31.7		-0.1 ± 19.5	
Strain (%)	7.2 ± 2.1	8.3 ± 2.1*	7.1 ± 2.3	8.5 ± 1.8*	7.6 ± 1.9	8.4 ± 1.7*
Δ%	22.8 ± 53.8		22.2 ± 40.2		13.9 ± 30.0	
Young’s modulus (MPa)	1012.9 ± 416.9	846.0 ± 316.3*	964.6 ± 302.1	852.9 ± 247.1*	1026.2 ± 299.1	675.5 ± 246.2*
Δ%	-8.8 ± 34.6		-4.4 ± 38.9		-31.3 ± 25.1$^\#$	

CTRL: control condition; FR90: foam rolling during three sets of 30s; FR180: foam rolling during three sets of 60s; CSA: cross-sectional area; *Different from PRE ($p \leq 0.05$); $^\#$Different from CTRL and FR90 ($p \leq 0.05$).

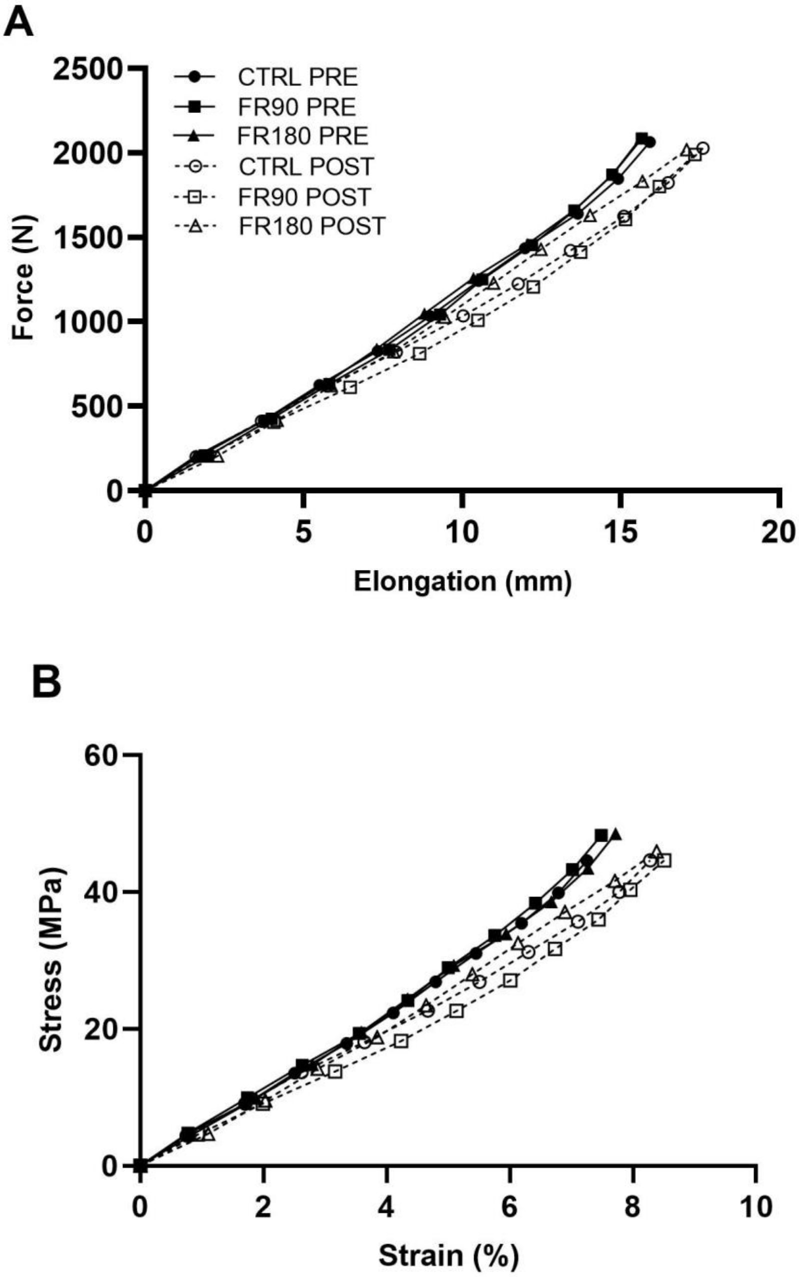


Figure 8 - Achilles tendon force–elongation (A) and stress–strain (B) relationships between pre and post evaluations. CTRL: control condition; FR90: foam rolling during three sets of 30s; FR180: foam rolling during three sets of 60s.

The individual responsiveness analysis for tendon stiffness and Young’s modulus changes showed that 65-85% of the participants responded to FR interventions. However, 50-60% also responded to CTRL condition. The values for all variables are described in Table 3.

Table 3. Individual responsiveness to conditions for all variables.

		TE	Responders n (%)			Non-responders n (%)			Adverse responders n (%)		
			CTRL	FR90	FR180	CTRL	FR90	FR180	CTRL	FR90	FR180
Tendon properties	TL	0.68	6 (30)	5 (25)	3 (15)	11 (55)	11 (55)	12 (60)	3 (15)	4 (20)	5 (25)
	CSA	0.30	1 (5)	4 (20)	3 (15)	16 (80)	10 (50)	12 (60)	3 (15)	6 (30)	5 (25)
	Stiffness	3.31	10 (50)	13 (65)	16 (80)	2 (10)	1 (5)	2 (10)	8 (40)	6 (30)	2 (10)
	YM	7.65	12 (60)	13 (65)	17 (85)	0 (0)	30 (0)	1 (5)	8 (40)	7 (35)	2 (10)
Passive stiffness	GM	0.02	18 (90)	18 (90)	18 (90)	0 (0)	0 (0)	0 (0)	2 (10)	2 (10)	2 (10)
	MTU	0.04	15 (75)	16 (80)	17 (85)	4 (20)	1 (5)	2 (10)	1 (5)	3 (15)	1 (5)
Jump height		0.15	1 (5)	5 (25)	6 (30)	2 (10)	2 (10)	3 (15)	17 (85)	13 (65)	11 (55)
Skin temperature	PF mean	0.01	16 (80)	14 (70)	17 (85)	3 (15)	0 (0)	0 (0)	1 (5)	6 (30)	3 (15)
	AT mean	0.29	12 (60)	7 (35)	8 (40)	8 (40)	10 (50)	12 (60)	0 (0)	3 (15)	0 (0)

TE: typical error; CTRL: control condition; FR90: foam rolling during three sets of 30s; FR180: foam rolling during three sets of 60s; TL: tendon length; CSA: cross-sectional area; YM: Young's modulus; GM: gastrocnemius medialis; MTU: myotendinous unit; PF: plantar flexors muscles; AT: Achilles tendon.

Gastrocnemius medialis and muscle-tendon unit passive stiffness

Table 4 presents the values for passive stiffness. The GM and MTU passive stiffness decreased in the post-intervention for CTRL (GM: ES = 1.66, $p < 0.001$; MTU: ES = 1.17, $p < 0.001$), FR90 (GM: ES = 1.86, $p < 0.001$; MTU: ES =1.3, $p < 0.001$), and FR180 (GM: ES = 1.6, $p < 0.001$; MTU: ES = 1.9, $p < 0.001$). There were no differences between conditions (Figures 9A, 9B). The individual responsiveness varied between 75-90% for FR conditions and for CTRL (Table 3). The ROM for dorsiflexion also did not change in any moment or condition ($p > 0.05$). No differences among conditions ($p > 0.05$) were found in percentual changes for GM (CTRL: -33.2 ± 31.9; FR90: -43.2 ± 36.4; FR180: -41.5 ± 29.0) and MTU passive stiffness (CTRL: -30.0 ± 29.2; FR90: -32.9 ± 48.8; FR180: -39.3 ± 24.9).

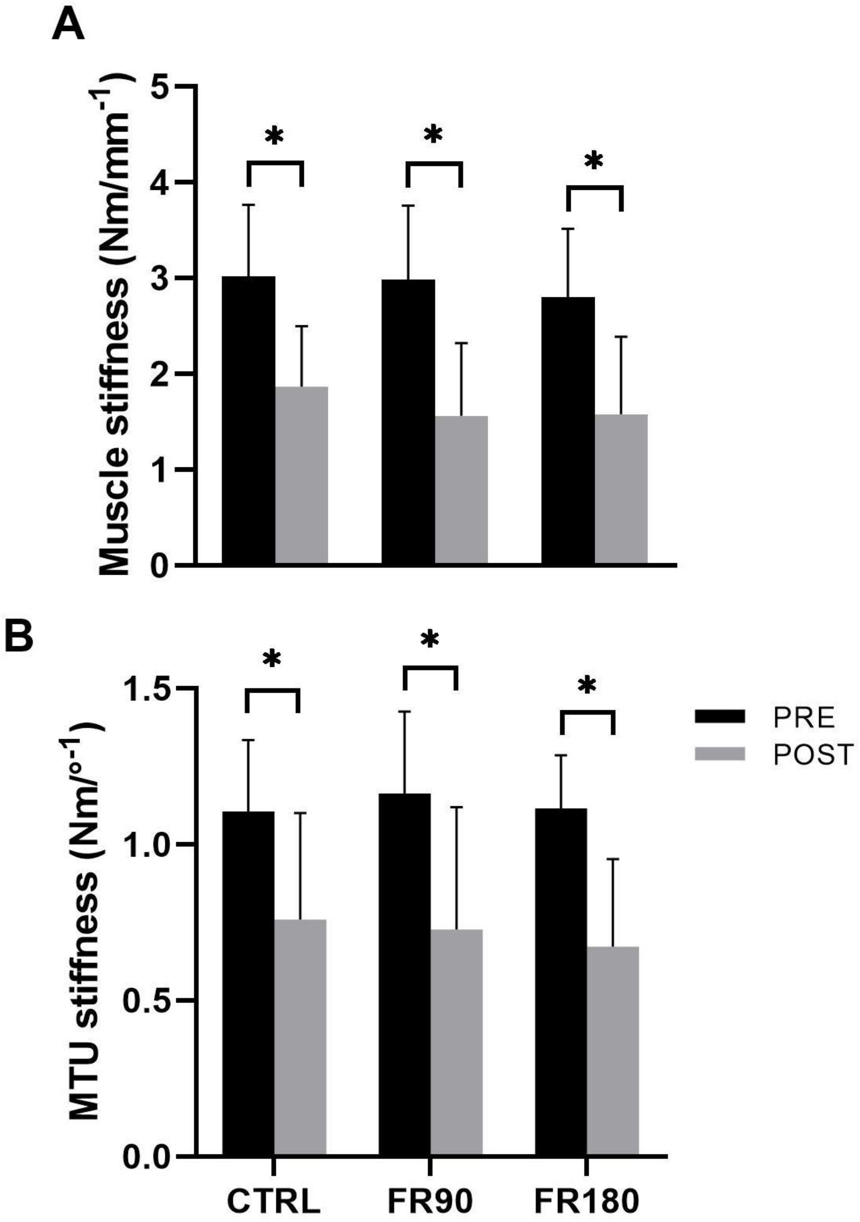


Figure 9 - Gastrocnemius medialis (A) and myotendinous unit (B) stiffness. CTRL: control condition; FR90: foam rolling during three sets of 30s; FR180: foam rolling during three sets of 60s; GM: gastrocnemius medialis; MTU: myotendinous unit. *Different from PRE ($p \leq 0.05$).

Unilateral countermovement jump height

The unilateral CMJ jump height decreased in post-evaluation for CTRL (ES = 0.27. $p < 0.05$), FR90 (ES = 0.09, $p < 0.05$), and for FR180 (ES = 0.17, $p < 0.05$) (Table 4). No changes between conditions were found ($p > 0.05$). The responsiveness to this decrease was 85% in CTRL, 65% in FR90, and 55% in FR180 (Table 3).

Table 4. Jump height in the unilateral countermovement jump (mean ± standard deviation).

	CTRL		FR90		FR180	
	PRE	POST	PRE	POST	PRE	POST
Jump height (cm)	20.4 ± 4.8	19.1 ± 4.7*	20.0 ± 5.1	19.6 ± 4.7*	20.0 ± 4.5	19.2 ± 5.0*
Δ%	-6.3 ± 5.6		-1.4 ± 8.0		-4.5 ± 9.8	

CTRL: control condition; FR90: foam rolling during three sets of 30s; FR180: foam rolling during three sets of 60s; *Different from PRE ($p \leq 0.05$).

The plantar flexor's pennation angles were similar among conditions and between moments ($p > 0.05$) for GM (CTRL - pre: 20.1° ± 4.2, post: 19.2° ± 4.0; FR90 - pre: 19.8° ± 4.2, post: 19.5° ± 3.5; FR180 - pre: 19.4° ± 3.3, post: 20.2° ± 3.5), gastrocnemius lateralis (CTRL - pre: 13.0° ± 2.3, post: 12.9° ± 2.0; FR90 - pre: 13.4° ± 2.0, post: 12.6° ± 2.3; FR180 - pre: 13.7° ± 2.4, post: 12.9° ± 2.2), and soleus (CTRL - pre: 19.5° ± 4.2, post: 20.8° ± 4.5; FR90 - pre: 20.6° ± 5.4, post: 20.6° ± 3.6; FR180 - pre: 20.7° ± 5.4, post: 20.2° ± 4.3).

Skin temperature

For PF region, the mean, maximum and minimum temperature increased for CTRL, FR90 and FR180 ($p < 0.05$). There were no differences between conditions ($p > 0.05$) (Table 5).

Table 5. Skin temperature in plantar flexors and Achilles tendon (mean ± standard deviation).

	CTRL			FR90			FR180		
	PRE	POST	ES	PRE	POST	ES	PRE	POST	ES
Plantar flexors (°C)									
Mean	29.5 ± 1.2	30.0 ± 1.1*	0.48	29.3 ± 1.2	29.7 ± 1.0*	0.45	29.4 ± 1.0	30.1 ± 0.9*	0.82
Δ%	1.8 ± 1.9			1.7 ± 2.8			2.6 ± 3.0		
Maximum	31.1 ± 1.3	31.7 ± 1.1*	0.48	30.9 ± 1.1	31.2 ± 1.1*	0.20	31.0 ± 1.0	31.6 ± 1.0*	0.66
Δ%	1.8 ± 2.2			0.8 ± 2.6			2.2 ± 3.0		
Minimum	26.1 ± 1.5	26.8 ± 1.0*	0.55	26.1 ± 0.8	26.1 ± 0.9*	0.05	26.0 ± 0.8	26.5 ± 1.1*	0.55
Δ%	3.0 ± 4.6			-0.1 ± 4.1			2.1 ± 4.6		
Achilles tendon (°C)									
Mean	27.8 ± 0.9	28.5 ± 0.9*	0.45	27.5 ± 1.1	27.9 ± 1.2*	0.36	27.5 ± 1.0	28.2 ± 1.3*	0.59
Δ%	2.5 ± 2.6			1.5 ± 3.6			2.5 ± 3.2		
Maximum	29.3 ± 0.8	29.8 ± 0.8*	0.52	29.2 ± 1.3	29.4 ± 1.2*	0.18	29.0 ± 1.0	29.6 ± 1.4*	0.42
Δ%	1.6 ± 2.0			0.8 ± 3.5			1.9 ± 3.8		
Minimum	26.4 ± 1.2	27.0 ± 1.8*	0.61	26.0 ± 1.6	26.6 ± 1.5*	0.33	26.0 ± 1.0	26.6 ± 1.3*	0.49
Δ%	2.4 ± 3.0			2.1 ± 4.4			2.2 ± 4.1		

CTRL: control condition; FR90: foam rolling during three sets of 30s; FR180: foam rolling during three sets of 60s; ES: effect size; *Different from PRE ($p \leq 0.05$).

For AT region temperature, the mean, maximum and minimum temperature increased for CTRL, FR90, and FR180 ($p < 0.05$). The conditions also did not present differences ($p > 0.05$) (Table 5). The responders for skin temperature were 40-80% for CTRL, and 50-80% for FR conditions (Table 3).

DISCUSSION

Our study aimed to investigate the effects of different FR protocols (longer and shorter application times) on AT stiffness, GM and MTU passive stiffness, and functional performance in unilateral CMJ. The main findings of our study demonstrated a reduction in soft tissue stiffness and CMJ height for both FR conditions and also for the CTRL condition. Additionally, FR180 exhibited a higher percentual changes in AT stiffness and Young's modulus compared to FR90 and CTRL.

The tissues' stiffness reduction found in all conditions indicates that this happened due to the common elements in the evaluation procedures and the stimuli during conditions. The viscoelastic materials (e.g., tendons) present a creep characteristic, i.e., the strain increase under a constant stress (Pearson *et al.*, 2007). Therefore, a constant force application can affect measured *in vivo* tendon elongation and, in consequence, the stiffness (Pearson *et al.*, 2007). The role of creep has been previously demonstrated in the patellar tendon, where even after preconditioning, a protocol with longer MVICs resulted in greater elongations compared to a protocol with shorter MVICs (at the same force level) (Pearson *et al.*, 2007). Previous studies found a reduction in AT stiffness after three (Kay; Husbands-Beasley; Blazevich, 2015) and six ramp MVICs (Kay; Blazevich, 2009). Also, it has been suggested that creep can occurs in tendon for even 160 contractions (Schatzmann *et al.*, 1988). Although tendon preconditioning was considered in these studies, creep occurs when the tendon is continuously loaded stimulated over an extended period of time (Pearson *et al.*, 2007). Our results agree with these findings, since the participants underwent continuous stress between the pre- and post-AT stiffness assessments, as follows: three passive dorsiflexion movements at maximum amplitude, five unilateral CMJ, and again the MVICs protocol for reassessment (a minimum of eight submaximal to maximal plantar flexion contractions), following the assessments protocols. Thus, the observed reduction in stiffness across all conditions in our findings may be attributed to the stimuli in all of our assessments. These results highlight the importance of a control group in studies examining AT stiffness adaptations after massage or self-massage techniques.

Although we did not identify differences between groups with absolute data, the relative changes in AT stiffness and Young’s modulus were greater in the FR180 condition, indicating that a longer period (three sets of 60s) of FR application reduces AT stiffness. In addition to creep, tendon structures also exhibit thixotropic properties, wherein the tissue becomes less viscous when subjected to specific stimuli, such as agitation, heat, or mechanical load (Behm; Wilke, 2019; Stromberg; Wiederielm, 1969). The shearing stress from rolling can decrease intracellular and extracellular fluid viscosity (Behm; Wilke, 2019; Behm, 2018), thereby reducing the tissue resistance to deformation (i.e., stiffness). Furthermore, the thixotropic response is time-dependent (Meyer *et al.*, 2011; Butler, 1978), thus, the longer intervention time (three series of 60s) resulted in greater reductions in stiffness and Young’s modulus. Contrary to our findings, Chang *et al.* (2021) used the same intervention time (three sets of 60s) and found no changes in AT stiffness. However, the weight bearing applying by Chang *et al.* (2021) was subjectively controlled (discomfort level 7, between 0 and 10) and the assessment method was different (myotonometer). Furthermore, in our protocol it was emphasized the intervention in the tendon region (i.e., the rolling started in the most distal region of the AT), which could explain these differences.

As with the AT, passive stiffness in both the GM and MTU decreased in all three conditions. The muscle tissue can also exhibit creep (Herda *et al.*, 2012), and, as with the tendon response, our evaluation protocols with continuous load possibly influenced the GM stiffness. A reduction in muscle stiffness can also occur due to a break in cross-bridges that persists in the muscle during rest, making the muscle more relaxed and less resistant to deformation (Proske; Morgan, 1999). This cross-bridges break would be justified by an increase in blood perfusion, which has already been found after FR (Hotfiel *et al.*, 2017). Moreover, the muscle also exhibits viscoelastic behavior and can become less viscous with certain stimuli, such as heat (Proske; Morgan, 1999). We evaluated skin temperature, which is related with muscle temperature (Hildebrandt *et al.*, 2010). Thus, the reduction in muscle stiffness aligns with the observed increase in skin temperature in all conditions. Furthermore, an inhibitory effect in nociceptors (i.e., pain receptors) also can occur after an increased blood perfusion, due nitric oxide release on endothelial cells after mechanical stress (Hotfiel *et al.*, 2017; Okamoto *et al.*, 2014; Bavencoffe *et al.*, 2014). Additionally, the decreased MTU passive stiffness reflect our findings for tendon and muscle decreased stiffness. The percentage reductions were higher in GM (38-48%), followed by MTU (30-39.6%), and AT (12.9-30.3%). This can be justified by the inherent stiffness of tendons compared to relaxed muscle tissue (Herda *et al.*, 2012; Kay; Husbands-Beasley; Blazevich, 2015). Furthermore, the higher relative reduction seen in tendon stiffness did not occur in passive assessments, indicating that the MTU response could be more defined by the muscular response. This may have occurred due to the similar forms of assessment between the two passive measures, as well as the local response of the region emphasized (AT).

The three conditions led to a reduction in unilateral CMJ height. In common, the three conditions presented a reduction in stiffness due to the creep effect. Considering this, it is plausible that an increase in hysteresis might have also occurred (Finni *et al.*, 2013). The hysteresis describes the energy dissipated during a stretch–shortening cycle in the viscoelastic tissues (Finni *et al.*, 2013) and the it can increase when a viscoelastic tissue is stimulated repetitively with constant load (Solomonow, 2009). Therefore, both hysteresis and creep are time-dependent effects (Solomonow, 2009). Thus, it is possible that energy dissipation during the jump also occurred after all conditions, reducing jump performance. In addition, a correlation between highly pennated PF and increased CMJ performance due to a greater production of maximal force was previously related in another studies (Earp *et al.*, 2010; Secomb *et al.*, 2015), but changes in pennation angle were not verified in our study, indicating that jumping performance may have been influenced by PF stiffness adaptations, but was not influenced by muscle morphology.

Similar to most variables, skin temperature increased in all three conditions, observed in both the PF and the AT region. This could be attributed to the stimulus during all the conditions. As an attempt to isolate the effects of rolling, our CTRL condition utilized the same position as the FR intervention (i.e., the participant was seated, with the contralateral lower limb crossed above the leg of interest). Although there was no rolling, the skin of the calf region received a compression stimulus during this moment, which can generate heat and compress the soft tissues, in the same way as rubbing (Drust *et al.*, 2003). Similar stimuli (compression, rubbing) can change skin temperature and it is recommended to be avoided for even 24 hours before the assessment (IACT, 2002; Quesada, 2017). Regarding the FR, both intervention time increased the skin temperature. In line with our findings, Drust *et al.*, (2003) found no differences in the decreased intra-muscular temperature after 5, 10, and 15min of massage. The authors justify that part of this heat is released from the muscle into the blood, as a self-regulation mechanism for muscle temperature. Although we did not assess intra-muscular temperature, it is plausible that self-regulation mechanisms also occur in the skin, stabilizing the temperature increase following massage techniques. Furthermore, since it was inevitable to generate several stimuli before the thermography pre-evaluation (e.g., ultrasound gel, MVICs, maximum dorsiflexions), it is possible that the skin temperature was already increased at the time of pre-evaluation.

Our study presents some limitations. Our assessments were quite long, making it impractical to conduct immediate reassessments after each condition. Additionally, the stimuli applied during the evaluations influenced the parameters measured. Future studies could use SWE, which is an assessment performed at rest, and it would avoid the creep effect. However, we followed recommendations for assessing the mechanical properties of soft tissues (Seynnes *et al.*, 2015). We also included a control condition, in order to mitigate theses evaluation-related effects. Furthermore, a previous study (Bohm *et al*., 2016) showed that the AT CSA, obtained through US, does not have high accuracy. However, we believe that using US to determine CSA was not a severe limitation of our study, because the determination of CSA showed high reliability. Authors of studies in which interindividual variation comes into play, such as when the aim is to determine normative data of tendon properties (e.g., CSA, stress, and Young's modulus) or to compare the effects of different intervention in different groups of subjects, should perhaps be more concerned about US accuracy in determining CSA.

Despite some limitations, our study also has strengths. To the best of our knowledge, our study was the first to investigate the FR effects on tendon stiffness using widely employed methods to assess the mechanical properties of soft tissues and also following the methodological recommendations to this approach. Moreover, we explored variables commonly hypothesized as FR mechanisms (muscle stiffness, MTU stiffness, skin temperature). Our study included control conditions, a distinguishing feature from several other studies with FR. We also diligently examined the role of different FR application times, controlling the weight bearing during rolling. In addition, our findings have implications for clinical practice, indicating that FR for longer time application (i.e., three sets of 60s) could reduce AT, enabling relaxation in healthy subjects. However, it must be considered that application for longer periods of time, with maximum weight bearing, and emphasizing the AT region can be considerably uncomfortable, and that these small structural changes in these circumstances would not justify the expected functional effects after FR.

CONCLUSION

Applying FR for a longer time (three sets of 60s) generated greater relative reductions in AT stiffness when compared to shorter time of FR or CTRL condition, with no differences in the changes for GM and UMT stiffness and jumping height. The decreases observed in these variables following all conditions may be attributed to assessment procedures and tissue’s creep characteristic.

FINAL CONSIDERATIONS

This master's degree dissertation aimed to identify the effects of different massage techniques described in the literature on soft tissue stiffness and to verify the effects of different FR protocols on the musculotendinous properties of plantar flexors and functional performance. For that, a systematic review and an experimental study was conducted.

The systematic review aimed to investigate clinical trials that tested the massage techniques' acute effects on the myotendinous structure's stiffness in healthy adults or athletes, in order to establish the massage techniques effects on soft tissue stiffness. As main results, we found that different massage techniques do not change the MTU and tendon stiffness, but it can reduce muscle stiffness, especially in the rectus femoris and GM, although with a small effect size. However, our review also showed that the quality of included studies and the certainty of evidence for these results are low. In addition, the FR was the massage technique most reported in the clinical trials.

In an attempt to change this scenario of studies with low methodological quality found in our review, as well as answer questions regarding the best protocol (volume of application) of the most reported technique in the literature (FR), our Chapter II investigated the effects of different FR application protocols on the musculotendinous properties of plantar flexors, functional performance and skin temperature. We found the same changes (lower tissue stiffness, lower jump height and higher skin temperature) for both protocols and also for the control condition, indicating that the reduction in soft tissue stiffness was influenced by the evaluation's stimuli and the creep characteristic, present in viscoelastic tissues.

Our findings also present considerations for clinical practice. Although FR is widely used, its effects on stiffness depend on a longer intervention time with maximum weight bearing, which may be very uncomfortable during the technique. Furthermore, reducing the stiffness of myotendinous structures can enable relaxation, which is a common effect of massage techniques. However, in our experimental study, the reduction in stiffness did not generate functional effects, such as jump increase performance.

Considering the findings from both studies, it is concluded that massage techniques can reduce muscle and tendon stiffness, especially in the rectus femoris, gastrocnemius medialis, and Achilles tendon. However, as the FR effects on AT depends on a longer application time, this may not bring benefits due to discomfort during rolling. In addition, the inclusion of a control group is essential when analyzing studies involving massage techniques since, in our experimental study, we observed a reduction in the stiffness of myotendinous structures even in the control condition. Our findings can be understood by the biomechanical characteristics of myotendinous structures (Proske; Morgan, 1999, Butler, 1978), such as the thixotropic property and the creep characteristic, which respond to load stimuli for long periods of time and mechanical stimuli (i.e., massage).

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APPENDIX A – PRISMA CHECKLIST

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	16
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	16
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	17-18
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	18
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	19
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	18
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	Table S2
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	18-19
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	19
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	19-20
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	19-20
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	21
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	20

Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	19-20
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	19-20
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	19-20
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe themodel(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	19-20
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	19-20
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	19-20
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	19-20
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	21
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	22
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	21
Study characteristics	17	Cite each included study and present its characteristics.	21-27
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	36
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	23-27
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	21-35
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	21-35
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	21-35

	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	21-35
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	21-35
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	37-38
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	38-42
	23b	Discuss any limitations of the evidence included in the review.	42
	23c	Discuss any limitations of the review processes used.	42
	23d	Discuss implications of the results for practice, policy, and future research.	42
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	18
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	18
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	NA
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	NA
Competing interests	26	Declare any competing interests of review authors.	NA
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	NA

APPENDIX B – SEARCH STRATEGY

Table S2. Search strategy.

ITEMS	SEARCH STRATEGY
#1	Massage OR "Myofascial Release" OR "Foam Roll*" OR "Manual Treatment" OR "Tissue Mobilization" OR Cyriax OR "Fascial Sling"
#2	"Connective Tissue" OR Tendon OR "Myotendinous Unit" OR "Tissue Stiffness" OR "Passive Stiffness" OR "Musculotendinous Stiffness" OR "Muscle Stiffness" OR "Muscle Rigidity" OR "Muscle Hardness"
#3	#1 AND #2

APPENDIX C - PRODUCTIONS RELATED TO THE DISSERTATION

In this section, the main productions and events that occurred concomitantly with the dissertation execution are presented.

Productions - Summary published in proceedings of conferences

- Soldatelli, I.; Rossato, C. E.; Löbell, R.; Nunes, T. D. L.; Mota, C. B; Lanferdini, F. J. Simetrias musculares em ciclistas treinados, 2023, Bauru-SP. Abstract Book for the XX Brazilian Congress of Biomechanics (CBB), 2023. v. 17. p. 119-119.
- Löbell, R.; Glanzel, M. H.; Vaz, M. A.; Geremia, J. M. Acute effects of different foam rolling protocols on the plantar flexors' musculotendinous properties in healthy individuals: a research project, 2022, Taquara-RS. Anais do XII Simpósio em Neuromecânica Aplicada. Taquara-RS : FACCAT, 2022. p. 148-149.
- Löbell, R.; Souza, G.S.; Rossato, C. E.; Silva, F. S.; Saccol, M. F.; Lanferdini, F. J. Comparação do torque de extensores e flexores de joelho entre atletas de futsal e futebol americano: dados preliminares, 2022, Taquara-RS. Anais do XII Simpósio em Neuromecânica Aplicada. Taquara-RS : FACCAT, 2022. p. 77-78.
- Rodrigues, D. R.; Glänzel, M. H.; Löbell, R.; Silveira, M. C.; Barbosa, I. M.; Lanferdini, F. J.; Vaz, M. A.; Geremia, J. M. Immediate effects of a muscle damage protocol on triceps surae properties and calf muscle strength: preliminary results, 2022, Taquara-RS. Anais do XII Simpósio em Neuromecânica Aplicada. Taquara-RS : FACCAT, 2022. p. 88-89.
- Prates, I. M.; Löbell, R.; Petter, G. N.; Glanzel, M. H.; Geremia, J. M. Acute effects of different massage techniques on musculotendinous structures' mechanical properties: a systematic review, 2022, Taquara-RS. Anais do XII Simpósio em Neuromecânica Aplicada. Taquara-RS : FACCAT, 2022. p. 101-102.
- Rossato, C. E.; Löbell, R.; De Souza, G. S.; Silveira, M. C.; Pranke, G. I.; Lanferdini, F. J.; Saccol, M. F. Razões de torque entre isquiotibiais/quadríceps em atletas de futsal, 2022, Taquara-RS. Anais do XII Simpósio em Neuromecânica Aplicada. Taquara-RS: FACCAT, 2022. p. 129-130.
- Sonda, F. C.; Lancanova, A. A. S.; Mallmann, S.; Gomes, D. C. S.; Machado, E.; Löbell, R.; Vaz, M. A. Effects of menstrual cycle phases on plantar flexor neuromechanical properties and achilles tendon mechanical properties of eumenorrheic women and hormonal contraception users, 2022, Taquara-RS. Anais do XII Simpósio em Neuromecânica Aplicada. Taquara-RS : FACCAT, 2022. p. 131-132.
- Sonda, F. C.; Lancanova, A. A. S.; Löbell, R.; Glanzel, M. H.; Vaz, M. A; Ramos, J. G. L. Is there correlation between perineometry and the modified oxford scale in women? A systematic review with meta-analysis and grade recommendations, 2022, Santa Cruz do Sul-RS. Anais do V Simpósio Sul Brasileiro de Atividade Física e Saúde, 2022. p. 157-157.

Research projects

- Rodrigues, D. R.; Glänzel, M. H.; Löbell, R.; Lanferdini, F. J.; Vaz, M. A.; Geremia, J. M. Efeitos da aplicação do foam rolling na recuperação de propriedades musculotendíneas e parâmetros funcionais de flexores plantares de indivíduos saudáveis. Universidade Federal de Santa Maria. 2022 – 2022.

- Saccol, M. F.; De Souza, G. S.; Löbell, R.; Rossato, C. E.; Lanferdini, F. J. Biomecânica e cinesiologia de esportes coletivos e individuais - GLAss UFSM. Universidade Federal de Santa Maria. 2022 – 2022.

Distinctions related to this research

- Schneider, B.; Löbell, R.; Geremia, J. M. Acute effects of different foam rolling protocols on the achilles tendon morphological properties and jump height in healthy individuals: preliminary results. "Destaque da sessão". Salão de Iniciação Científica da UFRGS. Universidade Federal do Rio Grande do Sul. 2023.

Other teaching and research activities

- Teaching internship: Estudos Anátomo-Funcionais - Cinesiologia. Undergraduate program in Physical Education (Universidade Federal do Rio Grande do Sul). Advisor: Geremia, J. M. 2022 – 2023.
- Workshop: Avaliação da arquitetura muscular e de propriedades mecânicas tendíneas. Evento de 50 anos do LAPEX. Universidade Federal do Rio Grande do Sul. 2023.
- Event organization: II Escola de Inverno em Biomecânica Musculoesquelética. Universidade Federal do Rio Grande do Sul. 2023.
- Event organization: UFRGS Portas Abertas. Universidade Federal do Rio Grande do Sul. 2023.
- Study group organization: Grupo de Estudos sobre Tendão de Aquiles. 2022 – 2023.
- Collaboration on undergraduate final project: Efeitos do exercício físico na dor, força e funcionalidade de adolescentes com dor patelofemoral: uma revisão sistemática. Author: Melo, M. A. Advisor: Geremia, J. M. Universidade Federal do Rio Grande do Sul. 2021.