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Pain Reduction Following Massage After Induced Fatigue Is Not Mediated by Changes in Muscle Stiffness or Intramuscular Fluid Content: A Randomized Controlled Trial With Mediation Analysis

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Context: Massage therapy is a common intervention for athletic recovery. However, its effects on fatigue, muscle stiffness, and intramuscular fluid content or the mechanism for pain reduction remain uncertain.

Objectives: To evaluate the immediate and subacute effects (24–72 hours) of massage on pain, fatigue, muscle stiffness, and intramuscular fluid content in university athletes after an induced fatigue protocol. Additionally, we investigated whether changes in muscle stiffness and intramuscular fluid content mediate the effects of massage on pain and fatigue.

Design: Randomized controlled clinical trial.

Setting: Laboratory.

Patients or Other Participants: Eighty-six university athletes, who completed a quadriceps fatigue protocol involving isokinetic concentric contractions.

Intervention(s): Participants received either 10-minute massage therapy targeting the quadriceps or a sham control intervention. They were applied immediately after the fatigue protocol and repeated 48 hours later.

Main Outcome Measure(s): Pain and fatigue were assessed using numeric rating scales. Muscle stiffness and intramuscular fluid content were evaluated using ultrasound elastography and echo intensity, respectively.

Results: Massage therapy reduced pain immediately after the intervention compared with the sham group by −1.4 points (95% confidence interval = −2.8, −0.1). No differences were observed for pain at subsequent time points or for fatigue, muscle stiffness, or intramuscular fluid content at any time. Mediation analysis did not reveal indirect effects of changes in muscle stiffness or intramuscular fluid content on changes in pain or fatigue.

Conclusions: Massage therapy provides immediate pain relief after induced fatigue but does not produce lasting effects on pain, fatigue, muscle stiffness, or intramuscular fluid content. The observed pain reduction was not mediated by changes in muscle stiffness or intramuscular fluid content.

Key Words: musculoskeletal manipulations, postexercise recovery, athletes, quadriceps muscle, sports

Key Points

- Massage reduces pain after induced fatigue.
- Massage does not influence perceived fatigue, stiffness, or intramuscular content.
- Massage's effect is not explained by change in stiffness or intramuscular content.

Team and high-speed sports often involve repeated high-load, explosive muscle actions, which results in muscle overload.[1] This can lead to nociceptor depolarization, reduced muscle performance, and the development of pain and fatigue, which are common complaints after exercise.[1,2] While the initial inflammatory response typically resolves within a short period, complete recovery may require several days.[3] For athletes, postexercise soreness and fatigue not only impair performance but also contribute to discomfort and hinder the ability to maintain optimal training loads.[4]

Various interventions have been proposed to support recovery and mitigate the adverse effects of exercise-induced soreness and fatigue.[5,6] Among these, massage therapy is one of the most commonly employed methods due to its practicality, ease of application, and high acceptance among athletes.[7] It is often applied immediately after exercise, with substantial evidence suggesting its role in enhancing recovery after intense physical activity. For instance, authors of two systematic reviews concluded that massage effectively alleviates acute exercise-induced pain.[2,5] However, findings regarding the effects of massage on perceived fatigue remain less clear. While authors of some studies reported significant fatigue reductions after events such as Ironman triathlons and ultramarathons, others, such as in investigations after a habitual 10-km run, found no detectable effect of massage on fatigue.[8–10] These inconsistent findings highlight the need for further research to clarify the effects of massage therapy on fatigue.

Despite conflicting findings regarding fatigue and the variable effect sizes for pain relief, massage is widely regarded as a useful tool for athletic recovery.[2,5] Nevertheless, questions remain about the underlying mechanisms by which massage influences exercise-induced impairments. Proposed mechanisms suggest that massage may exert its effects through biomechanical and vascular pathways.[11] Biomechanical effects include increased muscle-tendon compliance and potential reductions in stiffness due to applied mechanical pressure.[11] Vascular effects may involve enhanced blood and lymphatic circulation, promoting the clearance of intramuscular fluids, metabolic byproducts, and pain-inducing substances.[11] For instance, reductions in blood creatine kinase levels after massage have been reported, suggesting a potential role in mitigating inflammation and exercise-induced muscle damage.[5,12] Yang et al explored the effect of massage on intramuscular fluid dynamics, such as a reduction in fluid content in the thoracolumbar fascia after massage-gun application, and found a significant reduction in the intramuscular content.[13] However, they presented notable methodological limitations, such as the absence of an intention-to-treat analysis and blinding of assessors.[13] Additionally, participants were not under a fatigue condition, limiting the generalizability of the findings.[13] Similarly, the evidence for massage-induced changes in muscle stiffness is promising but inconclusive.[14,15] Crommert et al found that massage significantly reduced muscle stiffness in treated legs compared with untreated legs.[14] Similarly, Jelen et al compared two massage techniques and found no difference between groups, but both techniques effectively reduced stiffness.[15] Despite these promising findings, methodological limitations and the absence of sport-related contexts prevent definitive conclusions about the effects of massage on muscle stiffness.

Given the theoretical nature of the proposed mechanisms, it remains uncertain whether massage therapy can truly induce changes in intramuscular fluids or stiffness and how these factors may influence clinical outcomes, such as pain and fatigue. To address these gaps, the aim of this study was to investigate the effects of massage therapy, applied immediately and 48 hours after fatigue induction, on pain, fatigue, intramuscular fluid content, and muscle stiffness in university athletes, compared with a sham technique. A secondary aim was to investigate whether acute changes in intramuscular fluid content and muscle stiffness mediate the effects of massage on pain and fatigue.

METHODS

Design

This randomized, parallel-group, single-blind, controlled trial was reported according to the CONSORT checklist.[16] Ethical approval was obtained from the Federal University of Santa Maria Human Research Ethics Committee (Registration No. CAAE 67652823.0.0000.5346), and the trial was prospectively registered in the Brazilian Registry of Clinical Trials (RBR-10wr9c7d) on September 15, 2023.

Participants were randomly allocated to either the experimental or control group (Figure 1) using a concealed randomization process. Allocation was achieved through sealed, opaque, and sequentially numbered envelopes, which were prepared by an independent individual with no involvement in the study procedures. To ensure allocation concealment during recruitment, the envelopes were opened only after the completion of baseline assessments, at which point the group assignment was disclosed. The data collection was conducted in a university biomechanics laboratory between October 2023 and May 2024.

Participants

Eighty-six university athletes participated in the study. The participants engaged in a variety of team and individual sports, including soccer, handball, volleyball, padel, taekwondo, dance, and track and field. Written informed consent was obtained from all participants before enrollment.

The inclusion criteria were (1) university athletes aged 18 to 30 years who trained at least three times per week with activities related to their respective sports; (2) no history of cardiovascular, pulmonary, or metabolic diseases; and (3) no use of performance-enhancing or recovery-boosting substances. Potential participants were excluded if they had discontinued sports activities in the past six months due to injury or reported neurological, musculoskeletal, cardiovascular, pulmonary, or metabolic conditions that could interfere with or contraindicate the research procedures. University athletes were defined as undergraduate students who had been training consistently for at least one year and had recently competed in sports events.

Throughout the data collection period, participants were instructed to maintain their regular routines while refraining from recovery techniques such as anti-inflammatory medications, stretching, cryotherapy, or massage therapy targeting the evaluated region. They were also advised to avoid engaging in high-intensity physical activities.

Procedures

After confirming eligibility, participants completed a questionnaire to provide anthropometric data and details about their sports practice. Baseline assessments included pain and fatigue levels, as well as ultrasound (US) evaluations of intramuscular fluid content and muscle stiffness via muscle echo intensity and elastography, respectively (A1). After these initial assessments, participants performed a quadriceps muscle fatigue protocol. Immediately after the fatigue protocol, pain and fatigue levels were reassessed, and US evaluations were repeated (A2).

Participants were subsequently randomized by a researcher uninvolved in the assessments. Randomization and interventions were conducted at a separate setting to ensure evaluator blinding. After the allocated intervention, pain and fatigue levels were reassessed, along with US parameters (A3). A blinded evaluator contacted participants remotely via phone message (ie, WhatsApp) 24 hours after the initial intervention to collect additional data on pain and fatigue (A4).

Forty-eight hours postfatigue protocol, participants returned to the laboratory for a follow-up assessment, which included pain, fatigue, and US parameters (A5). Participants subsequently received the second intervention

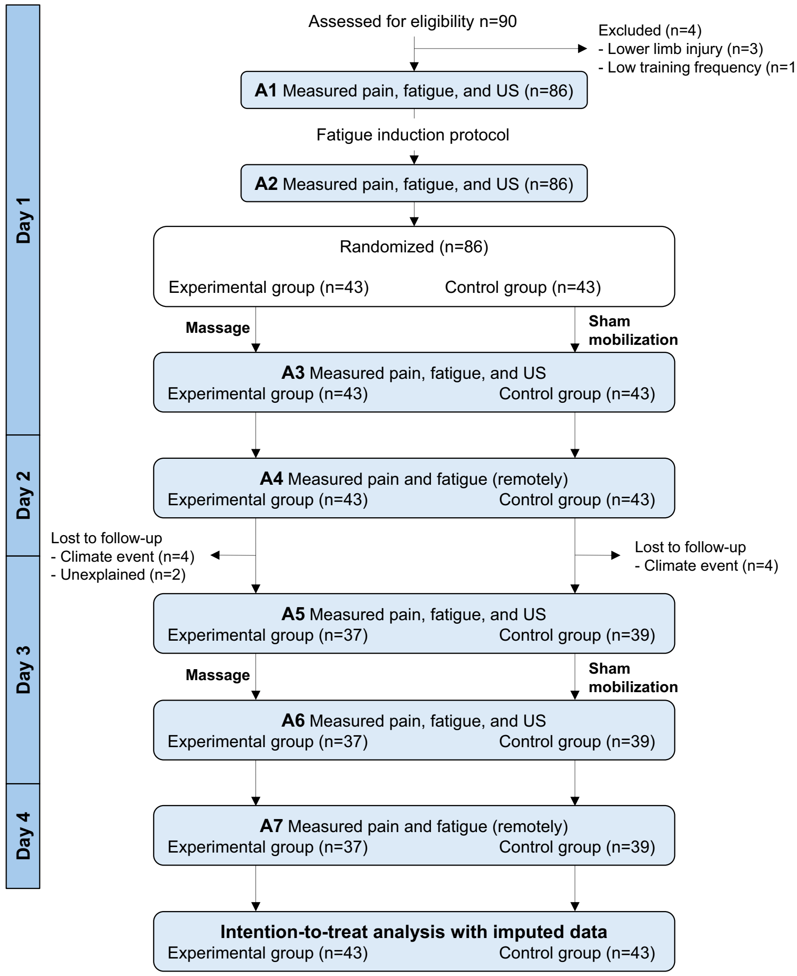

Figure 1. Study design and flow of participants.

according to their original allocation. Post-intervention assessment of pain, fatigue, and US parameters were then performed (A6). Finally, 72 hours after the first interventions, the same blinded evaluator conducted remote assessments of pain and fatigue (A7). The flowchart summarizing the study timeline and procedures is presented in Figure 1.

Induced Fatigue Protocol. The fatigue protocol targeted knee extensor muscles and was performed using a Biodex System isokinetic dynamometer (Biodex Medical Systems). Participants first completed a warm-up set of four submaximal repetitions. This was followed by four sets of 20 maximal concentric contractions at a velocity of 60°/s, with a 2-minute rest interval between sets.[17] This protocol effectively reduced maximal strength, with an approximate 60% decline in performance over the course of the protocol (Figure 2). Additionally, the protocol significantly increased pain and fatigue levels while reducing muscle stiffness. However, no significant changes were observed in intramuscular fluid content (Supplementary Material A).

Interventions

Participants in the experimental group received a 10-minute massage applied by a trained physiotherapist who was not involved in the assessments (Figure 3A). The massage protocol included the following steps: one minute of superficial effleurage, during which the therapist performed gentle gliding movements from distal to proximal along the muscle fibers of the quadriceps; three minutes of deep effleurage, which involved the same technique but with increased pressure; three minutes of petrissage, in which the therapist used the palms to compress, lift, and knead the tissue sequentially; one minute of tapotement, during which the therapist applied rhythmic, oscillatory movements to the thigh using cupped hands; and two minutes of superficial effleurage to conclude the intervention.[10] A neutral, soap-based foam was applied to facilitate smooth gliding during the massage. The massage protocol was designed to replicate common postrace clinical practice, prioritizing short, low-intensity interventions. Techniques were selected to promote relaxation and venous return with minimal discomfort and were applied in order of increasing intensity, ending with the least intense technique.

Participants in the control group underwent sham joint mobilizations targeting the knee (five minutes, Figure 3B) and hip (five minutes, Figure 3C) while lying supine with the knee flexed at 90° and the sole of the foot resting on the table.[10] These mobilizations involved gentle traction using a belt, designed exclusively to provide tactile sensation without inducing actual joint movement. The therapist avoided direct contact with the participants' legs. For the sham hip joint mobilization, the belt was positioned across the participant's inguinal region and around the therapist's

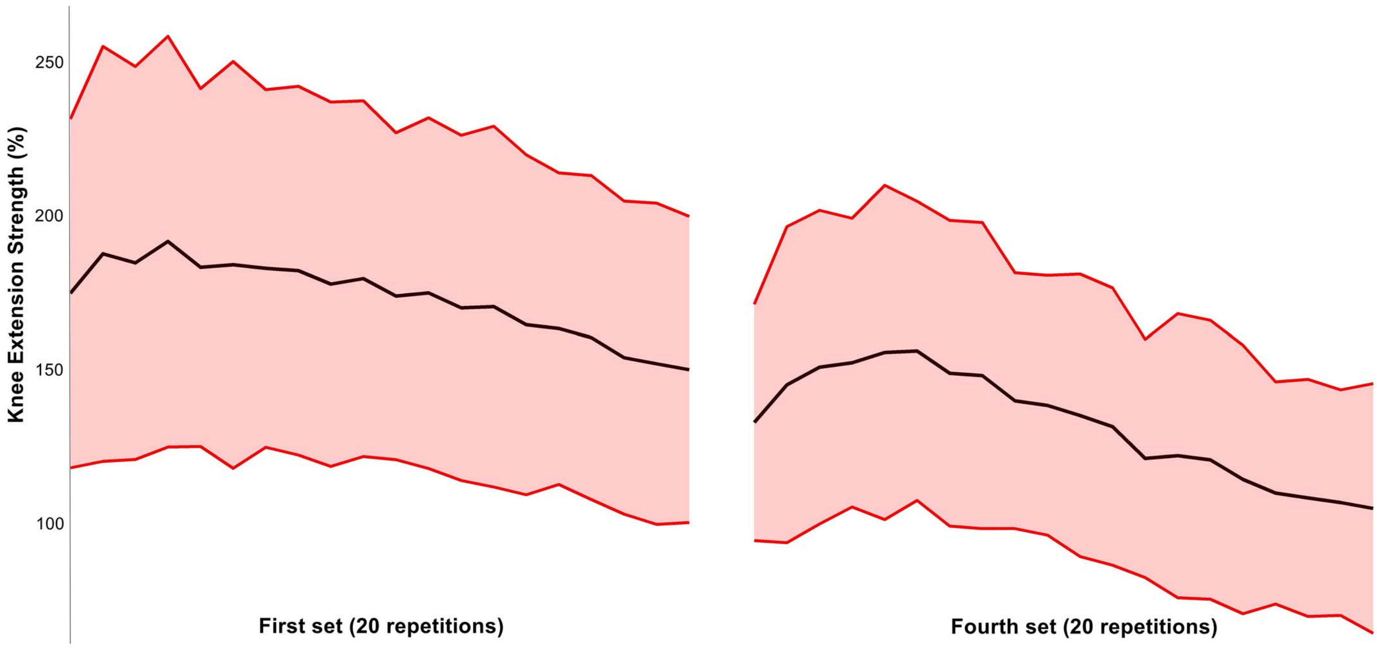

Figure 2. Normalized muscle torque during the fatigue protocol (the black line represents mean values, and the red shaded area indicates SDs).

lower back. For the sham knee joint mobilization, the belt was placed around the proximal tibial region of the participant and secured around the therapist's lower back. In both cases, the therapist applied a slight, oscillatory movement by shifting the body away from the participant, producing mild pressure on the skin. To ensure evaluator blinding, the same neutral foam used in the massage group was applied to the participants in the control group at the end of the intervention.[8]

The same intervention procedures were repeated 48 hours after the fatigue protocol for the second application. The Template for Intervention Description and Replication (TIDieR) checklist is reported in Supplementary Material B.

Assessments

All assessments were conducted by a trained evaluator who was blinded to the group allocation.

Pain and Fatigue. Pain and fatigue levels were measured using the numeric rating scale (NRS).[10,18] This scale demonstrates high reliability and is responsive to changes in both pain and fatigue.[8,18] Participants were asked the following questions: "What is your current pain level in your thigh?" on a scale from 0 (*no pain*) to 10 (*worst possible pain*), and "How much fatigue do you feel in your thigh right now?" with 0 indicating *no fatigue* and 10 indicating *extreme fatigue*.

Ultrasound Measurements. Echo intensity (intramuscular fluid content) and shear-wave elastography (muscle stiffness) were assessed using an US system (Siemens Healthineers, Acuson S2000, Germany) equipped with a 40-mm linear transducer (Siemens Healthineers, Acuson 9L4, Germany). Three US images were obtained for each assessment, with a depth of five cm, an intensity of eight MHz, and a general gain of 0 dB/DR65. The transducer was placed longitudinally, aligned with the direction of the vastus lateralis muscle fibers, at 50% of the distance between the greater trochanter and the lateral femoral condyle, in the anterolateral region.[19] All images were captured with the quadriceps at rest, with participants in the supine position and the lower limbs in a neutral position.[19] To ensure proper transducer coupling during each measurement, excessive pressure on the assessed region was avoided.[19]

Echo intensity refers to the amount of US waves reflected by muscle tissue, quantified on a grayscale histogram ranging from 0 (black) to 255 (white).[20] Higher echo intensity values reflect a greater presence of noncontractile components, including intramuscular fluid content,[20] which may be influenced by massage interventions.[11] Image analysis was performed using ImageJ Software 1.42q (National Institute of Health), with the region of interest defined within the vastus lateralis muscle.[20] To minimize the potential overestimation of echo intensity due to US attenuation by subcutaneous fat, values were normalized based on adipose layer thickness.[20] This thickness was defined as the distance between the superficial aponeurosis of the vastus lateralis and the superficial boundary of the adipose layer.[20] Corrected echo intensity was calculated by adjusting the mean grayscale value obtained from the region of interest according to the measured adipose thickness, using a validated correction formula.[20] For each participant, the average of three images, expressed in arbitrary units, was used for analysis. Before data collection, reliability was assessed on 20 images, reporting high reliability (intraclass correlation coefficient [$ICC_{3,1}$] = 0.96–0.98).

Shear-wave elastography quantifies tissue stiffness by measuring the velocity of shear waves propagated through the muscle.[21] For the measurement, a rectangular region of interest was placed in the belly of the vastus lateralis, between the superficial and deep aponeuroses.[19] Within each US image, 10 regions of interest were placed longitudinally along the muscle fibers to obtain shear modulus values, which were expressed in kilopascals.[19] Higher values in kilopascals indicate greater muscle stiffness, reflecting increased resistance to deformation, which may be modulated by massage interventions.[11] These values were used to generate an elastogram, a color-coded visual map in which red indicates higher stiffness and blue indicates lower stiffness (Supplementary Material C). Muscle

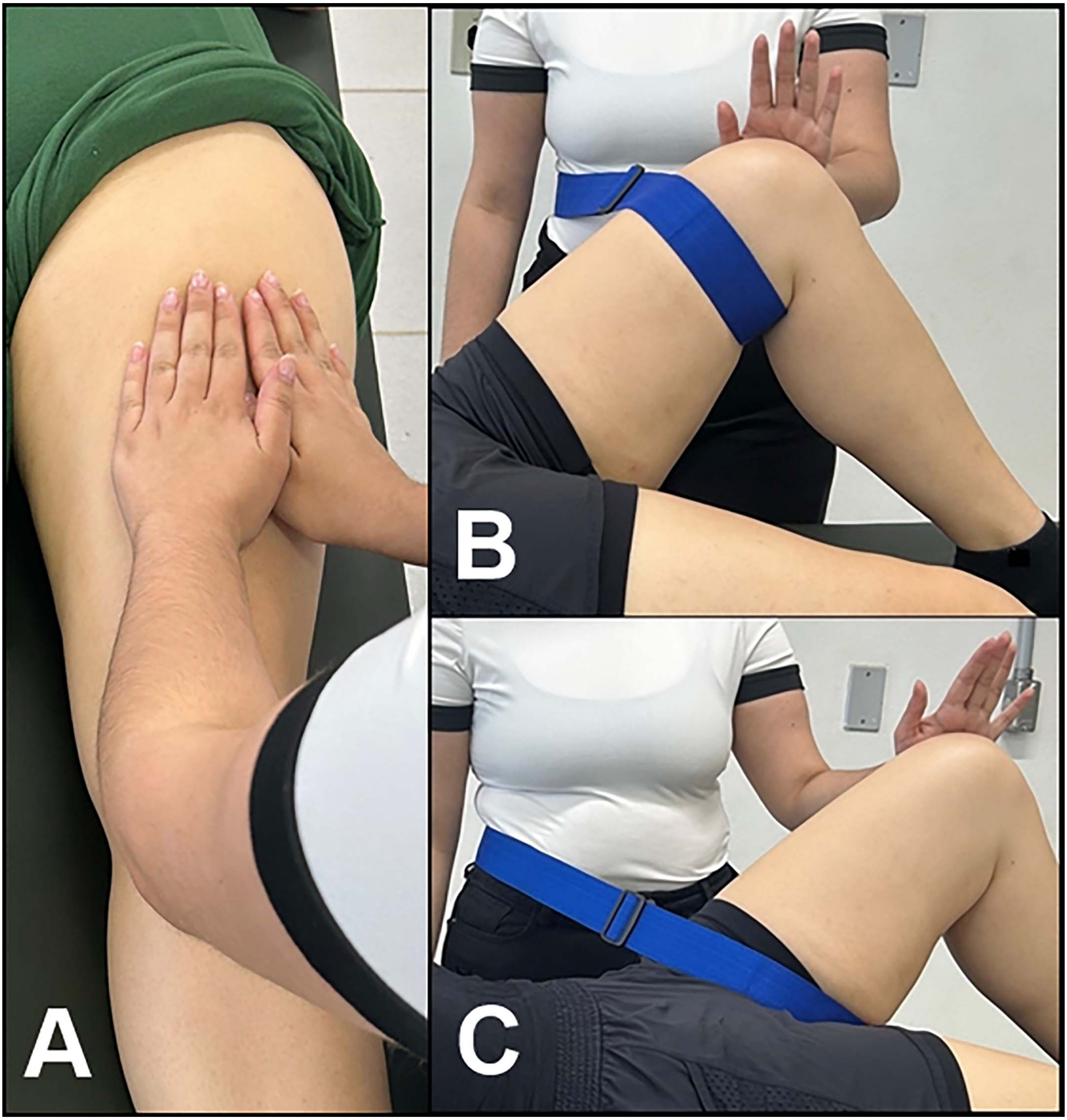

Figure 3. **A, Massage therapy. B, Sham knee mobilization. C, Sham hip mobilization.**

stiffness was calculated as the average across three images, totaling 30 regions of interest. This method has been reported as a reliable assessment.[21]

Statistical Analysis

The sample size was calculated to ensure 80% statistical power ($\beta = 0.20$) and a 5% significance level ($\alpha = 0.05$), accounting for a potential 15% dropout rate. The calculation indicated that a minimum of 42 participants would be required to detect a 2-point difference in pain (SD = 3 points) and a 1-point difference in fatigue (SD = 1.5 points), using the NRS.[22,23] The calculation was performed using G*Power software.

An intention-to-treat approach was employed for all analyses, with participants analyzed according to their initial randomization. Missing data were addressed using the multiple imputation method, generating 20 imputed datasets based on all available variables to predict missing values.[24]

All data are presented as mean ± SD. Data normality was evaluated using the Shapiro-Wilk test. To evaluate the effect of massage therapy across time points, a mixed linear model analysis of variance was conducted for pain, fatigue, muscle stiffness, and intramuscular fluid content. Differences from baseline (A2) were considered in the analysis of acute effects (A3) as well as after 24 hours (A4), 48 hours (A5), and 72 hours (A7) postfatigue induction. The efficacy of the second massage therapy, applied 48 hours after induced fatigue, was assessed using an independent *t*-test, comparing the change in outcomes from preintervention to post-intervention (A6 − A5). For each comparison, the mean difference and 95% confidence interval were calculated. Additionally, effect sizes (Cohen *d*) were computed for significant comparisons and interpreted as *small* ($d = 0.2$), *moderate* ($d = 0.5$), or *large* ($d \geq 0.8$).[25] Statistical analyses were conducted using IBM SPSS Statistics, version 26.0.

Mediation analyses were performed to investigate whether the acute effects of the massage (A3 − A2) on pain and fatigue were mediated by changes in muscle stiffness and intramuscular fluid content. This statistical approach assesses whether the effect of an independent variable (massage) on an outcome (pain or fatigue) occurs directly or indirectly through 1 or more mediating variables. In this context, direct effects represent the portion of the effect of the intervention not explained by changes in the mediators (muscle stiffness or intramuscular fluid content), while indirect effects quantify the portion mediated by them. Analyses followed the framework outlined by MacKinnon et al, using 1000 bootstrapped simulations to estimate the total, direct, and indirect effects, with the corresponding 95% CI.[26] All mediation analyses were conducted using the R software (mediation package), with statistical significance set at $P \leq .05$ for all analyses.

RESULTS

Participants Flow

Of the 86 participants initially enrolled in the study, 10 were lost to follow-ups after the A5 assessment. Consequently, 37 participants in the experimental group and 39 in the control group completed the study protocol. Baseline characteristics were comparable between experimental and control groups, including age, body mass, height, and gender distribution (Supplementary Material D).

Protocol Adherence

All randomized participants met the eligibility criteria. The assessors remained blinded throughout the study to ensure unbiased evaluations. All participants received the assigned intervention, and data were analyzed according to their original group allocation, adhering to the intention-to-treat principle.

Massage Effects

Descriptive statistics (means ± SDs) for pain, fatigue, muscle stiffness, and intramuscular fluid content across all assessments are provided in Supplementary Material E. Massage was associated with a reduction in pain levels immediately postintervention compared with sham mobilization (Table 1). Specifically, the experimental group presented a mean pain reduction of 1.4 points (95% CI = 0.1, 2.8) relative to the control group, corresponding to a moderate effect size ($d =$

Table 1. Between-Groups Comparisons Across the Study Protocol

	Difference Within Groups, Mean ± SD								Difference Between Groups, Mean Difference (95% CI)			
	Post minus baseline		24 h minus baseline		48 h minus baseline		72 h minus baseline		Post minus baseline	24 h minus baseline	48 h minus baseline	72 h minus baseline
Outcomes	Exp	Con	Exp	Con	Exp	Con	Exp	Con	Exp minus Con	Exp minus Con	Exp minus Con	Exp minus Con
Pain, points	–2.7 ± 2.5	–1.3 ± 2.2	–3.6 ± 3.5	–3.9 ± 3.3	–3.8 ± 3.5	–4.0 ± 3.7	–4.1 ± 3.2	–4.3 ± 3.7	–1.4 (–2.8, –0.1)[a]	0.3 (–1.0, 1.7)	0.2 (1.1, 1.6)	0.2 (–1.2, 1.5)
Fatigue, points	–3.8 ± 2.4	–3.5 ± 2.4	–6.4 ± 2.8	–5.7 ± 2.3	–7.0 ± 2.2	–6.7 ± 1.9	–7.3 ± 2.2	–7.0 ± 2.1	–0.3 (–1.3, 0.7)	–0.7 (–1.6, 0.4)	–0.3 (–1.3, 0.7)	–0.3 (–1.3, 0.7)
Stiffness, kPa	–0.9 ± 3.6	–0.2 ± 4.1	NA	NA	–0.4 ± 3.6	0.0 ± 3.7	NA	NA	–0.7 (–2.2, 0.9)	NA	–0.4 (–2.0, 1.2)	NA
Echo intensity	3.1 ± 5.4	3.5 ± 5.4	NA	NA	–0.9 ± 5.1	–0.8 ± 4.6	NA	NA	–0.4 (–2.5, 1.9)	NA	–0.1 (–2.3, 2.1)	NA

Abbreviations: Con, control group; Exp, experimental group; NA, not applicable.
[a] Significant difference.

Table 2. Between-Groups Comparisons for Intervention Reapplication at 48 Hours

	Difference Within Groups, Mean ± SD (Post Minus Preintervention at 48 h)		Difference Between Groups, Mean Difference (95% CI)
	Exp	Con	Exp minus Con
Pain, points	–0.1 ± 0.5	0.0 ± 0.3	–0.1 (–0.3, 0.1)
Fatigue, points	–0.2 ± 0.6	–0.1 ± 0.6	–0.1 (–0.4, 0.2)
Stiffness, kPa	–0.1 ± 2.7	0.4 ± 3.0	–0.5 (–1.7, 0.8)
Echo intensity	1.3 ± 4.3	1.9 ± 3.4	0.6 (–2.2, 1.1)

Abbreviations: CI, confidence interval; Con, control group; Exp, experimental group.

0.59). No significant differences between groups were observed for pain at subsequent time points.

Similarly, no significant between-groups differences were detected for fatigue, muscle stiffness, or intramuscular fluid content across any assessments (Table 1). After the second massage application, conducted 48 hours after fatigue induction, no significant between-groups differences were found for any of the outcomes analyzed (Table 2).

Mediation Analysis

Mediation analyses did not identify a significant mediating effect of muscle stiffness or intramuscular fluid content on the immediate post-intervention changes in pain and fatigue (Table 3). Muscle stiffness accounted for approximately 4% of the total effect on both pain and fatigue, while intramuscular fluid content contributed about 1% of the total effect on pain and 5% of the total effect on fatigue. These effects were minor and not statistically significant, indicating that the interventions primarily exert direct effects on pain and fatigue, independent of changes in muscle stiffness or intramuscular fluid content.

DISCUSSION

The findings indicated that massage reduced pain levels immediately after the intervention compared with the sham technique group. However, no significant differences in pain outcomes were observed between groups during subsequent follow-up assessments. Furthermore, massage had no effect on fatigue, muscle stiffness (elastography), or intramuscular fluid content (echo intensity) at any assessment point. The reapplication of massage 48 hours postfatigue induction also did not affect any of the measured outcomes. In terms of potential mechanisms, the results suggest that changes in muscle stiffness or intramuscular fluid content do not mediate the observed decreases in pain or fatigue.

The pain-relieving effects of massage in fatigued athletes aligns with prior literature.[2,5] This confirms massage as an effective and clinically relevant intervention for supporting postexercise recovery in athletes. Despite these benefits, the magnitude of the effect should be carefully considered in clinical decision-making. In this study, we reported a 1.4-point reduction in pain, which may be an arguable magnitude. Additionally, the lower bound of the CI suggests that, for some athletes, the actual effect of massage could be close to null effect, consistent with previous studies.[8,10] Nonetheless, massage remains a low-cost, noninvasive, and well-accepted intervention with no reported adverse effects, justifying its use for immediate pain relief in appropriate cases. Clinicians are encouraged to assess each case individually, considering not only pain modulation but also other potential benefits, such as psychological and relaxation effects.

The current findings refute previous theories suggesting that pain reduction caused by massage after strenuous activity is mediated by reductions in muscle stiffness or intramuscular fluid content.[11] To understand these results, some points must be considered. In the context of muscle fatigue, muscle stiffness was reduced after fatigue, consistent with prior literature.[27] Thus, pain reduction from massage cannot be attributed to decreased stiffness if stiffness was not elevated. Although authors of some studies have suggested massage reduces muscle stiffness measured via elastography, these findings were observed in nonfatigued individuals.[13,14] Furthermore, massage did not alter muscle stiffness but remained effective for pain reduction, suggesting that the effects of massage in fatigue conditions are likely unrelated to stiffness. Regarding intramuscular fluid content, the fatigue protocol did not significantly affect echo intensity, preventing conclusions about the influence of massage on intramuscular fluid levels. Massage effects on fluid mobility might be more pronounced in cases of fluid accumulation, such as inflammation, lymphatic alterations, or postexercise situations involving extreme exertion. Overall, these results suggest that the analgesic effects of massage in fatigued athletes are not explained by mechanical or vascular factors. Instead, neurophysiological mechanisms may play a role. Rapid pain reduction may be linked to massage-induced mechanoreceptor activation through pleasant tactile stimulation, which modulates pain.[28,29] Massage may also increase oxytocin, an endogenous mediator with antinociceptive and analgesic properties.[30] However, these are theoretical suggestions, and further research is needed to elucidate the mechanisms of massage.

Massage did not reduce fatigue compared with the sham technique. This finding supports existing evidence that the effect of massage on fatigue may be influenced by the initial level of fatigue, with its benefits potentially being more pronounced in situations of severe fatigue.[8–10] Although our fatigue-induction protocol increased participants' perceived

Table 3. Estimates (95% CI) for Mediation Analysis

	Average Causal Mediation Effect		Average Direct Effect		Average Total Effect	
	Estimate (95% CI)	*P* Value	Estimate (95% CI)	*P* Value	Estimate (95% CI)	*P* Value
For pain						
Stiffness	0.1 (–0.2, 0.3)	.60	–1.4 (–2.5, –0.5)	.01	–1.4 (–2.4, –0.4)	.01
Echo intensity	0.0 (–0.2, –0.2)	.99	–1.4 (–2.5, –0.5)	.01	–1.4 (–2.5, –0.5)	.01
For fatigue						
Stiffness	0.0 (–0.2, 0.1)	.76	–0.3 (–1.3, 0.8)	.61	–0.3 (–1.3, 0.8)	.56
Echo intensity	0.0 (–0.2, 0.2)	.88	–0.3 (–1.3, 0.7)	.59	–0.3 (–1.3, 0.7)	.57

fatigue by nearly 7 points, this level of fatigue and the duration of fatigue state may not have been sufficient to trigger a metabolic response that could be modulated by massage. This could also explain the absence of significant subacute effects of massage on pain and fatigue at 24, 48, and 72 hours postintervention. Similarly, the reapplication of massage at 48 hours did not yield significant reductions in pain or muscle fatigue. One possible explanation for this lack of effect is that the fatigue-induction protocol was designed to simulate typical levels of fatigue experienced by athletes, without inducing muscle damage. As a result, the protocol may not have caused delayed onset muscle soreness or elevated fatigue levels throughout the procedure. Consequently, participants reported relatively low pain, and fatigue scores the next day, which may have reduced the magnitude of the effect of the massage intervention. This suggests that the protocol employed could not induce persistent pain or fatigue symptoms. While authors of previous studies have demonstrated positive effects of massage in reducing delayed onset muscle soreness, the current findings imply that the therapeutic benefits of massage are most effective in acute pain scenarios and may be dependent on the intensity and physiological effect of the exertion involved.[12]

Limitations

This study was conducted with healthy university athletes who were accustomed to high training loads. As such, the findings may be limited to populations with similar characteristics. Although we included a sham technique, this did not guarantee the blinding of the participants. As a result, individuals with prior knowledge of massage therapy may have been biased in their responses, despite the use of a sham technique. Additionally, while the fatigue protocol affected performance and simulated a strenuous sports condition, it involved only concentric contraction and did not precisely replicate the movements and loads typically encountered by athletes during their sport-specific training and competition.

Therefore, in this study, we demonstrated that quadriceps massage reduces pain in university athletes immediately after intervention compared with a sham technique but has no effect on pain at later time points (24–72 hours postfatigue induction). Massage also showed no significant effects on perceived fatigue, muscle stiffness, or intramuscular fluid content at any time point. Reapplication of massage at 48 hours postfatigue had no additional effect. Moreover, changes in muscle stiffness (elastography) and intramuscular fluid content (echo intensity) did not mediate acute changes in pain and fatigue caused by massage.

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CRediT Author Statement

G.S.N. was responsible for the study conceptualization; G.S.N. and F.J.L. designed the study; B.D.G., A.P.D., A.C.O.M., N.K.R., and V.S.T. carried out the data collection; B.D.G., A.P.D., and A.C.O.M. performed the data analyses; G.S.N. and F.J.L. performed all the statistical analyses; and G.S.N., B.D.G., A.P.D., and A.C.O.M. drafted the manuscript. G.S.N. is the study guarantor. All authors reviewed and edited the draft, read and consented to the content of the final manuscript, and agreed with the order of presentation of the authors.

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SUPPLEMENTAL MATERIAL

Supplementary Material A. Effect of the fatigue protocol (mean ± SD).
Supplementary Material B. Template for Intervention Description and Replication (TIDieR) checklist.
Supplementary Material C. Sample of the shear-wave elastography analysis.
Supplementary Material D. Characteristics of participants.
Supplementary Material E. Descriptive analyses for the outcome (mean ± SD).

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