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Clinical and Histologic Manifestations of a Novel Rectus Femoris Myotendinous Junction Injury in Rats

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SUMMARY

Background. Animal models of muscle injury have primarily relied on methods which do not mimic the chronic scarring that typically occurs adjacent to the myotendinous junction (MTJ). The goal of this study was three-fold: (i) to create a strain-induced in vivo model of rectus femoris MTJ injury in rats; (ii) to document clinical manifestations of injury using longitudinal tracking of individual animals via voluntary and compulsory (treadmill) mobility analyses and (iii) to validate and assess the model for persistent scarring through serial histologic assessment and development of a semi-quantitative grading scheme to characterize injury response over time.

Methods. Strain-induced MTJ injury was generated in male Sprague Dawley rats via needle tension directed along the transverse axis between the rectus femoris muscle and distal tendon that attaches to the patella. Animals received mobility assessments (gait analysis using a DigiGait Treadmill System and weight bearing using a Tekscan Rodent Walkway System) at days 0, 1, 3, 6, 13, 20, and 27 of the experimental protocol. Rats were euthanized at 1, 3, 7, 14, and 28 days post-injury (n = 6 rats per time-point) and hindlimbs were processed for histology.

Results. Significant changes in locomotor parameters included injured and contralateral limb paw area, max dA/dt (limb deceleration/breaking time), stride time, stance time, force time impulse, and fore/hind symmetry, and injured limb maximum force. The most significant and consistent histologic finding was a pathologic fibrotic adhesive lesion at the muscle and tendon interface along the proximal aspect of the patella just distal to the injury site. This lesion was composed of reactive fibroblasts, disorganized collagen fibers, vascular profiles, and a myxomatous ground substance stroma.

Conclusions. This work is the first to characterize the clinical and pathologic development of a chronic model of rectus femoris MTJ injury, which resulted in altered mobility likely caused by a strain-induced fibrotic scar along the anterior patella. Notably, both the functional and pathologic changes recapitulated the course of injury progression similar to what is described in humans. This work provides a unique model to study MTJ injury mechanisms for the identification of enhanced treatment options for patients who suffer from activity-related muscle conditions.

KEY WORDS

Animal model; gait analysis; histopathology; myotendinous junction; quadriceps rectus femoris.

ABBREVIATIONS

MTJ: Myotendinous Junction
EDTA: Ethylenediaminetetraacetic Acid
H & E: Hematoxylin and Eosin

BACKGROUND

The myotendinous junction (MTJ) is as a highly specialized transition zone composed of many interacting tendinous and muscular filaments that contribute to physiologic adhesion and anchoring (1). This site is responsible for the transmission of force between the muscle, tendon, and bone, which ultimately dictates skeletal movement and gait patterns. As the connection between two different tissue types, the MTJ represents the weakest link along the greater muscle-tendon unit, making it highly vulnerable to tension-induced impairment (2, 3). Activities that induce eccentric loading, such as high speed running and kicking, pose an increased risk of MTJ injury (4). While the mechanical contributions of MTJ strains (stretch) during eccentric loading are fairly well described (4, 5), little is known about the clinical (gait and weight bearing parameters) or structural (histopathologic) responses to injury.

The quadriceps, hamstrings, and gastrocnemius muscles are uniquely prone to MTJ injury due to their superficial location and extension across two joints (2, 6). Indeed, lower limb orthopedic injuries affect 25-48% (7-9), 62.4% (10), and 32.3% (11) of military, athletic, and occupational populations, respectively, with strains of the aforementioned muscles accounting for the majority of these conditions. Depending on the severity of MTJ injury, patients present with swelling, tenderness, and pain (12). Due to the complicated structural organization and cell signaling at the MTJ, endogenous healing for structural and functional recovery is difficult. Current treatment regimens for MTJ injuries remain conservative and include the RICE method (rest, ice (cold), compression, elevation) followed by gradual exposure to physical exercise modalities (2). If not addressed, persistent scarring within the muscle directly adjacent to the MTJ can lead to reduced flexibility, weakness, and muscle atrophy, making previous injuries a leading risk factor associated with recurrence (7, 13, 14). Therefore, the controlled study of MTJ injury progression in a clinically relevant pre-clinical model may allow for the development of novel therapeutics and treatment strategies for patients who suffer from such injuries.

Animal models of muscle injury include chemical (15), traumatic (16, 17), acute high intensity exercise (18), contusion (19-21), contraction (22), and ischemia-reperfusion (23) methods (for a summary of published models see **appendix 1**). While valuable, the majority of current models do not specifically target the MTJ, a common site of clinical strain-induced activity-related injuries (2, 3). Recently, a model of gastrocnemius MTJ injury was developed in the rat (24). Through a needle core puncture directed along the transverse axis of the MTJ, grade I-II muscle lesions were generated as early as 1 day post-injury. Of note, muscle lesions in this model resolved by 26-46 days post-injury. Similarly, models that utilize mechanically and tetanically induced strain injuries (25, 26) replicate acute muscle damage near the MTJ without longer term lesion development. While these provide suitable models of inherent muscle regeneration and healing, a model that recapitulates the persistent scarring seen clinically would be advantageous for testing new methods of clinical management and reduction of re-injury rates. Therefore, the development of a chronic animal model is needed to better characterize factors leading to high re-injury rates and investigate interventions that may expedite patient recovery.

While gastrocnemius MTJ injury (24) has been studied to date, no published reports exist that employ an animal model of quadriceps MTJ injury. The most commonly reported site of quadriceps MTJ injury in humans is the distal MTJ of the rectus femoris muscle belly where it inserts on the proximal aspect of the knee joint (4, 27, 28). More specifically, given its short length, MTJ injuries of the rectus femoris usually occur near the osteotendinous junction approximately 2cm from the insertion of the quadriceps tendon on the patella (29). Indeed, as a bipennate muscle with short muscle fibers, a steep oblique tendon attachment, and a large MTJ transition zone (30), the rectus femoris is highly susceptible to strain during stretching (31). Owing to this, the goal of this study was to create a strain-induced *in vivo* model of rectus femoris MTJ injury in rats. Findings were characterized by: (i) documenting clinical manifestations and progression of injury using longitudinal tracking of individual animals via voluntary and compulsory (treadmill) mobility analysis; and (ii) validating and assessing the model for persistent scaring through temporal histologic assessment and establishment of a semi-quantitative grading scheme to characterize injury response over time. The controlled study and application of this model of MTJ injury may allow for the development of novel therapeutics and treatment strategies for patients who suffer from such injuries.

MATERIALS AND METHODS

Animals

All procedures were approved by the University's Institutional Animal Care and Use Committee (Protocol #16-6927A),were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals, and were in accordance with the MLTJ guidelines (32). Thir-

ty (30) male Sprague Dawley rats were purchased from a commercial vendor (Charles River Laboratory, Wilmington, MA) at 70 days of age and allowed to acclimate to the vivarium for 14 days. A single sex was chosen to standardize the model as (i) injury severity was dictated by the needle size relative to the targeted region, (ii) rectus femoris injury in males is better characterized clinically, and (iii) due to their size, males offered a larger targeted area for initial model development. Animals were monitored daily by a veterinarian. All rats were housed singly in solid bottom cages with corncob bedding and were maintained at 22-24 °C on a 12 h light/dark cycle. Commercially available irradiated water and food were available ad libitum during the experiments.

Rectus Femoris Injury at the Myotendinous Junction

Animals were anesthetized using a mixture of isoflurane and oxygen (2-4%) and both limbs were shaved and surgically prepped using chlorohexidine and isopropyl alcohol. The surgical region of interest was visualized via a 1 cm incision along anterior aspect of the knee. Strain-induced MTJ injury was generated on the right hind limb via needle tension (using an 18G hypodermic needle) that was directed along the transverse axis between the rectus femoris muscle and quadriceps tendon (**figure 1 A** and see **appendix 2**). Specifically, this injury focused on tissue plane separation and stretching (6) to induce strain between the tendon/muscle and surrounding structures, as contrasted to a coring needle puncture biopsy utilized in previous reports (24). For matched comparisons and clinical translatability, the left limb was utilized as a sham surgery control, receiving an anterior superficial surgical incision without subsequent needle injury. Rats were randomized to end-points and sacrificed at either day 1, 3, 7, 14, and 28 days post-injury (n = 6/time-point; see **figure 1 B**) in accordance with approved protocols (CO_2 inhalation with confirmatory cervical dislocation).

Mobility assessments

The 28 day time-point rats underwent compulsory gait analysis using a DigiGait Treadmill System (Mouse Specif-

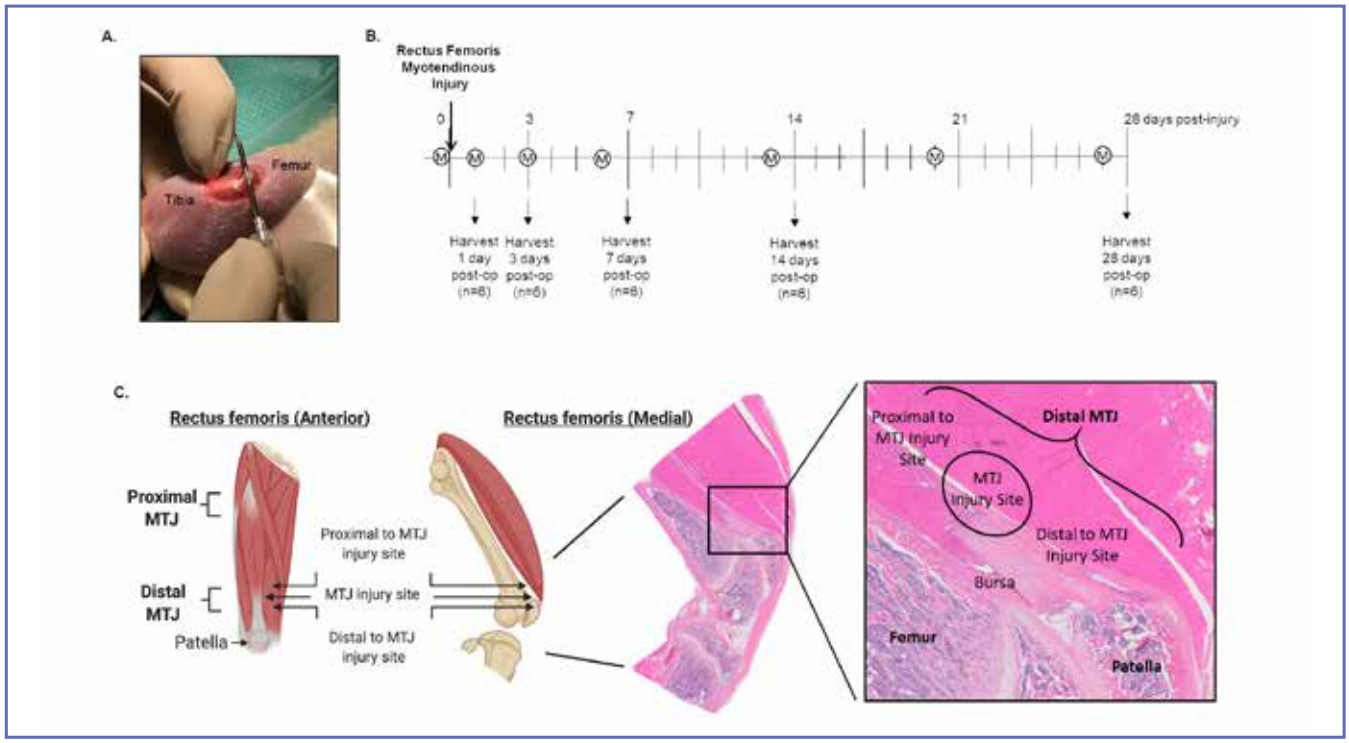

Figure 1. (**A**) Strain induced MTJ injury generated via 18G needle tension directed along the transverse axis of the MTJ. (**B**) Experimental time-course following injury. Rats were euthanized at 1, 3, 7, 14, and 28 days post-injury (n = 6/time-point) and their limbs taken for histology. Animals harvested for the 28 day time-point received mobility assessments (marked with M) at days 0, 1, 3, 6, 13, 20, and 27 of the experimental protocol. (**C**) Anatomic location of quadriceps distal MTJ injury (from BioRender) with a representative H&E stained section demonstrating a naïve/control MTJ. The injury site in this model occurred where the MTJ anchors the distal quadriceps muscle belly to the proximal patellar tendon.

ics, Framingham, MA) and voluntary weight bearing using a Tekscan Rodent Walkway System (South Boston, MA) on Days 0, 1, 3, 6, 13, 20, and 27 of the experimental protocol. Prior to start of the experiments, rats were acclimated to both systems over one week. For gait analysis, rats were run for three consecutive replicates per time-point on a flat treadmill at 30 cm/sec, and videos were analyzed for 22 gait parameters including stride length, % swing stride, % stance stride, % brake stride, % propel stride, and stride frequency. For weight bearing, rats were allowed to walk voluntarily over the Tekscan walkway for 3 times/day, and videos were analyzed for 12 weight bearing and gait parameters including maximum force, force time impulse, and maximum force symmetry values (front/hind, left/right, left front/right front, left hind/right hind). For both gait analysis and weight bearing, the three runs taken at each time-point were averaged and utilized for statistical comparisons. For information regarding how specific mobility parameters were calculated see equipment specific manuals.

Histology and Histopathology

Following euthanasia, hind limbs were removed at the coxofemoral joint and placed into 10% neutral buffered formalin for 48 hours. Limbs were then transferred to a 10% solution of ethylenediaminetetraacetic acid (EDTA) at pH 7 for decalcification. EDTA was replaced twice weekly for 8-10 weeks. Limbs were trimmed in the sagittal plane, routinely processed and embedded, sectioned at 5um, and stained with hematoxylin and eosin (H&E). Histologic sections were evaluated by one author blinded to the groups. A semi-quantitative grading scheme was developed to characterize injury-related histologic changes associated with needle puncture. The three sites assessed included: 1) the MTJ at the needle insertion/injury site; 2) the MTJ immediately distal to the injury site; and 3) the MTJ immediately proximal to the injury site (**figure 1 C**). At each location, the following parameters were scored: 1) lesion size, 2) degree of fibrosis (a measure of scarring), 3) ground substance (myxomatous degeneration), and 4) vascularity, with each having an allowable score of 0-3. Additionally, secondary changes were documented including: bursal cellularity/vascularity, and the presence or absence of reactive synovium and joint space effusion, with each having an allowable score of 0-2. Animals were assessed for the aforementioned histologic changes at days 1, 3, 7, 14, and 28 post-surgery (n = 6 males per group). Based on the degree of injury-associated pathology in each individual animal, the minimum possible score was 0 and the maximum possible score was 41. The histopathology grading scheme is provided in **appendix 3**.

Statistics

The experimental sample size (6 animals per time-point) was calculated using GPower Version 3.1.1 (33). Specifically, an *a-priori* power analysis was conducted using pilot gait data (stride length) obtained using this injury model in rats. This power analysis resulted in a power of 80%, using a 95% confidence interval and typical standard deviation of 1.00 within groups. All post-hoc statistics were conducted in GraphPad Prism 8.3.0 (San Diego, CA). For gait analysis and weight bearing parameters that quantify individual limb changes (stride length, % swing stride, *etc.*), groups were compared using a repeated measures, mixed effects analysis with limb (injured right hind, sham left hind, right fore, and left fore), time (days 0, 1, 3, 6, 13, 20, and 27 post-op) and limb/time interaction factors. Tukey's post-hoc tests were used to compare individual groups when the factors (limb, time, and limb/time) showed significance. For gait analysis and weight bearing parameters that demonstrate the relationship between limbs (*e.g.* stance factor, step angle, overlap distance, paw placement positioning, and weight bearing symmetry parameters), a repeated measures One-Way ANOVA with Tukey's post-hoc tests was utilized. For all graded pathology parameters, time-points were compared using a Kruskal-Wallis non-parametric One-Way ANOVA with Dunn's post-hoc tests. For correlation between parameters, a Pearson Correlation Analysis was conducted. Significance was set to $p < 0.05$ for all comparisons.

RESULTS

Mobility assessments

For treadmill-based gait analysis parameters, paw area significantly increased over time for both the injured right hind and sham left hind limbs (**figure 2 A**)**.** This was associated with a greater Max dA/dt (increased deceleration or breaking time during the stance phase) over time for both hind limbs (**figure 2 B**). Representative paw area output curves qualitatively demonstrate altered stance phase parameters up to 27 days post-injury (**figure 2 C**). No statistically significant differences were found for stride length (cm), %swing stride, %brake stride, %brake stance, %propel stride, %propel stance, %stance stride, stance/swing, stride frequency (steps/sec), absolute paw angle (degrees), min dA/dt (cm^2/sec), gait symmetry, ataxia coefficient, midline distance (cm), axis distance (cm), stance width (cm), stance factor, step angle (degrees), overlap distance (cm), or paw placement positioning (cm).

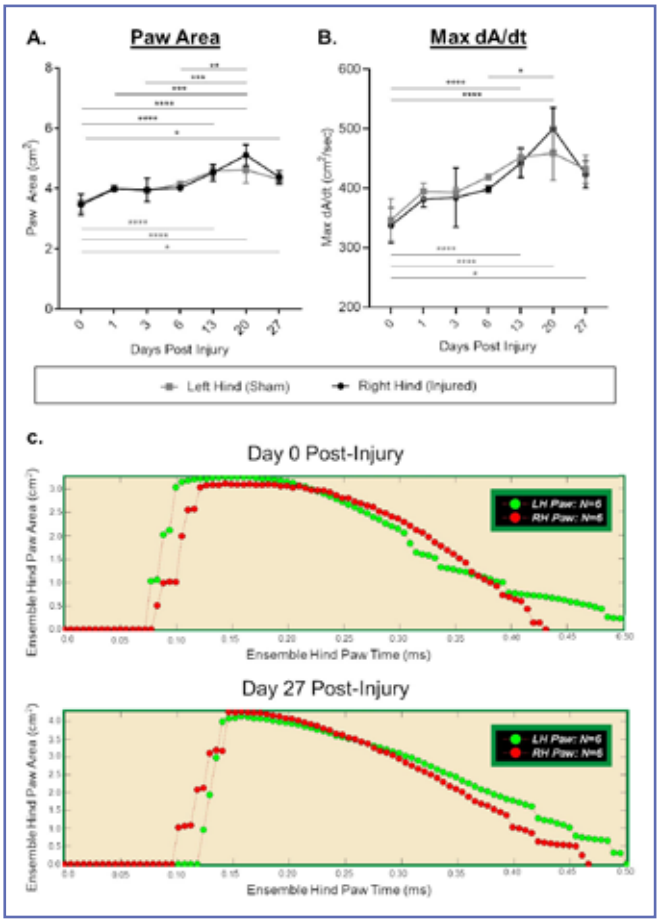

Figure 2. Treadmill based gait analysis parameters (Digigait Treadmill System) following quadriceps MTJ injury in the right hind limbs of rats. Significant spatiotemporal parameters include (**A**) paw area (cm^2) and (**B**) Max dA/dt (cm^2/sec). Significance differences over time marked (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001) with right hind (injured) differences in black at the top and left hind (sham) differences in grey at the bottom. No statistically relevant differences between hind limbs were noted. Values are represented as Mean +/- Standard Deviation. (**C**) Representative output paw area curves demonstrating altered stance phase parameters at 27 days post-injury.

For voluntary weight bearing parameters, a decrease in maximum force was seen in the injured right hind limb out to 27 days post-injury, but not the sham left hind limb (**figure 3 A**). Importantly, no appreciable correlation ($p = 0.36$, $R^2 = 0.02$) was found between maximum force and maximum velocity, demonstrating that animal speed did not dictate force measurements. Increases in the force time impulse increased for both the injured right hind and sham left hind limbs; however, this normalized by 27 days post-injury (**figure 3 B**). Interestingly, altered hind limb loading was associated with an increase to the fore/hind limb symmetry value, suggesting decreased hind limb loading was associated with increased fore limb loading (**figure 3 C**). In contrast to obligatory treadmill walking at a set speed (where no differences in temporal parameters of the stride and stance phases were appreciated), voluntary walking demonstrated a significantly increased stride time (**figure 3 D**) and stance time (**figure 3 E**) out to 27 and 20 days post-injury, respectively, with peaks at 6 days post-injury. These changes were associated with a decreased stride velocity (**figure 3 F**) at 6 days post-injury with normalization by 27 days post-injury. No significant differences were found for maximum peak pressure (MPa), maximum force left/right symmetry, maximum force left hind/right hind symmetry, stride length (cm) swing time (sec), stride acceleration (cm^2/sec).

Histology and Histopathology

For rectus femoris MTJ injury, a novel grading scheme was developed that separately and cumulatively assessed the following regions of the MTJ: 1) needle injury site; 2) MTJ proximal to the injury site; and 3) MTJ distal to the injury site. The joint, synovium, and bursa were also evaluated (see **appendix 3**). Of note, the following descriptions are for the injured right hind limbs, with no pathologies appreciated in the sham left hind limbs at all time-points (score of 0 for all parameters).

Overall, the most significant and consistent qualitative finding was a pathologic fibrotic adhesive lesion at the muscle and tendon interface along the anterior/cranial aspect of the patella (**figure 4**) at the MTJ distal to the injury site. This lesion typically measured 25-400 um and was composed of reactive fibroblasts and disorganized collagen fibers, variably prominent myxomatous ground substance stroma, and vascular profiles. When focusing on individual parameters that may contribute to these findings, lesion size ($p = 0.34$), vascularity ($p = 0.33$), and fibrosis ($p = 0.06$) at the MTJ distal to the injury site were not significantly different between time-points. However, the ground substance ($p = 0.02$) (see **appendix 4**) score was significant at the MTJ distal to the injury site, suggesting that this parameter may have the greatest contribution to the injury response and the overall pathology score.

In spite of the finding that the MTJ distal to the injury site progressively worsened over time, no significant differences were appreciated for the total injury score (**figure 5 F**, $p = 0.18$), the MTJ proximal to the injury site (**figure 5 A,** $p = 0.10$), MTJ injury site (**figure 5 B,** $p = 0.54$), or MTJ distal to the injury site (**figure 5 C**, $p = 0.11$). When considering the individual components which made up these scores,

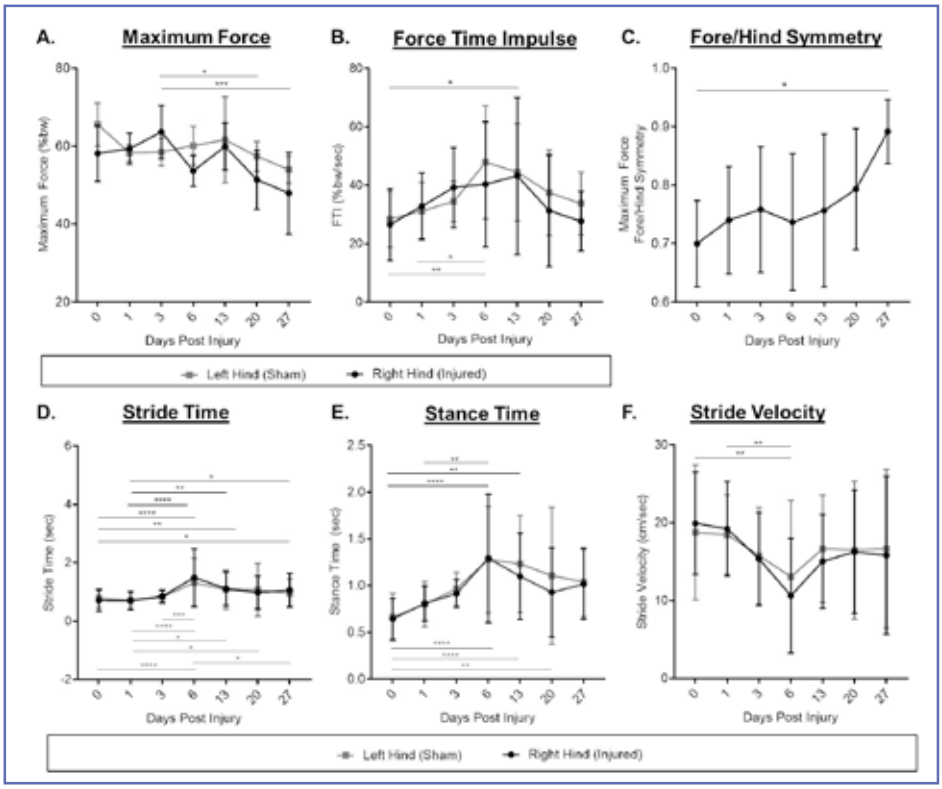

Figure 3. Voluntary weight bearing and gait parameters (Tekscan Rodent Walkway System) following quadriceps MTJ injury in the right hind limbs of rats. Significant parameters over time include (**A**) maximum force (% body weight (bw)), (**B**) force time impulse (FTI; % bw/sec), (**C**) maximum force fore/hind symmetry, (**D**) stride time (sec), (**E**) stance time (sec), and (**F**) stride velocity. Significance differences over time marked (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001) and no statistically relevant between limb differences were noted. Values are represented as Mean +/- Standard Deviation with right hind (injured) differences in black at the top and left hind (sham) differences in grey at the bottom.

no significant differences in lesion size, fibrosis, ground substance, and vascularity were appreciated at the MTJ injury site. For the MTJ proximal to injury site, a significant decrease in fibrosis ($p = 0.02$; between 1 day post-injury and both 7 and 28 days post-injury) was seen, with no significant changes in lesion size ($p = 0.15$), vascularity ($p = 0.20$), and ground substance. It was observed that the changes at the MTJ injury site and MTJ proximal to the needle site were subtle and the biological relevance of these findings remains undetermined.

With respect to secondary pathology changes, no significant differences between time-points were appreciated for the synovium ($p = 0.84$). However, significant differences were appreciated for joint space effusion **(figure 4 D** and see **appendix 5)** and bursa (**figure 4 E** and see **appendix 6**), although the pathology at both these sites seemed to decrease over time.

DISCUSSION

This animal model utilized needle tension to produce strain along the tendon-MTJ-muscle interface, a common site of impairment clinically (2, 3). Importantly, this work has resulted in a chronic model (up to 28 days post-injury) that histologically recapitulates muscle lesions adjacent to the MTJ in contrast to published models which focus on acute muscle injury (24-26). Therefore, this translational model of MTJ injury can be used for the study of aberrant or delayed healing and chronic scarring that may lead to high re-injury rates. Importantly, this strain-induced model also results in mobility changes associated with injured limb disuse, which can be used to study how therapeutics and rehabilitation strategies not only effect injury pathology but also mitigate decreased activity related to pain in clinical populations.

The major symptom and chief complaint of patients with muscle injuries is altered and/or painful mobility, yet there are few pre-clinical models which study functional alter-

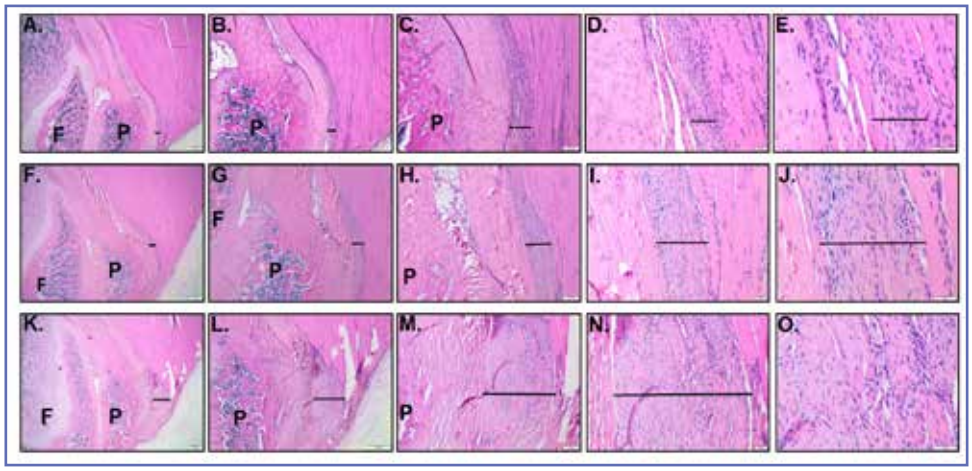

Figure 4. Representative photomicrographs of injury-associated pathology at the MTJ distal to the injury site. (**A-E**) Representative photomicrographs of an MTJ distal pathology score of 4. (**A**) Distal to the injury site along the anterior surface of the patella the MTJ and associated fascial planes are expanded by a linear proliferation of fibrous connective tissue, H&E, 2X. (**B**) Higher magnification, H&E, 4X. (**C**) The fibrous connective tissue proliferation measures 75 um, H&E, 10X. (**D**) Higher magnification, H&E, 20X. (**E**) Higher magnification, H&E, 40X. (**F-J**) Representative photomicrographs of a MTJ distal pathology score of 7. (**F**) The MTJ distal to the injury site is expanded by a large adhesion composed of fibrous connective tissue, H&E, 2X. (**G**) Higher magnification, H&E, 4X. (H) The fibrous adhesion measures 100um, H&E, 10X. (**I**) Higher magnification, H&E, 20X. (**J**) Higher magnification, H&E, 40X. (**K-O**) Representative photomicrographs of an MTJ distal pathology score of 9. **(K)** Distal to the injury site the MTJ is markedly expanded and effaced by haphazardly arranged fibrous connective tissue, H&E, 2X. (**L**) Higher magnification, H&E, 4X. (**M**) Fibrous connective tissue is accompanied by a myxomatous stroma which disrupt collagen fibers and support few vascular profiles, H&E, 10X. (**N**) Higher magnification, H&E, 20X. (**O**) Higher magnification, H&E, 40X. F = femur; P = patella.

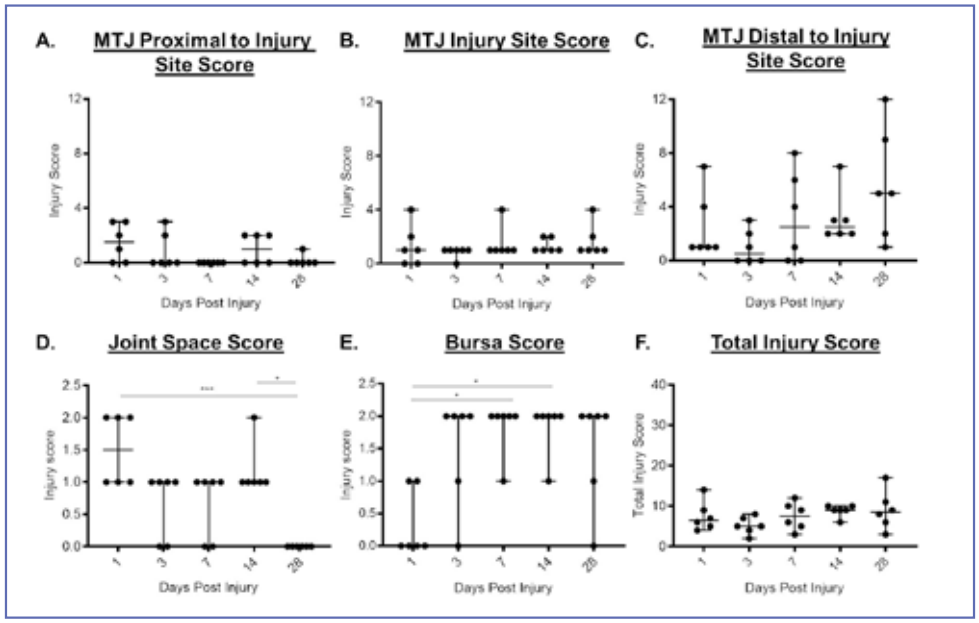

Figure 5. Individual MTJ Site Scores and Total MTJ Injury Score for the injured right hind limbs at 1, 3, 7, 14, and 28 days post-surgery. Injury scores for (**A**) MTJ proximal to the injury site, (**B**) MTJ at the injury site, (**C**) MTJ distal to injury site, as well as secondary changes within the (**D**) joint space and (**E**) bursa. (**F**) Total MTJ injury score reflects the sum of all pathology scores, with the maximal/maximum range of the scoring scheme provided on the y axis. Values are represented as Median with Range and individual animals marked. Significant differences over time marked (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$).

ations in gait and/or weight bearing. Many studies focus on changes to muscle force production (22, 24-26); however, a direct association to limb alterations has not been established. Indeed, previous studies have shown that locomotive changes following injury precede muscle force changes (20), suggesting that re-establishment of functional mobility may initiate muscle activation and subsequent force production. Therefore, mobility measures may more accurately demonstrate early symptom modifications post-injury. Mobility in pre-clinical models can be studied using spatial (position-based), temporal (time-based), or kinetic (force-based) methods. To the authors' knowledge, this is the first study quantitatively analyzing all forms of functional mobility alterations using both voluntary and compulsory methods (temporal, spatial, and weight bearing) in an animal model of MTJ injury. In this study, the longitudinal tracking of individual animals throughout the experimental time-course using multiple methods has provided a comprehensive understanding of clinical manifestations of rectus femoris-specific MTJ injury.

With respect to temporal and spatial movement parameters, there were decreases in temporal parameters with voluntary movement (decreased velocity and increased stance time and stride time which peaks at 6 days post-injury) in the current model. Indeed, non-invasive kinetic methods to evaluate weight bearing distribution have been shown to sensitively predict limb dysfunction post-injury (34). With respect to weight bearing, subjective gait scoring following muscle contusion injury demonstrated decrease limb loading in a mouse model (21). As a less severe model was utilized in the current study, quantitative dynamic weight bearing was undertaken, and similar results were obtained. Rats placed less weight on their hind limbs over time (decreased maximum force and increased force time impulse) and shifted their weight to their forelimbs (increased maximum force for fore/hind limb symmetry). Clinically, rectus femoris injury of the quadriceps muscle group typically occurs during the swing phase due to eccentric contractions of the muscle that occur during that portion of the gait cycle (4). Following rectus femoris MTJ injury in this study, animals were avoiding limb rotation (swing phase), which was associated with decreased animal movement. Therefore, it is likely that animals post-injury are attempting to avoid this swing phase to minimize rectus femoris muscle contraction. Of note, the functional mobility changes seen in the injured hind limb are also occurring in the contralateral hind limb that received a sham surgery.

Interestingly, no changes in temporal parameters were seen post-injury with compulsory treadmill-based gait analysis. Given the set treadmill speed and the fact that only data where the animal maintained speed with the treadmill were utilized for analysis, it can be difficult to appreciate natural gait differences. However, alterations in spatial parameters were documented with this obligatory method. Specifically, rats exhibited increased paw area and altered spatiotemporal parameters including increased Max dA/dt (impaired braking capacity). Collectively, our results suggest that following strain-induced MTJ injury, rats are favoring the stance phase to minimize limb rotation and movement during the swing phase. These results are corroborated by previous reports. Changes in ankle angle and calcaneus height have been shown in contusion models of anterior tibialis muscle injury (20), suggesting impaired joint rotation/movement. Additionally, voluntary walking impairment has previously been shown (19) in a rat model of traumatic muscle injury. In this study, treadmill-based walking trials were performed in triplicate to assess reproducibility in our clinical assessments. This necessitated 10 minutes of animal activity per timepoint, which was divided into short segments of non-strenuous compulsory movement followed by resting periods. We do not anticipate this level of movement modulated tissue remodeling in our model as more intense long term (> 60 minutes/day for 3-16 weeks) running protocols are typically utilized to induce muscle and tendon injury in rodents (18, 35, 36). Along this vein, more intense running protocols may be employed in future investigations to assess if chronic MTJ scarring, a unique characteristic of this model, may lead to high re-injury rates.

To determine a relationship between the observed mobility alterations and histoanatomic manifestations of injury, we created a semi-quantitative grading scheme to characterize the inflammatory and structural response to MTJ injury throughout the experimental time-course. Our approach was to evaluate not only the MTJ but also nearby structures to gain a comprehensive understanding of how the entire region is responding to needle injury and to identify key contributors to injury progression. The grading scheme included evaluation of the needle puncture site, the region proximal and distal to the needle site, as well as nearby fat pads, bursa, synovial membranes, and knee joint spaces. Currently there is a lack of histopathologic data describing the acute and chronic manifestations of MTJ injuries in humans. Clinicians tend to rely on magnetic resonance imaging for injury evaluation and studies have demonstrated the presence of hemorrhage, edema, fatty infiltration, and muscle atrophy directly adjacent to the MTJ (6). Similarly, we have documented acute mixed inflammation and edema acutely after injury. In the later stages of healing, we see more chronic changes including: fibrosis, vascularization, and secondary changes to nearby structures including the joint space, bursae, and synovium.

The most striking and consistent qualitative histologic finding was a fairly large (50-300 um) adhesive lesion along the MTJ distal to the needle injury site 28 days post injury,

which resulted in a significantly increased ground substance score at this time-point. It is anticipated that the development of this lesion was associated with an aberrant healing response resulting in early chronic injury progression (2). In regards to normal tissue healing, an initial injury (independent of severity) is followed by: (i) an inflammatory cell reaction and formation of a hematoma; (ii) phagocytosis of the damaged tissue (iii) repair and/or regeneration of myofibers; and lastly (iv) reorganization and remodeling with the aim of full functional recovery (2). Tissue regeneration at the MTJ can be particularly difficult due to the complex organization and anchoring between muscle myofibers and tendon collagen fibers (1). Indeed, in the current model, an excessive and disorganized fibroblastic response with diminished remodeling was seen as evidenced by the generation of a fibrotic scar 28 days post injury, which mimics the repair phase following muscle injury (2).

When identifying associations between histology and gait pattern outcome measures, it is noteworthy that the bursa score peaks at 7 days post-injury, which corresponds to decreases in voluntary movement at 6 days post-injury. Therefore, this may be a critical timeframe to target the local microenvironment towards a reparative *vs* delayed/dysregulated healing process. Cellular and tissue level variations at this time-point may identify pathways that could be manipulated for organized healing. Future work is needed to investigate the functional role of these structures and the influence they may have on resident progenitor cells such as skeletal muscle satellite cells, fibroadipogenic precursors, interstitial tenocytes, fibroblasts, nerves, endothelial cells and inflammatory cells. As many of these cells types were present in both injured and sham limbs natively and at different time points post-injury their specific role in driving the histoanatomic manifestations of injury progression requires further study.

Rats were chosen due to their large size (relative to mice), inherent exercise ability for mobility outcomes, and the fact that the Crl:CD(SD) rats are an outbred strain. Weight and skeletal measurements taken during the experimental time-course also demonstrate that rats were still growing, with skeletal maturity obtained at 16 weeks of age. Therefore, the results presented here are particularly relevant for adolescents and young adults (13-22 years of age) who experience MTJ associated sports-injuries. Notably, multiple prospective studies have identified that age is not a risk factor for quadriceps MTJ specifically, unlike in other MTJ etiologies (14, 37, 38). Future studies will determine whether age plays a significant role in quadriceps MTJ injury and healing with this model.

Caveats of this study include the use of the contralateral limb as a control for both mobility and histopathological assessments. While this was undertaken due to clinical applicability, no significant changes between the injured right hind and contralateral sham limb were appreciated. Therefore, further study on the compensatory effects in quadruped animals is warranted to fully characterize this model. Additionally, all outcomes demonstrate sustained changes out to 28 days post-injury. Future directions include characterizing the injury at later (56 and 120 day) time-points to evaluate histologic changes (we suspect there will be adhesion contracture and remodeling) and mobility alterations later on throughout the repair process. Based on our findings out to 28 days post-injury, we anticipate that, while mobility measurements may normalize at 56 and 120 day time-points, pathologic and scored features may be more distinct during these later stages of chronic tissue remodeling. The authors acknowledge that the exact strain which was induced during injury was not directly measured. However, the use of an 18G needle provided consistency among animals and dictated the degree of tissue plane separation as no excessive force was utilized.

The results from this study demonstrate the development of a chronic model of rectus femoris MTJ injury, altered mobility, and strain-induced fibrotic scarring along the anterior patella. Notably, these pathologic and functional changes recapitulate the course of injury progression similar to what is described in humans (4, 27, 28). Collectively, this work provides a unique pre-clinical model to study quadriceps MTJ injury mechanisms for the identification of enhanced treatment options for patients who suffer from such activity-related muscle conditions.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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SUPPLEMENTS

Appendix 1. Comparison of published rodent and rabbit animal models targeting muscle injury.

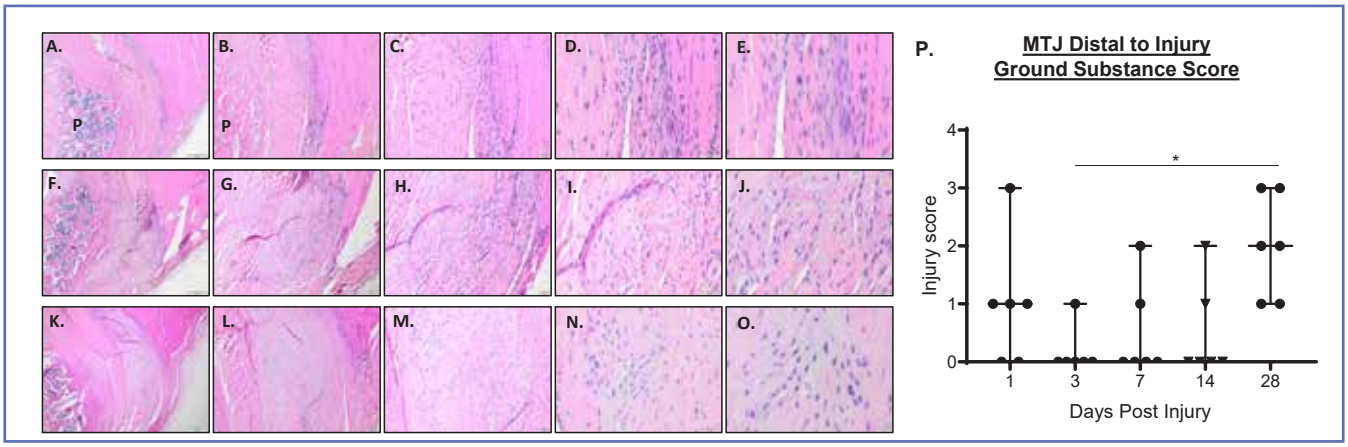
Reference	Species	Injury Induction	Model Characteristics			
			Muscle Group	Targets MTJ (Y/N)	Muscle Lesion (Y/N)	Muscle Lesion Resolution Time-Point (days)
(15)	Mouse	Chemical	Tibialis Anterior	N	Y	14 days
(16)	Mouse	Traumatic	Tibialis Anterior	N	Y	6-10 days
(17)	Mouse	Traumatic	Quadriceps	N	Y	Not Determined, >28 days
(18)	Mouse	Acute High Intensity Exercise	Quadriceps	N	N	N/A
(19)	Rat	Contusion	Tibialis Anterior	N	N	N/A
(20)	Rat	Contusion	Gastrocnemius	N	Y	28 days
(21)	Mouse	Contusion	Tibialis Anterior	N	Y	Not Determined, > 7 days
(22)	Mouse/Rat	Contraction	Quadriceps	N	Y	8 days
(23)	Mouse	Ischemia-Reperfusion	Gastrocnemius	N	Y	28 days
(24)	Rat	Traumatic	Gastrocnemius	Y	Y	26-46 days
(25)	Rabbit	Mechanical	Tibialis Anterior	Y	N	N/A
(26)	Mice	Tetanic	Gastrocnemius	Y	N	N/A
Current	Rat	Strain	Quadriceps	Y	Y	Not Determined, >28 days

Grey shading denotes model characteristics that match the current model.

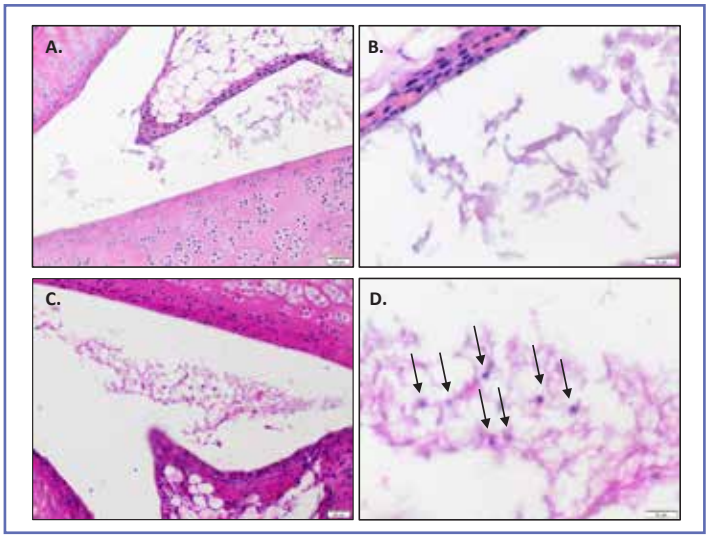
Appendix 2. Representative image of the generation of strain-induced MTJ injury in the quadriceps.

Appendix 3. Histopathologic grading scale for quadriceps MTJ injury pathology. The MTJ injury site, MTJ proximal to the injury site, and MTJ distal to the injury site were each graded independently on a scale of 0-3pts, with a maximum score of 12 possible for each site. The synovium, bursa, and joint were graded separately on a scale of 0-2pts each. Total MTJ injury score consisted of the sum of all sites and parameters with a maximum score of 41 possible.

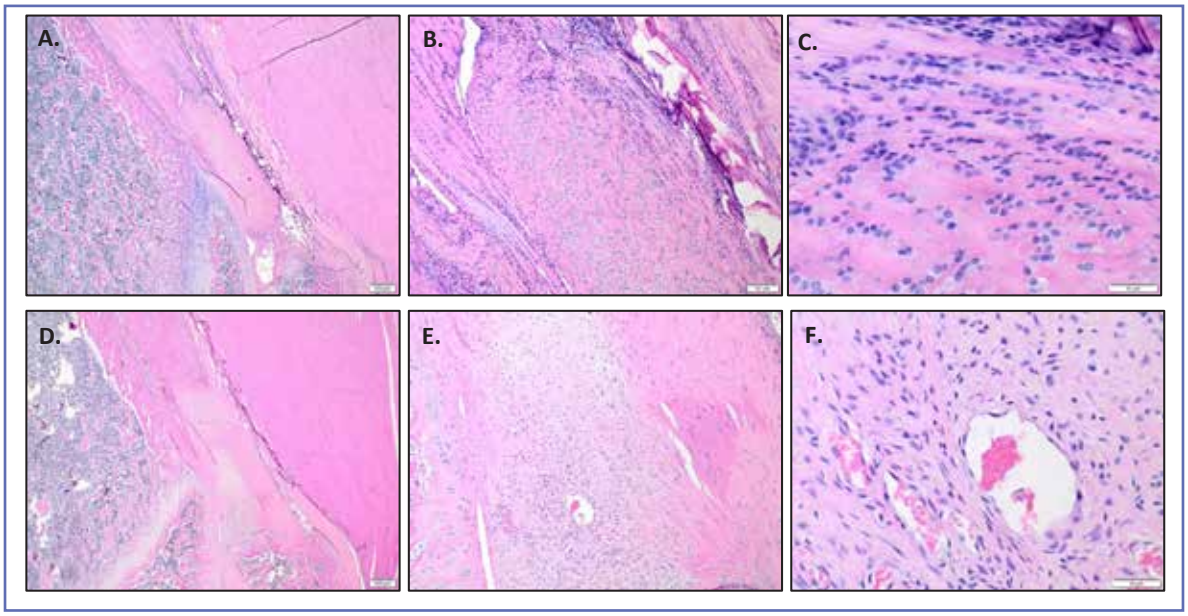
Score	0	1	2	3
MTJ Injury Sites (0-3pts)				
Size	Normal/ Inconspicuous	Mild (lesion measured between 0-75um)	Moderate (lesion measured between 75-150um)	Marked (lesion measured greater than 150um)
Ground Substance	Absent/ Normal	Mild myxomatous degeneration mildly separating collagen bundles	Moderate myxomatous degeneration disrupting or effacing collagen fiber organization	Marked myxomatous degeneration effacing collagen bundles with cartilaginous and/or osseous metaplasia and or enthesophytes at tendon insertion
Fibrosis	Normal	Mildly increased numbers of interstitial fibroblasts with maintenance of fiber organization	Fibroblasts disrupt collagen fiber organization and/or fiber polarization	Fibroblasts efface collagen fibers
Vascularity	Normal	1-2 cross-sectional vascular profiles per high powered field	3-6 cross-sectional vascular profiles per high powered field	Over 7 cross-sectional vascular profiles per high powered field
Joint Space, Bursa, and Synovium (0-2pts)				
Joint Space Effusion	Normal	Proteinaceous fluid	Intracapsular leukocytes +/- proteinaceous fluid	N/A
Bursa	Normal	Hypercellularity	Vascularization +/- hypercellularity	N/A
Synovium	Normal	Hypertrophied/reactive and/or hyperplastic synoviocytes	N/A	N/A



Appendix 4. Representative photomicrographs of MTJ ground substance pathology distal to the injury site. (A-E) Representative photomicrographs of MTJ ground substance score of 1. (**A**) Distal to the needle injury site along the anterior surface of the patella (marked with P) the MTJ is expanded by a linear proliferation of fibrous connective tissue which is accompanied by a mild basophilic myxomatous stroma, H&E, 4X. (**B**) Higher magnification, H&E, 10X. (**C**) Higher magnification, H&E, 20X. (**D**) Higher magnification, H&E, 40X. (**E**) Myxomatous degeneration mildly separates collagen fibers, H&E, 60X. (**F-J**) Representative photomicrographs of MTJ ground substances score of 2. (**F**) The MTJ distal to the injury site is effaced by a bed of fibrous connective tissue, H&E, 4X. (**G**) Higher magnification, H&E, 10X. (H) Higher magnification, H&E, 20X. (**I**) Higher magnification, H&E, 40X. (**J**) Fibrous connective tissue is accompanied by a moderate amount of myxomatous ground substance, H&E, 60X. (**K-O**) Representative photomicrographs of MTJ ground substance score of 3. (**K**) The MTJ distal to the injury site is markedly expanded and effaced by haphazardly arranged fibrous connective tissue, H&E, 4X. (**L**) Higher magnification, H&E, 10X. (**M**) Fibrous connective tissue is accompanied by a prominent basophilic myxomatous stroma, H&E, 20X. (N) Higher magnification, H&E, 40X. (**O**) Higher magnification, H&E, 60X. (**P**) MTJ distal to the injury site ground substance scores for the injured right hind limbs at 1, 3, 7, 14, and 28 days post-surgery. Values are represented as Median with Range and individual animals marked. Significant differences over time marked (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).



Appendix 5. Representative photomicrographs of joint effusion pathology. (**A-B**) Representative photomicrographs of joint effusion score of 1. (**A**) Within the joint space is a moderate amount of eosinophilic proteinaceous fluid, H&E, 20X. (B) Higher magnification, H&E, 60X. (**C-D**) Representative photomicrographs of joint effusion score of 2. (**C**) Within the joint space is a moderate amount of eosinophilic proteinaceous fluid which supports few variably degenerate leukocytes (arrows), H&E, 20X. (**D**) Higher magnification, H&E, 60X.



Appendix 6. Representative photomicrographs of bursal pathology. (**A-C**) Representative photomicrographs of bursal pathology score of 1. A. The proximal, anterior, and distal aspect of the bursa is hypercellular, H&E, 2X. (**B**) Higher magnification, H&E, 10X. (**C**) Higher magnification, H&E, 40X. (**D-F**) Representative photomicrographs of bursal pathology score of 2. (**D**) The distal and proximal aspect of the bursal contains increased numbers of reactive spindle cells accompanied by moderate numbers of vascular profiles, H&E, 2X. (**E**) Higher magnification, H&E, 10X. (**F**) Higher magnification, H&E, 40X.

REVIEW

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Effects of Prolonged Sitting Interventions on Chronic Low-Grade Inflammation in Adults: a Protocol for a Systematic Review

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LEVEL OF EVIDENCE: 2A

ABBREVIATIONS:
CSI: Chronic Systemic inflammation
SB: Sedentary behaviour
PA: Physical activity;
MVPA: Moderate to vigorous physical activity
LIA: Low intensity activity
CRP: C reactive protein
IL: Interleukins
IFN: Interferon
PAF: Plasminogen activating factor
IGF: Insulin like growth factors
TNF-α: Tumour Necrosis Factor alpha
TLF-4: Toll like factor-4

SUMMARY

Background. Chronic systemic inflammation (CSI) is linked with pathogenesis of chronic disease risk including type 2 diabetes, obesity, cardiovascular diseases and cancer. However, there is dearth of evidence to inform the stakeholders about the pooled effect of excessive sedentary behaviour or its interruptions, which may alter the CSI in adults. Our systematic review will aim to find the evidence behind the sedentary behaviour interventions on CSI.
Methods. Five databases (Scopus, PubMed, Web of Science, Cochrane Central Register of Controlled Trials, Ovid Medline and CINAHL) will be searched for studies examining the influence of excessive sitting or its interruptions on CSI markers (Interleukin; C-Reactive Protein, Cytokines), its dose, gender differences and context specific settings. Studies that included healthy working, adult population will be examined by two independent reviewers.
Results. The study quality will be assessed by QualSyst tool and Cochrane Risk of Bias tools using Revman 5.4. The mean effect size of the sitting interventions on CSI markers will be presented after exploring for potential publication bias. Appropriate visualisation of the effects of the outcome measures of interest will be assessed through Forrest plots to assess the direction, consistency and size of the intervention.
Conclusions. Potential associations between excessive sitting and the effects of interruption interventions on CSI will be explored after assessing the quality of the studies.

KEY WORDS

Chronic systemic inflammation; sedentary behaviour; interleukin; C-reactive protein; cytokines; prolonged sitting; interrupted sitting.

OPERATIONAL DEFINITIONS: adapted from (1)

Metabolic equivalent (MET)s. Physiological measure of energy expenditure expressing the intensity of physical activities. One MET is expressed as energy expenditure at rest ≈ 3.5 ml/kg/min.
Sedentary behaviour (SB). Any waking behaviour (sitting, lying, reclining) that is characterised by low energy expenditure (< 1.5 METs).
Moderate to vigorous physical activity (MVPA). Any bodily movement or exercise that increases energy expenditure by > 3 METs and exertion level of 5-6 on a scale of 10.
Light Intensity physical activity (LIA). Physical activity performed at an intensity between 1.5 and 3 METS at one's personal capacity and exertion level of 2-4 on a scale of 10.
Physical inactivity. Insufficient physical activity *i.e.*, non-compliant with the global activity recommendations of at least 150 minutes of MVPA per week.
Microbreaks. Transient short breaks during the typical workday not lasting for more than 2-3 minutes.

INTRODUCTION

Chronic low-grade inflammation attributed to sedentary behaviour (SB) can have deleterious effects on work, quality of life, and in severe cases eventuate into a metabolic syndrome. Chronic systemic inflammation (CSI) is associated with the incidence of a myriad of chronic diseases including cardiovascular diseases, type 2 diabetes, fatty liver diseases, cancer, musculoskeletal disorders, depression, dementia and Alzheimer's disease (2). Persistent circulating markers such as proinflammatory cytokines [interleukins (IL-6, IL-8), tumour necrosis factor (TNF-α)], acute phase proteins [C-reactive protein (CRP) and plasminogen activating factor (PAF)] may perpetuate underlying chronic disease risk via possible mechanisms such as high postprandial hyperglycaemia, insulin resistance, oxidation stress, adipose tissue dysregulation, neurodegeneration, triglyceridemia and atherosclerosis (2, 3). Hence, these immunomodulatory pathways become the potential target for SB interventions that increase daily physical activity (PA) to reduce disease risk and morbidity common in clinical populations.

Anti-inflammatory effects of PA or SB interventions

The anti-inflammatory effects of PA are evident in epidemiological studies that report low CSI in primitive, non-industrialised populations such as Hadza of Tanzania, Shuar of Amazon and the Tsimane foragers of Bolivia (4, 5). Hence, adding PA to reduce SB, in any form or volume, may aid in reducing the cardiometabolic risk associated with CSI in the mechanised world.

Large epidemiological studies have consistently shown that high SB and low levels of PA lead to visceral adiposity and affect inflammatory mediators (listed above) adversely leading to chronic disease (5, 6). Though limited and inconsistent, early empirical evidence claims that moderate to vigorous PA (MVPA) such as aerobic exercise training could help in reducing CSI and resulting cardiometabolic risk in contemporary men (7). Recent systematic reviews in this area have reported a moderate reduction of IL-6, TNF-α and CRP with leisure time MVPA (8, 9). Skeletal muscle contraction, β-oxidation, increased sensitivity of adipose tissue to epinephrine associated lipolysis and increased oxidation of intramyocellular triglycerides are proposed to reduce inflammation through toll like receptor (TLF4) activation, limiting adipose expansion and limiting proinflammatory signalling activation (10). Nonetheless, the uptake of recommended weekly levels of MVPA in contemporary men is less than optimal.

Emerging evidence of light activities on inflammation

While only a small amount of waking hours is spent in MVPA (0.58 hrs/day), more of the day is spent in light intensity activities (LIA) that include standing or stepping (5.19 hrs/day). Most of the day is spent in SB (9.53 hrs/day) including prolonged bouts of sitting (11, 12) (**figure 1**). As standing or stepping is more ubiquitous than the MVPA, LIA has become an appealing intervention target for reducing CSI (12, 13). Many consider prolonged sitting an independent disease risk factor from general SB, making the workplace a primary target for lifestyle behavioural interventions (6, 14). Nonetheless, the anti-inflammatory effects of such lifestyle interventions have yet to be systematically reviewed.

For a decade now, sizable experimental trials have utilised SB interventions or LIA to interrupt or replace prolonged sitting to investigate cardiometabolic risk factors (15) such as postprandial hyperglycaemia (16), triglyceridemia (17), blood pressure (18) and anthropometric measures such as waist circumference and body mass index (19, 20). Despite evidence suggesting that interrupting or replacing sitting with LIA, both acutely (< 7 days) (6) and chronically (> 2 weeks) (19), can have a moderate reduction in cardiometabolic risk factors. Nonetheless, inconsistencies in the reporting of CSI markers remain. For example, Henson and colleagues (2018) found that iso-temporal substitution of sitting with 60 mins of stepping yielded better reduction (-

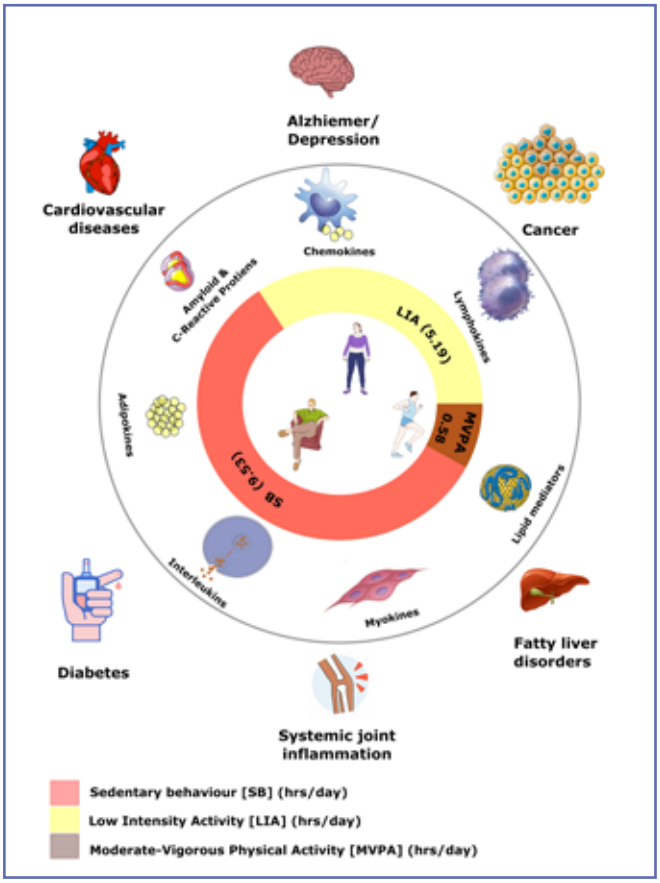

Figure 1. Association of daily wake hour activity, chronic inflammation and chronic diseases. (central circle representing daily physical activity is drawn from the data source) (11).

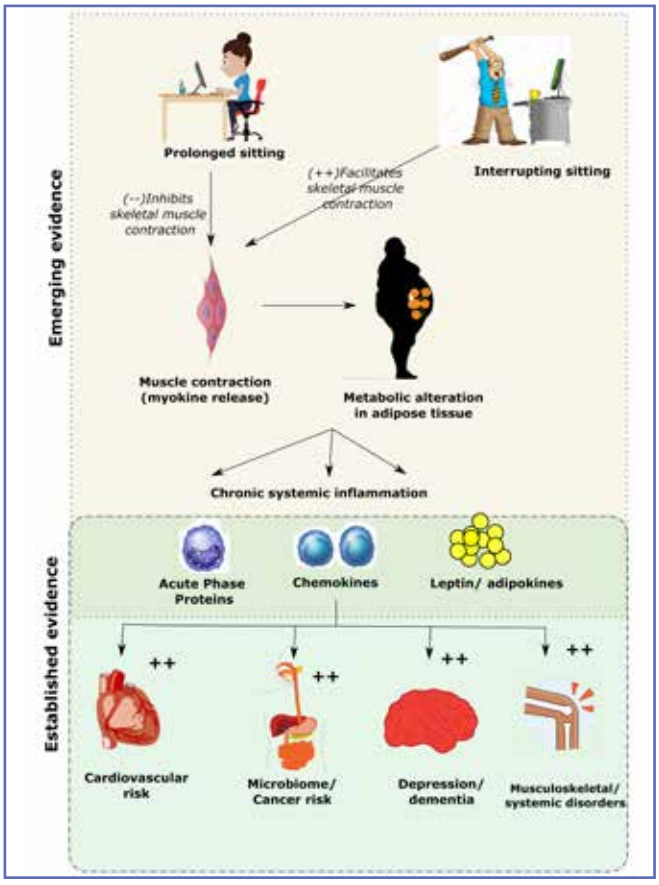

Figure 2. Graphical summary of the problem statement presented in the systematic review.

28%) in IL-6 compared to standing (- 5%). Regular sitting breaks also reduced IL-8 by 0.19 pg/µg, whereas uninterrupted sitting increased IL- 8 by 0.31 after a four hour trial period (21). These findings warrant further investigation.

Problem statement

While substantial evidence exists to claim the chronic disease risk due to CSI and scheduled exercise or MVPA on inflammation, the evidence regarding altered inflammatory physiology with SB and LIA is still emerging (22) (**figure 2**). Hence there is a need to establish a systematic review to pool the inflammatory effects of SB and LIA to inform the policy makers for better SB or PA interventions in community and context specific settings for combating inflammation and resulting chronic disease risk. This problem statement is illustrated in **figure 2**.

This review will aim to consolidate the existing evidence to determine the effects of both uninterrupted and interrupted sitting on systemic inflammatory markers (proinflammatory cytokines and acute phase proteins). We aim to determine if uninterrupted sitting is associated with increased proinflammatory cytokines [IL-1, TNF, adipokines, tissue plasminogen activator inhibitor (t-PAI)], chemokines (IL-8) and acute phase proteins in contemporary men, and negatively associated with IL-6 and CRP. Further, we will investigate whether and to what extent do sitting interruptions alter the proinflammatory cytokines (IL-1, IL-6, CRP, TNF, adipokines, t-PAI), chemokines (IL-8) and acute phase proteins in adults.

METHODS

Reporting methods and registration

The methodology of the present systematic review protocol is reported based on the guidelines of the Preferred Reporting Items for systematic reviews and Meta-Analyses Protocol (PRISMA-P, 2015). A completed copy of PRISMA-P checklist

is provided as **appendix 1**. The review protocol is prospectively registered in the International Prospective Register of Systematic Reviews (CRD42020216611; www.crd.york.ac.uk/PROSPERO). The research review was conducted ethically according to international standards and as required by the journal as described in Padulo *et al.* (2018) (23).

Data sources and search criteria

A comprehensive search of peer reviewed electronic databases (Scopus, Web of Science, Ovid Medline, Embase, PubMed Medline, Cochrane Central Register of Controlled Studies, Scientific Electronic Library Online, Cumulative Index to Nursing and Allied Health Literature) will be performed by the primary author with the assistance of librarian from inception until May 15th, 2020. Besides trial registries including International Trials Registry Platform (https://www.who.int/clinical-trials-registry-platform), meta Register of Controlled Trials (http://www.isrctn.com/page/mrct) and Clinical Trials.gov (https://www.clinicaltrials.gov/) will be checked for relevant registered trials and published studies for possible inclusion to the review. Further non peer reviewed databases such as OpenGrey, Google scholar will also be searched. The Medical Subject Heading (MeSH) terms and appropriate combinations will be identified for problem (SB), intervention (interrupt or replace), comparison (prolonged sitting or sedentary position) and outcome (inflammatory markers as exploded from MeSH browser of US National Library of Medicine) with appropriate qualifiers and MeSH tree structures. Appropriate combination of the keywords to identify the article that investigated prolonged sitting or interrupting prolonged sitting on inflammatory markers in healthy adult participants will be utilised. Appropriate wildcards not limiting to *, ?, / and proximity search using "N/n, adj n, Pre/n will be used appropriately for retrieving larger searches. For example, search terms such as "behavio?r", "sedentar*", "activit*", "inflammat*" will be framed as appropriate to the PICO search mentioned below. The reference list of the articles meeting the inclusion criteria including earlier systematic reviews will also be scanned for possible additional eligible studies. Example search strategy for Scopus, Ovid Medline and CINAHL are provided as **appendix 2**.

Eligibility criteria

The study question, search, extraction of the studies will be guided by the Population, Intervention or Exposure, Comparison, Outcome and Study (PICOS) design criteria to be used as the 'yardstick' for study eligibility.

Population

Studies which included apparently healthy adults (≥ 18 years; non-smokers; not taken any anti-inflammatory medications) will be added to the review. Studies which conducted experiments on healthy adults who worked full time or part time in desk-based jobs will be included.

Intervention

We will include any studies that aimed to reduce SB or increase PA in isolation or in combination of various intervention strategies as outlined in **table I**. Studies which have

Table I. Strategy categories and workplace intervention.

Strategy categories for replacing or interrupting occupational sitting	Interventional activities
Restructuring physical environment	Active workstations such as sit-stand, treadmill, biking desks; adding gym facilities, bike parking spaces.
Changing organisation culture and norms	Office environment that supports schedule breaks, standing and walking meetings, stair use, lunch walks and games, annual sports meet.
Information and counselling	Provide group or individual counselling strategies like goal setting, strategies based on self-determination and health belief models like dangers of sitting, benefits of move more at work, details of online information (websites, online/text messaging).
Prompts/cues	Computer based, sensor-based goal setting prompts for promoting walk and office-based activities.
Material reward/incentivization	Pedometer step based; stair climb challenges.
Monitoring of outcome	Activity logs and monitoring workplace SB.
Demonstration of behaviour (modelling)	Workplace champion/therapist showing model of the target behaviour (demonstration of exercises) face-face or through tele-health platforms at workplaces.

administered SB interventions for acute periods (at least 1 hour to ≤ 7 days) or chronic periods (≥ 7 days) will be included. Studies that have administered any form of the intervention intended to interrupt or replace the sitting period (walk, stand, calisthenics, resistance exercises, treadmill walk, stair climb time, steps, exercise, dance) will be grouped into strategy categories and interventions will be mapped with the behaviour change techniques as mentioned in **table I**.

Comparator

The studies that investigated occupational SB within groups and compared with parallel groups that received other treatments, comparison for a specified time, or without exposure of sitting interventions, or any form of intervention meant to interrupt occupational SB will be included. Since workplace interventions are often administered in groups, control groups may be a usual work group (often perceived to continue their routine work or received standard information for workplace wellbeing).

Outcome measures

Studies that have measured explicitly any of the following outcome measures in an isolated form or combined forms: adipokines (leptin, adiponectin), pro inflammatory cytokines (IL-6, IL-8, TNF-α), acute phase proteins including CRP, leptin, t-PAI and insulin like growth factor. Studies should report odds ratio in cases of exposure, mean differences or effect sizes in cases of interventional studies.

Study design

We will consider any type of study (experimental, non-experimental observational studies) that explored association between SB and any of the above-mentioned inflammatory markers. Thus, included studies will be randomised, non-randomised, cluster randomised trials, single group before-after studies, repeated measures or interrupted time series as defined by Cochrane's Effective Practise and Organisation of Care taxonomy (24).

Nature of publication

The potential studies to be included should be published in English and involved humans. The publications will not be limited to context specific settings as laboratory settings mimicking contextual settings have been explored. Conference proceedings, abstracts, editorials, case reports will be excluded.

Article selection

All the retrieved study references will be imported into a collaborative systematic review software, "Rayyan web application" (Qatar Computing Research Institute, Qatar, https://rayyan.qcri.org/welcome). After the removal of duplicates, two authors (AS, BC) will independently screen the titles and abstracts of the retrieved articles from the systematic search to include potentially relevant studies using PICO as proposed earlier. If the potential studies are labelled as 'included' or 'may be' by both the reviewers (AS and BC), full text articles will be downloaded and screened for eligibility based on the inclusion criteria mentioned above. All the possible reasons for exclusion of the studies will be documented. In case of any missing data in the included studies, the authors will be contacted for necessary information. Any discrepancies will be resolved through mutual agreement between both the authors and if not resolved, the third reviewer (SP) will be consulted. The results of the selection process with the included and excluded studies and possible reasons for exclusion will be illustrated using Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram. A completed PRISMA checklist will be provided at the final reporting.

Data extraction and quality check

A customised data extraction sheet will be used to extract the following details:

1. author, year, country of the study origin, design of the study, setting (location, environment);
2. duplicate study, study protocol publication or registration number provided;
3. participant characteristics (gender, number contacted, allocated intervention, completed the intervention, lost to follow-up, age, job nature, inclusion/ exclusion criteria), sedentary behaviour (measurement technique, time of measurement);
4. intervention (break time, frequency, intensity, duration, type of break, washout between the breaks, follow-up);
5. behavioral change technique embeded in the intervention and theoretical model underpinning the behavioral change technique;
6. outcome measures (type of the inflammatory marker, method of measurement, unit of the measure, validity and reliability, blinding, mean change or association after the intervention or exposure);
7. intervention effects (effect size, hazard or risk ratio, 95% confidence interval, standard deviation), intention to treat or per-protocol analysis, acknowledged limitations, possible bias (selection, reporting), conclusions and funding or any other source of conflicts of interest.

The primary investigator will extract data from the articles and the other two investigators (BC and CRR) will verify the extracted data in the customised, a priori data extraction sheets. First ten articles will be extracted to the data sheet independently by the three reviewers (AS, BC, CRR) as a pilot test. Effect sizes and the estimates of CSI markers (acute phase proteins, chemokines, interleukins, cytokines, t-PAI, CRP, TNF) will be calculated from the observational and randomised trials according to Higgins and colleagues (2020). In case of any incomplete or missing data in the included studies, the corresponding author of the included studies will be contacted by email to retrieve appropriate information.

The quality assessment tool, QualSyst (Alberta Heritage Foundation, Canada) for qualitative studies will be used to assess the quality of the behavioural interventions and observational studies (25). The QualSyst tool for qualitative studies includes 10 questions for which the reviewer will be scoring 'yes' (2), 'partial' (1) and 'no' (0) for each question on the checklist. Items not applicable to the 10 questions will be excluded from the calculation of summary score. A summary score for each study is determined by adding the number of "yes" scores x2 plus the number of "partial" scores x1 then dividing by 20 (the maximum number) minus the number of "not applicable" scores x2 (26). Thus, the quality of the study can be illustrated as: strong (summary score of > 0.80), good (summary score of 0.71-0.79), adequate (summary score of 0.50-0.70) and limited (summary score of < 0.50) (27).

Although appropriate for evaluating the quality of the study, QualSyst has been suggested to lack the ability to detect the biases present within a study (27). Hence, we will also assess the risk of bias present within studies using the Cochrane Risk of Bias tools (28) as elaborated below.

Risk of bias

After the quality check, the studies will be examined for apparent risks of publication bias including imprecision, inconsistency and indirectness. Risk of bias will be assessed using the Cochrane Risk of Bias tools (ROB-2 for RCTs; 2016 and ROBINS-I tool for non-randomised studies; 2016) (28). Bias (selection, attrition, detection, reporting) will be assessed using seven domains: 1) random sequence generation, 2) allocation concealment, 3) deviations from intended interventions, 4) missing outcome data, 5) selective outcome reporting, and 6) blinding of participants; 7) blinding of outcome assessors as reported in *Cochrane Handbook for systematic reviews, Section 7.6* (29). Based on the criteria of the signalling questions as outlined by the Higgins *et al.*, 2020 (29), the primary investigator (AS) will rate the risk of bias in each domain as 'low', 'unclear' or 'high' along with the justification of the reviewer's decision on the excluded study.

Sub-group analysis

Subgroup analysis will be performed to further understand the dose-response relationship of the frequency of prolonged sitting interruptions and the resultant inflammatory effects. We will consider intervention type (19, 30), intensity of the movement breaks (30, 31) and any differences attributed to gender (32). Further, our investigation will also include studies designed for working adults with specific conditions including hypertension, diabetes, arthritis, obesity and cardiovascular diseases. Other variables of interest will include: region of origin (US, Australia, European countries), age criteria (young adults: 19 to 35 *vs* older adults: > 35 years), follow-up duration (weeks, months, years), sitting assessment (objective *vs* self-reported) and the effect of above variables on the inflammatory markers will be individually explored.

Data analysis

Meta-analyses will be performed if two or more studies are homogenous in population, methodology (micro breaks, breaks administration), outcome measurements and reporting. The mean and standard deviations of the inflammatory markers (mg/dL, pg/dL) during the pre-post trial periods or mean differences with their standard deviations of both intervention and comparator groups will be entered into the Revman 5.4.1 (The Nordic Cochrane Centre, Copenhagen, Denmark). If meta-analyses are not possible, the study findings such as characteristics, sitting time (h/day), dosage of intervention (break mode, time and the duration), comparison of methods in assessing inflammatory markers, quality of the data presented, magnitude of change reported, bias, intention to treat analysis or per-protocol analysis will be presented as qualitative narrative syntheses.

The quality of evidence for each outcome of interest will be assessed using Grading of Recommendations, Assessment, Development and Evaluation (GRADE) levels of evidence as 'very low', 'low', 'moderate', and 'high'. The quality of evidence will be imported from the Cochrane workspace into GRADE Profiler version 3.6 (GRADEpro working group, McMaster University, ON, Canada) for rating the quality of evidence and the final evidence summary table will be created.

The statistical heterogeneity across the included studies will be assessed using tests of Chi square (χ^2) and homogeneity (I^2) with $I^2 < 50$ indicates a low risk of homogeneity whereas $I^2 > 50$ indicates a high risk of homogeneity. We will use a fixed

effects model if $I^2 < 50\%$ and random effects modelling if $I^2 > 50\%$ using an inverse variance method. DerSimonian-Laird method calculates the random effects by measuring standard errors of the adjusted study estimates as outlined in *Cochrane Handbook for systematic reviews,* section 9.4.3.1 (29). Pooled effects of the risk ratios will be calculated for observational studies enquiring the risk of sitting time with chronic low-grade inflammation. For intervention studies (to answer the second question), pooled mean differences effects will be estimated from the individual intervention effects (mean differences within and between the groups). Effect sizes will be calculated as standardized mean differences, where < 0.2 was defined as trivial, 0.2 to 0.4 as small, 0.4 to 0.7 as moderate, and > 0.7 as large (29).

Leave-one-out sensitivity analyses will be performed by excluding one trial at a time to test the robustness of the pooled results and to prevent conclusions from being too dependent on an individual study (*Cochrane Handbook for systematic reviews, Section 10* (29)). Possible publication bias will be visually analysed by contour enhanced funnel plots as guided by Begg's rank correlation test (33). If the Beggs test is underpowered, we will use non-parametric Tweddie's trim and fill method to calculate bias-corrected estimates (33). If enough homogenous data (method of intervention, outcome measure) is available, meta-analyses and forest plots will be created by Revman 5.4.1 to compute the pooled mean differences or the effect size of the intervention effect of interrupting sitting on each of the inflammatory markers.

DISCUSSION

To our knowledge, the present systematic review will be the first to examine the effectiveness of SB interventions specifically on chronic low-grade inflammation in context specific and laboratory settings of sedentary workplaces. There is a need in pooling the effects of SB and its interruptions on CSI which could facilitate human resources policy development to reduce sitting time for ameliorating the chronic disease risk. This systematic review will provide a rigorous examination of SB interventions on the CSI while considering the challenges and limitations associated with measuring inflammatory markers in a context specific workplace setting.

Potential limitations

The SB interventions may vary across different settings and countries using variety of behaviour change components; hence we anticipate a high heterogeneity in the potential studies that may be included in our review (19). Unpublished studies will not be included in the present review which may bias the results and may potentially limit the generalisation of the review results. Nevertheless, Hawthorne effects of the control group (where the participants may increase in the outcome of interest due to increased awareness of being observed over the trial period) may be inevitable in the studies as implemented in organisation not in 'real world setting'. Further we could probably expect only modest effect size between control and intervention groups due to the social desirability bias (participants of potential studies with tendency to report societal norms) and effect of measurement (high biomarker in control group causing behaviour change in absence of intervention). Further mixing of subjective and objective measure of SB assessment at context specific setting may affect the results of the review generalisation, however we are planning to conduct a different subgroup analysis for the subjective and objective SB measurements in the included studies.

HUMAN RIGHTS

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

CONTRIBUTIONS

SP, CRR & BC conceived and designed the research, AS & BC framed the protocol for the systematic review. BC drafted the manuscript. SP and CRR proofread the manuscript.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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SUPPLEMENTS

Appendix 1. PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol*.

Section and topic	Item No	Checklist item	Reported page
ADMINISTRATIVE INFORMATION			
Title:			
Identification	1a	Identify the report as a protocol of a systematic review	1
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	NA
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	6
Authors:			
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	Title Page
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	Title Page
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	NA
Support:			
Sources	5a	Indicate sources of financial or other support for the review	Title Page
Sponsor	5b	Provide name for the review funder and/or sponsor	NA
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	Title Page
INTRODUCTION			
Rationale	6	Describe the rationale for the review in the context of what is already known	3&4
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	5
METHODS			

Eligibility criteria	8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	7-9
Information sources	9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	6-7
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	Appendix 2
Study records:			
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	10
Selection process	11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	10
Data collection process	11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	10-11
Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	10-11
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	8
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	11

Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised	9-10
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I^2, Kendall's τ)	12-14
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	12-14
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	13-14
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	13-14
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	13

*It is strongly recommended that this checklist be read in conjunction with the PRISMA-P Explanation and Elaboration (cite when available) for important clarification on the items. Amendments to a review protocol should be tracked and dated. The copyright for PRISMA-P (including checklist) is held by the PRISMA-P Group and is distributed under a Creative Commons Attribution Licence 4.0.

From: Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart L, PRISMA-P Group. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ. 2015 Jan 2;349(jan02 1):g7647.

Appendix 2. Search strategy in the databases.

1) Database: Ovid MEDLINE(R) ALL <1946 to December 14, 2020>
Search Strategy:
1 sitting.mp. or Sitting Position/
2 "prolonged sitting".mp.
3 (prolong* adj3 (sedentar* or sit* or sitting)).mp.
4 ((uninterrupt* or excessive) adj2 (sit* or sedent*)).mp.
5 ((workplace or office) adj2 sit$).mp.
6 ((workplace or office) adj2 seat$).mp.
7 (sedentar* adj2 (lifestyle or behavio?r)).mp.
8 (excessive adj2 (sit* or sedentar*)).mp.
9 (workplace adj2 (sedentar* or sit*)).mp.
10 (sedentar* adj2 (behavio?r or position or posture)).mp.
11 "sedentary behavio?r".mp. or exp *Sedentary Behavior/
12 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11
13 (break* adj3 (sedentar* or sit*)).mp.
14 (interrupt* adj2 (prolong* or sedentary or sit*)).mp.
15 (walk* adj2 (activit* or bout*)).mp.
16 ((walk* or activit* or cycl* or exercis*) adj2 break*).mp.
17 (break* adj3 (up or sedentary or prolong* or sit* or behavio?r*)).mp.
18 ((interrupt* or disrupt* or replac*) adj3 (sedentar* or sit* or "prolonged sitting")).mp.
19 (desk adj3 (office or job or work)).mp.
20 microbreak*.mp.

21 (movement adj2 break*).mp.
22 13 or 14 or 15 or 16 or 17 or 18 or 20 or 21
23 12 or 19
24 (inflammat* adj2 (marker* or risk*)).mp.
25 (acute adj2 protein).mp.
26 ((Inflammat* or biochemi*) adj3 marker*).mp.
27 (chronic adj3 (inflammat* or "systemic inflammat*" or "low grade inflammation")).mp.
28 (immun* adj2 (reaction or response*)).mp.
29 (interleukin or IL-6 or IL-8).mp.
30 (C adj3 (reactive protein* or RP)).mp.
31 C-Reactive Protein/
32 *Adipokines/
33 (Tumo?r adj2 necrosis).mp.
34 Tumor Necrosis Factor-alpha.mp. or exp *Tumor Necrosis Factor-alpha/
35 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 34
36 23 and 35 [For objective one]
37 22 and 23 and 35 [For objective two]

2) Database: Scopus <Inception to December 17, 2020>

Objective 1

((((TITLE-ABS-KEY (*sitting*) OR TITLE-ABS-KEY (*«sitting position»*) OR TITLE-ABS-KEY (*«sedentary position»*) OR TITLE-ABS-KEY (*«prolonged sitting»*))) OR ((TITLE-ABS-KEY (*«excessive sitting»*) OR TITLE-ABS-KEY (*«prolonged sitting»*) OR TITLE-ABS-KEY (*«uninterrupted sitting»*))) OR ((TITLE-ABS-KEY (*sedentar**) OR TITLE-ABS-KEY (*sit**) OR TITLE-ABS-KEY (*«uninterrupted sitting»*))) OR ((TITLE-ABS-KEY (*lifestyle*) OR TITLE-ABS-KEY (*«lifestyle behavio?r»*))) OR ((TITLE-ABS-KEY (*«workplace sitting»*) OR TITLE-ABS-KEY (*«office sitting»*) OR TITLE-ABS-KEY (*«workplace seat*»*) OR TITLE-ABS-KEY (*«desk-based»*))) OR (TITLE-ABS-KEY (*prolong** PRE/3 (*sedentar** OR *sit** OR *sitting*))) OR (TITLE-ABS-KEY (*uninterrupt** PRE/2 (*sit** OR *sedent**))) OR ((TITLE-ABS-KEY ((*workplace* OR *office*) PRE/2 *sit$*) OR TITLE-ABS-KEY ((*workplace* OR *office*) PRE/2 *seat$*))) OR ((TITLE-ABS-KEY (*excessive* PRE/2 (*sit** OR *sedentar**)) OR TITLE-ABS-KEY (*sedentar** PRE/2 (*lifestyle* OR *behavio?r*))) OR ((TITLE-ABS-KEY (*workplace* PRE/2 (*sedentar** OR *sit**)) OR TITLE-ABS-KEY (*sedentar** PRE/2 (*behavio?r* OR *position* OR *posture*)))) OR ((TITLE-ABS-KEY (*«sedentary behavio?r» *sedentary* AND *behavior*) OR TITLE-ABS-KEY (*«*Sedentary Behavior»*)))) OR (TITLE-ABS-KEY (*desk* PRE/2 (*office* OR *job* OR *work*)))) AND ((TITLE-ABS-KEY (*inflammat** PRE/2 (*marker** OR *risk**))) OR (TITLE-ABS-KEY (*acute* PRE/2 *protein*)) OR (TITLE-ABS-KEY ((*inflammat** OR *biochemi**) PRE/2 *marker**)) OR (TITLE-ABS-KEY (*inflammat**)) OR (TITLE-ABS-KEY (*chronic* PRE/2 (*inflammat** OR *«systemic inflammat*»* OR *«low grade inflammation»*))) OR (TITLE-ABS-KEY (*chronic* PRE/2 (*inflammat** OR *«systemic inflammat*»* OR *«low grade inflammation»*))) OR ((TITLE-ABS-KEY (*cytokine*) OR TITLE-ABS-KEY (*immun** PRE/2 (*reaction* OR *response**)))) OR ((TITLE-ABS-KEY (*interleukin* OR *il-6* OR *il-8*) OR TITLE-ABS-KEY (*«C Reactive Protein»*) OR TITLE-ABS-KEY (*«C-Reactive protein»*))) OR (TITLE-ABS-KEY (*crp*)) OR (TITLE-ABS-KEY (*adipokine*)) OR (TITLE-ABS-KEY (*tumo?r* PRE/2 *necrosis*)) OR ((TITLE-ABS-KEY (*«Tumor Necrosis Factor-alpha»*) OR TITLE-ABS-KEY (*tnf**) OR TITLE-ABS-KEY (*«*Tumor Necrosis Factor-alpha»*)))) AND (LIMIT-TO (LANGUAGE , *«English»*)) AND (LIMIT-TO (SRCTYPE , *«j»*)) AND (EXCLUDE (EXACTKEYWORD , *«Nonhuman»*) OR EXCLUDE (EXACTKEYWORD , *«Animals»*)) AND (LIMIT-TO (DOCTYPE , *«ar»*))

Objective 2

((((TITLE-ABS-KEY (*sitting*) OR TITLE-ABS-KEY (*«sitting position»*) OR TITLE-ABS-KEY (*«sedentary position»*) OR TITLE-ABS-KEY (*«prolonged sitting»*))) OR ((TITLE-ABS-KEY (*«excessive sitting»*) OR TITLE-ABS-KEY (*«prolonged sitting»*) OR TITLE-ABS-KEY (*«uninterrupted*

sitting»))) OR ((TITLE-ABS-KEY (*sedentar**) OR TITLE-ABS-KEY (*sit**) OR TITLE-ABS-KEY (*«uninterrupted sitting»*))) OR ((TITLE-ABS-KEY (*lifestyle*) OR TITLE-ABS-KEY (*«lifestyle behavio?r»*))) OR ((TITLE-ABS-KEY (*«workplace sitting»*) OR TITLE-ABS-KEY (*«office sitting»*) OR TITLE-ABS-KEY (*«workplace seat*»*) OR TITLE-ABS-KEY (*«desk-based»*))) OR (TITLE-ABS-KEY (*prolong** PRE/3 (*sedentar** OR *sit** OR *sitting*))) OR (TITLE-ABS-KEY (*uninterrupt** PRE/2 (*sit** OR *sedent**))) OR ((TITLE-ABS-KEY ((*workplace* OR *office*) PRE/2 *sit$*) OR TITLE-ABS-KEY ((*workplace* OR *office*) PRE/2 *seat$*))) OR ((TITLE-ABS-KEY (*excessive* PRE/2 (*sit** OR *sedentar**)) OR TITLE-ABS-KEY (*sedentar** PRE/2 (*lifestyle* OR *behavio?r*)))) OR ((TITLE-ABS-KEY (*workplace* PRE/2 (*sedentar** OR *sit**)) OR TITLE-ABS-KEY (*sedentar** PRE/2 (*behavio?r* OR *position* OR *posture*)))) OR ((TITLE-ABS-KEY (*«sedentary behavio?r» *sedentary* AND *behavior*) OR TITLE-ABS-KEY (*«*Sedentary Behavior»*)))) OR (TITLE-ABS-KEY (*desk* PRE/2 (*office* OR *job* OR *work*)))) AND ((TITLE-ABS-KEY (*inflammat** PRE/2 (*marker** OR *risk**))) OR (TITLE-ABS-KEY (*acute* PRE/2 *protein*)) OR (TITLE-ABS-KEY ((*inflammat** OR *biochemi**) PRE/2 *marker**)) OR (TITLE-ABS-KEY (*inflammat**)) OR (TITLE-ABS-KEY (*chronic* PRE/2 (*inflammat** OR *«systemic inflammat*»* OR *«low grade inflammation»*))) OR (TITLE-ABS-KEY (*chronic* PRE/2 (*inflammat** OR *«systemic inflammat*»* OR *«low grade inflammation»*))) OR ((TITLE-ABS-KEY (*cytokine*) OR TITLE-ABS-KEY (*immun** PRE/2 (*reaction* OR *response**)))) OR ((TITLE-ABS-KEY (*interleukin* OR *il-6* OR *il-8*) OR TITLE-ABS-KEY (*«C Reactive Protein»*) OR TITLE-ABS-KEY (*«C-Reactive protein»*))) OR (TITLE-ABS-KEY (*crp*)) OR (TITLE-ABS-KEY (*adipokine*)) OR (TITLE-ABS-KEY (*tumo?r* PRE/2 *necrosis*)) OR ((TITLE-ABS-KEY (*«Tumor Necrosis Factor-alpha»*) OR TITLE-ABS-KEY (*tnf**) OR TITLE-ABS-KEY (*«*Tumor Necrosis Factor-alpha»*)))) AND (((TITLE-ABS-KEY (*break** PRE/2 (*sedentar** OR *sit**)) OR TITLE-ABS-KEY ((*interrupt** OR *disrupt**) PRE/2 (*prolong** OR *sedentary* OR *sit**)) OR TITLE-ABS-KEY ((*reallocat** OR *replac**) PRE/2 (*prolong** OR *sedentary* OR *sit**)))) OR ((TITLE-ABS-KEY (*walk** PRE/2 (*activit** OR *bout**)) OR TITLE-ABS-KEY ((*walk** OR *activit** OR *cycl** OR *exercis**) PRE/2 *break**) OR TITLE-ABS-KEY (*break** PRE/2 (*up* OR *sedentary* OR *prolong** OR *sit** OR *behavio?r**)))) OR ((TITLE-ABS-KEY ((*interrupt** OR *disrupt** OR *replac**) PRE/3 (*sedentar** OR *sit** OR *«prolonged sitting»*)) OR TITLE-ABS-KEY (*microbreak**) OR TITLE-ABS-KEY (*movement* PRE/2 *break**)))) AND (LIMIT-TO (LANGUAGE , *«English»*)) AND (EXCLUDE (EXACTKEYWORD , *«Nonhuman»*) OR EXCLUDE (EXACTKEYWORD , *«Animals»*))

3) Database: CINAHL <Inception to December 18, 2020>

#	Query
S14	(S6 AND S9 AND S13)
S13	(S6 AND S12)
S12	(S10 OR S11)
S11	"Tumor Necrosis Factor-alpha" OR *Tumor Necrosis Factor-alpha
S10	(inflammat* N2 (marker* or risk*)) OR acute N2 phase N2 protein OR ((Inflammat* or biochemi*) N3 marker*) OR (chronic N2 (inflammat* or "systemic inflammat*" or "low grade inflammation")) OR (immun* N2 (reaction or response*)) OR (interleukin* or IL-6 or IL-8) OR "plasminogen activator" OR leptin OR ("c-reactive protein*) or crp OR "C reactive protein") OR *Adipokines OR "low grade inflammation" OR ("Tumo?r N2 necrosis N2 factor) OR TNF)
S9	(S7 OR S8)
S8	((Cycl* OR exercis* OR danc* OR walk* OR calisthenic*) adj2 (activit* or bout*)) OR ((walk* or activit* or cycl* or exercis*) N2 break*) OR ((break* N3 (up or sedentary or prolong* or sit* or behavio?r*)) OR ((interrupt* or disrupt* or replac*) N3 (sedentar* or sit* or "prolonged sitting")) OR micro* N2 break OR microbreak* OR (movement N2 (break or interrupt*))

S7	(break* N3 (sedentar* or sit*)) OR ((interruptions or distraction or disruption) N2 (sit* OR seat* or sedentar*)) OR ((interrupt* N2 (prolong* or sedentary or sit*)) OR ((replac* OR reallocat*) N2 (prolong* or sedentary or sit*))
S6	(S1 OR S2 OR S3 OR S4 OR S5)
S5	desk N3 (office or job or work)
S4	(sedentar* N2 (behavio?r or position or posture)) OR ("sedentary behavio?r" OR *Sedentary Behavior/) OR (sedentary lifestyle or sedentary behavior or inactivity) OR (physical inactivity or physically inactive or sedentary)
S3	(desk* N2 (based OR bound) N2 (office OR job or Work*)) OR (sedentar* N2 (lifestyle or behavio?r)) OR (excessive N2 (sit* or sedentar*)) OR (workplace N2 (sedentar* or sit*))
S2	((uninterrupt* or excessive) N2 (sit* or sedent*)) OR ((workplace or office) N2 sit$) OR ((workplace or office) N2 seat$)
S1	(sitting OR "Sitting Position") OR "prolonged sitting" OR (prolong* N2 (sedentar* or sit* or sitting)

ORIGINAL ARTICLE

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Resveratrol Treatment Protects Tendons from Obesity and Insulin Resistance Effects

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SUMMARY

Background. Obesity and Insulin resistance (IR) are conditions that cause metabolic disorders and secondary effects, including musculoskeletal complications making tendon prone to lesions. Antidiabetic drugs presents several side effects, making fundamental the pursue of alternative treatments, to improve patient's quality of life. Resveratrol (RSV) can re-establish insulin sensitivity and regulate glycemic levels. It also presents a range of other biological properties such as: antioxidant, anti-inflammatory, neuroprotective, anti-aging, anti-cancer, prevention of cardiovascular disease, and improvement in mitochondrial function. The study evaluates RSV's effects in the calcaneal tendon in insulin-resistant obese animals. **Methods.** 40 male mice (C5B16) were distributed in 8 groups: Non-obese Control; Non-obese Insulin-treated; Non-obese RSV-treated; Non-obese Insulin and RSV-treated; Obese; Obese insulin-treated; Obese RSV-treated; Obese Insulin and RSV-treated. IR was induced by a hyperlipidemic AIN-93 diet and attested through an insulin tolerance test. RSV was administered daily via gavage (100 mg/kg) for two weeks, starting on the 10th week of life until euthanasia, when tendons were removed for testing. **Results.** Resveratrol treatment demonstrated a protective effect over tendons in obese, insulin-resistant animals, reducing the activity of different MMPs, MMP-2, and MMP-9, and avoiding protein content reduction, maintaining its levels similar to healthy animals. **Conclusions.** Resveratrol treatment reduces the activity of, MMP-2 and MMP-9, and also avoids protein content degradation. Its effects over inflammation could be helpful addressing IR patients, helping not only to control the disease, but also making the tendon lesion treatment more efficient.

KEY WORDS

Diabetes; extracellular matrix; insulin resistance; metalloproteinases; non-collagenous protein.

BACKGROUND

Obesity and insulin resistance (IR) impairs the ability of muscle cells to uptake and store glucose and triglycerides, which generates a high level of glucose and triglycerides in the bloodstream (1). IR leads to decreased sensitivity and responsiveness to insulin, therefore, it plays a significant pathophysiologic role in DM2. DM2 is a metabolic dysfunction, in which the insulin function is impaired,

whether because of the IR or the pancreas beta cells are not producing enough insulin. This chronic disease results in systemic inflammation and high blood glucose levels: hyperglycemia (2). IR is usually present in older adults but has become prevalent at all ages on individuals who are overweight and sedentary, which are also the chief risk factors of DM2. Several studies have demonstrated the association between these diseases and musculoskeletal disorders (2-5).

It is believed that IR impairs inflammatory response and alters different connective tissues constitution, compromising its functions, and damaging structures like tendons (4). Furthermore, hyperglycemia contributes to the formation of advanced glycation end-products (AGE), which modifies tendon physical properties (5). AGEs and oxidative stress mediators alter the organization of the tendons ECM and modifies its thermal stability by directly inducing collagen crosslinks. In addition, the effects of AGEs on cells promote an increase in the activity of transaglutaminase enzymes, contributing to the formation of calcification in diabetic tendons (6).

According to Bedi *et al.*, the risk of tendon lesion recurrence is higher in diabetic patients without glycemic control, showing that proper blood glucose levels can preserve the biologic traits of the tendons and reduce the risks of infection and other complications following tissue repair (4). In that matter, another study showed that muscle work during aquatic excercises improves the consumption of corporal glucose, therby reducing blood glucose levels. Nonetheless, physical exercise was not enough to solve the imbalance that leads to alteration of the tendons structural homogeneity and its biomechanical properties (7).

The calcaneal tendon is one of the most susceptible to lesions and the most prone to spontaneous rupture. Several factors such as age, gender, obesity, insulin resistance, and diabetes can increase the odds of lesion incurrence (8). Animals with IR present impairment in tendon mechanical properties and collagen bundles disorganization (6). The repair process of such structures is characterized by accretion of cicatricial tissue with increased cellularity. These factors affect the tendon attributes, necessary to fulfill its functions (2). Usual treatments against diabetes and IR are based on drugs, but weight gain and hypoglycemia are recurrent side effects. To avoid these complications, several alternative therapies are currently under investigation, including phytotherapy (9). Recent studies demonstrated that resveratrol (RSV), a polyphenolic compound, may be employed in IR treatment due to its properties (9).

RSV is a natural stilbene polyphenol found especially in grapes and red wine. It presents anti-inflammatory, anti-diabetic, and anti-tumoral properties, also able to help against neurodegenerative diseases (10). This substance may have the capacity to assist in the repair of tendon lesions caused by IR, since it might aid in diabetes and IR treatment – risk factors that impairs tendon recovery – and also act against the inflammatory process.

Tendon repair and mechanical properties are altered in IR and patients also present articular limitations due to tendon stiffness. It is common for IR patients to develop foot ulcers, resulting from pathological changes in nerves and tendons. The etiology of this ulcer is a combination of neurological degeneration and changes in the tendon structure. Although not fatal, it is a debilitating condition that may result in amputation as a form of treatment. Thus, the prevention or even the efficient treatment of tendon injuries could be the best way to prevent the occurrence of ulcers (11). The longevity of these patients has been increasing, but also has the incidence of tendon related problems, reflecting the urgency of new therapies (12).

MATERIALS AND METHODS

Animal care was in accordance with the Basic principles and recommendations in clinical and field Science Research (13) and is consistent with the ethical principles of animal experimentation adopted by the Brazilian College of Animal Experimentation (COBEA). The present study was approved by the Committee on Ethics in Animal Experiment (CEEA) of the State University of Campinas – UNICAMP (Protocol nº 3502-1).

Animals and experimental groups

Male 4-week-old mice from the C57BL/6 line were used. The animals were obtained from the Multidisciplinary Center for Biological Research (CEMIB) at UNICAMP and kept at 24 ºC, with a daily lighting period of 12h, with water and food ad libitum in the vivarium of the Pharmacology Department of FCM/UNICAMP. A total of 40 young adult male mice (C57Bl6) were distributed in 8 groups:

- non-obese control group (untreated);
- non-obese Insulin-treated group;
- non-obese Resveratrol (RSV) treated group;
- non-obese Insulin + RSV group;
- obese group;
- obese Insulin-treated group;
- obese Resveratrol (RSV) -treated group;
- obese Insulin + RSV group.

After the appropriate treatments, the animals suffered euthanasia through the deepening of anesthesia with isoflurane followed by cervical dislocation. The calcaneal tendons were removed for further analysis.

Inducing obesity with hyperlipidic diet

Obesity was induced by replacing the standard mice feed (AIN-93) with a high-fat diet of the Pragsoluções brand, for 12 weeks. The lean groups received a standard commercial diet (10% lipid, 70% carbohydrate and 20% protein) from NUVILAB (14). Both diets and water were offered *ad libitum*.

Resveratrol treatment

Obese and lean mice were treated daily with resveratrol (100 mg/kg) per gavage, for two weeks (15). The treatment started concomitantly with the first immunization (10th week), ending after the last sensitization and challenge with ovalbumin (12th week).

Insulin tolerance test (ITT)

The animals were kept at fast for 6 hours and their blood was collected through the caudal vein. The blood glucose level was assessed using a glucometer (Accu-Chek Performa, Roche Diagnostics).

Animals received, after fasting, an intraperitoneal injection of insulin (1 IU/kg). Blood was collected from the tail at 0, 5, 10, 15, 20, 25 and 30 min, to measure glucose levels. The rate of glucose decay was then calculated using the linear regression curve of the Neperian logarithm of glucose versus time. This value was assumed as the glucose decay constant after ITT and expressed as a percentage per minute (16).

Insulin administration and euthanasia

Animals were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg). Then the abdominal cavity was opened and the animals received an intravenous injection of insulin (Humulin regular; 1 UI per animal in the inferior vena cava). After 5 minutes, the animals were euthanized by cervical displacement and the calcaneal tendons removed for analysis.

Extraction procedures

Tendons were cut into small pieces and homogenized with extraction buffer (Tris-HCl 50 mM, pH 7.4, NaCl 0.2 M, Triton X-100 0.1%, $CaCl_2$ 10 mM and protease inhibitor 10 µl/ml). For every 30 mg of tissue, 100 µl of buffer was used. Proteins' extraction took place in ice, under stirring, for 2 hours, after which the material was centrifuged for 20 minutes at 4000 rpm, under refrigeration. After supernatant removal, the precipitate was resuspended with 1/3 of the initial volume, heated for 5 minutes at 60°, and centrifuged again. The supernatants of both steps were mixed (17).

Non-collagenous proteins quantification

Non-collagenous proteins (NCP) were quantified following the Bradford method (18) using Coomassie Brilliant Blue G-250. Bovine serum albumin (BSA) was used as standard and readings were performed using an HP 8452 A Diode Array spectrophotometer at 595 nm.

Zymography

For the electrophoresis following the extraction, researchers used a 10% polyacrylamide gel containing 2 mg/ml gelatin and applied 20 µg of protein (15). After the run, gels were placed in incubation buffer for 24 h (Tris-HCl 50 mM pH 8.4, $CaCl_2$ 5 mM, and $ZnCl_2$ 1 µM). To visualize the bands, the gels were stained with Coomassie Brilliant Blue R-250 and destained using methanol 30% and acetic acid 10% solution in water.

The gels obtained were imaged and band densitometry was measured in pixels using Li-Cor Image Studio Lite 5.2.5.

Biomechanical analysis

Tendon biomechanics were analyzed using a texturometer TA.XT plus, from Stable Micro Systems. Five tendons from each experimental group were measured in their three dimensions using a digital caliper and the transversal section of the tendon (A) was calculated.

During the test, both the ends of the tendons are fixed in the machine, which applies an increasing load at a constant rate of 20 mm/min until tendon rupture. The force necessary to rupture the tendon was designated as failure load (F). With this parameter and the tendon displacement, it was possible to calculate the Stress in MPa ($\sigma = F/A$); the strain in % (ε = displacement/initial length), and the Young Modulus ($E = \sigma/\varepsilon$).

Statistical analysis

All results are here expressed as mean ± standard deviation. Data from different experimental groups were analyzed by analysis of variance (oneway ANOVA) followed by the Tukey test. The level of significance was $p < 0.05$. The analysis were carried out in GraphPad Prism® (GraphPad Software, La Jolla, CA, USA).

RESULTS

Data obtained through zymography can be observed in **figure 1**. The zymogram revealed the presence of metalloproteinase-2 and -9 (MMP-2 and MMP-9). The densitometries are plot in **figure 2**. It is possible to observe that obese

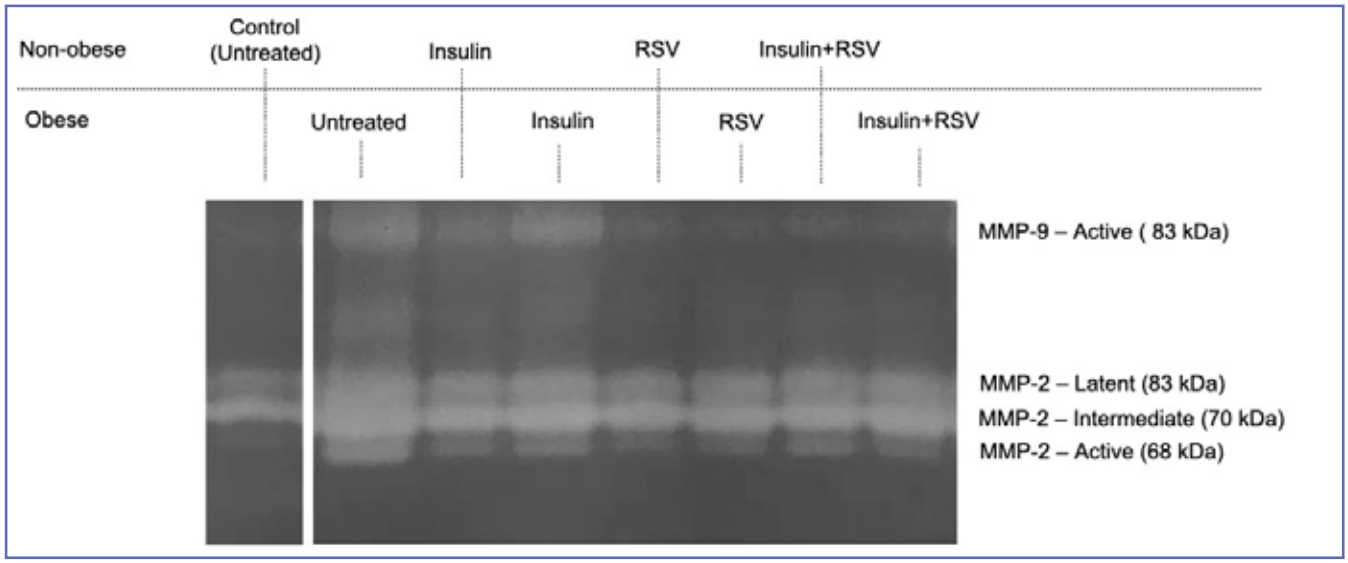

Figure 1. Zymography of the different groups revealing the presence of different MMPs.

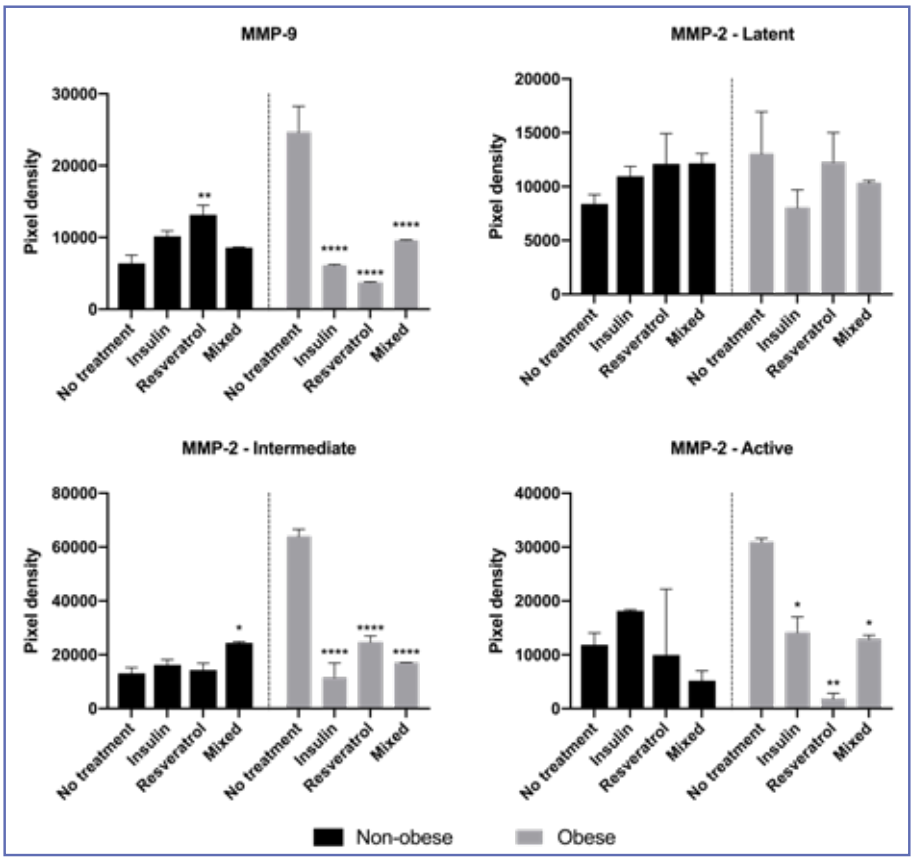

Figure 2. Band densitometry obtained from zymography of MMPs - 2 and - 9.

The * means the treatment caused a significative difference compared to the untreated group inside the same set (obese/non-obese). *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001.

animals, without any kind of treatment, presented the highest amount of most MMPs found. Insulin and RSV were both able to reduce the concentration of MMP-9 and the intermediate and active isoforms of MMP-2. Resveratrol was particularly able to reduce the concentration of the active MMP-2.

In **figure 3** we can see that obesity by itself caused a reduction in the protein content compared to the control group. Interestingly, any treatment containing insulin (even on non-obese animals) caused a reduction in the protein content compared to the control group (**figure 3**). Nonetheless, animals receiving only RSV (both obese and otherwise) presented no reduction in the protein content.

The biomechanical results are plotted in **figure 4**. As it can be observed, the strength necessary to cause tendon rupture (Stress) was much lower in obese animals, but insulin was able to counter the obesity effect. The Young Modulus followed the same pattern as the Stress. No difference was observed in any group regarding tendon Strain.

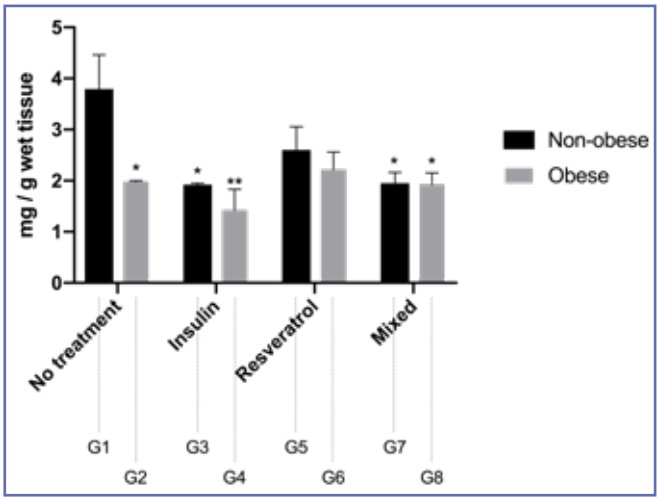

Figure 3. The concentrations of non-collagenous proteins (NCP).
Asterisk designates statistical difference from non-obese and untreated animals. *p < 0.05; **p < 0.01.

It was observed that the obese untreated animals present higher quantities of MMPs since obesity establishes a chronic tendon lesion (2) which will need intense matrix degradation to repair. The obese group treated with resveratrol had a lower amount of active MMP-2 compared to the obese group treated with insulin. Samples with lower MMPs concentration are probably undergoing less degradative and inflammatory processes, revealing RSV anti-inflammatory efficiency (10).

Injured tendons present reduction in the concentration of NCPs (11). Obesity alone caused a significant decrease, which was also detected in non-obese animals that received insulin (associated or not with RSV). Alternatively, the mice treated only with RSV (obese or otherwise) did not present any reduction in the concentration of NCP. When comparing non-obese animals with the use of insulin and without, it is possible to notice that insulin alone, just like obesity, cause a reduction in the concentration of NPC in the tendon. RSV

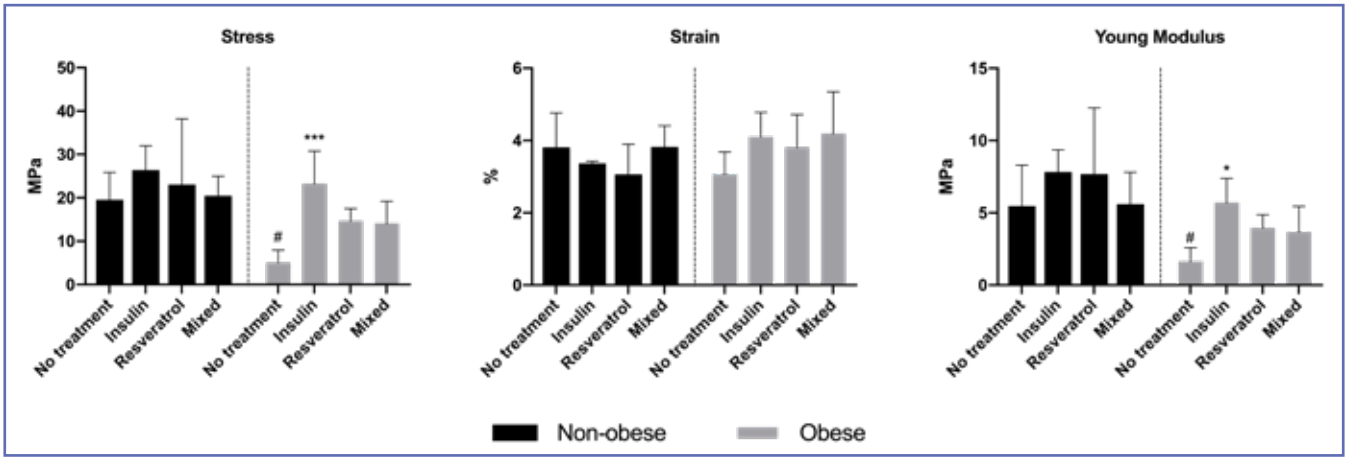

Figure 4. Stress and Strain values of the evaluated tendons. No significative difference could be observed in the strain of the different groups. Obesity alone decreased the tendon strength when compared to all non-obese animals (#). Insulin treatment made the tendon as resistant as the non-obese animals' tendons.
The * mean the value is different from obese untreated animals. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001.

DISCUSSION

In this study, the connection between the obese insulin-resistant mouse and changes in calcaneal tendon was explored. Obese, with or without treatment, have alterations in MMP-2 and MMP-9 isoforms activity which can cause modification in molecular levels in tendons. Interestingly, the activity of MMP-9 increases in the tendons of obese mice in comparison with lean mice, while treatment with resveratrol was able to decrease it. It was noticed that RSV acts reducing the activity of important MMPs in the tendons of insulin-resistant obese animals.

on the other hand demonstrated a protective effect, preserving the content of this component.

IR may cause complications in tissues rich in collagen due to non-enzymatic glycosylation – reduction of sugars in the proteins – which generates structural changes in collagen, resulting in biomechanical damage (7). In the biomechanical tests performed, the parameters analyzed were: stress, which corresponds to the strength needed to cause the tendon rupture; strain, refers to how much that the tendon could be stretched before the rupture; finally, The Young Modulus, analyzes whether the tendon is elastic or if its structure is rigid. A high-

er Stress means the tension resistance is better preserved due to the insulin treatment of obese animals (19). When it comes to strain, no significant difference was observed between the groups. The results observed from The Young Modulus shows that insulin treatment is more efficient to maintain the tendon's elasticity. Obesity, as expected, was able to decrease the tendon strength, and Young Modulus; which is also related to the fact that IR patients are more susceptible to tendon lesions and have their biomechanical properties compromised (19). Other studies also demonstrated that tendons of IR animals are thickened, presents decreased Young's modulus and tensile stress, in addition to a reduced resistance to maximum load with a lower peak failure force (6, 11).

Biomechanical results demonstrated that insulin treatment improved tendon properties, while resveratrol did not bring significant changes. The obese animals receiving the combined treatment showed no improvement compared to the control. These data suggest a possible interaction between insulin and RSV that led to the reduction of insulin positive effects. It is possible that RSV caused an insulin reduction, thus reverting its positive effects over tendon biomechanics (20). According to Zhu, even though RSV regular consumption improves glucose homeostasis and reverses insulin resistance, the results available in the literature are inconsistent, possibly due to a lack of standardization in the doses used (21).

Due to its biological properties, RSV plays a fundamental role in tendon healing on IR and DM2 patients. The anti-diabetic effects can increase the activity and number of GLUT – involved in glucose transportation, protects pancreatic cells from oxidative stress and reverses insulin resistance by reducing body fat mass (3). Also, RSV enhanced the production of matrix components, such as collagen types I and III, whereas it inhibited gene products involved in inflammation and apoptosis (22). In this context RSV effects over inflammation and diabetes could be helpful addressing DM2 patients, helping not only to control the disease but also to make the tendon lesion treatment more efficient.

According to Ackerman *et al.* (2017) (2) a diabetic tendon will have an increase in thickness and number of ECM cell numbers, since DM2 alters tendon homeostasis and impairs the healing process. In an attempt of recovering, there is an overexpression of MMPs genes. During repair three phases can be observed: inflammatory, proliferative, and remodeling phase. During the inflammatory phase, there is intense ECM degradation, explaining the presence of MMP-2 and -9. Throughout the remodeling phase, collagen III (synthesized during proliferative phase) is replaced by collagen I, with the presence of MMP-2 (23).

A study using human tenocyte culture model, with IR induced by TNF, demonstrated the process of tenocytes homeostasis in diabetic individuals with tendinopathy. It was observed that tenocytes from injured tendons produce a greater amount of type III collagen, when compared to an intact tendon. In addition, tendon homeostasis is modulated by MMPs and TIMPs (tissue inhibitors of MMPs), which regulates the production of collagen and non-collagen matrix by tenocytes. Thus, it was shown that IR positively regulates the expression of MMP-9, highlighted here, and MMP-13 (24). Another study demonstrated that RSV, as an antioxidant, can decrease MMP-9 expression not only by suppressing MMP-9 production but also by augmenting tissue inhibitors of metalloproteinases (TIMPs) production (25).

The mechanism through which RSV acts over obese tendons is still unclear and needs further clarification. Also, future studies evaluating different RSV doses over animal treatment are necessary.

CONCLUSIONS

Resveratrol treatment demonstrated a protective effect over tendons in obese insulin-resistant animals, reducing the activity of different MMPs, MMP-2, and MMP-9, and avoiding protein content reduction. In addition to that, it was noticed that tendons from obese mice had alterations in the profile of the tested MMPs, and strain and elasticity compromised. Overall, this study showed that the response of MMP-2 and MMP-9 are high in obese, yet RSV treatment was able to reduce it. Nonetheless, insulin and RSV combination reduced insulin efficiency over the biomechanical properties tested.

In order to propose an alternative treatment that could benefit the patients, more studies are necessary to elucidate how RSVs antidiabetic properties act. It's also important to determine efficient doses and the best means of administration.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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Does the Antimalarial Drug Quinine Contribute to Muscle Fibro/Adipogenic Progenitor Fibrogenesis?

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SUMMARY

Background. In many resource-limited countries, children are routinely given intramuscular (IM) injections of medication to treat pain or illness. IM injections are suspected in the development of gluteal fibrosis (GF) in children, a condition that limits normal hip motion and function. The mechanism by which GF develops is not understood. Our study examines a commonly IM administered antimalarial in Uganda, quinine, to assess its ability to cause fibrogenesis of muscle fibro/adipogenic progenitors (FAPs), the cellular source of muscle fibrosis. The purpose was to evaluate if quinine itself could alter muscle cells and cause fibrosis.

Methods. FAPs were isolated from skeletal muscle in wildtype C57BL/6 mouse with florescence-activated cell sorting (FACS). After sorting, the FAPs were cultured in standard media until they reached 80% confluence. The cells were then cultured in a series of quinine concentrations. Fibrogenesis of FAPs was determined with RT-qPCR of fibrogenic markers.

Results. The RT-PCR results showed increased αSMA, vimentin and collagen-1 expression in quinine exposed cells. At lower quinine dosages, expression increased in stemness markers; Sox2, cMyc, Oct-4, and Nanog. Conversely, at higher dosages quinine decreased stemness marker expression. Lastly, quinine increased TGFβ1 and Ki67 and decreased BMP7.

Conclusions. Our findings suggest that quinine induces FAP fibrogenesis and reduces FAP stemness. Further study is needed, but if confirmed that Quinine induces fibrogenesis, limiting the use of Quinine may be an effective public health intervention to reduce cases of gluteal fibrosis and resultant childhood disability.

KEY WORDS

Fibro/adipogenic progenitors; gluteal fibrosis; intramuscular injections; TGFβ1; quinine.

BACKGROUND

In many resource-limited countries, children are routinely given intramuscular (IM) injections for the delivery of medication to treat febrile illnesses/communicable diseases (1-10). IM injections are thought to be the underlying cause of gluteal fibrosis (GF) – a fibrotic infiltration of the gluteal muscles that results in a loss of flexion and adduction of the hips (1, 2, 4-6). The resultant abduction contracture of the hips impacts children's ability to sit and squat normally. Additionally, this condition limits their ability to attend school and carry out normal daily activities such as using the toilet and performing chores. In certain regions of Uganda, such as the District of Kumi, there are many children with this condition – with as many as 30% of visits to a musculoskeletal clinic and 40% of outreach visits being for an injection related injury (1, 4). In this particular region of Eastern Uganda, some of the local medical providers suspect that quinine is the culprit in their high number of GF cases. Studies have indicated that over 80% of children affected by GF had received multiple IM quinine injections prior to their post injection complications

(1, 4). However, the exact mechanism of how GF develops through the administration of IM injections is still not understood. As such, we have sought to investigate the basic science aspects of GF in order to better understand this condition and impact future prevention and treatment efforts.

Our study examines a commonly IM administered antimalarial in Uganda, quinine, to assess its ability to cause fibrotic changes in skeletal muscle cells (1, 2, 4, 5, 7-9, 11, 12). We studied quinine in particular because, through the senior author's (CS) work on GF in Uganda, quinine has been anecdotally implicated as the main agent in inducing GF. Should it induce changes at the cellular level, this can further our understanding of the mechanism by which GF develops in this population. Using a cell model, we exposed fibrogenic/adipogenic progenitor (FAPs) cells in culture media conditions to different concentrations of quinine in order to determine its effects on FAP fibrogenic differentiation and filament production (4, 11, 13-16, 18). This investigation allowed us to begin to uncover the microscopic events that potentially lead to macroscopic muscle fibrosis and loss of function (4).

FAP's are integral regulators of satellite cells (skeletal muscle stem cells) in acute injury states (14, 15). During times of cellular stress, FAP's assimilate environmental conditions into signals that regulate satellite cell activities (14, 15). Interestingly, the abnormal activity of FAP's has been linked to the development of pathologic states within skeletal muscle such as impaired muscle regeneration and fibrosis (14, 15). We anticipate that quinine will significantly alter FAP gene expression leading to the abnormal healing of skeletal muscle and ultimately resulting in fibrosis.

MATERIALS AND METHODS

FAP sorting and isolation

In order to study the effects of quinine on FAP differentiation and filament production, we isolated the cell population of $CD31^-$, $CD45^-$, $Integrin\alpha7^-$, $Sca\text{-}1^+$ and $PDGFR\alpha^+$ FAPs from skeletal muscle of 3-month-old male wildtype C57BL/6 mice (Jackson laboratory Corp., Sacramento, CA). After C57BL/6 mice were sacrificed, skeletal muscles were harvested and minced into 1 mm chunks with sterile scissors in cell culture hood. We then incubated the minced skeletal muscle with 0.2% Collagenase II for 90 minutes in a 37 °C sterile water bath. Next, forty milliliter washing buffer (F/10, 10% Horse Serum, 1 × HEPES) was added into the mixture and then centrifuged at 1500 rpm for 5 minutes at room temperature. The supernatant was then transferred to a new 50 mL centrifuge tube and set aside. The remnants were then rinsed with washing buffer and spun down for 5 minutes at 1500 rpm. The supernatant then collected and combined with the supernatant from the first round of centrifuging. Then D2 solution (0.06% Collagenase II, 0.15% Dispase with washing buffer) was added to the combined supernatant and incubated at 37 °C for 30 minutes. This solution was then passed through a 70 µm cell strainer (VWR International) and then a 40 µm cell strainer (VWR International). The filtered cells were collected and washed with 40 mL FACS buffer (2.5% FBS, 20 mM EDTA, 1 × PBS) and centrifuged at 1500 rpm for 5 minutes. The filtered cell supernatant was discarded, and the cell pellets were re-suspended with 500 µL of FACS buffer. The cell solution was then incubated with anti-CD31-FITC (BD bioscience), anti-CD45-FITIC (BD bioscience), anti-integrin α7-APC (R&D systems) PE-Cy7-Sca1 (BD bioscience, Clone. E13-161.7) and anti-cd140a(PDGFRα)-BV421 (BD bioscience, Clone APA5) for 30 minutes before being sorted with FACSAria™ II sorter (BD biosciences). FAPs were collected as the $CD31^-/CD45^-/Integrin\alpha7^-/Sca\text{-}1^+/PDGFR\alpha^+$ cell population. The SFVAMC Institutional Animal Care and Use Committee (IACUC) approved all procedures and handling of the animals.

Cell culture

24 well plates were coated with 1% Matrigel in DMEM for 1 hour at room temperature before cell seeding. After sorting, the FAPs were seeded into Matrigel pre-coated 24 well plates and at a density of 5,000 cells per well. Cells were cultured for one week with standard cell culture medium (Ham's F-10, 10% fetal bovine serum, 10 ng/mL βFGF and 1% antibiotic-antimycotic solution, Thermo Fisher Scientific, MA USA). Then one 24 well plate of cells was set aside to be used as our negative control. The rest of the cells were then cultured in 0.5 mL of standard culture media with the addition of a fixed concentration of serially diluted quinine for two weeks before processing for RT-qPCR. Using a logarithmic serial dilution, we exposed the cells to quinine's injection dosage concentration (IDC), the concentration that quinine is injected into the patient, and successive diluted concentration until we ended at one thousand times below the IDC (IDC, IDC × 0.1, IDC × 0.01, IDC × 0.001) (1, 3, 4, 8, 9, 12, 17, 18). Lastly, our negative control group was exposed to 0.5 mL of standard culture media for 2 weeks. We also tested the effect of quinine on the cells at its peak serum concentration (SC), the highest concentration of quinine found in the blood stream after administration (12, 17, 18). Six biological replicates per condition were run.

Real Time qPCR

Total RNA was extracted from the cells on day 14 using a Trizol reagent (Applied Biosystems) according to the

manufacturer's instructions. Real-time qPCR was run with Fast SYBR Green Master Mix (Applied Biosystems) on a Viia7 Real Time Detection System (Applied Biosystems). This test was performed to quantify the expression of TGFβ-1, BMP7, and Ki67, the fibrotic markers collagen-1, vimentin, and αSMA expression and the stemness markers Nanog, Sox2, Oct-4 and cMyc (13, 14). The ΔΔCt method was used to compare gene expression between the different quinine concentrations using GAPDH as the housekeeping gene.

Statistical analysis

For all analyses, ANOVA was used to assess for significance. Significance was defined as $p < 0.05$. Data are presented as mean ± standard error of measurement.

Ethical standards

Our study meets the ethical standards of Muscle Ligaments and Tendons Journal. From our cell line development and authentication to the reporting of our molecular results obtained through basic science practices (19, 20).

RESULTS

Quinine induces fibrogenesis

The RT-PCR results showed an increase in αSMA expression (+ 3.02-fold average, $p < .05$, SE = .46) and vimentin (+ 81.89-fold average, $p < .05$, SE = 3.89) across all quinine concentrations – except IDC – at the end of the 14 days compared to the control (Graph 1). Similarly, there was a significant average fold increase in collagen-1 (+ 16.55-fold average, $p < .05$, SE = 1.22) across all quinine concentrations (**figure 1**).

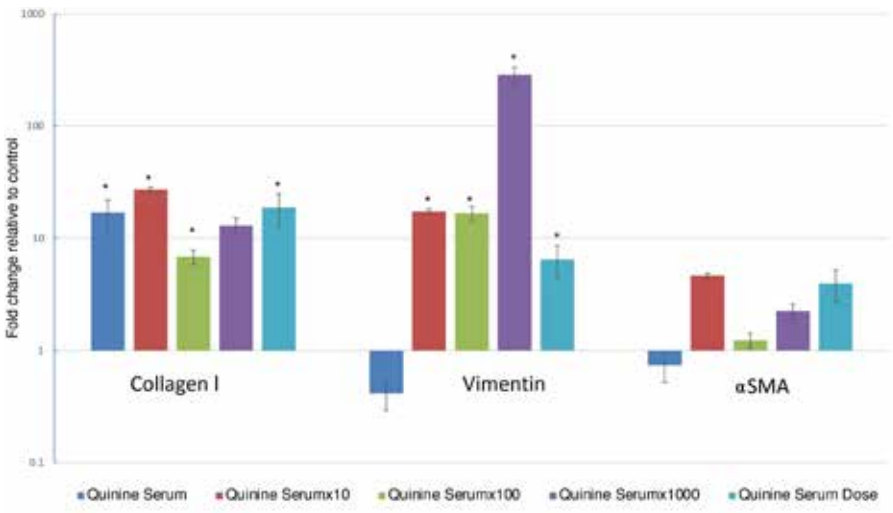

Figure 1. Quinine increases fibrogenesis marker expression significantly in FAPs.

Quinine reduces stemness

Furthermore, at lower dosages quinine increased stemness markers in Sox2 (+ 3.57-fold, $p < .05$, SE = .3528), cMyc (+ 7.38-fold, $p < .05$, SE = .712), Oct-4 (+ 3.58-fold), and Nanog (+ 11.58-fold) (Graph 2). Conversely, at higher dosages quinine decreased stemness markers compared to the control after 14 days: Sox2 (+ .51-fold), cMyc (+ .16-fold), Oct-4 (+ .09-fold, $p < .05$, SE = .003), Nanog (+ .06-fold, $p < .05$) (**figure 2**).

Quinine induced TGFβ1 and Ki67 but decreased BMP7 expression

Lastly, quinine increases TGFβ1 across all quinine concentrations – except at IDC × 0.01 (+ 9.06-fold average, $p < .05$, SE = 1.48). and decreases BMP7 significantly at IDC × 0.001 (+ .12-fold, $p < .05$, SE = .001). Quinine also increases Ki67 at IDC × 0.1 (+ 17.66-fold, $p < .05$, SE = .5299) (**figure 3**).

DISCUSSION

Gluteal fibrosis (GF) is unfortunately quite common in certain areas of Uganda and many other countries and has a significant negative impact on the hip function in affected children and adults. As many as 28% of visits to a musculoskeletal clinic in the Kumi District of Uganda are related to injection-induced GF (2). Various studies have indicated that over 80% of children affected by GF had received multiple IM quinine injections prior to the development of the fibrosis (1, 4). Few studies have investigated the microscopic phenomena that leads to the development of this disabling condition. Furthermore, no studies have been conducted investigating the role that antimalarial drugs, such as quinine, play in gluteal fibrosis' development. As such, we investigated a commonly IM administered antimalarial in Uganda, quinine, and found that it plays a key role in causing fibrotic changes to skeletal muscle

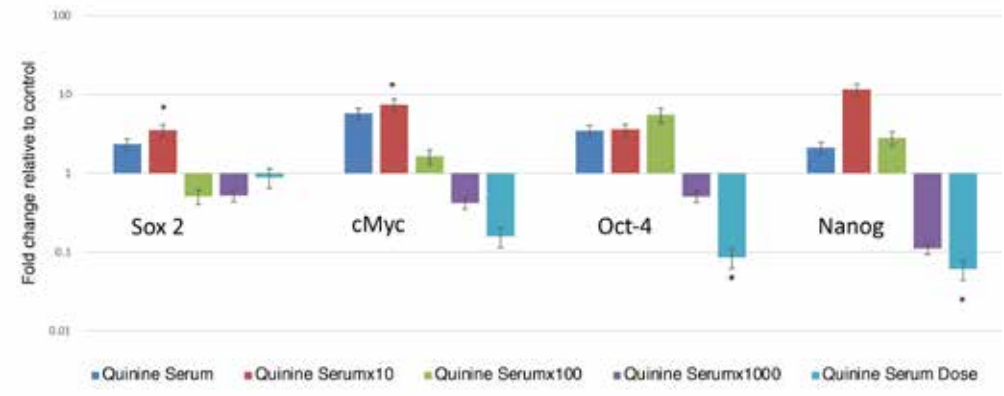

Figure 2. Quinine increases stemness markers at lower doses but decreases those markers at higher doses in FAPs.

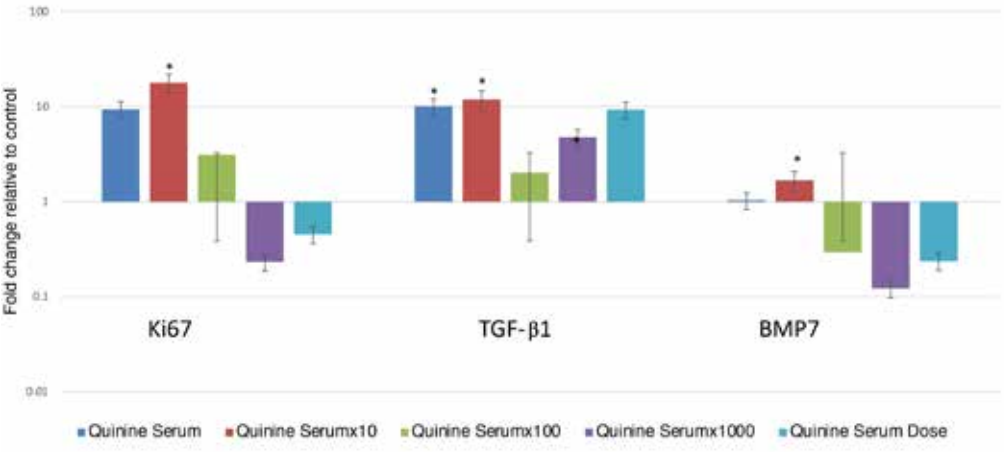

Figure 3. Quinine increases TGFβ1 and Ki67 expression but decreases BMP7 expression in FAPs.

cells (1, 2, 4, 5, 7-9, 11, 12). Quinine ultimately promotes skeletal muscle fibrotic changes by inducing FAP fibrogenesis and reducing FAP stemness via the TGF-β1/BMP7 pathway. Furthermore, our findings suggest that quinine's microscopic effects on human myocytes potentially account for the development of macroscopic gluteal contractures (4-6, 11, 13-16).

Despite decades of studies reporting the presence of, and treatment for GF, little information is available about the cellular mechanisms by which GF develops. Recently, the Transforming Growth Factor-β (TGF-β) signaling pathway has been under investigation in relation to its effects on skeletal muscle FAP's. This pathway has been found to play a pivotal role in the development of pathologic fibrosis in various tissues and organs (13-15). Specifically, studies have shown that TGF-β is associated with increased fibrosis of injured skeletal muscle by preventing FAP apoptosis (13-15). Furthermore, FAP's have been identified as integral cell precursors for skeletal muscle adipocyte infiltration and fibrosis (14, 15). FAP's are integral regulators of satellite cells (skeletal muscle stem cells) in acute injury states. They have been linked to the development of pathologic states within skeletal muscle such as impaired regeneration, fatty infiltration and fibrosis (14, 15). We believe that the TGF-β pathway is the key regulator of the fibrotic changes seen in skeletal muscle during pathologic injury states, such as GF, due to its role in regulating FAP apoptosis.

The Transforming Growth Factor-β (TGF-β) signaling pathway has been identified as a superfamily of polypeptide ligands (14, 15). Components of the TGF-β superfamily include: TGF-β-like ligands, bone morphogenetic proteins (BMPs) and activins. The TGF-β superfamily of ligands is incredibly important for the proliferation, differentiation and development of skeletal muscle cells (14, 15). TGF-β's cellular effects depend on the duration of its activation. In an acute setting, TGF-β has been found to assist in skeletal muscle repair (14, 15). However, when skeletal muscle is chronically exposed to TGF-β, pathologic fibrosis develops (14, 15). Similarly, our results demonstrate that chronic activation of the TGF-β pathway expression results in pathologic muscle fibrosis seen in FAPs exposed to quinine. Throughout almost every quinine exposed condition, TGF-β1 was found to be upregulated and BMP7 downregulated. These results along with the supporting literature on the TGF-β pathway's role as a key regulator in skeletal muscle fibrosis leads us to believe that quinine activates this pathway, which in turn leads to pathologic muscle fibrosis.

RT-qPCR confirmed that quinine induces FAP phenotypic changes and increased fibrogenesis as determined by the up-regulation of collagen-1, vimentin, and αSMA expression

(11, 13, 14, 16). Furthermore, at high dosages quinine downregulates Nanog, Sox2, Oct-4 and cMyc stemness markers, but at lower dosages quinine increases stemness markers in FAPs (16). These results highlight that at lower concentrations quinine has a therapeutic effect on cells by increasing stemness, at higher concentrations, quinine can be especially determinantal to the cells. We postulate that quinine's ability to decrease stemness markers and increase fibrogenic markers is due to the upregulation of TGF-β1 and the downregulation of BMP7 based on our RT-qPCR findings (14, 15).

Because this is a pilot study, our lab is aware that there are limitations to our initial investigation of this topic. Limitations include that the cells we used were mouse cells, not human muscle cells and therefore they may respond differently than human cells do. Further, this study was not conducted *in vivo*, and our samples lacked exposure to mechanical stress from syringe administration and fluid infiltration which may also be factors in development of gluteal fibrosis.

In an effort to better understand the etiology of GF we desire to repeat these studies with quinine in comparison to other commonly injected medications (1-4, 7). Furthermore, we will explore options for an *in vivo* mouse model. These approaches will allow us to gain a better understanding of this condition and the factors leading to the development of GF. Further studies to explore the effects of the mechanical act of delivering the injection, the potential role of micro-abscess formation after sub-sterile preparation and if similar results are seen with other commonly injected medications would be beneficial, as the development of GF may be multi-factorial in nature.

CONCLUSIONS

Overall, our findings suggest that quinine induces FAP fibrogenesis and reduces FAP stemness. Furthermore, we postulate the TGF-β1/BMP7 pathway is the underlying mechanism for quinine-induced muscle fibrosis. In a clinical sense, these findings suggest that quinine contributes to the fibrosis of human myocytes by increasing collagen-1, vimentin and αSMA production in FAPs while also causing FAP's to differentiate into their fibrogenic counterparts (4, 11, 13, 15, 16). The microscopic phenomena we found lead us to believe that quinine can potentially contribute to the development of macroscopic gluteal contractures through the same mechanisms (4, 5, 6, 11, 13-16). However, these findings do not prove that quinine is solely responsible for gluteal contractures but suggest that quinine may contribute.

Future work is needed to demonstrate how quinine interacts with TGF-β1 and BMP7 pathways in FAPs.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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Mechanical Properties of Muscles and Tendons in Asymptomatic Individuals with Generalized Joint Hypermobility

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SUMMARY

Background. The purpose of the present study was to investigate changes in mechanical properties of muscles and tendons in asymptomatic individuals with generalized joint hypermobility (GJH).

Methods. This cross-sectional study was conducted in 126 participants aged 19-40 years. The Beighton score was used to determine whether the participants had GJH. An experienced physiotherapist screened all participants using the Beighton score to inquire about the presence of GJH. At the end of the clinical evaluations, 36 asymptomatic participants with GJH (age, 24.6 ± 6.1 years) and 34 age- and sex-matched controls (age, 24.6 ± 6.8 years) were included in the present study. The oscillation frequency (indicator of tone), dynamic stiffness (indicator of stiffness), and logarithmic decrement (related to elasticity) of the medial and lateral gastrocnemius, biceps brachii, and brachioradialis muscles, and the Achilles and patellar tendons were measured with a portable myotonometer (MyotonPRO, Myoton AS, Tallinn, Estonia).

Results. The oscillation frequency, dynamic stiffness, and logarithmic decrement of the biceps brachii, brachioradialis, and medial and lateral gastrocnemius muscles were similar in GJH and control groups ($p > 0.05$). In addition, there was no significant difference between groups in terms of the oscillation frequency, dynamic stiffness, and logarithmic decrement of the Achilles and patellar tendons ($p > 0.05$).

Conclusions. The elasticity, stiffness, and/or tone of the biceps brachii, brachioradialis, and medial and lateral gastrocnemius muscles were similar in individuals with and without GJH. The results obtained suggest that the mechanical properties of muscles and tendons are not associated with GJH.

KEY WORDS

Generalized joint hypermobility; muscle; tendon; stiffness; elasticity.

BACKGROUND

Generalized joint hypermobility (GJH) describes a condition involving joints that actively or passively demonstrate excessive movement beyond the expected or normal physiological range (1). The prevalence of the GJH is reported to vary between 12.5% and 39% in the general population (2, 3). GJH is associated with many factors such as sex, age, and race. It is well known that females or younger age groups have a higher hypermobility compared to males or older age groups (4). On the other hand, GJH is related to musculoskeletal pathological conditions such as back pain, sprains, dislocations, or balance problems (2). There are various explanations about high prevalence of musculoskeletal pathological conditions in individuals with GJH. It is

suggested that higher tissue elasticity may cause a decrease in passive or active joint stability (5). The decrease in joint stability may alter force production and transmission related to musculotendinous structures (6, 7). These changes in joint stability or transmission related to musculotendinous structures may result in an overload on the joint or related structures in the long run.

There are some factors associated with joint hypermobility such as hormonal imbalance, genes encoding collagen, or environmental factors (8-10). These factors have the potential to cause changes in mechanical properties of soft tissues, such as muscles and tendons (11-13). The changes in mechanical properties of soft tissues may be a factor in excessive movements involving joints in individuals with GJH. Mechanical properties such as tone, stiffness, and elasticity of muscles and tendons are important components of joint stability or joint controls (14, 15). Changes in mechanical properties of muscles and tendons may be an important factor causing the joint excessive movement in individuals with GJH. There exist very few studies presenting the changes in mechanical properties of muscle and tendon structures in symptomatic patients with the Ehlers-Danlos syndrome (16-18). Mechanical properties of muscles and tendons would be different in asymptomatic individuals with GJH. It is well known that orthopaedic conditions or orthopaedic conditions related pain may change the mechanical properties of muscles and tendons (19-21). In addition, the stiffness of muscles, tendons, or musculotendinous structures has been investigated only in these studies; however, other parameters such as tone or elasticity of muscles and tendons has not been investigated. Identifying a possible change in mechanical properties of muscles and tendons may help practitioners better understand the pathomechanics of increased prevalence of musculoskeletal pathological conditions in asymptomatic individuals with GJH.

There are several methods for the measurement of muscle and/or tendon stiffness, such as myotonometry, elastography, shear-wave elastography, and magnetic resonance. Myotonometry has some advantages over magnetic resonance elastography and shear-wave elastography. For example, the myotonometer is a portable device that allows measurements in different environments. On the other hand, it is relatively low-cost compared to magnetic resonance and/or ultrasonography devices. Measurement made with myotonometry is easily learned and it is simple to apply compared to magnetic resonance elastography and shear-wave elastography. In addition, it has been reported that the reliability and validity of myotonometry are similar to those of magnetic resonance and shear wave elastography (22-25). In addition, while magnetic resonance and shear-wave elastography allow only stiffness measurements in soft tissue, besides measuring stiffness, the myotonometer can also measure other mechanical properties such as tone and elasticity.

The purpose of the present study was to investigate the change in the elasticity, stiffness, and/or tone of muscles and tendons in asymptomatic individuals with GJH. It was hypothesized that (1) muscle and tendon stiffness would be lower in asymptomatic individuals with GJH, and (2) muscle and tendon elasticity would be higher in individuals with GJH compared to controls.

METHODS

Ethics permission

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with 1964 Helsinki declaration and its later amendments or comparable ethical standards. To conduct the study, permission was obtained from the ethics committee of Toros University (Protocol no: 2021-5/58). Prior to the study, oral and written informed consents were provided by all participants.

Sample size calculation

Prior to the study, a power analysis was conducted using a statistical analysis program (IBM Corporation, SPSS Sample Power 3.0 Software Armonk, NY, USA) to identify the minimum required sample size. The minimum required sample size was calculated to be at least 27 participants for each group for the desired power (β) of 80% with an assumed alpha level (α) of 0.05 to detect a minimum clinical difference of 40 N/m muscle stiffness when the average muscle stiffness value in the control group is 257 N/m with a standard deviation of 53 N/m (22).

Individuals

This cross-sectional study was conducted in 126 participants aged 19-40 years, who were students/staff of Alanya Alaaddin Keykubat University and Toros University. The Beighton score, which was reported as a valid and reliable tool for assessing GJH (26, 27), was used to determine whether the participants had GJH. The Beighton score consists of 5 items as follows: 1) the fifth metacarpophalangeal joint passive dorsiflexion score is positive if ≥ 90° (bilateral testing), 2) the thump opposition score is positive if the thump reaches the forearm volar aspect (bilateral testing), 3) the elbow passive hyperextension score is positive if ≥ 10° (bilateral testing), 4) the score of the knee passive hyperextension is positive if ≥ 10° (bilateral testing),

and 5) the spinal hypermobility score is positive if the hand palms rest easily on the floor with straight knees (26, 27). The Beighton score ranges from 0 to 9. A Beighton score of ≥ 5/9 indicates the presence of GJH for adults up to age of 50 years (28). An experienced physiotherapist screened all participants using the Beighton score to inquire about the presence of GJH. At the end of the clinical evaluations, 36 asymptomatic participants with GJH (age, 24.6 ± 6.1 years) and 34 age- and sex-matched controls (age, 24.6 ± 6.8 years) were included in the present study (**figure 1**). Individuals were excluded from the study if they reported any of the following conditions: 1) having a history of a major trauma, lower extremity fracture or surgery, 2) having an upper or lower orthopedic disorder, such as tendinitis, muscle strain/sprain, or ligaments injury, 3) having multiple joint pain for longer than 3 months, 4) having a systemic disease, such as diabetes mellitus, 5) having a neurological or cardiopulmonary disorders, or rheumatic diseases, 6) having a body mass index more than 30 kg/m^2, and 7) having performed any strenuous exercises within 48 h prior to measurements.

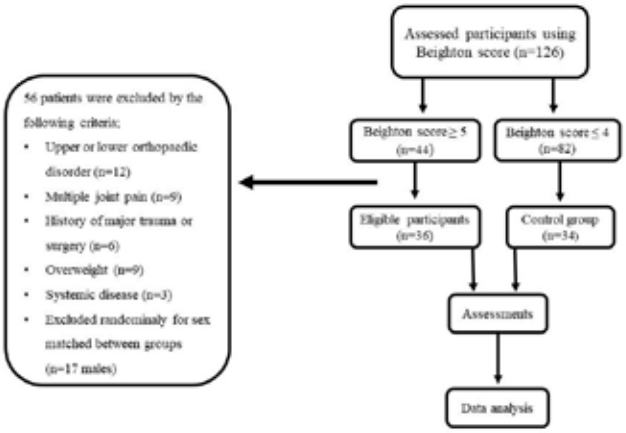

Figure 1. Sample selection fluxogram.

Mechanical properties measurements

Mechanical properties of the medial and lateral gastrocnemius, biceps brachii, and brachioradialis muscles, and the Achilles and patellar tendons were measured with a portable myotonometer (MyotonPRO, Myoton AS, Tallinn, Estonia) (**figure 2**). The MyotonPRO has been reported as a reliable and valid device for measuring mechanical properties of muscles and tendons (22, 23, 29). The MyotonPRO applies a mechanical impulse with a constant mechanical force (up to 0.6 N) and short duration (15 milliseconds) to the target structure. After this mechanical impulse, measuring the mechanical oscillations in the target structure by the device provides the following data: 1) logarithmic decrement, 2) dynamic stiffness (N/m), and 3) oscillation frequency (Hz). Logarithmic decrement shows the elasticity of the target soft tissue. Dynamic stiffness (N/m) gives important information about the resistance capacity of the soft tissue against an external force. Oscillation frequency (Hz) provides information about the tone of the muscle in passive or resting state.

Figure 2. MyotonPRO was used for measurements of the mechanical properties of the selected muscles.

Mechanical properties of the selected tissues were measured by a physiotherapist with a 3-year experience with myotonometric measurements, and who was blinded to the groups. The dominant hand of the individuals was determined by questioning about the hand they used while writing (30), and the dominant leg of the individuals was determined by questioning about the leg they used while kicking the ball (31). Similar to previous studies (22, 32), the mechanical properties of the Achilles tendon were measured on the point 3 cm above the calcaneal tuberosity. Based on previous studies (22, 32), the measurements of the medial and lateral gastrocnemius muscle were performed at a point 70% off of the lower leg length in line with the popliteal crease to the lateral malleolus. The Achilles tendon and medial and lateral gastrocnemius measurements were performed while the individuals were in prone position, the knees at full extension, and the ankle at neutral position. The patellar tendon measurements were performed at midpoint between the inferior pole of the patella and tuberositas tibiae while the individuals were sitting with their legs over the edge of the bed and knees flexed to 90 degrees (33). For the measurements of the biceps brachii muscle, the participants lay in supine position with the wrists

supinated, the elbows at 15° flexion, and the shoulder at neutral position and externally rotated. The measurements of the biceps brachii were performed at midpoint between the acromion and cubital fossa (34). The measurements of the brachioradialis were performed at one-thirds distance from the styloid process to the lateral supracondylar ridge, while the participants lay supine with forearm pronated and elbow extended (35) (**figure 3**). Each test was performed three times, and the average of the three measurements was noted.

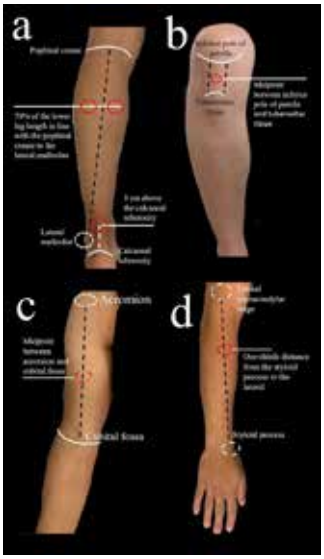

Figure 3. Locations of the myotonometric measurement: (**a**) Achilles tendon, medial and lateral gastrocnemius muscle, (**b**) patellar tendon, (**c**) biceps brachii muscle, (**d**) brachioradialis muscle.

Statistical analyses

Statistical analyses were performed using a statistics software program (SPSS for Windows version 22, IBM Corporation, Armonk, NY, USA). Analytical (Kolmogorov–Smirnov/Shapiro–Wilk's test) and visual methods (histograms, probability plots) were performed to decide whether the parameters were normally distributed. Mean and standard deviation are used to present the normally distributed variables. Student's t-test was used to compare the parameters between the GJH and control groups. A p-value of less than 0.05 was considered to show a statistically significant result.

RESULTS

Both groups had similar age (p = 0.940), height (p = 0.437), weight (p = 0.290), and body mass index (p = 0.700) (**table I**). The oscillation frequency, dynamic stiffness, and logarithmic decrement of the biceps brachii, brachioradialis, and medial and lateral gastrocnemius muscles were similar in both GJH and control groups (p > 0.05). In addition, there was no significant difference between groups in terms of the oscillation frequency, dynamic stiffness, and logarithmic decrement of the Achilles and patellar tendons (p > 0.05) (**table II**).

DISCUSSION

The purpose of the present study was to investigate changes in mechanical properties of muscles and tendons in indi-

Table I. Demographic data of GJH and control groups.

Parameter	Control group (n = 34)	GJH group (n = 36)	P value
Age (year)	24.6 ± 6.8	24.6 ± 6.1	0.940
Height (m)	1.64 ± 0.08	1.66 ± 0.08	0.437
Weight (kg)	58.4 ± 12.2	61.0 ± 15.5	0.290
BMI (kg/m²)	21.5 ± 3.2	21.9 ± 4.3	0.700
Beighton score	1.6 ± 1.0	6.1 ± 1.3	**< 0.001**
Sex			
Male, n (%)	6 (17.6 %)	6 (16.6 %)	
Female, n (%)	28 (82.4 %)	30 (83. 4 %)	
Dominant hand			
Right, n (%)	32 (94.1 %)	35 (97.2 %)	
Left, n (%)	2 (5.9 %)	1 (2.8 %)	
Dominant limb			
Right, n (%)	31 (91.2 %)	35 (97.2 %)	
Left, n (%)	3 (8.8 %)	1 (2.8 %)	

*p < 0.05. Data are presented as mean ± standard deviation.

Table II. Comparisons of the mechanical properties of the assessed tissues between GJH and control groups.

Parameters	Control group (n = 34)	GJH group (n = 36)	P value
Medial gastrocnemius muscle			
Frequency (Hz)	15.0 ± 1.8	14.7 ± 1.6	0.462
Stiffness (N/m)	261.7 ± 44.4	249.1 ± 43.4	0.236
Logarithmic decrement	1.0 ± 0.1	1.0 ± 0.1	0.763
Lateral gastrocnemius muscle			
Frequency (Hz)	15.2 ± 1.9	14.9 ± 2.1	0.486
Stiffness (N/m)	271.5 ± 55.2	262.49.0	0.386
Logarithmic decrement	1.0 ± 0.1	1.0 ± 0.2	0.661
Biceps brachii muscle			
Frequency (Hz)	13.4 ± 1.0	13.1 ± 0.9	0.177
Stiffness (N/m)	199.8 ± 21.1	200.7 ± 21.3	0.851
Logarithmic decrement	1.2 ± 0.2	1.2 ± 0.2	0.977
Brachioradialis muscle			
Frequency (Hz)	15.5 ± 1.0	15.2 ± 0.8	0.100
Stiffness (N/m)	252.1 ± 26.0	244.1 ± 19.0	0.143
Logarithmic decrement	0.9 ± 0.1	1.0 ± 0.1	0.509
Achilles tendon			
Frequency (Hz)	30.8 ± 2.2	30.9 ± 2.0	0.939
Stiffness (N/m)	783.4 ± 66.7	774.7 ± 69.8	0.596
Logarithmic decrement	0.8 ± 0.1	0.8 ± 0.1	0.311
Patellar tendon			
Frequency (Hz)	22.0 ± 3.2	21.8 ± 3.5	0.888
Stiffness (N/m)	623.1 ± 143.7	640.5 ± 136.4	0.606
Logarithmic decrement	0.9 ± 0.1	0.9 ± 0.2	0.941

Data are presented as mean ± standard deviation.

viduals with GJH. It was hypothesized that the elasticity of muscles and tendons would be higher, and their stiffness would be lower in individuals with GJH compared to controls. The hypothesis was based on the idea that mutation in the genes encoding collagen and deficiency or hormonal imbalance in individuals with hypermobility-related disorders (9, 10) may cause a collagen deficiency in musculoskeletal structures, and it may cause a change in mechanical properties of muscles and tendons. Changes in mechanical properties of muscles and tendons may be a factor for the excessive movements involved in joints in individuals with GJH, because mechanical properties of muscles and tendons are important components of joint stability or joint controls (14, 15). In addition, potential changes in mechanical properties of muscles and tendons may be related to the increase in the prevalence of musculoskeletal pathological conditions in individuals with GJH (5-7). Different from the hypothesis, it was found that elasticity, stiffness, and tone of the Achilles tendon, patellar tendon, biceps brachii muscle, brachioradialis muscle, and the medial and lateral gastrocnemius muscle were similar in individuals with and without GJH. The results obtained show that the mechanical properties of muscles and tendons were not associated with GJH. There are some studies investigating the mechanical properties of muscles or tendons. Similar to the results obtained, Magnusson *et al.* (36) reported that passive properties of the muscle-tendon unit were similar in women with benign joint hypermobility syndrome and controls. On the other hand, Alsiri *et al.* (16) conducted a study to assess the changes in mechanical properties of muscles and tendons in hypermobility spectrum disorders by strain elastography. They reported that the elasticity of the brachioradialis muscle, Achilles, and patellar tendon was lower in hypermobility spectrum disorders; however, the elasticity of the deltoid, biceps brachia, rectus femoris, and gastrocnemius muscles was similar in individuals with hypermobility spectrum disorders and controls (16). Different from our results, Rombaut

et al. (17) investigated the passive properties of the plantar flexors muscle-tendon tissue in patients with the hypermobility type of Ehlers-Danlos syndrome by measuring the passive muscle tension with an isokinetic dynamometer. They found that patients with the hypermobility type of Ehlers-Danlos syndrome had a lower passive muscle tension in plantar flexors and Achilles tendon stiffness (17). Moreover, Nielsen *et al*. (18) investigated patellar tendon stiffness in patients with Ehlers-Danlos syndrome by force and ultrasonographic measurements during a ramped isometric knee extension. They indicated that patellar tendon stiffness was lower in patients with Ehlers-Danlos syndrome compared to healthy controls (18). There are some potential causes related to the differences in results reported in the literature. It seen that different techniques, such as measuring passive resistive torque (17, 18, 36), or equipment, such as strain elastography, have been used to measure the mechanical properties of muscles and tendons. Using different techniques or equipment may have caused differences in the results. For example, almost all of the studies in the literature calculated stiffness by measuring passive resistive torque and tendon/muscle-tendon tissue elongation. In this technique, tendon/muscle-tendon tissue elongation is measured during maximal isometric contraction and tension of the tendon/muscle-tendon tissue (17, 18, 36). Contrary to these studies, the passive mechanical properties of the assessed tissues were carried out in full rest position using a myotonometer. On the other hand, studies reporting a change in mechanical properties of muscles and tendons have been conducted with symptomatic participants with Ehlers-Danlos syndrome or hypermobility spectrum disorders (16, 17). The reported changes in the mechanical properties of muscles and tendons in these studies (16, 17) may be related to orthopaedic disorders or chronic multiple-joint pain of the study participants. It is well known that orthopaedic conditions or orthopaedic conditions related to pain may change the mechanical properties of muscles and tendons (19-21).

The study has some limitation. First, the study was conducted with young participants. Mechanical properties of muscles and/or tendons would be different in middle aged or geriatric individuals with GJH. Second, the mechanical properties of muscles and tendons were only measured in the passive state. They may be different in tension or loading conditions in individuals with GJH. Further studies are needed to investigate the mechanical properties of muscles and tendons in tension or loading conditions in individuals with GJH. Lastly, the assessed muscles are global movers of the related joint. The mechanical properties of the stabiliser muscles may be different in individuals with GJH.

CONCLUSIONS

It was found that the elasticity, stiffness, and/or tone of the biceps brachii, brachioradialis, and medial and lateral gastrocnemius muscles were similar in individuals with and without GJH. The results obtained show that the mechanical properties of muscles and tendons are not associated with GJH. The results also suggest that mechanical properties of muscles and tendons are not associated with the excessive movements involving joints in individuals with GJH.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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Whole-Body Vibration, Morphological and Antioxidant Effects on the Diaphragm Muscle of Obese Rats

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SUMMARY

Background. This study aimed investigating the effects of whole-body vibration (WBV) on morphological parameters and the antioxidant system of the diaphragm muscle of Wistar rats with monosodium glutamate (MSG) obesity induction.

Methods. 28 animals were separated into two groups: obesity (administration of MSG) and control group. At 80 days of age, the groups were subdivided and training with WBV was started for eight weeks, constituting the control (GC), WBV control (GCP), obese (GO) and obese WBV (GOP) groups. At the end of the experimental period, when the animals were 136 days old, euthanasia and removal of the diaphragm muscle occurred. The right muscle anthem was prepared for histological analysis, quantification of total proteins and oxidative stress.

Results. Obese animals exhibited a reduction in the cross-section area of fiber types IIA, IIB, and total proteins; as for the enzymes of oxidative stress, there was a reduction in catalase activity and an increase in glutathione S-transferase. On the other hand, in the animals trained, there was a reduction in the amount of fiber type IIB, inferring possible deleterious effects on the contractile capacity of strength and an increase in the activity of the glutathione S-transferase enzyme, which was also verified in the group of association of obesity and training, besides a reduction in the activity of glutathione reductase.

Conclusions. Obesity caused a reduction in the total proteins of the diaphragm muscle, as well as a decrease in the area of type IIA and IIB fibers and enzymes in the antioxidant defense system. The WBV has not been able to reverse the effects caused by obesity.

KEY WORDS

Oxidative stress; exercise; hypothalamic obesity; muscle tissue; diaphragm.

INTRODUCTION

The excessive or abnormal accumulation of fat from obesity has been associated with numerous comorbidities and medical complications and is considered an important public health problem (1). Among the damages to health, cardiovascular diseases, type 2 diabetes, and several types of cancers stand out (2). Thus, it is verified that obesity compromises the health and daily activities of the individual, which makes weight loss a necessity in the recovery of quality of life (3).

The regular practice of physical exercises has been pointed out as a positive factor associated with health, contributing to the prevention and control of diseases such as obesity and its comorbidities (4). The planned, structured and repetitive physical activity, which characterizes physical exercise, brings numerous benefits to health, such as improved physical conditioning, decreased loss of bone and muscle mass, improved cardiorespiratory fitness and increased strength, coordination and balance (4-7).

There is a diversity of types and modalities of exercises, including activities in which equipment is used, such as those that produce vibration (8), which can be local, in a specific limb, or whole-body (WBV) and generate muscle strength gains (9-11). The WBV causes alterations in the musculoten-

dinous length, by transmitting mechanical stimuli to receptors, leading to stimulation of the muscle spindles, which results in reflex neural activity with consequent muscle contraction (12). This physiological mechanism promotes an increase in energy expenditure and, consequently, an increase in calorie burning. When used in strength training, the vibrations show an increase in these parameters, which provides an improvement in physical performance, physical rehabilitation, and an increase in muscle mass (7, 13). Furthermore, according to Zago *et al.* (14) WBV is a promising form of intervention for obese women and can improve cardiac autonomic function, reduce peripheral and central arterial stiffness, increase insulin sensitivity and glucose regulation, improve muscle strength, and reduce body weight. Alavinia *et al.* (15) also in a meta-analysis points to a reduction in body weight and fat mass, especially when WBV is associated with other measures such as diet and exercise.

In an obese condition, the muscle system is compromised because the inspiratory muscles – especially the diaphragm – receive deep influence and work overload, which can lead to a reduction in respiratory strength (16). Thus, the training of this musculature aiming at improving resistance is of utmost importance, and exercise with WBV can become advantageous due to the effects on the improvement ventilation and its systemic action (13, 17).

Few studies investigate the influences of physical exercise on the morphological structure of the inspiratory diaphragmatic muscle, which is considered the most important in the act of breathing. Reid *et al.* (18) analyzed in hamsters the morphology of the diaphragm using swimming exercise; however, no significant differences were observed in the cross-sectional area of muscle fibers and also in the percentage of fiber types, showing that its effect on the diaphragm seems to be less than other interventions, such as running on a treadmill or the inspiratory resistance load. Luciano and Mello (19) verified in diabetic rat diaphragms a reduction in total protein levels, with the improvement in this parameter after swimming exercise. Even if swimming, also considered an aerobic exercise involving the limbs, as occurs with WBV, has presented relevant data, these are still controversial (20). In this context, the effects of WBV on morphological parameters and the antioxidant system of the diaphragm muscle were investigated in an experimental model of monosodium glutamate (MSG) obesity induction.

METHODS

A total of 28 male Wistar rats were kept under controlled light conditions (light/dark cycle 12 h) and temperature (23 ± 2 ºC), in standard cages (41 cm long × 34 cm wide × 17 cm high), with access to standard rodent feed (BioBase, Santa Catarina, SC, Brazil) *and ad libitum* water.

All experiments were conducted following national and international legislation (21) and with the approval of the Ethics Committee on the Use of Animals at the Universidade Estadual do Oeste do Paraná (Unioeste): experimental certificate on the use of animals in research number 08/18 - CEUA.

Experimental groups

The animals were initially separated into two groups: control (GC, n = 14) and obese (GO, n = 14). During the first five days of life, GO animals received injections of MSG at a dose of 4 mg/g body weight and, to go through the same stress, GC received a subcutaneous saline solution at a dose of 1.25 mg/g body weight (22). The day of birth was considered day zero and the injections started on day one (**figure 1**).

At 70 days of age, the training with WBV was initiated, causing both groups to be subdivided into two more: the control group submitted to the vibration platform (GCP, n = 7) and the obese group submitted to the vibration platform (GOP, n = 7).

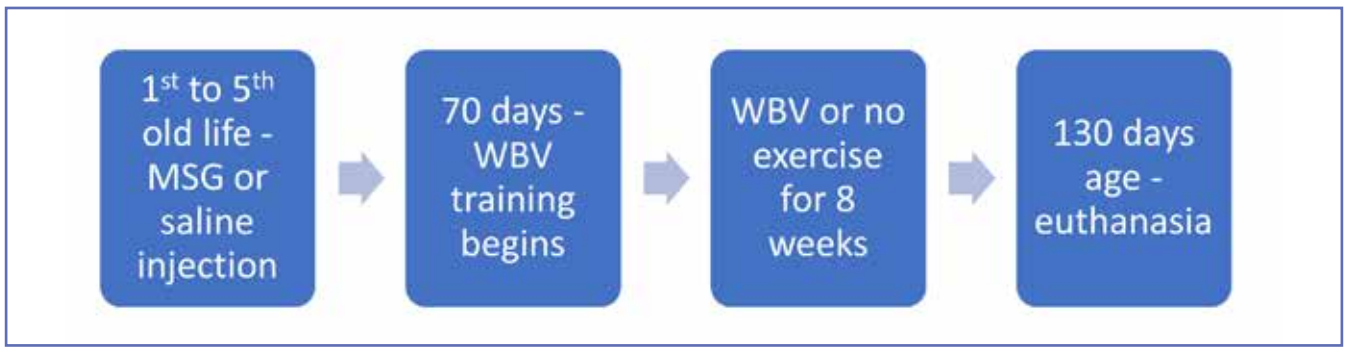

Figure 1. Time scheme for performing different steps of the experiment with the live animal.

WBV Protocol

The commercial Vibro-oscillatory platform (Arktus, Brazil) was used three times a week for 10 minutes for eight consecutive weeks, with at least 48 hours of rest between sessions. The exercise protocol was adapted to a 60 Hz frequency with an amplitude of two millimeters (23).

The animals were positioned and contained in an MDF wooden support, with compartments 13 cm wide, 19 cm long and 25 cm high. In each training, the animals were rotated in the enclosures to minimize biases resulting from the difference in vibration amplitude in the different areas of the platform.

Collection of the diaphragm muscle

At 130 days of age, after finishing the training with the vibration platform, the animals were desensitized in a CO_2 chamber and eutanasiated with the aid of the guillotine. The animals were kept in a ventral position and an incision in the median region was made just below the thorax, with the skin and muscles subsequently folded. The removal of the diaphragm consisted only of its costal part, being divided into right and left anterms.

Histoenzimological analysis

A sample of the right antler of the diaphragm muscle was kept at room temperature for a period of 30 to 40 minutes. After this time, the sample was covered with neutral talc and frozen in liquid nitrogen for subsequent storage in Biofreezer at - 80 ºC. During processing, the frozen muscle samples were transferred to a cryostat chamber (LUPETEC CM 2850 Cryostat Microtome) at - 30 ºC and kept for 30 minutes. These samples were then one end glued to a metal support using Tissue Freezing Medium (Leica, Jung, Germany) and sectioned transversely at 7 µm thickness.

The cross-sections were submitted to the reaction of Nicotinamide Adenine Dinucleotide - Tretrazolium Reductase (NADH-TR), according to Pearse's technique, modified by Dubowitz and Brooke (24), which allows the analysis of the oxidative and glycolytic metabolism of the three types of muscle fibers. With this material, the quantification of the three types of fibers and the measurement of their respective cross-sectional areas were performed using the Image Pro Plus 6.0® program (Media Cybernetics, Maryland, USA), with four microscopic fields (20X objective) being used for each animal.

Oxidative stress analysis

In the oxidative stress analysis, the other part of the sample from the right antymere of the diaphragm muscle was frozen in liquid nitrogen and stored in a freezer - 80 ºC for subsequent homogenization. The samples were homogenized in 1 ml of Tris HCl, pH 7.4 buffer and centrifuged at 13,680 G for 10 minutes at a temperature of 4 °C. The protein quantification of the samples was determined by the method of Bradford (25) using bovine serum albumin as the standard. All samples were then normalized to 1 mg of protein/ml.

To evaluate the enzyme dosage, associated with the antioxidant system, the activity of Superoxide Dismutase (SOD), the activity of Catalase (CAT), the reaction of Lipoperoxidation (LPO), the activity of Glutathione S-Transferase (GST) and the activity of Glutathione Reductase (GR) were analyzed.

In the case of SOD, the method proposed by Crouch *et al.* (26) modified was used. The principle of this analysis consists in quantifying the complex formed between superoxide and tetrazolium blue (NBT), measured at 560 nm during 1 h 30 min. An aliquot of 0.75 mg/ml of protein in 25% ethanol was prepared in a volume of 800 µL and centrifuged at 13,680 G (4 °C) for 20 minutes. From the supernatant, the reaction medium was prepared in 96-well microplate, in triplicate, with a final volume of 200 µL, containing 0.1 mg of protein × mL^{-1}, 0.09 mM of NBT, 0.015 mM of EDTA, 34.78 mM of hydroxylamine sulfate, 79 mM of sodium carbonate buffer pH 10.2 and the plate read at 22 °C, and a unit of SOD in nmol × min^{-1} × mg of $protein^{-1}$.

The activity of CAT was accompanied by a decrease in absorbance at 240 nm (27), from the principle of peroxide dismutation, whose molar extinction coefficient is 40 $M^{-1} \times cm^{-1}$. The duplicates, in 2 mL of solution in a quartz bucket presented with a final concentration of 0.01 mg of protein × mL^{-1} and the reaction medium presented with final concentrations of 13.5 mM of H_2O_2, 50 mM of TRIS-HCl pH 8.0 and 0.25 mM of EDTA. The results of the catalase enzyme activity were expressed in mmol × min^{-1} × mg of $protein^{-1}$.

The determination of the LPO reaction was performed to indirectly quantify the peroxides, thus reflecting the intensity of lipid peroxidation. The method of thiobarbituric acid (TBARS) (28) was performed by a comparison of absorbance with the pattern curve of Malondialdehyde (MDA), the main by-product of cellular lipid peroxidation. For sample preparation, the medium, containing an aliquot of 0.33 mg/ml of sample protein in 6.7% trichloroacetic acid in a final volume of 180 µL, was stirred in a vortex, left in an ice bath for 5 minutes and centrifuged for 5 minutes at 13,680 G at 4 ºC. For the dosage of TBARS reactive substances, 40 µL of the supernatant, as well as different concentrations of MDA, were added in a microplate, in triplicate, in the reaction medium, containing 21.42 mM of TBA, 17.86 mM of NaOH (used for solubilization of TBA), 0.73 M of TCA, 0.032 mM of BHT, 3% ethanol (used for

solubilization of BHT) in PBS. The reaction was read at 22 °C; after 60 minutes of incubation at 60 °C at an absorbance of 535 nm. The results of lipid peroxidation were expressed in nmol of MDA × mg of protein^{-1}.

The detoxification evaluation was performed by analysis of the GST enzyme activity. With the methodology of Keen *et al.* (29), the activity of this enzyme was measured over 5 minutes at 30-second intervals, evaluating the increase in absorbance due to the formation of a thioether at an absorbance of 340 nm. The reaction composition is potassium phosphate buffer pH 6.5, GSH 1.5 MM, CDNB 2 mM in 1 mL of ethanol. The activity of GST is expressed in µmoles of thio-ether × min^{-1} × mg of protein^{-1}.

The activity of GR was evaluated according to the technique proposed by Sies *et al.* (30). The reaction system was constituted of phosphate buffer 100 mmol × L^{-1} (pH 7.0), EDTA 1 mmol × L^{-1}, GSSG 0.66 mmol × L^{-1}, NADPH 0.075 mmol × L^{-1}. The reaction was initiated by the addition of GSSG and monitored for 5 minutes at 340 nm. The results were expressed in NADPH oxidized × min^{-1} × mg of protein^{-1}.

Statistical analysis

In the case of NADH, the data were expressed as mean ± standard deviation of the mean. For the statistical evaluation, it was used the analysis of variance (ANOVA) of two factors. When F is significant, the differences between the means were evaluated with a p-value corrected for Tukey. Values with $p < 0.05$ were considered significant. The software used for statistical analysis was GraphPad Prism version 5.0 for Windows (GraphPad Software©) (La Jolla, USA).

For the data on the activity of oxidative stress enzymes, the integrated biological response test version 2 (IBR2) was performed, which combines mathematical value with the graphic results and was applied in this study based on the evaluation of the enzymatic activities of the antioxidant system together (31). Data processing was as follows:

$$A_i \text{¼} Z_i - Z_0 \; Z_i \text{¼} \partial Y_i - \mu P = s \; Y_i \text{¼} log \; X_i = X_0 \; \partial P$$

A_i, Z_i, Z_0, µ, s, X_i, and X_0 represented the biomarker deviation index, standardized biomarker response mean, reference biomarker data mean, general Y_i mean, Y_i standard deviation, individual biomarker data, and reference mean data, respectively.

For each separate factor, the parameters are reported in a star graph to represent the reference deviation of each enzyme activity investigated. Values above zero represent enzyme induction, and values below zero indicate inhibition of enzyme activity.

RESULTS

The histoenzyme study of the NADH-TR activity revealed the presence of muscle fibers of types I (small diameter and intense oxidative activity), IIA (medium diameter and moderate oxidative activity) and IIB (large diameter and low oxidative activity) in all groups studied (**figure 2 A-D**).

The cross-sectional area of the muscle fibers showed a reduction of 21% in type IIA fibers ($F_1 = 6.44$; $p = 0.02$) and 31% in type IIB ($F_1 = 11.35$; $p = 0.002$) in the GO and GOP groups when compared to GC and GCP (**figure 3 B, C**). In the area of type I fibers, there was no significant difference between the groups studied and no interaction between the two factors, obesity and platform ($F_{1,3} = 0.41$; $p = 0.53$) (**figure 3 A**).

Regarding the count of the number of different types of muscle fibers, in the animals of GCP and GOP, a reduction of 21% was observed only in type IIB ($F_1 = 7.66$; $p = 0.01$), when compared to the groups GC and GO (**figure 3 F**). In the analyses of type I and IIA count, no significant differences were observed between the studied groups and there was also no interaction between the factors, obesity and platform (fiber type I - $F_{1,3} = 0.75$; $p = 0.40$; fiber type IIA - $F_{1,3} = 0.04$; $p = 0.84$) (**figure 3 D, E**).

The quantification of total protein in the samples in the GO and GOP groups, showed lower mean values ($F_1 = 6.96$; $p = 0.01$) than the GC and GCP groups. The analysis of the effect of WBV

training revealed no difference ($F_1 = 0.18$; $p = 0.67$) or interaction between the factors, obesity and WBV ($F_{1,3} = 0.17$; $p = 0.68$) (**figure 4**).

Through the IBR2 test, it was possible to verify the activity of enzymes in the oxidative system, observing that GST had greater activation in obesity and in WBV training, as well as in the association between these factors (**figure 5 A-C**). Additionally, the activity of CAT suffered a small reduction in obesity (**figure 5 B**) and the GR enzyme had its activity reduced in the association of obesity factors and vibratory platform (**figure 5 C**). On the other hand, in the evaluation of the SOD enzyme and the LPO reaction, no differences were observed in any of the factors evaluated (**figure 5 A-C**).

DISCUSSION

The administration of MSG in animals in their neonatal period causes lesions in the hypothalamic arched nuclei, resulting in the destruction of 70% to 90% of neural cell bodies, being one of the damages to the decrease in the secretion of growth hormone (GH) (32), which, in turn, demonstrates to be a mediator of the metabolism of lipids, carbohydrates and proteins in the tissues of the body, as well as in the growth processes (33). Due to its relationship

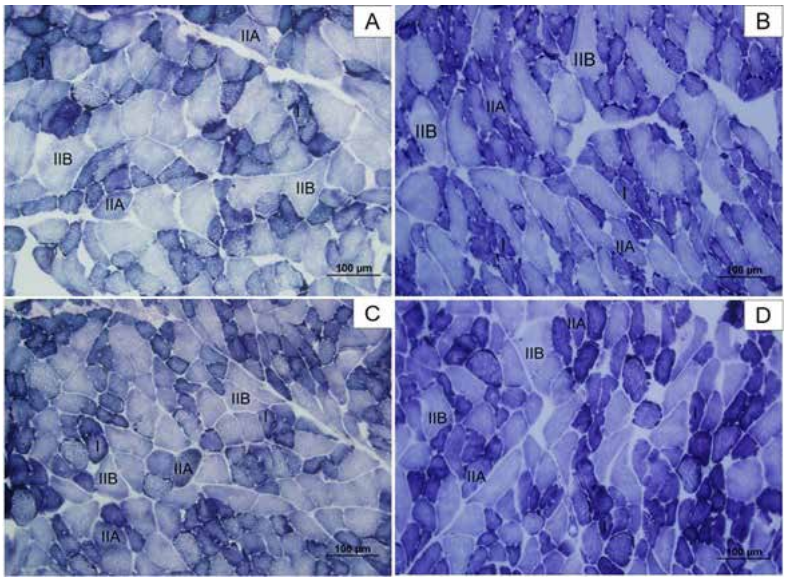

Figure 2. Photomicrographs of the diaphragm muscle of 130-day old Wistar rats. Cross-section. Type I, IIA and IIB muscle fibers. Nicotinamide Adenine Dinucleotide - Tetrazolium Reductase (NADH-TR) reaction.
A: Control Group (GC); **B**: Control Group exercised in WBV (GCP); **C**: Obese Group (GO); **D**: Obese group exercised in WBV (GOP).

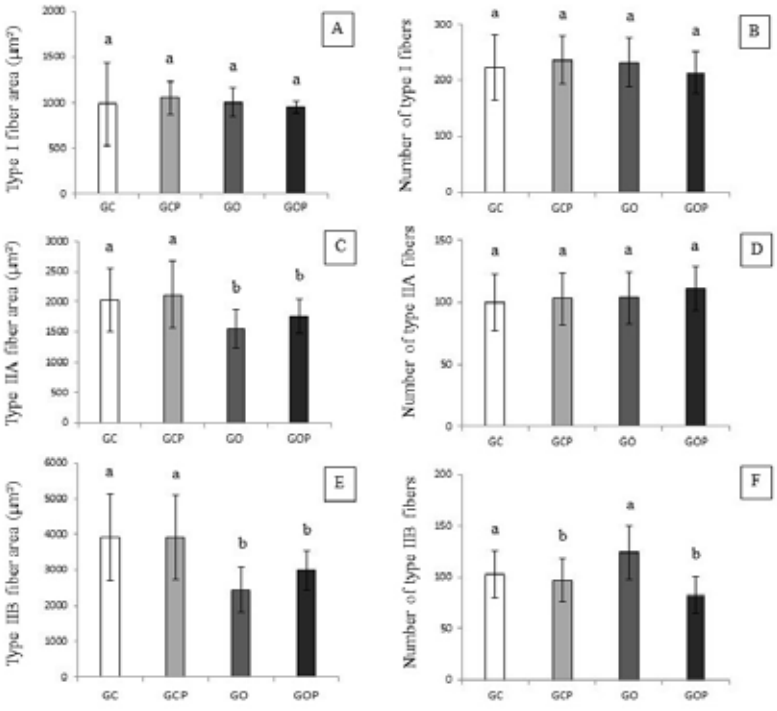

Figure 3. Morphometric analysis of the muscular fibers of the diaphragm muscle of 130-day-old Wistar rats in the control (CG), WBV trained (GCP), obese (GO) and WBV trained (GOP) groups, respectively.
A: type I fiber area; **B**: area of type IIA fibers; **C**: area of type IIB fibers; **D**: number of type I fibers; **E**: number of Type IIA fibers; **F**: number of type IIB fibers. Values expressed as mean and standard deviation. Different letters indicate significant differences. Two-way ANOVA variance analysis test ($p < 0.05$).

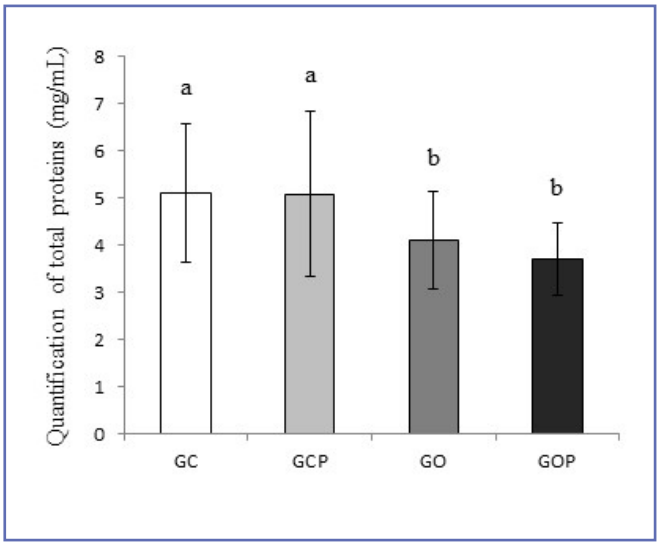

Figure 4. Analysis of the quantification of total diaphragm muscle proteins from 130-day-old Wistar rats in the control (GC), WBV trained (GCP), obese (GO), and WBV trained (GOP) groups, respectively.

Values expressed as mean and standard deviation. Different letters indicate significant differences. Two-way ANOVA variance analysis test ($p < 0.05$).

with protein synthesis, the decrease in the concentration of this hormone leads to protein deficiency (34), corroborating with the results of this study, considering that the animals that underwent administration of MSG presented a reduction in the analysis of the quantification of total proteins of the diaphragm muscle.

In this model, through the reduction of GH secretion, causes protein deficiency, thus harming the development of the muscle, resulting in a reduction in the area of muscle fibers (34), an aspect observed in the present study, in which obesity, through MSG, led to a reduction in the area of type IIA fibers and the area of type IIB fibers. Additionally, the response of the muscle fibers to different stimuli is specific to the type of fiber, and more atrophy of the type II fibers is observed than of the type I fibers in conditions associated with the loss of muscle mass in disease (20), which also corroborates the findings of this study.

The muscle fibers can change their physiological and biochemical properties according to the stimuli to which they are submitted (35), and physical exercise can promote changes in muscle fibers, altering these properties, because different types of exercise can alter the amount of slow or fast fibers (36). When the number of each type of muscle fibers

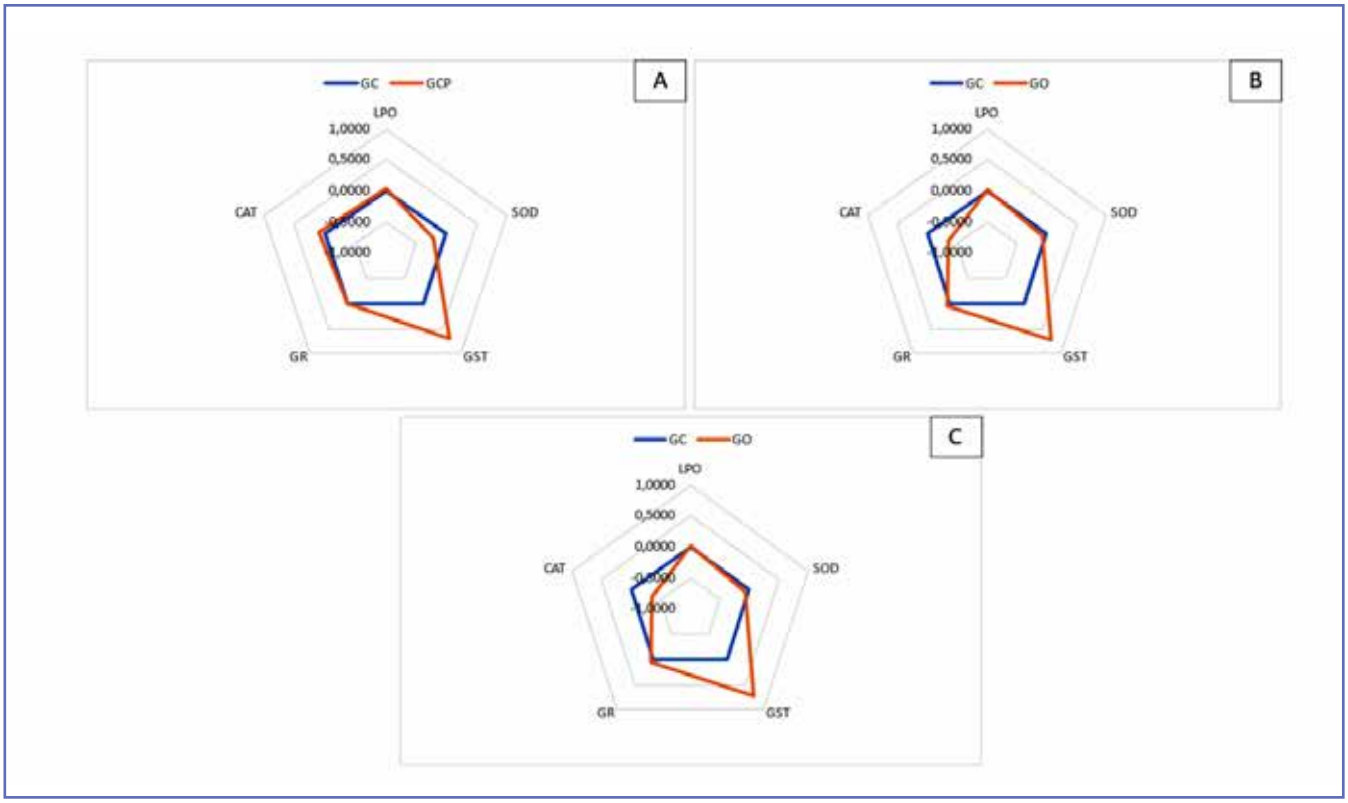

Figure 5. IBR stellar portions of the activity of enzymes present in the antioxidant defense system.
A: animals trained in WBV (GCP); **B**: obese animals (GO); **C**: obese animals trained in WBV (GOP).

was counted, a reduction was observed only in the fibers of type IIB, due to the vibratory platform. Endurance or aerobic exercises, as is the case of WBV, promote an increase in the myoglobin, in the number and size of mitochondria and also in the activity of oxidative enzymes (37), bringing an improved metabolic capacity and allowing the guarantee of activity for prolonged periods without excessive fatigue, recruiting more type I fibers (20, 38), which could be associated to a reduction in the number of type IIB fibers.

The data on the reduction in the amount of type IIB fibers show the adaptations of the skeletal muscle submitted to the vibratory platform exercise and reveal an important measure of its plasticity, which is the high capacity that this tissue has to undergo adaptation both structurally and functionally, considering the workload, activities and pathological conditions (38). The WBV, Park *et al.* (39) highlight brings positive results in the condition of a strength exercise, and the protocol used in this study was adapted from Butezloff *et al.* (23) in which they observed anabolic effects of WBV; however, the results obtained in this study infer possible deleterious effects on the contractile strength capacity of the diaphragm. Other studies investigating the diaphragm muscle after performing physical exercise have not revealed significant differences in the morphological parameters of percentage and cross-sectional area of muscle fibers (18). Thus, there is a need for new studies varying the time of intervention, as well as the level of intensity of exercise with WBV. It should be noted that the findings of this study are valid for the diaphragm muscle, because analyzing the lipid profile in a similar model and protocol, Andrade *et al.* (40) observed lipid mobilization indicating WBV as a method for metabolic rehabilitation. It was also observed by Boaretto *et al.* (41, 42) with a similar protocol, a reduction of intramuscular connective tissue, an increase in muscle fibers, and neuromuscular junction measurements, despite the absence of changes in cholinesterase concentration in the synaptic cleft of soleus.

Regarding oxidative metabolism, it has been discussed in the literature that obesity causes increased lipid peroxidation and decreased cytoprotective enzymes, resulting in progressive cellular damage generated by oxidative stress (43). In the present study, a reduction in CAT activity was observed to the detriment of obesity, corroborating the study of Antunes Neto and Paula (44) with obesity and sedentary men, in which a reduction in the activity of this enzyme was also observed. Due to the important role of CAT in the process of hydrogen peroxide breakdown, which is increased by obesity, the decrease in its activity causes, consequently, a reduction in the efficiency of the antioxidant defense system and increases the probability of growth in oxidative stress (45).

Some of the enzymes that make up the antioxidant defense system seem to work in an integrated way, and as the activity of one increases, the activity of another decreases, as is the case of glutathione reductase and catalase (46). It goes with that was observed in this study, because in obese animals, unlike Catalase, the activity of Glutathione S-Transferase presented increased and may have occurred as a protective action of cells due to its detoxifying action.

It is known that high muscle activity, promoted by physical exercises, leads to an increase in reactive oxygen species (ROS); however, regular exercises performed at moderate intensity are known to diminish a pro-inflammatory state in the body and may reinforce antioxidant defenses by adapting to oxidative stress (47). When the activity of GST in animals trained with WBV was analyzed, it was increased and may be an adaptation to combat the oxidative stress initially caused by WBV exercise.

The same result of increased GST activity was observed in the group with obese animals trained in WBV, indicating that the two factors together also influence the activity of this enzyme. However, this same group presented a reduction in the activity of GST, showing that the association of both factors also influences the activity of this enzyme. In the same way of this study, Watson *et al.* (48) observed the reduction of this enzyme in obese ob/ob rats, and Prada *et al.* (49) reported a reduction in RG in rats submitted to swim training, and no studies were found analyzing the activity of this enzyme with both associated factors. The drop in GR activity may be related to the reduction in nicotinamide adenine dinucleotide phosphate (NADPH) by pentose cycles (50), as studies show that some physical exercises, depending on their intensity, reduce the concentration of NADPH, which is one of the substrates of GR, without which the reaction catalyzed by this enzyme does not occur (51).

In the analysis of SOD activity and LPO reaction, no differences were observed in any of the factors evaluated. Studies with analysis of the effects of WBV on the activity of oxidative enzymes are very scarce in the literature. In a study that investigated the effects of training with WBV on oxidative stress in diabetic animals, Liu *et al.* (52) did not find differences in the activity of SOD, but the exercise was able to reduce the LPO. However, the protocol used by Liu *et al.* (52) was different from the present study, and the muscle under analysis as well, which may have interfered with the differing results.

CONCLUSIONS

Based on the above results, it is concluded that obesity through MSG has caused a reduction in the total proteins of the diaphragm muscle, as well as a decrease in the area

of type IIA and IIB fibers and enzymes in the antioxidant defense system. Furthermore, it is understood that training with WBV does not seem to be a recommended resource since it has not been able to reverse the effects caused by obesity.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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Whole-Body Vibration Promotes Beneficial Changes on the Anterior Tibial Muscle Histomorphometry of Hypothalamic Obese Rats

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SUMMARY

Background. Due to the deleterious effects of obesity on muscle tissue and the search for tools to reverse these losses, it is important to understand the effect of physical exercises on the muscle structure of obese individuals. This study aimed to analyze the effect of whole-body vibration (WBV) on the histomorphological parameters of the anterior tibial muscle using the monosodium l-glutamate (MSG) obesity model.

Methods. MSG-obese rats that were exposed to WBV on a vibrating platform with a frequency of 60 Hz, the amplitude of 2 mm, three times/week, 10 min/day, for eight weeks (from postnatal day (PN) 80 to PN136). The histomorphology of the anterior tibial muscle was evaluated.

Results. When performing a WBV exercise, the animals showed altered structural responses in the MSG animals, such as reduced muscle mass, increased connective tissue, and nuclear activity. The WBV reduced the extracellular matrix and the nuclear activity in the MSG animals, showing efficiency in the protocol.

Conclusions. Even with the aggressive character of the MSG model, the WBV exercise was able to induce repair to the muscle tissue of these animals, thus being a safe protocol for use in similar conditions.

KEY WORDS

Exercise therapy; extracellular matrix; monosodium glutamate; skeletal muscle; Whole-Body Vibration.

BACKGROUND

According to the World Health Organization, obesity is a pandemic that affects approximately 650 million people (1), who are subject to several associated comorbidities. The accumulation of adipose tissue can negatively influence the process of muscle remodeling, promoting the reduction of muscle mass, potentiating the deleterious effects on the locomotor system (2). Overweight caused by obesity has several negative effects on the locomotor system, including joint problems, pain, and reduced locomotion (3).

This excess of adipose tissue is one of the main responsible for the metabolic changes of obesity (4), one of the main characteristics of which is the increase in the secretion of pro-inflammatory cytokines. The constant maintenance of high levels of these inflammatory molecules stimulates the reduction of synthesis and increase of protein degradation, consequently leading to a reduction in muscle mass and strength (5). With persistent muscle inflammation, satellite cells, responsible for the regeneration of muscle fibers, have their recruitment reduced and there is a greater proliferation of fibroblasts, and thus, there is an increase in

intramuscular connective tissue that contributes to muscle changes and weakness (6).

To minimize part of the deleterious effects of obesity and its comorbidities, several experimental methods are employed, and physical exercise is effective and has few side effects. As they are low-invasive and low-cost modalities, they are easily adopted in the treatment of obesity leading to improved quality of life (7). The Whole-Body Vibration (WBV), obtained through vibrating platforms, is an alternative for people who, due to their physical conditions, do not adhere to traditional forms of physical exercise, and dependent on a low-impact activity (5).

WBV acts directly on the muscle, increasing the capacity for muscle contraction and relaxation (8), stimulating protein synthesis, accelerating regeneration, and improving vascularization (9). However, even knowing the effects of WBV on some muscle and bone parameters, the effects of this exercise modality associated with obesity models are not well understood. Thus, the present study aimed to evaluate the effects of WBV on the histomorphology of the anterior tibial muscle of Wistar rats with obesity induced by monosodium glutamate (MSG).

METHODS

Animals and experimental model

This study had an experimental character with a completely randomized design (DIC), with a level of evidence 1b. For the present study, 32 male Wistar rats were used, kept in standard polypropylene boxes, in an environment with a temperature of 22 ± 1 ºC, with a photoperiod of 12 hours, with free access to water and feed. Initially, the animals were randomly separated into two experimental groups (n = 16): 1) CTL, whose animals received hyperosmotic saline, and 2) MSG, whose animals were induced to obesity with monosodium glutamate. Of these, each group was subdivided into two other experimental groups (n = 8): a) CTL-SED – control group, b) MSG-SED – obese group c) CTL-WBV – control group trained with WBV and d) MSG-WBV – an obese group trained with WBV (5). All procedures were conducted according to the ethics parameters described in this journal (10).

In the very first days of life, with an initial body weight between six and seven grams and that comprised the MSG-SED and MSG-WBV groups received intradermal injections of monosodium glutamate (MSG) at a dose of 4 mg/g of body weight during the first 5 days deity. For those composing the CTL-SED and CTL-WBV groups, during the same period, hyperosmotic saline solution (12.5%) was administered at a dose of 1.25 mg/g of body weight.

Whole-Body Vibration protocol

The platform used was the professional tri-plane Vibro Oscillatory model of the Arktus brand, with adapted protocol a frequency of 60 Hz, the amplitude of two millimeters, for 10 minutes (5, 7), from the animals' 80 days of age, three times a week, on alternate days, making a total of eight weeks of treatment (11). To place the animals on the platform, to contain them and carry out the training with several animals simultaneously, a white wooden MDF support was used, compartmented in eight stalls 13 cm wide, 19 cm long, and height of 25 centimeters. At each training, the animals were rotated between the bays to minimize bias due to different vibration frequencies in different areas of the platform (**figure 1**).

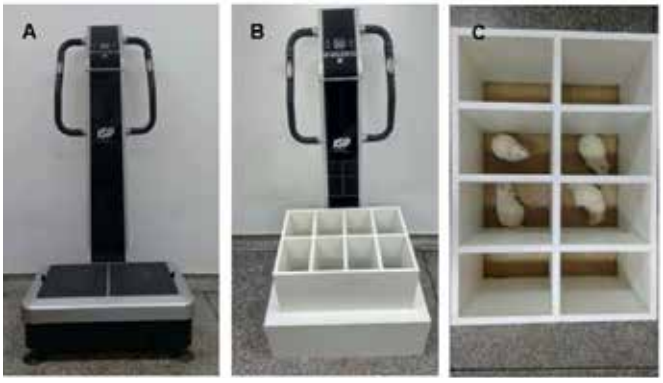

Figure 1. (A) Vibrating platform, **(B)** front view and **(C)** top view.

Collection and preparation of the Tibialis Anterior Muscle

At 142 days of age, the animals were weighed, desensitized in a CO_2 chamber, and euthanized by guillotining. The retroperitoneal (RETRO) and perigonadal (PERIG) fats were removed, their weight measured and normalized to 100 g-1 g body weight, and the Lee Index ($\sqrt[3]{}$ bodyweight / nasal-anal length × 1000) was calculated (7). The Tibialis Anterior muscle was dissected and fixed in metacarn for 24 hours, stored in 70% alcohol until processing for inclusion in paraffin blocks, where they were first dehydrated in increasing alcoholic series and diaphanized in N-butyl alcohol. The blocks were cut transversely in a microtome to a thickness of five µm and stained in Hematoxylin and Eosin, and Masson's Trichromic (5, 12).

Histomorphological analyzes

The materials, stained with Hematoxylin and Eosin and Masson's Trichromic, were photomicrographed using a

photomicroscope with an Olympus BX60® coupled camera (Tokyo, Japan) with a 40x objective, totaling 12 images per animal in each technique. The morphological analyzes were performed in the Image ProPlus 6.0 program, and in each image the muscle fasciculus was scanned to randomly select ten fibers, thus totaling 120 fibers per animal (5).

From these fibers, the shape and organization and density (DENS) the positions of the nuclei, and the architecture of the connective tissue were analyzed, and the cross-sectional area (CSAF), larger (LDF), and smaller (SDF) diameters, was measured. The numbers of central (NC%) and peripheral nuclei were also quantified, as well as the area of these nuclei (CSAN). The number of fibers and nuclei were used to calculate the nucleus to fiber ratio (N/F) and the area ratio of the nuclei to the sarcoplasm (AN/AF) area (5, 11).

As for the analysis of connective tissue, the GIMP 2.0 program was used, and the quantification and comparison of the total number of pixels in the image with the number of connective tissue pixels were performed, to define the total connective tissue present in the visual field. Thus, this quantification considered the percentage of pixels referring to the total connective tissue (CON), epimysium (EPI), perimysium (PER), and endomysium (END) (11, 12).

Statistical analysis

All variables were analyzed by the normality (Shapiro-Wilk test) and homoscedasticity (Bartlet test) and those that were in agreement with such assumptions, were analyzed by Two-Way Analysis of Variance (ANOVA) follow by the posthoc Tukey-HSD test, to examine the interaction of obesity (MSG) and whole-body vibration (WBV). When the assumptions were not in agreement, the Kruskal-Wallis test was performed followed by the posthoc Dunn test. All analyses were performed with a level of significance $\alpha = 0.05$.

Then, the matrices of the variables were standardized and analyzed using the principal component analysis (PCA). With the PCA, factorial loads are established for each variable and analyzed in response components. The data provided by the PCA is reduced, the data overlays are removed, and the most representative linear units of the data are known. The factor loads of the main components were evaluated in terms of statistical significance using a Two-Way Analysis of Variance (ANOVA) follow by the posthoc Tukey-HSD test (7). As the main components (PC) are ordered in decreasing order of importance for a structure of variance of the data set, the greater the retention of the total variance in a smaller number of linear formulas, the better the application of the procedure to the experimental data. All procedures were performed in software R version 4.0.3 (13).

RESULTS

Body Parameters

Bodyweight was affected only by MSG ($p < 0.00001$), with no modification by the WBV factor. The Lee index showed a significant difference due to the fixed effects of MSG and WBV. An increase in means was observed in the MSG groups ($p < 0.0001$) and a significant reduction in the WBV groups ($p = 0.01$; **table I**). Only the MSG resulted in an increase in weight of the retroperitoneal and perigonadal fats compared to the respective controls ($p < 0.0001$; $p < 0.0001$, respectively; **table I**). There was no significant interaction between the fixed factors, which means that exercise did not change the effect of MSG on these variables.

Morphological analysis

The CTL-SED (**figure 2 A**) animals have polygonal fibers, with the nuclei in a peripheral location, just below the sarcoplasmic membrane. The extracellular matrix (**figure 2 E**) organized in endomysium, intimately in contact with the sarcoplasmic membrane, individually covers each muscle fiber. The fibers are organized in muscle fascicles, delimited by the perimysium that allows the entry of blood vessels and nerves. Meanwhile, the animals of the MSG-SED (**figure 2 B**) present both polygonal fibers and fibers with a rounded shape and of smaller size, with a greater occurrence of central nuclei. It is also possible to observe a greater presence of connective tissue in all muscle wraps (**figure 2 F**).

However, the CTL-WBV (**figure 2 C**) and OBS-WBV (**figure 2 D**) animals showed predominantly polygonal muscle fibers, with a smaller caliber than the respective sedentary groups, and less occurrence of central nuclei. In the case of connective wraps (**figure 2 G, F**), it is also possible to notice less deposition throughout the extracellular matrix of these groups.

Morphometrical analysis

When assessing the CSAF, there was no interaction between the factors ($p = 0.457$). However, MSG animals showed lower values when compared to CTL ($p < 0.0001$). Meanwhile, WBV animals showed a decrease in CSAF when compared to SED ($p = 0.003$). Likewise, there was no interaction between the factors in the LDF analysis ($p = 0.083$). However, MSG animals showed lower values when compared to CTL ($p < 0.0001$). Following the same pattern, WBV animals showed a reduction in LDF when compared to SED ($p < 0.0001$). When evaluating the SDF, there was an interaction between the factors ($p < 0.0001$), with no difference between the CTL and MSG animals ($p = 0.992$). However, CTL-WBV animals

Table I. Average ± Standard Deviation of the values of body and morphometric parameters of Wistar rats.

	GROUPS				p VALUES		
	CTL-SED	CTL-WBV	MSG-SED	MSG-WBV	MSG	WBV	INT
BW	407.33 ± 12.85^{Aa}	400.00 ± 17.49^{Aa}	317.60 ± 9.03^{Ba}	307.78 ± 11.51^{Ba}	< 0.0001	0.875	0.248
LEE	293.18 ± 11.31^{Ba}	279.17 ± 8.28^{Bb}	307.67 ± 15.91^{Aa}	296.71 ± 9.59^{Ab}	< 0.0001	0.005	0.704
RETRO	1.20 ± 0.36^{Ba}	1.22 ± 0.28^{Ba}	1.97 ± 0.43^{Aa}	2.05 ± 0.45^{Aa}	< 0.0001	0.728	0.817
PERIG	1.31 ± 0.14^{Ba}	1.16 ± 0.36^{Ba}	1.70 ± 0.40^{Aa}	1.85 ± 0.41^{Aa}	0.0001	0.975	0.229
CSAF	3011.60 ± 335.03^{Aa}	2785.38 ± 258.74^{Ab}	2533.19 ± 179.74^{Ba}	2162.21 ± 268.54^{Bb}	< 0.0001	0.003	0.457
LDF	71.98 ± 4.57^{Aa}	66.50 ± 3.04^{Ab}	63.08 ± 3.04^{Ba}	52.47 ± 4.96^{Bb}	< 0.0001	< 0.0001	0.083
SDF	43.80 ± 2.07^{Aa}	36.24 ± 1.95^{Bb}	40.571 ± 1.70^{Ba}	39.46 ± 1.92^{Aa}	0.992	< 0.0001	< 0.0001
DENS	227.36 ± 35.39^{Bb}	275.56 ± 22.34^{Ba}	308.65 ± 36.93^{Ab}	392.32 ± 47.49^{Aa}	< 0.0001	< 0.0001	0.182
N/F	2.10 ± 0.10^{Aa}	1.92 ± 0.03^{Ab}	1.74 ± 0.09^{Ba}	1.61 ± 0.17^{Bb}	< 0.0001	< 0.0001	0.519
NC%	0.89 ± 0.19^{Bb}	0.77 ± 0.20^{Bb}	1.31 ± 0.38^{Aa}	0.54 ± 0.17^{Bb}	0.289	< 0.0001	0.001
CSAN	14.91 ± 1.45^{Ab}	16.89 ± 2.03^{Aa}	14.63 ± 0.99^{Ab}	16.24 ± 1.01^{Aa}	0.365	0.001	0.723
AN/AF	0.010 ± 0.001^{Ab}	0.011 ± 0.002^{Aa}	0.010 ± 0.001^{Ab}	0.012 ± 0.001^{Aa}	0.968	0.01	0.524
CONJ	9.14 ± 0.63^{Bb}	8.56 ± 0.64^{Bb}	16.51 ± 0.86^{Aa}	10.56 ± 0.61^{Ab}	< 0.0001	< 0.0001	< 0.0001
EPI	2.32 ± 0.22^{Bb}	2.15 ± 0.16^{Bb}	4.05 ± 021^{Aa}	2.59 ± 0.12^{Ab}	< 0.0001	< 0.0001	< 0.0001
PER	1.08 ± 0.14^{Bb}	1.22 ± 0.08^{Bb}	2.37 ± 0.16^{Aa}	1.56 ± 0.14^{Ab}	< 0.0001	< 0.0001	< 0.0001
END	1.15 ± 0.07^{Ba}	0.89 ± 0.23^{Bb}	1.82 ± 0.23^{Aa}	1.12 ± 0.09Ab	< 0.0001	< 0.0001	0.001

CTL-SED: sedentary control group; CTL-WBV: trained control group; MSG-SED: sedentary obese group, MSG-WBV: trained obese group. Capital and different letters represent isolated differences from the MSG factor; lowercase and different letters represent isolated differences from the WBV. BW: body weight (g); LEE: Lee index (g/cm³); RETRO: retroperitoneal adipose tissue (g/100 g); PERIG: perigonadal adipose tissue (g/100 g); CSAF: cross-sectional area of the muscle fiber (µm²); LDF: larger diameter of the muscle fiber (µm); SDF: smaller diameter of the muscle fiber (µm); DENS: density of muscle fibers (fibers / mm²); N/F: core to fiber ratio; NC%: percentage of central nuclei; CSAN: cross-sectional area of the nucleus (µm²); AN/AF: a ratio of the nucleus area to sarcoplasm area; CON: total connective tissue (% pixels); EPI: connective tissue of the epimysium (% pixels); PER: perimysium connective tissue (% pixels); END: connective tissue of the endomysium (% pixels).

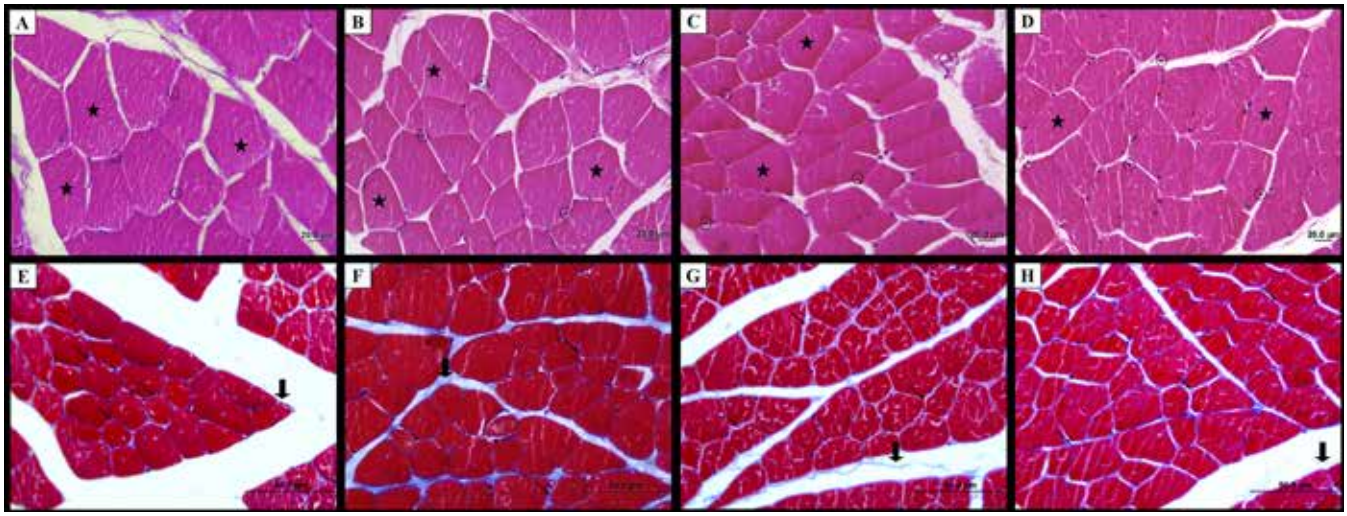

Figure 2. Photomicrographs of the anterior tibial muscle of Wistar rats at 142 days of age.

Cross-section, hematoxylin, and eosin staining (A-D), and Masson's trichrome (E-H). (A, E) Sedentary control group (CTL-SED); (B, F) Sedentary obese group (MSG-SED); (C, G) Trained control group (CTL-WBV); (D, H) Trained obese group (MSG-WBV). Polygonal-shaped fibers (stars); peripheral nuclei (circles). Connective tissue corresponding to perimysium (thick arrows) and endomysium (thin arrows).

showed a reduction in SDF when compared to CTL-SED ($p < 0.0001$). In the case of DENS, there was no interaction between the factors ($p = 0.182$). However, there was an increase in MSG animals when compared to CTL ($p < 0.0001$). Likewise, the WBV animals showed higher averages than the SED ($p < 0.0001$) (**table I**).

When comparing the N/F values, there was no interaction between the factors ($p = 0.519$). However, there was a reduction of this ratio in MSG animals when compared to CTL ($p < 0.0001$). Likewise, there was a reduction in N/F in WBV animals compared to SED ($p < 0.0001$). In the case of NC%, there was an interaction between the factors ($p = 0.001$), but there was no difference between the CTL and MSG animals ($p = 0.289$). However, WBV reduced NC% in MSG animals ($p < 0.0001$). When comparing the CSAN values, there was no interaction between the factors ($p = 0.723$), and no difference between the CTL and MSG animals ($p = 0.365$). However, the WBV promoted an increase in CSAN in trained animals ($p = 0.001$). Similarly, there was no interaction between factors ($p = 0.524$) in AN/AF, and there was also no difference between CTL and MSG animals ($p = 0.968$). However, WBV promoted an increase in AN/AF when compared to SED animals ($p = 0.01$) (**table I**).

When assessing connective tissue variables, there was an interaction between factors in the CON ($p < 0.0001$), EPI ($p < 0.0001$), PER ($p < 0.0001$) and END ($p = 0.001$). In all envelopes, MSG animals had higher connective tissue averages than CTL ($p < 0.0001$). Likewise, the exercise promoted a reduction in all connective wraps in MSG-WBV animals when compared to MSG-SED ($p < 0.0001$). Only in END, the exercise promoted the same reduction in CTL-WBV animals when compared to CTL-SED ($p < 0.0001$) (**table I**).

Principal components analysis

After creating the PCs, it was observed that only 3 dimensions correspond to 84.47% of data exploration. Therefore, these 3 components were used for the development of the analysis. The first dimension was called the Extracellular Matrix (CON, EPI, PER, END), the second was called Muscle Mass (CSAF, LDF, SDF, DENS, AN/AF, N/F), and the third of Nuclear Activity (CSAN, NC%, N/F, AN/AF). Comparing the animals in terms of the Extracellular Matrix and Muscle Mass components (**figure 3 A**), it is possible to notice that the CTL-SED animals are determined by the values of Muscular Mass, while the MSG-SED animals show direction governed by the increase in the Extracellular Matrix. Meanwhile, the exercise brought the animals CTL-WBV and MSG-WBV closer together, both in reducing muscle mass and in the extracellular matrix.

Meanwhile, the animals as a function of the Muscle Mass and Nuclear Activity components (**figure 3 B**) it is possible to notice that the CTL-WBV animals have greater Nuclear Activity and less Muscular Mass than the CTL-SED. Likewise, MSG-WBV animals have reduced Muscle Mass and Nuclear Activity. Even with different results of Nuclear Activity, it is possible to notice that the exercise promoted changes that bring the two WBV groups closer together. It is possible to observe this characteristic of approximation in the components Nuclear Activity and Extracellular Matrix (**figure 3 C**), where it is possible to notice that even with an increase in Nuclear Activity in CTL-WBV animals and a reduction in MSG-WBV animals, the approximation promoted by the reduction of Extracellular matrix in the MSG-WBV promotes overlapping of these animals with the CTL.

When evaluating the distribution of the Extracellular Matrix data (**figure 3 A'**), there was an interaction between the factors ($p < 0.0001$; $F_{(1,28)} = 34,183$). The MSG animals showed greater variation when compared to the CTL ($p < 0.0001$; $F_{(1,28)} = 165,931$). Likewise, the exercise promoted a reduction in variations in WBV animals compared to SED ($p < 0.0001$; $F_{(1,28)} = 87,613$). Conversely, when evaluating the distribution of Muscle Mass data (**figure 3 B'**), there was no interaction between the factors ($p = 0.92968$; $F_{(1,28)} = 0.008$). However, it is possible to observe that MSG animals have lower averages than CTL ($p < 0.0001$; $F_{(1,28)} = 42.617$). Likewise, WBV animals show reduced Muscle Mass when compared to SED ($p < 0.0001$; $F_{(1,28)} = 44,798$). Meanwhile, when assessing the distribution of Nuclear Activity data (**figure 3 C'**), there was an interaction between the factors ($p = 0.0001$; $F_{(1,28)} = 18.6430$). Even though there was no difference between the CTL and MSG animals ($p = 0.185873$; $F_{(1,28)} = 1.8393$) and the SED and WBV animals ($p = 0.168550$; $F_{(1,28)} = 1.9978$), the interaction of the two factors promoted the opposite effect in the animals. While CTL-WBV showed an increase in Nuclear Activity compared to CTL-SED, animals MSG-WBV showed a reduction when compared to MSG-SED.

DISCUSSION

The obesity model used in the present study promoted negative changes in the structure of muscle tissue, increasing the conjunctival extracellular matrix and reducing muscle mass, and an increase in nuclear activity in MSG animals, demonstrating a harmful effect on tissue functionality. Nevertheless, when the MSG animals were submitted to WBV, these changes were reversed, with a reduction in the extracellular matrix and nuclear activity, suggesting muscle repair. Also,

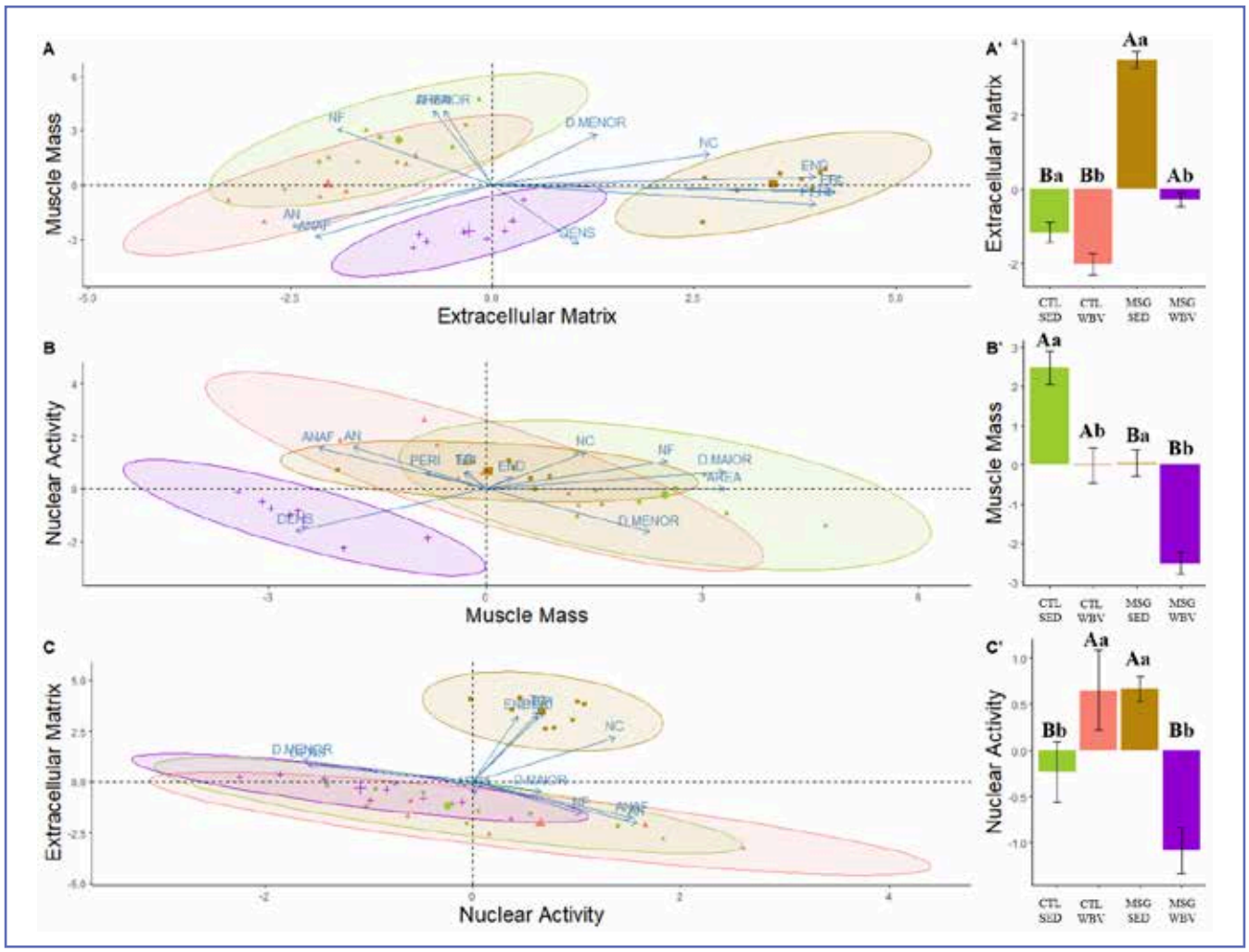

Figure 3. Principal Component Analysis (**A-C**) and Variation of data in each Component (**A'-C'**).
CTL-SED: sedentary control group; CTL-WBV: trained control group; MSG-SED: sedentary obese group, MSG-WBV: trained obese group. Capital and different letters represent isolated differences from the MSG factor; lowercase and different letters represent isolated differences from the WBV. Extracellular Matrix (CON, EPI, PER, END), the second was called Muscle Mass (CSAF, LDF, SDF, DENS, AN/AF, N/F) and the third of Nuclear Activity (CSAN, NC%, N/F, AN/AF), these three components exploit 84.47% of the data.

CTL animals increased nuclear activity, which may indicate the effect of exercise on protein synthesis.

The MSG-induced obesity model promoted an increase in the Lee index, as well as the deposition of retroperitoneal and perigonadal body fats, that corroborates with studies which, that have already demonstrated a significant increase in these parameters and, therefore, the efficiency of the model for the induction of obesity in rats (14, 15). The effectiveness of this experimental model is justified by the injuries that occur in several central structures in the paraventricular region, with the arcuate and ventromedial nuclei of the hypothalamus being the most affected sites, with 80% to 90% of the neurons responsible for controlling energy expenditure, food consumption and glycemic homeostasis (16). Thus, due to the damage to the hypothalamus caused by MSG, there is a decrease in the production and secretion of hormones such as GH, resulting in a model of dwarfism due to low systemic growth derived from this low hormone (17).

When analyzing the histomorphometric parameters of the anterior tibial muscle, there were a reduction in the cross-sectional area, larger and smaller diameters of the muscle fiber in MSG animals, showing that the model was able to reproduce the expected hypotrophic characteristics. There is a direct relationship of inhibition of factors that

repress muscle development, such as myostatin, in the presence of GH. Since these animals do not have the normal secretion of this hormone, there is an increase in the activity of myostatin, leading to less growth of muscle fibers during the development of the animal (18, 19).

The connective wraps are responsible for the structural maintenance of muscle fibers and for allowing the individually generated contraction force to act on the entire muscle and, finally, transmit it to the tendon and bone to generate movement. Also, the connective tissue serves as a support for nerves and blood vessels and which facilitates sliding for these structures. It is important to note that almost all pathological changes in the muscle are also associated with some degree of thickening of the extracellular matrix (6, 20). In this study, it was observed that obesity increased the percentage of all connective tissue variables in MSG-SED animals, possibly due to the reduction of protein synthesis and sedentary stimuli, which promotes muscle disuse, and consequently, atrophy (5, 7). Therefore, due to the obesity model causing muscle damage, there may be an imbalance in the proliferation of growth factors involved in muscle regeneration and mesenchymal growth, among them the fibroblast growth factor (FGF), which works by stimulating the synthesis of connective tissue (21). The connective tissue fills the area that would initially be occupied with muscle fiber, which justifies the increase in the connective tissue of the MSG-SED when compared to the CTL-SED. Besides, it was shown that the most significant increase in MSG-SED was concerning the perimysium, responsible for cell signaling (20, 22). In this sense, a structural change, such as an increase in fibrotic tissue, can negatively impact the arrival of muscle growth signaling factors.

The increase in the percentage of connective tissue observed in MSG-SED possibly occurred due to the high proliferation of fibroblasts and synthesis of type III collagen, which consequently led to the proliferation of type I collagen, which determines the tensile strength and rigidity of the tissue (23). This is also described in immobilization models (12) with an increase in intramuscular connective tissue in the anterior tibial, suggesting that there is a change in fibroblast metabolism and, consequently, an increase in collagen synthesis, which would be similar to what happened in the animals of the MSG-SED. Boaretto *et al.* (2020) (5), obtained similar results in the soleus muscle of Wistar rats, in which the sedentary obese group also obtained higher averages of muscle connective tissue. This highlights the role of a sedentary lifestyle and muscle disuse in the accumulation of connective tissue between muscle fibers.

However, the WBV animals showed a reduction in the amount of connective tissue in the extracellular matrix, suggesting that the exercise used was able to alter the synthesis and remodeling of the muscle connective wraps. Polizello *et al.* (2011) (23), when assessing the distribution of collagen fibers in the wraps, observed that the exercise promoted a reduction in the proportion of type I collagen, which is mainly responsible for the rigidity and fibrotic characteristics of the tissue. In this sense, the remodeling promoted by the modality, in addition to reducing the amount of connective tissue, promotes increased flexibility and malleability. Also, WBV has shown promise in reducing obesogenic and inflammatory parameters in humans and improved insulin resistance. According to the authors, these results, which were also achieved in eight weeks, are largely driven by the reduction of body fat through the increase in fat oxidation promoted by the WBV (24).

The use of WBV in obese humans has beneficial effects on the modulation of body mass, promoting a reduction in body weight and fat deposits. Also, it promotes increased muscle mass and, consequently, increased muscle strength. The main mechanism of action of WBV on skeletal muscle involves the activation of neuromuscular spindles and α motor neurons (25). In this sense, the WBV promotes involuntary contractions through the tonic vibratory reflex. These stimuli promote, in addition to the induction of muscle protein synthesis and neural activation, modulation of cardiovascular activity, increased lipid mobilization, paracrine and endocrine activation (7, 26). The reduction in the cross-sectional area of CTL-WBV and MSG-WBV animals may be related to the muscle used (27). Although in the present study we presented a reduction in the size of the muscle fiber, other studies with the soleus muscle, an agonist of this movement, showed improvement in the cross-sectional area of animals trained with the same protocol (11).

Melo *et al.* (2019) (28) state that, unlike the safety limit parameters in the work environment, the use of therapeutic vibration, that is, by medical devices, is not yet standardized. According to the parameters of their revised protocols, and following the review by Zago *et al.* (2018) (26), in humans, an average of 15 minutes of daily exposure, 30-50 Hz and magnitude from 2.25 to 7.98 grams, amplitude around 2 millimeters (mm) with a gradual increase in intensity during the intervention, can be safe, with positive effects for that population. On the other hand, data regarding the use of this therapeutic modality in obese rats is limited. In the work by Boaretto *et al.* (2020) (5), using the same protocol as the present study, the soleus muscle also showed a decrease in the cross-sectional area in the sedentary and obese groups submitted to the WBV. According to the authors, the result found was possibly due to the parameters used in the treatment protocol (alternate days per week, 60 Hz, 2 mm for 10 minutes), suggesting that higher frequencies are potentially harmful.

Despite the positive results found in the present study, the MSG model is a limitation, as it differs significantly from human obesity, which is mainly induced by excessive intake of caloric foods. In contrast, one of the strengths of the present study was the ability of the WBV exercise to promote

improvement in the structural condition of the anterior tibial muscle, even in a model of such significant changes.

CONCLUSIONS

The results of the present study showed that treatment with WBV-exercise was able to promote significant improvements in the muscle structure of obese MSG animals.

ACKNOWLEDGMENTS

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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The Effect of Heel Height of Shoe on Ankle Muscle Activation Pattern in Women with Functional Ankle Instability during Stair Descending

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SUMMARY

Background. Wearing high-heeled shoes (HHS) increases the risk of musculoskeletal disorders, especially in unstable situations. This study was conducted to evaluate the electromyographic (EMG) activity of the ankle muscles during stair descent with HHS in women with and without functional ankle instability (FAI).

Methods. In this experimental study, Thirty-six subjects were surveyed in 4 groups: The group 1: Ten non- habitual HHS wearer with FAI, group 2: Eight habitual HHS wearer with FAI, group 3: Ten non-habitual HHS wearer without FAI, and group 4: Eight habitual HHS wearer without FAI. The EMG activity was obtained from the tibialis anterior (TA), peroneus longus (PL), and gastrocnemius medialis (GM) muscles during stair descent with HHS and barefoot.

Results. In non-habitual and habitual FAI group, onset latency of the TA and PL muscles was longer in the HH compared to barefoot. However, in healthy groups, onset latency of the selected muscles was longer only in the non-habitual healthy subjects. In the non-habitual FAI group there was a significant increase in TA and PL muscles onset latency compared to that in the non-habitual healthy group. EMG activity of the TA, PL and GM muscles were significantly greater in the HHS group compared to the barefoot in FAI and healthy groups during stair descending.

Conclusions. The findings of this study suggest that wearing HHS may put people with FAI and without the experience of wearing HHS at greater risk for developing ankle sprains.

KEY WORDS

Functional ankle instability; surface electromyography; high-heeled shoes; stair walking.

INTRODUCTION

Wearing high-heeled shoes (HHS) makes walking unstable (1, 2) and may place individuals at greater risk for imbalance and falling (3, 4). Excessive ankle plantar flexion due to wearing HHS, changes in kinematics, and kinetics of walking (3, 5, 6). Long-term use of HHS limits ankle range of motion (7) and changes the ankle muscle activation pattern (8). It also could increase the muscular activation and loading, which in turn can accelerate muscle fatigue and joint instability (1, 2, 5-7). Wearing HHS could be more likely harmful during less stable situations such as walking on uneven surfaces or stair ascending- descending.

Stair walking is a common activity of daily life that demands greater muscle activation of the lower extremities compared with level walking (9, 10). During Stair ascending-descending, single-leg support time is longer (11), and it needs more muscle control and activation. So, fatigue and discoordina-

tion of the muscles may subsequently happen. This condition could raise the risk of imbalance and fall, particularly with HHS (12, 13). In fact, the excessive plantar flexion coupling with the foot inversion, especially in stair descent, is likely to increase the possibility of instability and lateral ankle sprain with HHS (14).

Most of the studies that investigated the stair walking activities with HHS, have been focused on healthy individuals. Though millions of women wear HHS only a small number of them get involved in ankle injuries or falling (1). Therefore, the purpose of this study was to determine the effects of wearing HHS on electromyography (EMG) activity of the ankle muscles during stair descent in women with and without FAI and with various experiences of wearing HHS. Two hypotheses were tested: 1) during stair descending with HHS, people with FAI would show different muscle activation pattern when compared with healthy subjects, 2) during stair descending with HHS, habitual HHS wearers would show different muscle activation pattern when compared with non-habitual HHS wearers.

MATERIALS AND METHODS

Subjects

Study design

Through a public announcement, 36 women (age range, 20-33 years) with recurrent chronic lateral ankle sprain, were recruited in this cross-sectional study. Volunteers were included if they had more than one ankle sprain in their dominant leg within the last year, and with the initial incidence more than one year before the study. Self-reported functional conditions determined and confirmed by foot and ankle ability measure (FAAM) questionnaire, and participants with the score of less than 85% were recruited (14).

The sample size of the study was estimated utilizing primary information from a pilot study on the ankle sprain in two different heights of shoes (mean1 = 1.88, SD1 = 0.68; mean2 = 0.81, SD2 = 0.51). Taking into account 80% power, 95% confidence, a two-tailed test the sample size was calculated to be at least 7 subjects per group, by G-Power Software (15). Considering a dropout rate of about 20%, the sample size increased to 9 participants per group.

All subjects completed a questionnaire consisting of their demographic information, medical history, and the time period they wore HHS in the past six months. Subjects were allocated to 4 groups based on the ankle health status (FAI or healthy) and the frequency of using HHS (non-habitual or habitual). The inclusion criteria for the FAI were a history of more than one ankle sprain in their dominant leg and the score of less than 85% in FAAM (14). Group one consisted of 10 subjects with FAI and experience of wearing HHS less than 10 times, each less than two hours, in the past 1 year (non-habitual FAI). Group 2 consisted of 8 subjects with FAI who habitually wore HHS at least 2 times a week, every 4 hours in the past 1 year (habitual FAI). Group 3 consisted of 10 subjects with healthy ankles and similar experience in using HHS as group one (non-habitual healthy). Group 4 consisted of 8 subjects with healthy ankles and similar experience in using HHS as group 2 (habitual healthy). The minimum heel height of the shoes the subjects used was 5 cm. The healthy groups 3 and 4 were matched according to age, height, weight, body mass index (BMI), and dominant leg with the FAI groups 1 and 2, respectively. The exclusion criteria were pregnancy, any neurological or musculoskeletal symptoms in the three months prior to data collection and any dysfunction which may preclude them from safe ambulation in shoes with up to 8 cm high heel, obese subjects (BMI> 30), any history of surgery in the musculoskeletal structures of the lower extremity or back, a history of fracture in the lower extremity, circulatory disorder, and serious joint conditions such as rheumatoid arthritis and osteoarthritis.

This study was approved by the regional ethical committee of Tabriz University of Medical Sciences, Code number: IR.TBZMEED.REC.1399.489. All volunteers were explained the study procedure and signed a written informed consent if they were agreeing to participant. The study was conducted according to the international ethical standards as well as the ethical principles of the Muscle, Ligaments and Tendons Journal (16).

Equipment

Electromyography signals were recorded from the tibialis anterior (TA), peroneus longus (PL), and gastrocnemius medialis (GM) using surface EMG equipment (ME6000, Mega electronics Ltd, Finland). The EMG electrodes (Ag/AgCl) were placed over the muscle bellies and aligned with the longitudinal axis of the muscles with a center-to-center distance of 2 cm. One reference electrode was placed over the lateral malleolus. The cables and interfaces were shielded to eliminate interference. Raw EMG signals were recorded at the sampling rate of 1,500 Hz, amplified (differential amplifier, CMRR = 96 dB, gain 1,000, noise < 1 uV), filtered (using Butter-worth filter, effctive band-width 1–500 Hz), converted with A/D board of 14 bit, stored in a PC computer, and analyzed by Megawin software.

Data processing

To determine the onset latency of each muscle, a threshold of two standard deviations from the mean value (mean

+ 2 SD) observed at baseline was calculated using Megawin software. The onset points were controlled visually to confirm the validity of the calculated points. The relative differences in the onset times between each muscle and the first activated muscle (*i.e.*, GM) were calculated. All EMG data were averaged over 3 repetitions of each test. Root mean square (RMS) of raw EMG signals calculated with 2ms sliding window and EMG signal amplitude of each muscle was expressed as a percentage of the 3 s maximum RMS values obtained during a maximal voluntary isometric contraction (MVIC) test for 5 s.

Subject preparation and procedure

Prior to data collection, the subjects performed several practice trials to get familiar with the task. Each subject was provided footwear in their respective shoe size and completed two practice trials for each HH to check for proper shoe fit and comfort. Subjects' skin was then prepared for electrode placement by shaving, cleaning with 70% alcohol, and slightly abrading the area with fine sandpaper. Electrode placement followed the guidelines as reported by Perotto *et al*. as follows (17):

- TA: the electrodes were placed four finger-breadths below the tibial tuberosity and one finger-breadth lateral to the tibial crest.
- PL: the electrodes were placed three finger-breadths below the fibular head directed toward the lateral aspect of the fibula.
- GM: the electrodes were placed as far as a palm below the popliteal fold on the medial side of the calf muscle. The electrode placement is presented in **figure 1**.

Figure 1. Electrode placement with high heeled shoe. Tibialis Anterior (TA): four finger-breadths below the tibial tuberosity and one finger-breadth lateral to the tibial crest, Peroneus longus (PL): three finger-breadths below the fibular head directed toward the lateral aspect of the fibula. Gastrocnemius medialis (GM): as far as a palm below the popliteal fold on the medial side of the calf muscle.

Raw EMG data were rectified, smoothed, and filtered prior to processing. After rectification, the EMG signal amplitude was normalized to the maximal activity level recorded during the trial. For normalization purposes, before the performance of the test, 5 s of the EMG data were recorded three times for each muscle, while the subjects performed MVIC. For the TA muscle, the subjects were in a supine position and performed maximum dorsiflexion with inversion, with the hallux in neutral position (not extended). The PL muscle was also assessed in supine position with the subjects performing eversion with plantar flexion. The GM muscle was assessed with the subjects in a prone position performing maximum plantar flexion.

EMG signals of the muscles were recorded at the dominant leg in healthy groups and at the injured dominant leg in FAI groups. The experimental staircase consisted of three steps (step height 18 cm and tread length 30 cm). The subjects stood on the third step of the staircase and were instructed to descend the stairs at a self-selected pace with barefoot and then with HHS (8 cm heel height with 3.5 cm diameter of the heel for each shoe), placing only one foot on each step (step-over-step) with arms freely moving at their sides. During stair descent, the stride cycle was defined by the dominant foot beginning at foot contact on the third step and ending at subsequent contact of the same foot on the first step. Each trial was performed three times, with the maximum amplitude of trials being averaged. To avoid muscle fatigue in the lower limbs, a 2-minute rest period was given between each trial.

Statistical analysis

In this study, according to the correlation structure among measurements, a Mixed Model test and an estimation procedure of Restricted Max Likelihood (REML) was utilized with the help of a first order autoregressive covariance structure (AR (1)). The age, weight, height, BMI data were expressed as the mean ± SE, and analysis of variance (ANOVA) and chi-square tests for dominant leg were performed to identify statistically significant differences between groups. The

Table I. Baseline demographic characteristics (mean ± SE) of the groups.

		Non-habitual FAI (n = 10)	Habitual FAI (n = 8)	Non-habitual healthy (n = 10)	Habitual healthy (n = 8)	
Age (y)		23.10 ± .57	23.88 ±1.18	22.70 ± .47	24.00 ± .95	0.607
Weight (kg)		59.50 ± 2.39	62.75 ± 1.68	57.00 ± 1.88	59.25 ± .89	0.218
Height (cm)		163.70 ± .97	164.88 ± 1.23	163.70 ± 1.57	160.75 ± 1.31	0.191
BMI		22.02 ± .73	23.04 ± .43	21.18 ± .6	23.00 ± .54	0.104
Dominant leg	R	7 (70%)	6 (75%)	7 (70%)	6 (75%)	0.974
	L	3 (30%)	2 (25%)	3 (30%)	2 (25%)	

significant level of P = 0.05 and confidence interval of 95% were taken into account by applying SPSS software, version 16 to analyze the data.

RESULTS

Table I displays the baseline demographic characteristics of the subjects of the present study. No statistically significant differences were found among the groups regarding the demographics.

EMG onset time

The overall EMG onset latency during stair descent is illustrated in **figure 2 a, b**. In FAI groups (non-habitual and habitual FAI) and healthy subjects (non-habitual and habitual healthy). In FAI group, the onset latency of the TA and PL muscles was longer in the HHS wearers compared to barefoot during stair descent ($p < 0.0001$).

However, in healthy groups, onset latency of the TA and PL muscles was longer only in the non-habitual healthy subjects ($p = 0.001$) with no significant increase in onset latency in habitual healthy subjects ($p > 0.05$).

In the non-habitual FAI group, there was a statistically significant increase in TA ($p = 0.002$) and PL ($p = 0.001$) muscles onset latency compared to that in non-habitual healthy group. The non-habitual FAI group displayed a significant increase in muscle onset latency for TA ($p = 0.002$) and PL ($p = 0.022$) muscles compared to the habitual FAI group.

EMG amplitude

Average RMS values of the TA, PL and GM muscles activity was found to be statistically greater in the HHS group compared to the barefoot in FAI and healthy groups when descending the stairs ($p < 0.0001$).

No significant differences were noted between habitual and non-habitual groups (healthy and FAI) for average RMS values in selected muscles during stair descend ($p > 0.05$).

A comparison of RMS of EMG signals among all groups illustrated in **figures 3, 4, 5**.

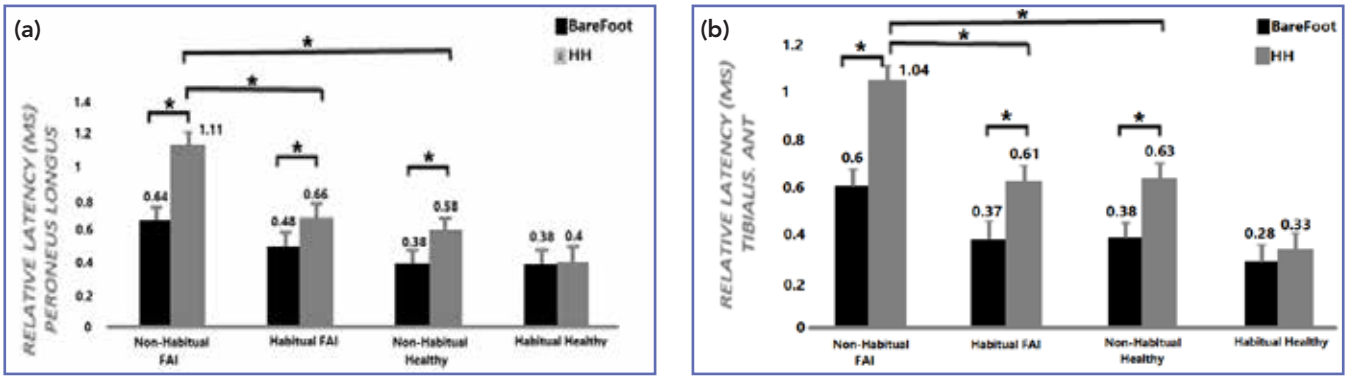

Figure 2. Between group changes (mean ± SE) in relative latency of the TA and PL. **(a)** Between group changes (mean ± SE) in relative latency of the TA muscle compared to GM muscle during stair descent. **(b)** Between group changes (mean ± SE) in relative latency of the PL muscle compared to GM muscle during stair descent.

*Significant difference between two groups ($p \leq 0.05$) according to Mixed Model test.

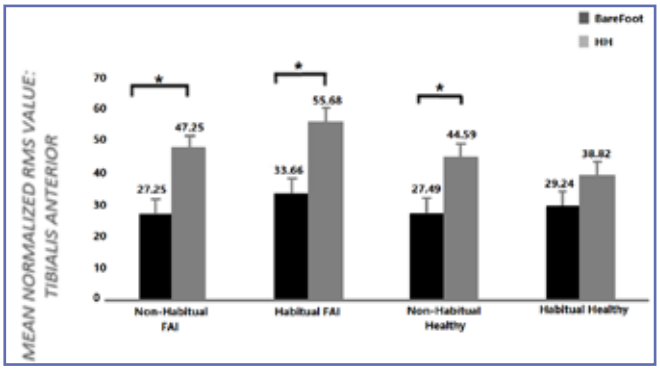

Figure 3. Between group changes (mean ± SE) in normalized RMS values (mean ± SE) of the TA muscle during stair descent.

*Significant difference between two groups ($p \leq 0.05$) according to Mixed Model test.

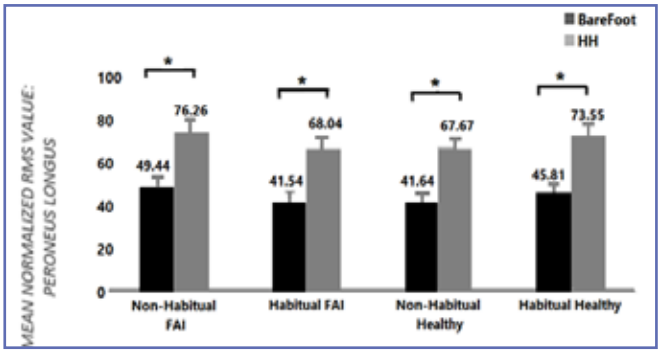

Figure 4. Between group changes (mean ± SE) in normalized RMS values of the PL muscle during stair descent.

*Significant difference between two groups ($p \leq 0.05$) according to Mixed Model test.

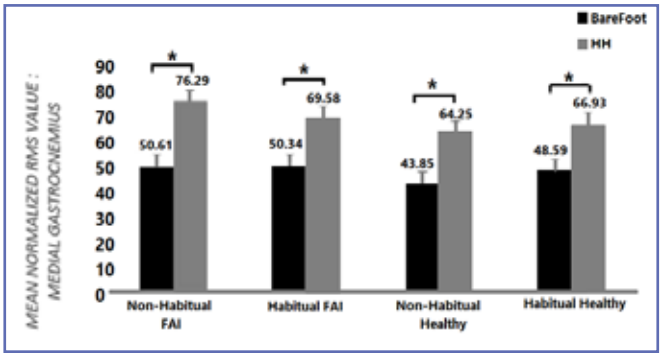

Figure 5. Between group changes (mean ± SE) in normalized RMS values of GM muscle during stair descent.

*Significant difference between two groups ($p \leq 0.05$) according to Mixed Model test.

DISCUSSION

Stair walking with HHS is an unstable situation which places the individual at greater danger of imbalance and falling (10). Risk of ankle inversion injuries (14), especially in subject with FAI could be increase during stair ascending-descending. The aim of the present study was to investigate the effects of the wearing HHS on EMG activity of the ankle muscles in the patient with FAI and during stair descending. We also examined the effect of the HHS wearing experience on muscle activity pattern. The muscle onset latency and electrical activity of the TA, PL, and GM, were measured in healthy subjects and patients with FAI. The result revealed that the muscle onset latency of the selected muscles, increased in non-habitual FAI, habitual FAI, and non-habitual healthy groups during stair descent with HHS compared to the barefoot or low-heeled shoes. This change may contribute to the lengthening of the tibiofemoral lever arm resulted from the lengthening of the lower limb and altered ankle posture (11, 20).

The result was also revealed that irrespective of the experience of waring HHS, during stair descending the onset latency of the muscles in FAI groups was prolonged than that in the healthy subjects. According to the previous studies, in healthy group excessive plantar flexion and decrease in the base of support due to wearing HHS increase the ankle eversion moment and changes the activity pattern of the muscles to stabilizing the joint and foot arc during different phases of the gate (13, 18). However, ankle sprain could cause damage to the joint structures and create some changes in muscle mechanoreceptors and γ motor neurons. These changes may lead to a reduction of the muscle spindle sensitivity and augmentation of the muscle onset latency, which appears to be demonstrated by the present study (19, 20).

The study presented that muscle activation pattern in healthy, non-habitual HHS wearers was prolonged compared with that in habitual HHS wearers during stair descending. According to the Herzog *et al.* (2003), muscles are the primary contributors after alteration in lower limb biomechanics (21). Wearing HHS is associated with biomechanical changes in ankle structure, joint reaction force and center of pressure specially in stair descending (22). So, wearing HHS could alter the muscle activation pattern in HHS wearers (18). However, the habitual healthy group presented similar muscle activation pattern with HHS to the BF. It implies that long term use of HH, likely contributes to the constant feed-forward postural response to the central nervous system (CNS) (23, 24) and subsequently could change the body postural reaction. It seems that following longer use of the HHS, the CNS may adapt to the change of the footwear and could adopt different movement strategy

of the habitual HHS wearers (1, 25). In a study by Aljear *et al*. (2012), it was suggested that the controlled HHS walking in healthy habitual HHS wearers rely more on an increased movement variability, as a neural strategy which is adapted by the CNS to maintain balance (1).

In the present study, wearing HHS caused a significant increase of TA, PL, and GM muscle activation in both healthy subjects and the patients with FAI. In accordance with our findings, Kermani *et al*. (2018) and Arnadottir *et al*. (2011), showed similar results for the PL and GM muscle activation in healthy subjects (26, 27). Isokinetic measurement of the ankle muscle during wearing high heeled also presented strengthening of these muscles in HHS wearers compare to the barefoot (28). This increase in muscle activation is presumably related to the plantar flexion position of the ankle during activities with high heel. In fact, the ankle plantar flexion is mechanically an unstable situation, so the increased activation might overcome the instability while wearing HHS. Thus, increased muscle activity is expected partly due to less stability at the talocrural joint and the smaller body base of support while walking on high heel (26). Su *et al*. (2012), also reported increased TA muscle activity related to the decrease in stability, which is caused by wearing HHS during walking (29).

Increasing the muscle activation in patients with FAI during wearing HHS, may be associated with the lateral displacement of the body center of pressure and inversion of the ankle joint before and after heel strike. These changes are considered as important risk factors for ankle instability in patients with FAI (20). So, the increased activation of the TA and PL muscles were expected during unstable positions such as stair descent with HHS. This muscle strategy was also explained by Hopkins *et al*. (2012) for increasing the TA and PL activity during walking in patients with FAI (20). Accordingly, it has been shown that the limitation in the dorsiflexion in patients with FAI compensated by increasing the TA muscle activation (30). Wearing HHS did not significantly affect the muscle activation in patients with FAI compared with that in healthy subjects with no ready explanation by the authors.

No significant differences were noted between habitual and non-habitual HHS wearers during stair descent with regards to the muscle activation. Foster *et al*. (2012) and Stefanyshyn *et al*. (2012) studied the effects of long term use of HHS on the kinematics, kinetics, and muscle activity of the ankle joint. They evaluated only healthy habitual HHS wearers while wearing HHS during walking and found an increase in the PL muscle activity, which was similar to our study. They reported that in the presence of an inversion moment, there might be a greater demand on the ankle evertor muscle activation. Similar to our results, they showed no significant increase in the TA muscle activation during walking, possibly due to the fact that the inversion control was shared by the tibialis posterior muscle (5, 31).

In contrary to our findings, Cronin *et al*. (2012) and Alkjear *et al*. (2012) showed that the activity of the TA and Soleus muscles, increased in healthy habitual HHS wearer during walking. They linked their results to co-activation of the TA and Soleus muscle in habitual HHS wearers (1, 32). But in the present study, habitual healthy group don't display a significant increase in TA muscle activation. One possible explanation for this controversy could be the different height of the HHS and the walking task compared to the present research. Similar to our study, Ingrid *et al*. (2014) attributed the high recruitment of TA muscle in non-habitual HHS wearers to the improvement of instability caused by wearing heels (33).

Considering the points above, findings from this study and those reported previously indicate that some muscle activation changes occur with increases to heel height. This could indicate a greater likelihood of injury or pain in infrequent wearers of HHS, and when wearing HHS for the first time. However, further research is required to confirm this.

CONCLUSIONS

In conclusion, the findings of the current study identified that activation and onset latencies of the TA, PL, and GM muscles increased with wearing HHS in both healthy and patients with FAI. Therefore, HHS may put people with FAI and without experience of wearing HHS at greater risk for developing ankle sprains.

Limitation

There are some limitations in this study that can be improved. We did not use electro goniometer or accelerometer to definitely compare the onset times of muscles. So, we had to use the first activated muscle as zero point to estimate muscle latency. Comparing the affected limb with unaffected side in patients with FAI is another limitation in this study. We also did not test shoes with different diameters of the heel to the ground in participants. Future studies are suggested to overcome these limitations.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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The Effects of SARS-CoV-2 Pandemic Countermeasures on Patients Receiving Infiltrative Treatment for Musculoskeletal Disorders: a Study from an Italian Cohort

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SUMMARY

Background. COVID-19 outbreak had a massive worldwide impact and several countermeasures to contain its spread have been adopted, such as the interruption of non-urgent outpatient clinics. We wanted to describe the effects of the national lockdown on the well-being of a cohort of Italian patients with musculoskeletal disorders receiving infiltrative treatment.

Methods. 40 patients who received intra- or peri-articular treatment were surveyed using a structuralised questionnaire that assessed their well-being during the first national lockdown.

Results. 24 out of 40 patients (60%) did not come back after the re-opening of the Clinic because they were afraid of the pandemic. Of these 24 patients, the 83.3% noticed increased pain and stiffness in the joint. Due to the lockdown, the patient's quality of life was reduced by 61.66 ± 15.72%. After the lockdown, for the 92% of patients, the infiltrative treatment was perceived as more important than before, the 83% of patients perceived long-term security of infiltrative therapy availability as very important, and the 72% of the patients perceived the lockdown as inadequate.

Conclusions. The infiltrative outpatient clinic's interruption was seen to significantly worsen the physical condition of subjects with musculoskeletal disorders, with an important increase in both articular pain and stiffness. Therefore, any kind of infiltrative treatment suspension or delay should be avoided.

KEY WORDS

Infiltrative treatment; musculoskeletal disorders; SARS-CoV-2; COVID-19; Coronavirus.

INTRODUCTION

The COVID-19 (COronaVirus Disease-19) pandemic, caused by the SARS-CoV-2 (Severe Acute Respiratory Syndrome CoronaVirus 2), had a massive impact all across the world during 2020, and marked our lives in an indelible way, changing the reality we were used to (1).

Italy has been the first European country to be hit by the pandemic, and it has also been among the most affected in the world after China, with the highest number of reported cases in Europe during the first outbreak of the pandemic (2-4). Consequently, several countermeasures to control the COVID-19's spread were implemented, such as a general lockdown and the suspension of public hospitals' outpatient clinics (2). This interruption put under pressure almost all the healthcare sectors, such as the Physical and Rehabilitation Medicine (PRM) ones, and negatively influenced the access to care of patients affected by musculoskeletal disorders (such as osteoarthritis) who periodically receive intra- or peri-articular infiltrative treatment.

The intra- and peri-articular infiltrative treatment were proven to be safe and effective (5-7), and they are widely used for the treatment of several musculoskeletal disorders, such as arthritides, tendinopathies and fasciopathies, that can cause significant pain and functional limitation in patients, reducing their quality of life (QoL) and impairing their activities of daily living (ADL).

At our infiltrative outpatient Clinic, injections are usually practiced using corticosteroids, corticosteroids and anesthetics, hyaluronic acid, and collagen.
After the first outbreak of the pandemic, at the re-opening of the injection Clinic, infiltrative therapy was carried out following the guidelines launched by the I.S.Mu.L.T. (Italian Society of Muscles, Ligaments and Tendons), that highlighted the importance of selecting patients not affected by COVID-19 (for example recognizing some musculoskeletal symptoms such as fatigue, myalgia and arthralgia that have been related to the novel Coronavirus (8)), to practice injections by implementing all the most appropriate measures to protect healthcare workers and patients from contagion, and to guarantee the maximum sterility and safety during the injection procedure (9). Furthermore, several studies showed how corticosteroid injections are safe and could be performed during the pandemic since they are not associated with a higher infection rate compared to the general population (10-13).
Infiltrative therapy has short-term effects on articular pain and stiffness, so it must be cyclically repeated, approximately every 6-12 months (depending on the underlying condition and on the used drug).
For this reason, we hypothesized that the suspension of infiltrative therapy due to the COVID-19 countermeasures could have an important impact on the physical condition of patients with musculoskeletal disorders, causing the reduction of their autonomy in complying with the ADL.
Therefore, the aim of this study is to describe how the countermeasures for COVID-19 influenced the well-being of a cohort of Italian patients with musculoskeletal disorders receiving intra- or peri-articular infiltrative treatment.

Countermeasures against Covid-19

The President of the Italian Republic enacted a decree on the 23 February 2020 to contain the SARS-CoV-2 contagion, stating that "urgent measures on the containment and management of the epidemiological emergency due to COVID-19" were needed (14). Following this national ordinance, the Campania region (with Naples being its capital and biggest city), which was one of the first regions in Italy to adopt all the measures against the COVID-19 spread, promulgated a regional regulation on the 5 March 2020 that established the immediate interruption of all the non-urgent outpatient activities provided by all hospitals (both public and private) until the 18 March 2020 (15).
The University Hospital Federico II of Naples, where our intra- and peri-articular injection treatment outpatient Clinic is based, and where this study was conducted, followed the regional regulation of the 5 March 2020 and then prolonged the suspension of deferrable outpatient activities until the 3 May 2020 (15).
From the 5 May to 17 June 2020, despite the re-opening of some outpatient Clinics, our intra- and peri-articular infiltrative treatment's outpatient Clinic was not operative since patients rejected to immediately resume the infiltrative treatment, being afraid of an increased risk of SARS-CoV-2 infection in the hospital setting.

MATERIALS AND METHODS

Design

This study is a survey on the lockdown's effects due to the SARS-CoV-2 pandemic on patients receiving intra- or peri-articular injections therapy. The survey is based on telephone interviews using a structuralised questionnaire (**table I**) like the one created by Dressler and Adib Saberi to assess the reduction of the quality of life of patients receiving botulinum toxin therapy during the first outbreak of the COVID-19 pandemic (16). Their questionnaire was modified to the aims of our study. The telephone calls were made in May 2021.
The study meets the ethical standards of the journal (17). This study was carried out following the guidelines given by the local ethics committee. All information from patients were treated anonymously, and all data were saved on a laptop which access password was given just to the authors involved in the study.

Treatment institution

The study was performed at the Rehabilitation Unit, Department of Public Health, University of Naples Federi-

Table I. Structuralised questionnaire to survey the effects of the anti-coronavirus lockdown on patients receiving infiltrative treatment.

1. Increased articular pain: YES, NO
2. Increased articular stiffness: YES, NO
3. Reduction of quality of life (from 0 to 100%): _____
4. Change of perception of the infiltrative treatment caused by lockdown: MORE IMPORTANT THAN BEFORE, NO CHANGE. IN PERCEPTION, LESS IMPORTANT THAN BEFORE
5. Perception of long-term security of the infiltrative treatment: IMPORTANT, VERY IMPORTANT, LESS IMPORTANT
6. Perception of the anti-coronavirus lockdown: ADEQUATE, NOT ADEQUATE

co II, Naples, Italy. Two Resident Doctors (D.T., R.G.) and two Professors of Medicine (F.S., B.C.) were involved in the study. In 2019, a total of 394 intra- or peri-articular injections were performed, while in 2020, due to the lockdown and the subsequent closure of the infiltrative treatment's outpatient Clinic, only 182 injections were performed.

Patients

Patients were retrospectively collected from the Department's database. A total of 40 patients were recruited for this study. The age range was 42-82 years. The inclusion criteria were the following: 1) patients who came once for treatment from October 2019 to the beginning of the first lockdown (8 March 2020), but then did not come back to the Clinic after its re-opening; 2) patients who cyclically came to the Clinic in 2019, but then did not return in 2020 at all; 3) patients who cyclically came to the Clinic from 2019 to the beginning of the first lockdown (8 March 2020) and then returned in 2020 but after a while since the re-opening of the Clinic.

The first inclusion criteria were established from October 2019 to the beginning of the first lockdown because it was a 6-month interval, so it was reasonable that patients who had their first cycle of treatment during that period, could then come back during the lockdown period.

The participation of patients in this study was entirely voluntary, and none of the invited patients declined their participation.

Intra- or peri-articular treatment

Intra- or peri-articular infiltrative treatments were performed using corticosteroids, corticosteroids and anaesthetics, collagen and hyaluronic acid. The musculoskeletal conditions treated were shoulder arthritis, rotator cuff tendinopathy, partial rotator cuff tear, epicondylitis, wrist arthritis, sacroiliac joint arthritis, hip arthritis, knee arthritis, Achilles tendinopathy, and plantar fasciitis.

Inter-injection intervals were usually settled between 6 to 12 months, and the choice of the most appropriate drug was tailored on patient's needs.

For the treatment of the arthritides, corticosteroids, corticosteroids plus anaesthetics, and hyaluronic acid were used. For the treatment of tendinopathies and fasciopathies, collagen was used.

Table II and **table III** report the treated anatomical regions, and how many intra-or peri-articular injections (and related used drug) were performed in 2019 and 2020, while in in **table IV** patient demographics are reported.

RESULTS

A summary of the results of the present survey is reported in **table V**.

Of the 40 patients selected for the study, the 5% did not answer the telephone. The 20% of patients said that they did not come back after the re-opening of the Clinic since they felt good after the first cycle of treatment. The 15% of patients did not have any benefits after the intra- or peri-articular infiltrative treatment, so they did not want to come back to the Clinic.

24 of 40 patients (60%) did not come back after the re-opening of the Clinic because they were afraid of the COVID-19 pandemic.

For the 83.3% of patients who did not come back to the Clinic, pain and stiffness in the joint increased, for the 8.3% only pain increased (but not stiffness), and for another 8.3% only stiffness increased (but not pain).

Due to the lockdown, the patient's quality of life was reduced by 61.66 ± 15.72% (range 30%-80%).

Table II. Anatomical regions treated and number of injections performed in 2019.

- ***Shoulder:*** 14 corticosteroid and anaesthetic, 20 corticosteroid, 6 hyaluronic acid, 128 collagen
- ***Sacroiliac joint:*** 4 corticosteroid and anaesthetic
- ***Hip:*** 2 corticosteroid, 50 hyaluronic acid
- ***Knee:*** 16 corticosteroid and anaesthetic, 18 corticosteroid, 122 hyaluronic acid
- ***Foot:*** 14 collagen (8 for plantar fasciitis, 6 for Achilles tendinopathy)

Table III. Anatomical regions treated and number of injections performed in 2020.

- ***Shoulder:*** 16 corticosteroid and anaesthetic, 22 corticosteroid, 16 hyaluronic acid, 30 collagen
- ***Elbow:*** 6 collagen (for epicondylitis)
- ***Wrist***: 12 corticosteroid and anaesthetic (for the trapeziometacarpal joint)
- ***Sacroiliac joint:*** 6 corticosteroid and anaesthetic
- ***Hip:*** 6 corticosteroid and anaesthetic, 8 hyaluronic acid
- ***Knee:*** 4 corticosteroid and anaesthetic, 44 hyaluronic acid
- ***Foot:*** 10 collagen (for Achilles tendinopathy)

Table IV. Patient demographics and administered doses.

Total number of patients [n]	40
Male patients [n]	28
Female patients [n]	12
Patient age in 2021 (mean ± standard deviation) [years]	64.55 ± 11.62
Injections performed in 2019 [n]	394
Injections performed in 2020 [n]	182
Reduction in performed injections between 2019 and 2020 [%]	54

After the lockdown, for the 92% of patients, the infiltrative treatment was perceived as more important than before, while for the 8% there was no change in perception.
The 83% of patients perceived long-term security of infiltrative therapy availability as very important, 17% as important and none as less important.
72% of the patients perceived the lockdown as inadequate, while the 28% perceived the lockdown as adequate.

DISCUSSION AND CONCLUSIONS

COVID-19 pandemic caused the interruption of hospitals' outpatient clinics in order to contain its spread, especially among the most fragile patients. The COVID-19 pandemic had a negative effect on the care of patients with musculoskeletal disorders, since it is well known that all chronic musculoskeletal conditions (especially osteoarthritis) require a regular and accurate follow-up (18).
PRM clinical activities were heavily affected, negatively influencing the access to care of subjects who receive intra- or peri-articular infiltrative treatment.
Consequently, patients' quality of life during the lockdown period was reduced by 61.66 ± 15.72%. In the 92% of patients, the lockdown confirmed the perception of the importance of infiltrative treatment. The 83% of

Table V. Effects of the infiltrative treatment outpatient Clinic's lockdown on patients according to the administered questionnaire.

Symptoms caused by lockdown	
• Increased joint pain [% of patients]	8.3
• Increased joint stiffness [% of patients]	8.3
• Increased both of them [% of patients]	83.3
Reduction of quality of life caused by lockdown (mean ± standard deviation) [%]	61.66 ± 15.72
Change of perception of intra-articular treatment caused by lockdown	
• Therapy is more important than before [% of patients]	92
• No change [% of patients]	8
• Therapy is less important than before [% of patients]	0
Perception of long-term intra-articular treatment security	
• Very important [% of patients]	83
• Important [% of patients]	17
• Less important [% of patients]	0
Perception of lockdown	
• Inadequate [% of patients]	72
• Adequate [% of patients]	28

patients felt the long-term infiltrative treatment security as very important for their health condition, and the 72% reported that their patient rights were not respected during the lockdown. Therefore, the pandemic and the subsequent interruption of intra- or peri-articular infiltrative therapy for patients with musculoskeletal diseases caused a worsening of their physical conditions, with a marked increase in articular pain and stiffness.

One limitation of our study is the relatively small sample and the lack of a control group, but it should be emphasized that, to our knowledge, this is the first survey that assessed the impact of COVID-19 countermeasures on patients receiving intra- or peri-articular infiltrative treatment.

In conclusion, the results of our study highlighted how the injections outpatient Clinic's interruption in 2020 worsened the physical condition of subjects with musculoskeletal disorders, causing them a considerable increase in their symptomatology. The importance of intra- and peri-articular infiltrative therapy to treat these patients and the need to administer it regularly and without any important interruption is essential to not lose its clinical benefits, so any kind of treatment suspension or delay must be absolutely avoided.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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Evaluation of Quadriceps and Hamstring Muscles' Elastrographic Properties after Anterior Cruciate Ligament Reconstruction

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SUMMARY

Background. Tissue stiffness measurement by ultrasound elastography is commonly used for internal organs and tendons. In this study, rectus femoris, vastus medialis, vastus lateralis, biceps femoris, and semitendinosus muscles' stiffness of patients who underwent anterior cruciate ligament reconstruction surgery was followed up by ultrasound elastography.

Methods. 19 male recreational athletes aged between 18-40 years who had undergone anterior cruciate ligament reconstruction surgery with semitendinosus grafting method were included in the study. All patients received a standardized home exercise program and a standardized supervised rehabilitation program. Thigh muscle stiffness measurements were performed at post-operative first-week, first-month, second-month, and third-month.

Results. There was a significant difference between non-operated and operated knees at first measurement ($p < 0.05$). The second measurement was higher than the first, and the third measurement was higher than the second for both legs ($p < 0.05$). Rehabilitation process has increased muscle stiffness of both legs, and there was still a significant difference between non-operated and operated knees at third month ($p < 0.05$).

Conclusions. Post-operatively decreased thigh muscle stiffness increases with physical rehabilitation. Therefore, ultrasound elastography can be used as an additional follow-up tool. Moreover, theoretically it might be a return to sports criteria if baseline values are available.

KEY WORDS

Anterior cruciate ligament reconstruction; follow-up; muscle stiffness; rehabilitation; ultrasound elastography.

BACKGROUND

Anterior cruciate ligament (ACL) reconstruction is one of the most common surgeries in recent years. Although the surgical technique is critical for the success of the operation, well-structured rehabilitation is as crucial as surgery (1, 2). Despite numerous studies on this topic, postoperative anterior cruciate rehabilitation protocols and objective criteria for follow-up have not yet been standardized (3-5). Ultrasound elastography is a method used to show the structural properties of the tissue. This method measures

the hardness of the tissue in terms of quality and quantity (6). The tissue hardness value is expressed in kilopascals (7). Increasing evidence (8-11) shows that shear-wave elastography can be used to measure the mechanical properties of musculoskeletal tissue in clinical practice (**figure 1**).
This study aims to measure the hardness of the rectus femoris, vastus medialis, vastus lateralis, biceps femoris, and semitendinosus muscles by elastography after the rehabilitation of anterior cruciate ligament repair and to investigate whether it can be used as an objective criterion for follow-up.

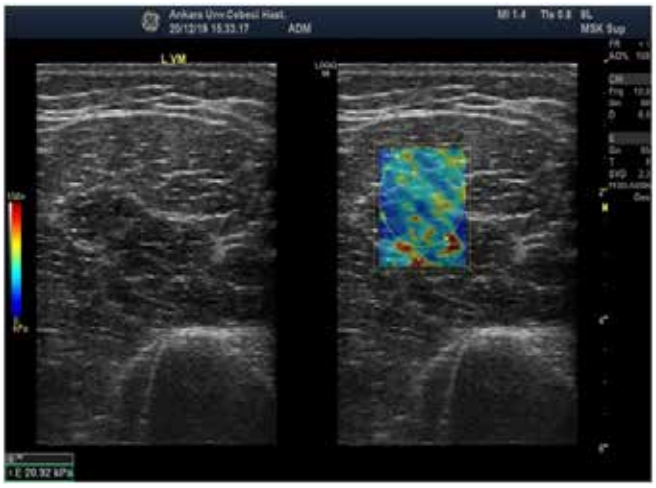

Figure 1. Elastographic evaluation of Vastus Medialis muscle.

MATERIALS AND METHODS

Study design and ethics approval

The study was designed as a prospective study to be held between April 2019 and August 2019. Participants were followed up at post-op first-week, first-month, second-month and third-month.
The study is approved by the Ethics Committee of Ankara University Faculty of Medicine (number: 05-410-19). The informed consents of all participants were obtained. The study meets the ethical standards of the journal (12).

Participants

The participants were recruited from the sports medicine outpatient clinic in Ankara University. 19 male recreational athletes aged between 18-40 who underwent ACL repair (with or without meniscal repair) were included in this study. The reconstruction methods other than the semitendinosus grafting method and patients with chronic illnesses (*e.g.* hypertension, diabetes mellitus, connective tissue disorders) were excluded.

Procedure

At the post-op first-week, elastography measurements, manual circumference measurements, and questionnaires were performed in the outpatient clinic, and home exercises were taught as a standard procedure to be applied until the third week. The patients started rehabilitation in our clinic in the third week. At the post-op first month, manual circumference measurements at mid-thigh and 5 cm superior to the patella, shear-wave elastography measurements (Logiq S8 XD Clear 2.0 Shearwave 9-12 Mhz linear probe, S., 2018) were performed. Also, International Knee Documentation Committee (IKDC) Score and LYSHOLM questionnaires were applied. The same procedures were repeated at the post-op second and third month. The measurements were made by a single person at the same time of the day, with 30 days between them, with clothes that would not tighten the thigh and with a similar level of fatigue.

Rehabilitation protocol

Rehabilitation protocol is presented in the **Supplements** section.

Elastography measurements

All elastosonography measurements were performed by a single musculoskeletal radiologist, who has ten years of experienc in the field, for five muscles around the thigh. In these measurements, the locations of the muscles were marked and muscle thickness was measured. The marking of the locations of the muscles was determined according to the Seniam Criteria (13). To achieve standardization, the points determined by anatomical markers were taken into consideration and the probe angle was placed perpendicular to the muscle orientation and skin as possible in 4 different muscles. For the vastus lateralis, measurements were obtained in the transverse-oblique plane, since the VL fibers in the distal 2/3 thigh were relatively oblique when the probe was positioned perpendicular to the skin.
3 measurements were taken from each region according to the literature(14) and their average was recorded. During the second and third measurements of the patient, the first USG images were simultaneously evaluated by the radiologist on a second computer screen. In consecutive USG examinations, it has been paid attention that the probe angle and the measurement area are the same as the first measurement area.

Questionnaires

The IKDC and LYSHOLM questionnaires are subjective scales that reveal the functional status of the knee. They were both adapted to Turkish (15, 16).

Statistical analyses

Statistical analyses were performed with the SPSS for Windows 20 package program. Spearman correlation coefficient was used to evaluate the elastography values of the operated and intact extremities and manual circumference measurements within themselves, and the Mann-Whitney U test was used to compare them with each other. The significance value for the results was accepted as $p < 0.05$.

RESULTS

Descriptives

The descriptive statistics for sociodemographic data are presented in **table I**.

Comparison of 1st, 2nd, and 3rd elastography measurements of rectus femoris, vastus lateralis, biceps femoris, and semitendinosus muscles

In the elastography measurements of these muscles, the second measurement for both knees was higher than the 1st and the 3rd measurement was higher than the second (**table II**). There was a statistically significant increase in both knees in the measurements ($p < 0.05$).

Table I. General characteristics of patients.

	Mean ± SD**	Minimum	Maximum
Age (years)	30.05 ± 7.84	18	40
Height (cm)	180.1 ± 5.63	171	189
Weight(kgs)	81.4 ± 16.38	57	108
BMI*(kg/m2)	25.1 ± 4.22	17.5	31.5

*BMI: Body-Mass Index; **SD: Standard Deviation.

Table II. Elastographic increase of the measured muscles.

	Operated knee (Mean ± SD*)			Non-operated knee (Mean ± SD*)		
	1st month	2nd month	3rd month	1st month	2nd month	3rd month
Rectus femoris	12.36 ± 3.14	14.63 ± 3.43	16.66 ± 4.46	14.66 ± 3.14	17.07 ± 3.73	17.59 ± 4.1
Vastus lateralis	6.04 ± 2.86	7.91 ± 3.20	8.98 ± 3.71	9.46 ± 4.44	10.54 ± 5.14	10.65 ± 5.01
Biceps femoris	15.44 ± 5.49	19.24 ± 5.49	20.08 ± 5.69	19.09 ± 7.16	21.73 ± 7.79	21.77 ± 7.77
Semitendinosus	16.05 ± 5.65	20.28 ± 5.72	21.37 ± 5.46	20.43 ± 5.85	24.56 ± 6.67	26.27 ± 7.07
Vastus medialis	11.58 ± 3.19	13.27 ± 3.52	15.06 ± 2.98	13.06 ± 2.95	16.07 ± 3.96	16.7 ± 4.29

*SD: Standard Deviation.

Comparison of 1st, 2nd, and 3rd elastography measurements of vastus medialis muscle

In the elastography measurements of the vastus medialis muscle, a statistically significant increase was found only in the operated knee ($p < 0.05$) (**table II**).

Elastographic increase of 5 different muscles in the operated knee

The hardness of 5 different muscles has increased by time (**figure 2**).

Figure 2. Elastographic Increase of 5 Different Muscles.

Manual circumference measurements

Circumference measurements over 5 cm above the patella and mid-thigh resulted in a statistically significant increase after rehabilitation ($p < 0.05$) (**table III**).

Table III. Circumference measurements of thigh in operated and non-operated knees.

	Operated knee (Mean ± SD*)			Non-operated knee (Mean ± SD*)		
	1st month	2nd month	3rd month	1st month	2nd month	3rd month
5 cm above patella	40.79 ± 3.55	41.97 ± 3.6	42.45 ± 3.58	42.74 ± 3.65	43.47 ± 3.8	43.92 ± 3.75
Mid-thigh	54.5 ± 6.12	55.95 ± 5.8	57.03 ± 5.42	56.68 ± 5.5	57.74 ± 5.5	58.53 ± 5.45

*SD: Standard Deviation.

Comparison of the 1st, 2nd, and 3rd measurements of IKDC and Lysholm Questionnaires

After rehabilitation, the second measurement was higher than the 1st and the 3rd measurement was higher than the 2nd in the questionnaires for both knees. These increases were statistically significant ($p < 0.05$).

Elastography-Questionnaire correlation

The correlation between elastography results and questionnaire scores after rehabilitation was not statistically significant ($p > 0.05$).

Percentage increase of 5 different muscles in the operated knee by elastography

After rehabilitation, the percentage increase between the 1st and 2nd measurements of the rectus femoris, vastus lateralis, biceps femoris, and semitendinosus muscles was higher than the percentage increase between the 2nd and 3rd measurements. For the vastus medialis muscle, this was found to be the opposite.

DISCUSSION

The most important finding of the study was the increase of thigh muscles' stiffness of patients who underwent rehabilitation. As percentage increased between the 1st measurement and the 3rd measurement, the highest increase was found in vastus lateralis. The semitendinosus muscle, which was used as a graft, was in the second row. The percentage increase between the 1st and 2nd measurements was higher than the percentage increase between the 2nd and 3rd measurements. So, it seems that ultrasound elastography might be a method for postoperative follow-up for anterior cruciate ligament reconstructed patients.

Circumference measurements made 5 cm above the patella and mid-thigh level, increased significantly in the follow-up. No statistically significant correlation was found between muscle stiffness and IKDC-Lysholm questionnaires, contrary to the study conducted by Zhang *et al.* (17). But, functional questionnaires include activities such as kneeling, squatting and jumping which are challenging activities for an early post-operative patient. Therefore, statistically insignificant increase in the questionnaires could be attributed to this.

The fact that the sample consisted of only males and that it included a relatively small number of patients can be considered as the limitations of the study. On the other hand, including patients with similar activity levels, all operated with the same technique and a close follow-up period for a relatively long time with a standard rehabilitation program can be considered as the strength of the study.

In a study by Zhang *et al.* (17), Achilles tendon elastography was performed at 12, 24, and 48 weeks after the operationon 26 patients who underwent Achilles tendon repair. There was an increase in elastography measurements in all measurements. In the same study, a statistically significant correlation was found between The American Orthopedic Foot and Ankle Score (AOFAS) and elastography results (17). While the current literature (7, 18-20) is mostly related to tendon elastography in the musculoskeletal field, our study shows elastographic changes in muscle groups and is thought to help identify deficiencies in the rehabilitation phase.

Andonian *et al.* (21) have shown the effect of endurance exercise on elastographic muscle measurement; 50 volunteers of marathon athletes were measured before, in the middle of the race, at the end of the race and 48 hours after the end of the race. Rectus femoris, vastus medialis and vastus lateralis muscles were examined. While the highest values were found in the measurements made in the middle of the run, it was observed that the stiffness values measured 48 hours after the end of the run returned to the pre-run levels. In the same study considering the mid-run values, the highest elastography values were obtained in the rectus femoris similar to our study. However, while the lowest values were obtained from the vastus medialis in that study, in our study it was obtained from the vastus lateralis.

Botanlıoğlu *et al.* (22) have compared patients with patellofemoral pain syndrome with a control group and it was found that the elastographic values of the vastus lateralis muscles were similar in both groups, but the vastus medialis' stiffness values of the patellofemoral pain syndrome group were significantly lower.

When these studies are evaluated together, it is thought that elastographic stiffness measurements of muscles may be a promising method in the future to follow rehabilitation progress, physiological or pathological variations and responses to certain type of exercises. Theoretically, if the baseline values for a person are available, it might be used as one of the criteria for return to sports, but more studies are needed to back up this idea.

CONCLUSIONS

With the progression of rehabilitation after anterior cruciate ligament reconstruction surgery, the stiffness of thigh muscles also increases, indicating that muscle stiffness may be a possible follow-up tool for patients.

ACKNOWLEDGMENTS

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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SUPPLEMENTS

Standardized home exercise program
First 3 weeks, repeated everyday.

Isometrics
Adduction with a towel between knees (30 repetitions, 5-10 seconds for each rep);
Adduction with a towel between ankles (30 repetitions, 5-10 seconds for each rep);
Extension with a towel under the knee (30 repetitions, 5-10 seconds for each rep);
Extension with a towel under the heel (30 repetitions, 5-10 seconds for each rep).

Straight Leg Raises (SLRs)
Hip Flexion (3 sets, 10 repetitions for each set);
Hip Extension (3 sets, 10 repetitions for each set);
Hip Abduction (3 sets, 10 repetitions for each set);
Hip Adduction (3 sets, 10 repetitions for each set).

Range of Motion Exercises
Assisted knee flexion with a towel (15 repetitions, 5-10 seconds for each rep);
Prone Hang for knee extension (total of 5 minutes with short breaks in between).

Standardized rehabilitation program in clinic
From 3rd week until 3rd month, repeated every other day.

Warm-up
With bicycle ergometer (5 minutes, 50 watts resistance and 50 rpm)

Straight Leg Raises (SLRs) with weights
Hip Flexion (3 sets, 10 repetitions for each set);
Hip Extension (3 sets, 10 repetitions for each set);
Hip Abduction (3 sets, 10 repetitions for each set);
Hip Adduction (3 sets, 10 repetitions for each set).

Resistance Band Exercises
Hip adduction (3 sets, 10 repetitions for each set);
Hip abduction (3 sets, 10 repetitions for each set);
Hip extension (3 sets, 10 repetitions for each set);
Hip flexion (3 sets, 10 repetitions for each set);
Hip external/internal rotation (3 sets, 10 repetitions for each set).

Open Kinetic Chain Exercises
Knee extension (beginning with assisted to active) (3 sets, 10 repetitions for each set);
Knee flexion (beginning with assisted to active) (3 sets, 10 repetitions for each set).

Closed Kinetic Chain Exercises
Squats (beginning with wall squats and box squats to active with weights) (3 sets, 10 repetitions for each set);
Leg press (3 sets, 10 repetitions for each set).

Proprioception Exercises
Standing on the wobble board with one and two legs (eyes open and closed) (30 seconds, 3 sets);
Half-squats on wobble board with double and one leg (eyes open and closed) (30 seconds 3 sets).

Gait Training
Walking forward, backwards and sideways for total of 10 minutes on treadmill.

Stretching
Hamstring Stretching (15 repetitions, 5-10 seconds for each rep);
Calf Stretching (15 repetitions, 5-10 seconds for each rep);
Quadriceps Stretching (15 repetitions, 5-10 seconds for each rep).

ORIGINAL ARTICLE

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Kinesiophobia Relates to Decreased Sports Capability Perceptions, and Altered Gait Following ACL Reconstruction

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LEVEL OF EVIDENCE: 2B

SUMMARY

Introduction. Kinesiophobia can negatively influence perceived knee function after anterior cruciate ligament reconstruction (ACLR). Less is known about its influence on perceived sports capability, and gait kinematics.

Methods. At 3 months after ACLR, 34 male patients completed the Tampa Scale of Kinesiophobia (TSK). Raw and grouped scores (group 1 = high kinesiophobia; group 2 = low kinesiophobia) were compared with perceived sports capability and gait kinematics over the initial 9 months after ACLR. The Tegner Activity Level Scale (TALS) was completed pre-injury, before ACLR, and at 6 and 9 months after ACLR. The Lysholm Knee Scoring Scale was completed pre-surgery, and at 6 and 9 months after ACLR. Walking and running gait analysis was performed at 3 and 6 months; and 6 months after ACLR, respectively ($P \leq 0.05$).

Results. Group TALS scores were similar pre-injury, however, Group 1 had lower TALS scores pre-surgery, and at 6 and 9 months after ACLR. Inverse relationships were observed for the 3 month TSK score and TALS scores before ACLR, and at 6 and 9 months afterwards. By 9 months following ACLR, kinesiophobia remained related to sports capability. Kinesiophobia had a stronger influence on side-to-side gait asymmetries than surgical side for hip adduction and mid-stance knee flexion when walking at 3 months after ACLR, for mid-stance knee flexion and terminal stance knee extension when walking at 6 months after ACLR, and for terminal stance knee extension when running at 6 months after ACLR.

Conclusions. High kinesiophobia following ACLR was related to walking and running gait kinematic characteristics and sports capability perceptions.

KEY WORDS

Knee joint; biomechanics; activity level; sports capability; fear avoidance.

INTRODUCTION

For athletically active patients, the primary reason for undergoing ACL reconstruction (ACLR) is to safely return to pre-injury sports participation (1). However, widely ranging return to sport rates (43%-93%) have been reported after ACL reconstruction (2-5). Successful return to sports after ACLR is influenced by multiple physical and psychological factors (6-8). Ardern *et al.* (2) reported that knee function at one-year after ACLR was not related to ultimate return to sports success. By 2 and 5 years after ACLR, Eriksson *et al.* (9) reported that neuromuscular, balance, and proprioception impairments continued to negatively influence lower extremity movements. Suboptimal neuromuscular recovery after ACLR is a contributing factor to increased ipsilateral knee re-injury or contralateral knee injury rates (10-12).

"Kinesiophobia" exists when a patient perceives excessive, irrational, and debilitating physical movement and activity fear due to perceived painful injury or re-injury vulnerability

(13). The Tampa Scale for Kinesiophobia (TSK) is a valid, reliable, and responsive kinesiophobia or movement fear perception measurement (13). This patient-reported questionnaire has a scoring range from 17-68 where higher scores indicate greater kinesiophobia (14). A TSK score of > 37 represents high kinesiophobia (15). The Lysholm Knee Scoring Scale, and the Tegner Activity Level Scale (TALS) are widely used patient-administered surveys for measuring perceived knee function, and sports capability, with minimal detectable change (MDC) values of 8.9, and 1, respectively (16-18).

An essential early rehabilitation plan component after ACLR is gait training. In a biomechanical comparison of 8 patients at 6 months following bone-patellar tendon-bone (BPTB) autograft ACLR, and 22 healthy subjects, Devita *et al.* (3) reported that patients had normal walking kinematics, however, stance phase hip extensor torque was increased to compensate for reduced knee extensor torque. Using kinematic and electromyographic methods to compare 25 patients with ACL deficiency prior to, and at 6 weeks, 4 months, 8 months and 12 months following BPTB autograft ACLR with 51 healthy subjects, Knoll *et al.* (4) reported that pre-injury walking gait kinematic restoration required at least 8 months.

While gait asymmetries are known to persist following ACLR, their relationship to kinesiophobia, knee function, and sports capability perceptions have not been fully elucidated. The purpose of this pilot study was to determine if patients with high kinesiophobia measured at the end of the acute care ACLR rehabilitation phase (3 months) had different walking (3 and 6 months) and running (6 months) gait kinematics, perceived knee function, and sports capability compared to patients with low kinesiophobia over the initial 9 months after ACLR.

MATERIALS AND METHODS

Subject selection

Following hospital medical ethics committee approval (SingHealth Institutional Review Board, Singapore), 34 consecutive male patients from a single institution who were about to undergo primary ACLR were recruited for study participation. Each subject provided written informed consent. All subjects had complied with the rehabilitation protocol. Revision ACLR, multi-ligament knee injury, subjects who displayed a grade 3, "explosive" pivot shift test prior to ACLR, or patients with co-morbidities other than partial meniscectomy or repair, or articular cartilage debridement were excluded from study participation (**figure 1**).

Surgical procedure

Femoral and tibial tunnels were individually prepared. All subjects received a four-strand hamstring autograft with cortical femoral fixation and tibial interference screw fixation with a back-up staple.

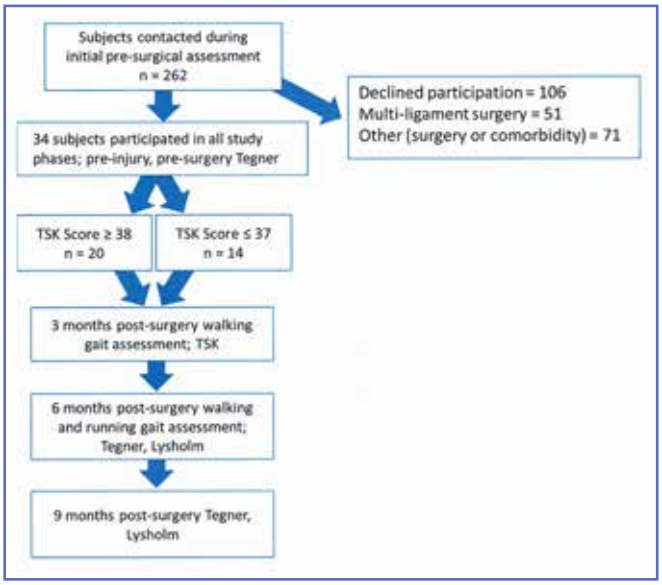

Figure 1. Subject recruitment and assessment progression.
TSK: Tampa Scale of Kinesiophobia.

Rehabilitation protocol

Over the first year following ACLR, patients participated in a standardized, three phase rehabilitation protocol: Acute care (0-3 months), strength training (4-6 months), and return to sports training (7-9 months). The protocol was adapted from previously reported guidelines (19, 20). Full release to unrestricted sports generally occurred near the end of the 9th post-surgical month. Most subjects initiated full weight bearing (FWB) walking during the first week after ACLR. Subjects who underwent concomitant meniscal repair or articular cartilage debridement initiated FWB 4 weeks following ACLR. Isometric quad-setting, and four-way progressive resistance straight leg raises were initiated during the first week. Mini-squats, and progressive resistance multi-directional lunges were added after FWB was initiated. At 3 months after ACLR, progressive resistance seated knee extension, hamstring curls, and single leg pressing was initiated. Stationary cycling, and elliptical device training were started at 3 weeks, and 8 weeks after ACLR, respectively. A jog-to-run progression was initiated at 4-5 months, when the surgical lower extremity achieved 80%, 10 repetition maximum bilateral resistance equivalency with the non-surgical lower extremity for seated knee extension (knee extensors), hamstring curls (knee flexors), and single leg press (composite lower extremity extension).

At 6 months following ACLR, progressive resistance hip abduction, single leg hip thrusts, hip extensions, squats, lunges, and neuromuscular core exercises were added. At 8 months after ACLR, progressive intensity plyometric jumping, and sudden directional change agility tasks were added. The study surgeon assessed knee laxity at 3, 6 and 9 months after ACLR using manual maximum Lachman and pivot shift tests. All subjects displayed normal (≤ 2 mm) anterior translational knee laxity and negative pivot shift tests over the course of the study.

Kinematic gait analysis

Two-dimensional walking (4 km/hr = 24 min/mile pace), and running (8 km/hr = 12 min/mile pace) kinematic gait analysis were assessed on a treadmill with digital video cameras placed on the same side of the treadmill as the surgical lower extremity (1.8 m away) facing the center (sagittal, camera #1), and at each end of the treadmill (anterior = 0.5 m away) facing the center (frontal, cameras #2) for motion capture (60 Hz) (SiliconCoach™ System, Tarn Group, Dunedin, New Zealand). With subjects wearing cycling shorts, 2.54 cm diameter skin markers with adhesive backing were placed on the shorts over the bilateral anterior (ASIS) and posterior (PSIS) superior iliac spines, the greater trochanter, lateral knee joint line, patellar center, and lateral malleolus of each lower extremity. After a 3 minute warm-up at volitional walking pace, a 30 sec duration data collection was performed for each condition.

Lower extremity joint displacements during gait were determined from the following angles.

- Camera #1*:* hip flexion-extension = midway point along the line between the ipsilateral ASIS-PSIS and the greater trochanter-mid-lateral knee joint line; knee flexion-extension = greater trochanter, mid-lateral knee joint line, lateral malleolus.
- Camera #2*:* hip abduction/adduction = intersection between the line formed by the bilateral ASIS, the stance lower extremity ASIS-patellar center. Two-dimensional kinematic joint angle measurements were performed by the primary investigator using the software cursor function (**figure 2**). Good measurement reliability has been reported using this system for the kinematic analysis of sports movement performance (21-23), and good-to-excellent reliability was identified for this study during pilot testing.

Self-reported patient outcome surveys

The Tampa Scale of Kinesiophobia (TSK) was administered at 3 months following ACLR at the end of the acute care rehabilitation phase (6-8). The Lysholm Knee Scoring Scale was administered before ACLR, 6 months (beginning of return to sports training phase), and 9 months (return to play decision-making phase) after ACLR. Patients were asked to retrospectively self-report their pre-injury TALS score based on perceived sports capability prior to sustaining the index ACL injury. Pre-surgery, 6 months (beginning of return to sports training phase), and 9 months (return to play decision-making phase) after ACLR TALS scores were collected as subjects self-reported their current highest sports activity level capability.

Statistical analysis

Shapiro-Wilk tests revealed data normality, therefore, parametric statistical tests were performed for continuous

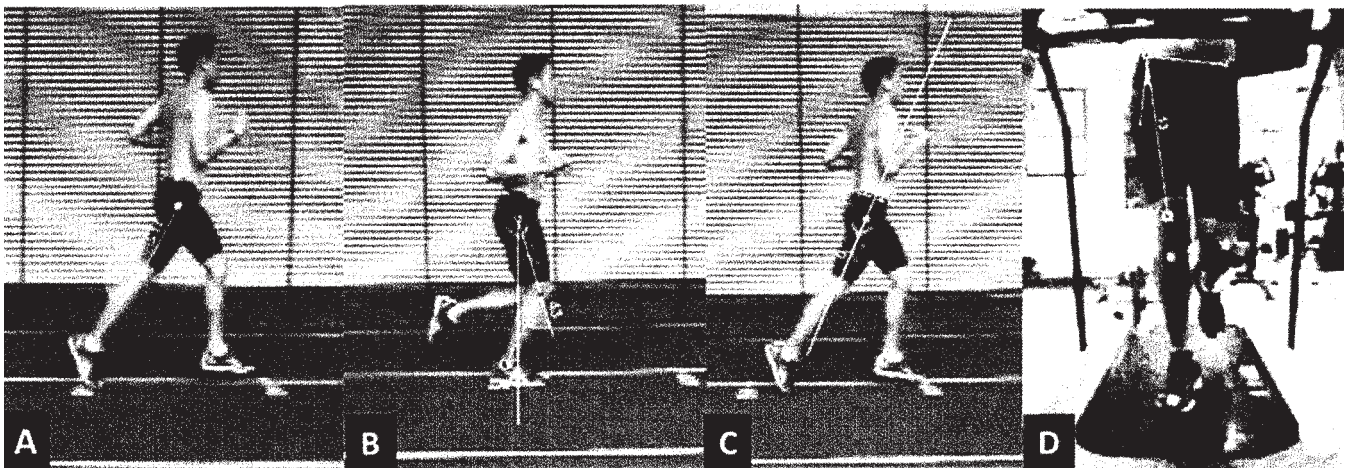

Figure 2. Two-dimensional gait analysis reference points. Knee extension at terminal stance (**A**), knee flexion at midstance **(B)**, hip extension at terminal stance **(C)**, hip adduction at midstance **(D)**.

data. A series of mixed model, two-way ANOVA (Kinesiophobia group, Side) were used to compare gait variables. Dependent sample t-tests were used to compare Lysholm Knee Scoring Scale score group differences. Chi-square tests were used to evaluate group frequency differences for the number of subjects who underwent concomitant partial meniscectomy, meniscal repair, or articular cartilage debridement, and to identify group TALS score response frequency differences. Pearson product moment correlations were used to more accurately delineate relationship strength between gait kinematic, and patient self-reported survey data (24). Statistical analysis was performed using SPSS ver. 26.0 software (SPSS-IBM, Armonk, NY, USA). An alpha level of $p \leq 0.05$ was selected to indicate statistical significance.

RESULTS

All subjects completed each study component. Overall subject age was 24.7 ± 6.9 years and body mass index (BMI) was 26.3 ± 6.9 kg/m^2. Kinesiophobia group subject demographics are displayed in **table I**. Although the high kinesiophobia group had greater Lysholm Knee Scoring Scale scores pre-injury compared to the low kinesiophobia group (64.2 ± 11.5 *vs* 57.1 ± 17.6, P = 0.03), this value did not meet the previously reported 8.9 MDC value (18), and statistically significant differences were not observed at 6 months (78.3 ± 15.8 *vs* 82.3 ± 15.6, P = 0.32), and 9 months (84.7 ± 14.2 *vs* 81.1 ± 13.9, P = 0.92) after ACLR.

Pre-injury TALS scores failed to reveal kinesiophobia group differences with both groups displaying scores indicating perceived recreational running, soccer, rugby or basketball playing capability. At pre-surgery, and at 6 and 9 months after ACLR the high kinesiophobia group had lower TALS scores with each group difference exceeding the one point MDC (18). Chi-square tests revealed that the high kinesiophobia group had significantly lower (3 *vs* 5, P = 0.03) pre-surgery TALS score frequencies suggesting that they only perceived light labor, or sports swimming capability, compared to the low kinesiophobia group who perceived heavy labor, competitive cycling, or recreational jogging capability. At 6 months following ACLR, TALS score frequencies remained lower in the high kinesiophobia group (4 *vs* 7, P = 0.04) suggesting that high kinesiophobia group subjects only perceived moderate labor, recreational cycling, or jogging capability, compared to the low kinesiophobia group who perceived the ability to play competitive tennis, running or handball, and recreational soccer, rugby, basketball or running. By 9 months after ACLR, TALS scores remained lower (6 *vs* 8, P = 0.04) in the high kinesiophobia group suggesting perceived sports capability of only being able to safely participate in recreational tennis, badminton, handball or jogging, compared to the low kinesiophobia group who perceived the ability to safely participate in competitive badminton, track and field athletics (including jumping), or racquetball.

Correlation analysis failed to identify significant relationships between Lysholm Knee Scoring Scale scores, kinematic gait measurements, or TSK scores. However, correlation analysis revealed consistently significant moderate-to-low inverse relationships between the 3 month TSK, and each TALS score (**figure 3**). Over the 9 month time period following ACLR, the TSK score measured at 3 months after ACLR (end of the acute rehabilitation phase) continued to influence sports activity capability perceptions (**figure 4**).

At 3 months following ACLR, walking kinematic gait analysis revealed that the high kinesiophobia group had greater peak stance phase hip adduction, and peak mid-stance phase knee flexion than the low kinesiophobia group (**table II**). At 6 months after ACLR, the high kinesiophobia group continued to display greater peak mid-stance phase knee

Table I. Subject demographics.

		Group 1 (High TSK)	Group 2 (Low TSK)	P
Study Entry	Age (years)	25.3 ± 6.9	24.0 ± 7.0	0.61
	BMI (kg/m2)	26.5 ± 8.0	26.1 ± 5.0	0.88
	# Partial Meniscectomy/Meniscus Repair Articular Cartilage debridement	3/2/3	2/2/2	0.64

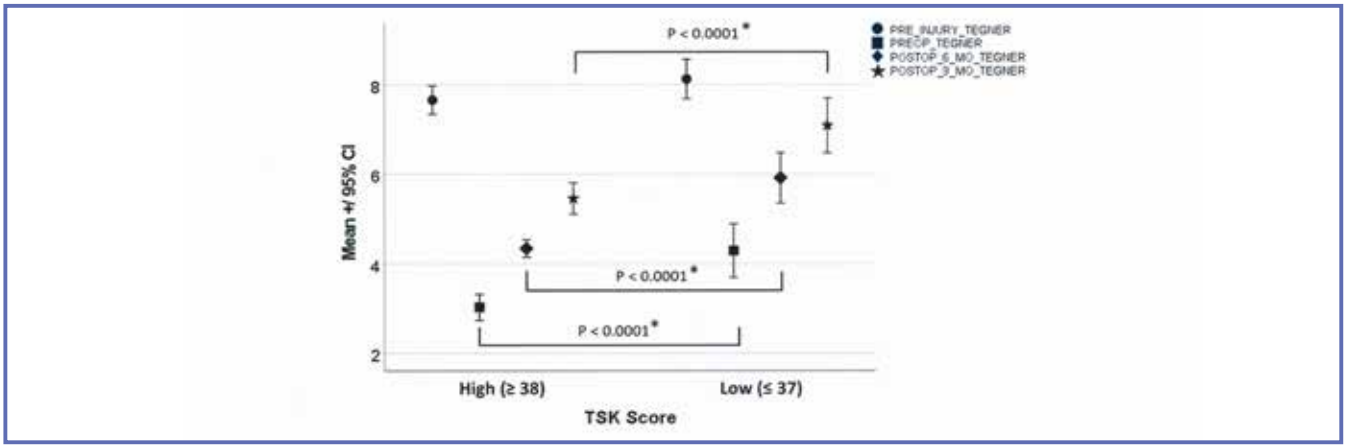

Figure 3. Although groups displayed comparable Tegner Activity Level Scale scores prior to the index ACL injury, the high kinesiophobia group had significantly lower scores prior to surgery and over the 9 month rehabilitation program.
TSK: Tampa Scale of Kinesiophobia; *p ≤ 0.05.

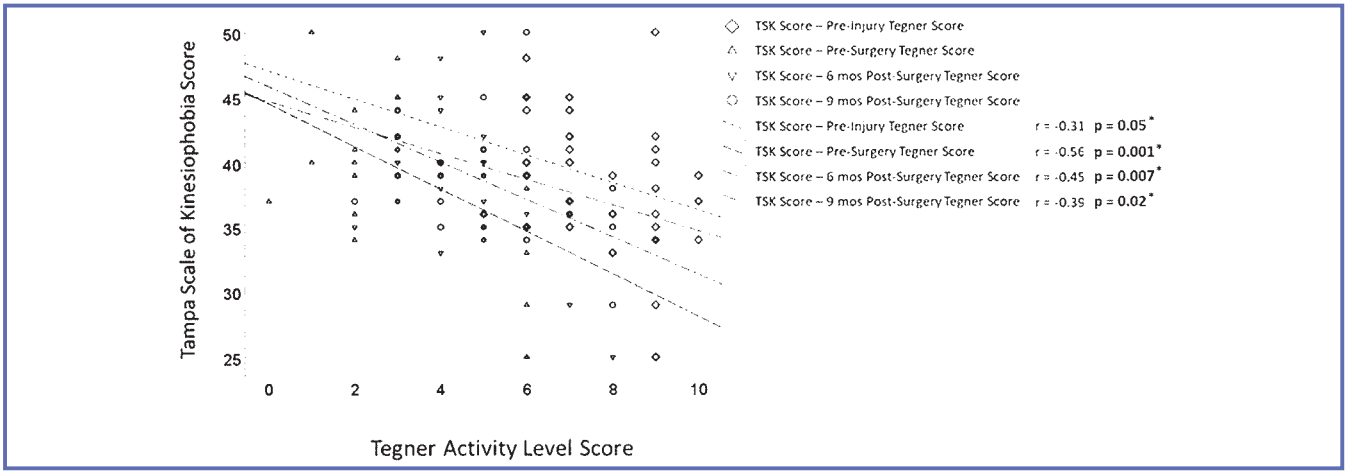

Figure 4. Significant inverse relationships existed between TSK score and Tegner Activity Level Scale scores over the 9 month study period.
TSK: Tampa Scale of Kinesiophobia; *p ≤ 0.05.

Table II. Three month walking kinematic gait comparison.

	3 Month Walking		Two-Way ANOVA		
	High TSK Group (surgical side/non-surgical side)	Low TSK Group (surgical side/non-surgical side)	Group	Side	Group × Side
Hip Extension at Terminal Stance	8.3 ± 3.4°/9.5 ± 3°	10.1 ± 3.6°/9.4 ± 4.8°	0.34	0.79	0.30
Hip Adduction at Mid-Stance	7.7 ± 2.3°/7.1 ± 2.3°	5.2 ± 2.8°/6.6 ± 2.4°	0.047*	0.51	0.45
Knee Mid-Stance Flexion	65.1 ± 20.4°/63.4 ± 23.4°	48.3 ± 13°/ 55.1 ± 10°	0.008*	0.58	0.35
Knee Terminal Stance Extension	- 31.6 ± 11.4°/- 28.7 ± 12.4°	- 27.7 ± 15°/-25.5 ± 9.1°	0.24	0.40	0.35
Step Length	48.9 ± 4.1 cm / 50.0 ± 3.9 cm	49.9 ± 5.6 cm / 48.6 ± 6.2 cm	0.91	0.96	0.32

*P < 0.05.

Table III. Six month walking kinematic gait comparison.

	6 Month Walking		Two-Way ANOVA		
	High TSK Group (surgical side/non-surgical side)	**Low TSK Group (surgical side/non-surgical side)**	**Group**	**Side**	**Group × Side**
Hip Extension at Terminal Stance	7.9 ± 4.6° / 9.1 ± 3.7°	7.6 ± 4.7° / 7.6 ± 3.3°	0.59	0.71	0.71
Hip Adduction at Mid-Stance	6.4 ± 3.4° / 6.3 ± 2.6°	4.8 ± 2.3° / 6.2 ± 4.1°	0.48	0.61	0.55
Knee Mid-Stance Flexion	59.8 ± 10.7° / 62.4 ± 6.9°	46.4 ± 10.9° / 57 ± 9.4°	0.018*	0.10	0.30
Knee Terminal Stance Extension	- 29.4 ± 11.5° / - 27.8 ± 10.1°	- 19.8 ± 10.8 / - 19.2 ± 12.8°	0.033*	0.71	0.99
Step Length	52 ± 5.7° / 52.3 ± 6°	54 ± 2.6° / 53.6 ± 4.2°	0.43	0.99	0.86

*P < 0.05.

Table IV. Six month running kinematic gait comparison.

	6 Month Running		Two-Way ANOVA		
	High TSK Group (surgical side/non-surgical side)	**Low TSK Group (surgical side/non-surgical side)**	**Group**	**Side**	**Group × Side**
Hip Extension at Terminal Stance	8.3 ± 3.4° / 8.5 ± 4.5°	9.1 ± 6° / 7.7 ± 6.3°	0.99	0.76	0.71
Hip Adduction at Mid-Stance	6.2 ± 2.9° / 6.2 ± 3.5°	5.6 ± 1.3° / 7.1 ± 3.4°	0.87	0.50	0.50
Knee Mid-Stance Flexion	82.2 ± 31.6° / 87.3 ± 32.8°	74.3 ± 20.8° / 77.9 ± 22.5°	0.42	0.68	0.94
Knee Terminal Stance Extension	- 26.3 ± 9.6° / - 30.2 ± 8.5°	- 18.7 ± 6.1° / - 23.6 ± 7°	0.03*	0.17	0.87
Step Length	56.7 ± 5.2 cm / 56.2 ± 7.5 cm	56 ± 5.2 cm / 57.1 ± 4.7 cm	0.95	0.90	0.75

*P < 0.05.

flexion during walking than the low kinesiophobia group, but now also displayed less terminal stance phase knee extension (**table III**). At 6 months, the high kinesiophobia group displayed less terminal stance phase knee extension during running than the low kinesiophobia group (**table IV**). For each data collection period, kinesiophobia, not surgical side, was the sole factor that displayed significant kinematic gait effects.

DISCUSSION

The most important study finding was that high kinesiophobia at 3 months after ACLR (end of the acute rehabilitation phase) was related to walking and running gait kinematics at 3 and 6 months following ACLR, respectively. Kinesiophobia scores during this rehabilitation phase were also related to subject sports activity capability perceptions at 6 months and 9 months after ACLR. Among a cohort of patients that received primarily BPTB autografts, Kvist *et al.* (6) reported that those who had not returned to sport by 3-4 years after ACLR had greater re-injury fear. Among 73 patients who underwent ACLR using different autograft or allograft types, Lentz *et al.* (8) reported that regardless of graft type, patients who did not return to sport at one year following ACLR had higher kinesiophobia.

Kinesiophobia measured at 3 months after ACLR displayed a sustained inverse moderate-to-weak relationship with patient perceived sports capability before ACLR, and at 6 and 9 months following ACLR. Several studies have reported that reduced kinesiophobia is predictive of patients returning to sports at pre-injury level goals after ACLR (6-8). Despite 80% or greater surgical lower extremity hip and knee muscle strength symmetry with the non-surgical lower extremity at 4-5 months after ACLR, the current study identified a prolonged inverse relationship between the kinesiophobia measured at 3 months after ACLR (end of acute rehabilitation phase), and perceived sports activity capability measured before ACLR, and at 6 and 9 months afterwards.

Increased mid-stance knee flexion when walking in the high kinesiophobia group at 3 and 6 months after ACLR may

suggest greater functional quadriceps femoris muscle group impairment, avoidance, and/or possible re-setting of normal sagittal plane knee kinematics to protect the healing soft tissue graft. The reduced terminal stance phase knee extension observed during running in the high kinesiophobia group at 6 months after ACLR further supports this premise. At 3 months after ACLR, the high kinesiophobia group displayed greater peak stance phase hip adduction during walking than the low kinesiophobia group. This suggests greater hip abductor neuromuscular impairment with compensatory, more central surgical lower extremity foot placement to maintain lower extremity stability during early stance phase. Impaired hip muscle function is a concern following ACL injury, particular as the patient progresses to running and jumping tasks (25). Increased peak mid-stance knee flexion in the high kinesiophobia group suggests the need to prolong eccentric quadriceps femoris muscle group activation during weightbearing, possibly, to mitigate the anteriorly directed translational tibial forces that increase graft loading during terminal knee extension.

Among 12 healthy recreational runners, Orendurff *et al.* (26) reported that peak internal hip, knee and ankle extensor moments are sequentially distributed with peak moments occurring at 4% (hip), 11% (knee), and 17% (ankle) of running stance phase. During running, peak sagittal plane knee power occurs at about 15% of stance phase with concentric-eccentric hip and knee coupling over the first 50% of stance phase. The current study suggests that peak knee extensor moments during running may occur later, and at lower magnitudes among patients with high kinesiophobia levels following ACLR. Others have identified gait compensations among patients after ACLR. Among 10 male patients of similar age, and BMI as patients in the current study, at 6.2 ± 3.2 months after ACLR with a hamstring autograft, Asaeda *et al.* (27) reported that compared to healthy subjects, patients displayed less running knee flexion and reduced internal knee extensor moments. Also, at 12 months after ACLR, maximal isokinetic knee extensor torque (180°/sec), and peak knee flexion angle during running stance phase displayed a strong inverse relationship r = - 0.745, and a strong direct relationship r = 0.83 was observed between maximal isokinetic knee extensor torque (180°/sec), and internal knee extensor moments when running. These findings support the direct relationship between increased knee flexion angle and decreased quadriceps femoris muscle group functional integrity. In comparing 12 patients at 4.5 years after ACLR with 12 healthy control subjects, Varma *et al.* (28) reported that during walking there was a tendency for the contralateral knee of the ACLR group to display greater peak knee flexion and lower peak internal knee extension moments compared with the ACLR knee, and both knees of the control group. They concluded that the ACLR changed bilateral knee function to reduce ACLR knee loading (28). In a study that compared 196 patients at 12 months after ACLR using BPTB autografts and 106 healthy control group subjects while walking, Davis-Wilson *et al.* (29) reported less knee flexion, bilaterally lower early stance phase vertical ground reaction forces, and greater midstance vertical ground reaction forces among the surgical group. Surgical group subjects also displayed lower magnitude, and earlier surgical knee stance phase flexion at 6 and 12 months after ACLR, with the non-surgical knee displaying similar characteristics by 12 months after ACLR. Compared to control group subjects, the surgical lower extremity of the ACLR group also produced lower magnitude peak stance phase internal knee extensor moments during walking at 6 and 12 months following ACLR, and the non-surgical lower extremity displayed lower magnitude peak stance phase internal knee extensor moments at 12 months after ACLR. Differences observed during walking between the non-surgical lower extremity of the ACLR group, and both lower extremities of the control group increased from 6 to 12 months post-ACLR. At one year after ACLR, bilateral impairments were evident, with the non-surgical lower extremity progressively resembling the surgical lower extremity, rather than the surgical lower extremity gradually returning to pre-injury characteristics (29).

Current study findings suggest that high kinesiophobia levels could potentially have a stronger influence on walking, and running lower extremity kinematic symmetry than surgical side. Based on current study findings, and the results of previous reports, biomechanical, and kinesiophobia-related compensations may develop in a manner that is somewhat unique to the patient, the rehabilitation progression, the graft that is used, and its related harvest site, and healing characteristics.

Higher TALS scores represent sport or vocational activities with progressively more intense, or more frequent knee loading challenges culminating in sports such as soccer, American football, or elite level rugby (level 10). In comparison, a TALS score of 5 represents competitive sports with lesser intensity or frequency, with more predictable knee loads such as cross-country skiing, cycling, jogging, or vocational expectations such as heavy construction labor. At the lower end of the TALS score range, level 1 represents comparatively sedentary, more desk-based work. As it increases from 1 to 10, the TALS score represents subject perceptions of a greater ability to participate in higher level sports or vocational activities of progressively greater, and less predictable knee loads, movement ranges, sudden pivoting/directional changes, and a greater likelihood for being exposed to

direct contact or collisions that increase the potential for further knee injury. From this perspective, the TALS score differences observed between the high and low kinesiophobia groups at each measurement period reveals how patients with seemingly comparable neuromuscular function based on current clinical assessment methods can display vastly different return to sports or vocational activity readiness perceptions. Across the entire study period, the high kinesiophobia group consistently had approximately 2-point lower perceived sports or vocational capability scores.

This study has several important limitations. The study group consisted entirely of men, therefore, these results may not generalize to women. Given the potential influence of monthly menstrual cycle on lower extremity biomechanics, connective tissue laxity, and neuromuscular control (30), the first component of this study focused exclusively on men. Although a prospective study design was used with comparable subject groups, kinematic gait comparisons were made solely between the surgical and non-surgical lower extremities of subjects who had undergone ACLR. The addition of three-dimensional kinematic, kinetic and electromyographic data may have identified more specific lower extremity impairments and compensations. Also, sagittal plane trunk position measurements may have better confirmed knee extensor function as increased forward trunk lean is known to compensate for reduced internal knee-extensor moments during running (31). Rehabilitation protocols often reference the non-surgical lower extremity when making decisions about safe return to unrestricted sports participation readiness. However, the non-surgical lower extremity may not be the best reference, as growing evidence supports bilateral neuromuscular control impairments following ipsilateral ACL injury (28). Ideally, an age, and activity-matched healthy control group would have been included in the study design. Obtaining safe return to sports readiness indices from matched healthy athletes may help establish the content and criterion validity needed to ensure that essential neuromuscular, and psychobehavioral recovery thresholds have been achieved. Lastly, study duration was only over the initial 9 months following ACLR. Recent reports suggest that functional recovery after ACLR may take upwards of 2 years (32). The study time period was selected to coincide with the rehabilitation program phases used at our institution which generally culminate with return to sports at 9 months following ACLR.

CONCLUSIONS

High kinesiophobia at 3 months after ACLR was related to walking and running gait kinematic characteristics 3 and 6 months post-surgery, respectively. They were also related to subject perceptions of sports activity capability at 6 months and 9 months after ACLR.

Main points

- Tegner Activity Level Scores were similar between groups prior to the index knee injury, however, the high kinesiophobia group had lower scores pre-surgery, and at 6 and 9 months after ACLR.
- By 9 months after ACLR, kinesiophobia continued to influence perceived sports capability.
- Kinesiophobia had a stronger influence on kinematic gait patterns than surgical versus non-surgical lower extremity differences.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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The Injured Limb Presents Lower Values in Foot Structure Measurements 6 Years After an Achilles Tendon Rupture

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SUMMARY

Background. It is not known if foot structure may change after an Achilles tendon rupture and if a possible change may have an impact on lower limb function.
The primary aim of the study was to explore the difference in foot structure between injured and healthy limb and between two treatment groups, at mean 6 years after an Achilles tendon rupture. A secondary aim was to explore if the differences in foot structure correlated with functional and clinical outcome.
Methods. Ninety patients (15 women) with the mean (SD) age of 49 (9) years were evaluated. They had all been randomized to be treated with (n = 45) or without (n = 45) surgery. Foot structure was evaluated with Navicular Drop (Ndrop) and Drift (Ndrift), Longitudinal Arch Angle (LAA) and standing Dorsiflexion with knee straight and bent (DFstraight) and (DFbent). Calf muscle performance was evaluated with Single-leg standing heel-rise test and tendon length with ultrasound. For Patient-reported outcome measurements, Achilles tendon Total Rupture Score (ATRS) and Physical Activity Scale (PAS) were used. Both limbs were evaluated and the limb symmetry index (LSI (%) = injured/healthy × 100) was calculated.
Results. In all patients, the injured limb demonstrated lower values (injured/healthy) in Ndrift (6.0/6.7 mm, p = 0.034), Ndrop (6.6/7.4 mm, p = 0.32) and DFbent (44/46°, p < 0.001). In the group treated with surgery, there was significant difference between limbs in DFbent (44/46°, p = 0.002). In the non-surgically treated group, the injured limb demonstrated significantly lower values in Ndrift (6.0/7.4 mm, p = 0.005), Ndrop (6.9/8.2 mm, p = 0.005) and DFbent (44/46°, p = 0.008). There was no difference between treatment groups in LSI-values.
Conclusions. An Achilles tendon rupture seems to have an impact on foot structure long time after the injury. There is a need to clarify if the injury influences both feet and if there is a difference between treatment groups.

KEY WORDS

Achilles tendon rupture; foot structure; long-term follow-up; recovery; rehabilitation.

BACKGROUND

The incidence of Achilles tendon ruptures has increased in the last decades and has been reported to be 55/100000 inhabitants in men and 14/100000 inhabitants in women (1). There is evidence that the re-rupture rate is equivalent between patients treated with surgery and patients treated with no surgery if early weightbearing and functional rehabilitation are performed (2). However, other studies have reported a lower re-rupture rate and a better functional outcome in surgically treated patients even if there are other risk factors with surgical treatments such as wound problems and nerve damage (3).

Permanent deficits in calf muscle strength, endurance and heel-rise height are reported several years after an Achilles tendon rupture (4). Therefore, an Achilles tendon rupture may also have a negative impact on gait, running and jumping activities even if these activities also are dependent on strength, coordination and core muscles as well as balance performance (5). However, there are evidence that an Achilles tendon rupture may cause deficits in jumping ability during a drop counter movement jump two years after the injury (6). Moreover, Willy *et al.* (7) proved that patients with an Achilles tendon rupture had decreased ankle joint power, but increased knee joint power in the injured limb during hopping six years after the injury.

Long term deficits in gait and running after an Achilles tendon rupture have also been reported (8, 9). Tengman *et al.* (8) concluded that a group of patients had shorter step length during walking in both limbs compared to a healthy control group 2-5 years after the injury. Additionally, the patients had lower work both in the ankle and in the knee bilaterally compared to the control group. At mean 6 years after the injury, Jandacka *et al.* (9) found that during barefoot walking and running, athletes that have had an Achilles tendon rupture showed increased internal knee abduction moments compared with a healthy control group. The deficits in gait and running after an Achilles tendon rupture are for sure due to both changes in muscle strength, ankle range of motion and endurance, but not much is known about what happens to foot posture and foot structure after this injury.

It has been reported that different types of foot structure may have an impact on developing overuse injuries in the lower limb in some individuals (10-13). The medial longitudinal arch in the foot has been described to be low, normal or high and depending on the height, it could cause malalignment such as *pes cavus* or *pes planus* (14). Navicular drop and navicular drift are clinical tests that have been used to identify a low or high medial longitudinal arch (15). Navicular drop describes the navicular tuberosity's vertical movement while navicular drift describes the horizonal movement from a non-weight bearing position to a weight bearing position (15). The evaluation of the longitudinal arch angle (LAA) has also been presented as a useful clinical test for evaluating the medial longitudinal arch angle (16). It has been suggested that a low medial longitudinal arch in the foot may lead to an increased risk for pain in the lower limb (10, 12, 13).

However, to our understanding, there is a lack of knowledge about if and how foot structure may change after an Achilles tendon rupture and if a possible change may have an impact on lower limb function.

Therefore, the primary aim of this study was to evaluate possible difference in foot structure between the injured and the healthy limb and between treatment groups six years after an Achilles tendon rupture. A secondary aim was to explore if the differences in foot structure between limbs correlated with functional and clinical outcome in the lower limb and if there was a difference between treatment groups.

METHODS

Population

In total 90 patients were included in the present study. They had originally been included in either of two randomized controlled trials (RCT) where they had been randomized to be treated with or without surgery (17, 18). At mean six years after their Achilles tendon rupture, these cohorts participated in two different long-term follow-up studies where evaluations of foot structure, lower limb function and tendon length were performed (4, 19). Inclusion criteria for these long-term follow-ups were that they had been included in one of the above mentioned RCT's and also had participated in the one-year follow-up. Exclusion criteria were re-rupture, bilateral Achilles tendon ruptures or any other injury in the ankle joint that prevented them to perform the functional evaluations. The demographics of the included patients are presented in **table I**.

The research protocols (S617-03/307-07/032-09 for the original RCT's and additional applications for the present study: T426-12/058-14) have been approved by the Regional Ethical Review Board in Gothenburg, Sweden. Both oral and written consent were obtained from all participants before recruitment and the study was conducted according to international standards as described by Padulo *et al.* (20).

For all 90 patients, the same experienced physiotherapist (AB) performed all evaluations.

Table I. Patient characteristics and group comparisons at mean 6 years after the Achilles tendon rupture.

Variables	Total	Surgery	Non surgery	P-Value
Age	N = 90	N = 45	N = 45	
Mean (SD)	49 (9)	50 (9)	48 (9)	.209
Median	48	49	45	
Min-Max	30-69	30-67	34-69	
Body mass index (BMI)				
Mean (SD)	27.0 (3.5)	27.1 (3.2)	26.9 (3.8)	.684
Median	26.3	26.2	26.4	
Min-max	21.8-44.9	22.3-35.2	21.8-44.9	
Year since injury				
Mean (SD)	6.3 (1.5)	6.4 (1.5)	6.3 (1.5)	.723
Median	7	7	7	
Min-max	4-9	4-9	4-9	
Sex (men/women)	75/15	36/9	39/6	.573

Body Mass Index (BMI) = weight (kg)/height $(m)^2$.

Surgical techniques

Forty-five patients were treated with surgery and 45 patients with non-surgery and all of them had been randomized to be treated either with surgery or without surgery. In all patients treated with surgery, an open surgical technique was used. The same modified Kessler technique (21) was used in all patients but in 11 of the 45 patients, semi-absorbable sutures (No.-2 OrthocordTM, Depuy Mitek, Norwood, MA) were used instead of absorbable 1-0 polydioxanone (PDS) sutures (PDS II, Ethicon, Somerville, New Jersey). In this study, no comparison was performed between the two different sutures.

Foot structure

Foot structure measurements were performed as described by Barton *et al.* (10). These measurements have not been proved to be valid for patients with Achilles tendon rupture but have been found to be reliable and sensitive on a group level in patients with patellofemoral pain syndrome (PFPS) (10). Both the intrarater and the interrater reliability were found to be good to excellent (ICC-values between 0.74-0.95 and 0.54-0.93 respectively) with higher values for experienced raters (10).

Navicular drop and drift

Navicular drop and drift tests are designed to describe how the navicular bone moves from an unloaded position – with the ankle joint placed in a subtalar joint neutral position – to a weightbearing position where both feet are loaded equally with body weight (10, 15). A business card was used to mark how much the navicular bone moved in both directions (**figure 1**).

The vertical navicular height (VNH) was marked in both positions and navicular drop and drift were calculated from the marks on the business card (10).

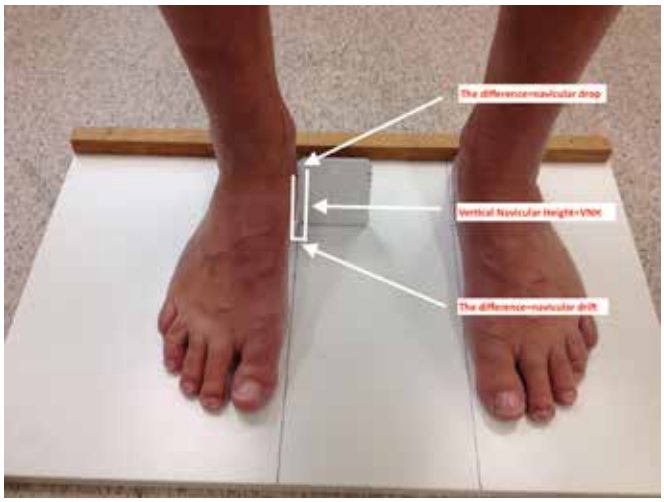

Figure 1. Vertical navicular height and navicular drop and drift.

Longitudinal Arch Angle (LAA)

The LAA is the angle formed by the two vectors with the origin on the tip of the navicular bone; one line goes through the medial malleolus and the other through the medial part of the first metatarsal head (16) (**figure 2**). The LAA was measured with a goniometer.

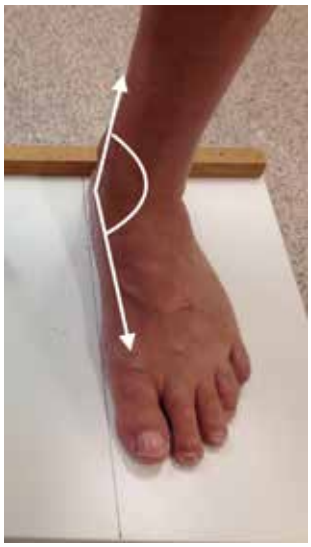

Figure 2. Longitudinal Arch Angle.

Dorsiflexion in the ankle

The dorsiflexion of the ankle was evaluated in a standing position, both with the knee straight and bent. A digital inclinometer placed on the anterior border of the patient's tibia was used to measure the dorsiflexion in the ankle joint (22) (**figure 3**).

Figure 3. Evaluation of the dorsiflexion in the ankle joint in a standing position with the knee straight.

Single-leg standing heel-rise test

A single-leg standing heel-rise test was used for evaluating calf muscle endurance. This test has been proved to be reliable and valid for evaluating patients with Achilles tendon ruptures (23). The patients were standing on a box with 10° incline on one leg and performed as many heel-rises at possible with a straight knee. A linear encoder was attached to the patients' shoe and, during the test, MuscleLab® system (Ergotest Technology, Oslo, Norway) was used to evaluate the performed heel-rise height (cm), number of repetitions (n), and total amount of work (Joule). The pace was set to 30 heel-rises per minute and the test ended either when the patient was unable to keep up the tempo or incapable to perform a heel-rise higher than 2 cm. The healthy leg was evaluated first in all evaluations.

Tendon length

The Achilles tendon length was evaluated with ultrasound using the extended field of view (Logiq E Ultrasound; GE Healthcare Sweden AB). Both the injured and healthy limb were measured between the calcaneal osteotendinous junction (OTJ) and the gastrocnemius musculotendinous junction (MTJ) as described by Silbernagel *et al.* (24).

Patient-reported outcome measurements

The Physical Activity Level was evaluated with Physical Activity Scale (PAS) (25) which is a 6 graded scale from 1-6 where 1 indicates hardly any physical activity and 6 indicates heavy and hard physical exertion several times a week. Achilles tendon Total Rupture Score (ATRS) (26) was used to evaluate patient-reported symptoms and function. ATRS is a reliable and valid score developed specifically for patients with Achilles tendon ruptures and is a widely used score in studies investigating patients with Achilles tendon ruptures. It consists of 10 questions and the maximum score is 100, which is an indication of no symptoms and full functional recovery.

Statistical analyzes

No power calculation could be performed since, to our knowledge, foot structure has not been evaluated in patients with Achilles tendon rupture before. Instead, all evaluations were performed by the same experienced physiotherapist (A.B.) and all patients were randomized to treatment with and without surgery.

Shapiro-Wilks test showed that some of the variables were not normally distributed, therefore non-parametric statistics were used. For comparison between limbs, Wilcoxon signed rank

test was used and for comparison between treatment groups, Mann-Whitney U test was used. Spearman´s correlation coefficient was used for exploring if interlimb differences in foot structure correlated with functional and clinical outcome in lower limb.
Level of significance was set to $p \leq 0.05$.
Limb Symmetry Index (LSI) was used to compare interlimb differences between groups and was calculated as a quote between the injured limb and the healthy limb × 100, expressed as percent (%).

RESULTS

Difference in foot-structure between injured and healthy limb and between treatment groups

Six years after the Achilles tendon rupture, the patients presented lower values in navicular drop and drift, and ankle dorsiflexion with the knee bent in their injured limb compared to the healthy limb (**table II**). There was no difference between treatment groups but comparison between the healthy limbs showed higher values in the group treated with non-surgery regarding LAA and navicular drop and drift (**table II**).

Difference in functional and clinical variables between injured and healthy limb and between treatment groups

The whole population and both treatment groups presented deficits in the injured limb in the functional and clinical variables except for numbers of repetitions in single-leg standing heel-rise test in the group treated with surgery (**table III**).

Differences in patient reported outcomes (PROMS) between treatment groups

The group treated with non-surgery reported a higher physical activity level compared to the group treated with surgery (**table IV**). No other differences were found.

Correlations

Non-surgery group (n = 45)

- LSI Nav_{DROP} correlated both with LSI HRheight ($r = 0.31$, $p = 0.044$) and LSI $DF_{STRAIGHT}$ ($r = -0.34$, $p = 0.023$).
- LSI Nav_{DRIFT} correlated also with LSI HRheight ($r = 0.46$, $p = 0.002$).

DISCUSSION

The most important finding in this study was that, still six years after the Achilles tendon rupture, the patients presented lower values in foot structure measurements in the injured limb compared to the healthy limb. The group treated with non-surgery presented interlimb differences in more foot structure variables compared to the group treated with surgery. However, there was no differences between treatment groups comparing the LSI-values. Interestingly, it was shown that the group treated with non-surgery had higher foot structure values in their healthy limb compared to the healthy limb in the group treated with surgery. There were no differences in foot structure values in the injured limb between groups.
In the present study, few correlations were found between foot structure and functional or clinical outcome and only in the group treated with non-surgery.
To our knowledge, foot structure has not been explored in patients with Achilles tendon rupture before. Additionally, very little is known about if these patients are at higher risk for other over-use injuries after their rupture. Neal *et al.* (27) showed limited evidence that a pronated foot posture was a risk factor for developing patellofemoral pain. In the present study, all participants were asked if they had any other injury that kept them from being physically active and in that case, which body part. Nine patients said they had symptoms from their knee, four from the hip/groin, two from the back, three from the shoulder, one from the chest and one from the stomach. However, from this data, it is not possible to draw the conclusion that a change in foot structure is the cause for symptoms from other body parts.
Foot length and gender have been suggested to influence navicular drop (28). In the present study foot length was not evaluated in all patients and therefore not included in the calculations, but for the 66 patients where foot length was evaluated, there was no difference between treatment groups and there was no difference in gender distribution.
It has been proved that early weight-bearing and accelerated rehabilitation are favorable after an Achilles tendon rupture (2). Being immobilized for 8 weeks may cause weakness in the intrinsic muscles as well as in the extrinsic muscles in the injured lower leg. In the present study, 66 of the patients were not allowed to weight-bear before the sixth week but they were allowed to actively move their foot in plantarflexion from the second week (17). For 24 of the included patients, they were allowed to weight-bear at once after the injury (18). All patients were exhorted to move their toes several times a day. To not be able to weight-bear on the injured limb may have caused an over-use reaction on the healthy limb but it is unknown if this could have a permanent effect on the foot structure.
In the present study, patients treated with non-surgery had interlimb differences in more foot structure variables than patients treated with surgery. However, the differences were presented in the healthy limb and not in the injured limb.

Table II. Results in foot structure between healthy and injured limb and between treatment groups. The differences between the injured limbs and the healthy limbs in the two treatment groups are also presented.

Variables	Injured limb	Healthy limb	P-value	Injured limb	Healthy limb	P-value	Injured limb	Healthy limb	P-value	P-VALUE groups (LSI-values)	P-value injured limbs	P-value healthy limbs
	Total			**Surgery**			**Non-surgery**					
LAA	n = 90	n = 90		n = 45	n = 45		n = 45	n = 45				
Mean (SD)	142.6 (13.4)	143.7 (12.5)	.071	138.8 (16.6)	139.6 (14.2)	.396	146.4 (7.7)	147.9 (8.9)	.093	.818	.060	**.016**
Median	144	145.5		142	144		146	148				
Min	100	98		100	98		136	128				
Max	168	168		162	160		168	168				
NavDrift (mm)	n = 90	n = 90		n = 45	n = 45		n = 45	n = 45				
Mean (SD)	6.0 (3.2)	6.7 (3.4)	**.034**	6 (3.3)	6 (3.7)	.946	6.0 (3.2)	7.4 (3)	**.005**	.100	.807	**.044**
Median	5	6		5	5		6	8				
Min	0	0		1	0		0	2				
Max	17	14		17	14		14	14				
NavDrop (mm)	n = 90	n = 90		n = 45	n = 45		n = 45	n = 45				
Mean (SD)	6.6 (3.4)	7.4 (3.6)	**.032**	6.4 (3.9)	6.6 (3.9)	.850	6.9 (3.4)	8.2 (3.2)	**.005**	.156	.383	**.019**
Median	6	7.5		6	6		6	9				
Min	0	0		1	0		0	2				
Max	17	17		17	17		17	15				
DFBent (°)	n = 90	n = 90		n = 45	n = 45		n = 45	n = 45				
Mean (SD)	44 (6.6)	46 (6.6)	**< .001**	44 (6.4)	46 (6.2)	**.002**	44 (6.8)	46 (7.0)	**.008**	.881	.945	.725
Median	44	46		44	46		44	46				
Min	30	33		30	36		32	33				
Max	65	65		58	62		65	65				
DFStraight (°)	n = 90	n = 90		n = 45	n = 45		n = 45	n = 45				
Mean (SD)	39 (6)	40 (6)	.336	39 (7)	40 (7)	.368	40 (6)	40 (7)	.678	.765	.487	.961
Median	38	40		38	40		39	40				
Min	25	27		27	29		25	27				
Max	61	55		55	55		61	55				

Significant differences are presented in bold.

Table III. Results in functional and clinical variables between healthy and injured limb and between treatment groups. The differences between the injured limbs and the healthy limbs in the two treatment groups are also presented.

Variables	Injured limb	Healthy limb	P-value	Injured limb	Healthy limb	P-value	Injured limb	Healthy limb	P-value	P-Value groups (LSI-values)	P-value injured limbs	P-value healthy limbs
	Total			**Surgery**			**Non-surgery**					
hr height (cm)	n = 89	n = 88		n = 45	n = 44		n = 44	n = 44				
Mean (SD)	11.1 (2.5)	13.2 (2.1)	**< .001**	11.2 (2.5)	13.4 (2.2)	**< .001**	11.1 (2.4)	12.9 (2)	**< .001**	.658	.504	.063
Median	11.3	13.2		11.5	13.6		11.1	12.7				
Min	4.8	7.3		4.8	7.9		6.4	7.3				
Max	17.7	20.4		16.9	18		17.7	20.4				
hR REPs (n)	n = 88	n = 87		n = 44	n = 43		n = 44	n = 44				
Mean (SD)	30 (12)	34 (13)	**< .001**	31 (14)	32 (12)	.199	30 (11)	35 (15)	**< .001**	***.031***	.970	.721
Median	28	30		28	30		28	30				
Min	10	16		10	18		11	16				
Max	85	81		85	77		54	81				
hR Work (J)	n = 88	n = 87		n = 44	n = 43		n = 44	n = 44				
Mean (SD)	2066 (826)	2723 (832)	**< .001**	2115 (851)	2737 (818)	**< .001**	2017 (808)	2709 (854)	**< .001**	.377	.478	.959
MedIAn	1966	2645		2040	2628		1954	2680				
Min	374	810		374	810		719	1131				
Max	4504	4592		4101	4592		4504	4386				
tendon length (cm)	n = 87	n = 87		n = 43	n = 43		n = 44	n = 44				
Mean (SD)	22.4 (3.3)	20.6 (2.8)	**< .001**	21.9 (3.6)	20.4 (2.7)	**< .001**	22.8 (2.9)	20.9 (2.9)	**< .001**	.296	.222	.492
Median	22.9	20.2		21.7	20		23.2	20.8				
Min	13.4	14.2		13.4	14.3		16.7	14.2				
Max	29.3	26.8		29.3	26.7		29.2	26.8				

Significant differences are presented in bold.

Table IV. Results in patient reported outcomes (PROMS) in the whole population and in the two treatment groups.

PROMS	TOTAL (n = 90)	SURGERY (n = 45)	NON- SURGERY (n = 45)	P-VALUE
Atrs				
Mean (SD)	90 (16)	90 (17.7)	89 (14)	.297
Median	95	95	94	
Min-Max	0-100	0-100	22-100	
Pas				
Mean (SD)	3.9 (1.1)	3.7 (1.1)	4.1 (0.9)	**.044**
Median	4.0	3.0	4	
Min-Max	2-6	2-6	2-6	

Significant differences are presented in bold.

Headlee *et al.* (29) found that fatigue in the intrinsic muscles of the foot, increased the pronation in healthy individuals. The reason for this could be that the intrinsic muscles support the medial longitudinal arch in the foot (29). It is unknown if treatment with non-surgery increase the load on the healthy foot compared to treatment with surgery and there is a need to further explore what impact treatment have both in the injured and the healthy limb after an Achilles tendon rupture.

It is possible that an acquired low medial longitudinal arch could develop over years and that a change in movement pattern could lead to a low medial longitudinal arch. Furthermore, this could lead to a decreased ability in heel-rise height, which may be a possible explanation for the correlations between navicular drop and drift and heel-rise height in the present study (30). It has also been proved that persons with a low longitudinal arch together with a mobile foot are at greater risk for injuries since it would lead to increased biomechanical demands during walking (11). However, Okamura *et al.* (31) proved that activation of the intrinsic foot muscles had an impact on the medial longitudinal arch during gait and standing in subjects who were flat-footed. Therefore, it is important to guide the patients with Achilles tendon ruptures in exercises designed for strengthening the foot muscles both in the injured and uninjured foot.

A limitation in this study is the lack of comparison in foot structure in healthy subjects. Moreover, it is not known what values the patients had in foot structure before the injury. Furthermore, there is a lack of studies comparing foot structure between the right and left foot in healthy subjects. Nevertheless, in a large study, navicular drop was evaluated in 500 healthy Indian men and women between 18-21 years. In this study, no difference was found between the right and left foot (median (IQR)); 6 mm (4-9) / 6 mm (4-9), p = 0.200 (32). This is the same median value as in the present study except for the healthy limb in patients treated with non-surgery.

There was also a difference in physical activity level between treatment groups which could be a limitation in the present study. The patients treated with surgery scored at median of 3 and patients treated with non-surgery scored at median of 4 on the 6-level questionnaire, PAS (25). Level 3 means "Light physical exercise around 2-4 hours a week, *e.g.*, walks, fishing, dancing, normal gardening, including walks to and from shops" while level 4 means "Moderate exercise 1-2 hours a week, *e.g.*, jogging, swimming, gymnastics, heavy gardening, home repairs or easy physical activities more than 4 hours a week". It cannot be concluded if this difference may have an impact on foot structure in the present study since physical activity also may change over time.

The reliability in navicular drop and drift have been found to be weak to moderate but higher reliability for experienced evaluators (10). However, in the present study, all foot structure measurements were performed by the same, experienced physiotherapist.

In the present study, foot structure evaluation was only performed once and that was several years after the injury. This is a limitation since evaluation over time would have given us more information how foot structure may change after the injury. It is also unknown if the two different sutures used in the surgically treated patients could have any impact on foot structure. Taken together, further studies are needed, mainly focusing on possible foot structure changes after an Achilles tendon rupture and of how foot structure is affected by different demanding activities such as running and jumping.

CONCLUSIONS

An Achilles tendon rupture seems to have an impact on foot

structure long time after the injury. There is a need to clarify if the injury influences both feet and if there is a difference between treatment groups.

Highlights

An Achilles tendon rupture seem to have an impact on foot structure.
Non-surgically treated presented higher foot structure values in the healthy limb.
Foot structure values did not differ between groups in the injured limb.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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Functional Performance of Ankles Between Male and Female Practitioners of Resistance Exercise

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SUMMARY

Background. Gender difference has shown that females exhibit a greater the ankle range of motion (ROM) than males. On the other hand, few studies have examined the link between the ankles ROM, muscle strength and functional performance (FP) in the gender difference.

Objective. The purpose of this investigation was to compare the ankles ROM, ankle isometric muscle strength and ankle PFP between males and females practitioners of resistance exercise.

Methods. Males (n = 20) and females (n = 20) healthy were recruited. All participants underwent three tests to assess the ROM, muscle strength and FP of the ankles, respectively. ROM measurements were taken in both ankles with a digital goniometer. Ankle isometric muscle strength was measured using a load cell. Ankle FP was assessed with the Single Leg Hop Test (SLHT) in both limbs.

Results. Plantar flexion ROM was significantly greater in the females than males for both ankles ($p < .01$). No significant difference ($p > .05$) was found between the groups for ankle-dorsiflexion ROM. Isometric muscle strength during dorsiflexion was significantly lower in the females for both ankles ($p < .01$). SLHT showed demonstrating better FP in the males ($p < .001$). Isometric muscle strength during plantar flexion was significantly correlated with plantar flexion ROM in males ($r = 0.52$; $p < .02$) and females ($r = 0.46$; $p < .03$).

Conclusions. This study showed the better ankles FP and greater isometric muscle strength during ankle-dorsiflexion and plantar flexion in males. On the other hand, plantar flexion ROM was greater in females.

KEY WORDS

Ankles; functional performance; range of motion; resistance training; sex differences.

INTRODUCTION

Resistance exercise (RE) is a systematic physical activity modality with the objective of increase muscle strength to overcome resistance (1, 2). Thus, RE have been suggested in sports guidelines aiming at improving physical conditioning and health (3). This physical activity modality is a combination of dynamic actions and static effort with the principle of increasing muscle strength and power from multiple variables, such as: exercise order, rest interval between sets, exercise mode, training frequency, movement velocity, training volume, repetitions per set, number of sets, type of muscle action, and the load intensity that can all be manipulated to meet the training goals and individual differences in training needs (1, 2, 4). But, interestingly, in regard to range of motion (ROM), muscle strength and functional performance of ankles there are a limited number of studies that have assessed gender difference (males *vs* females) of practitioners of RE.

The ankle joint complex is formed by the dome of the talus fitting into a mortise formed by the tibia and the fibula where this joint produces movements of dorsiflexion and plantar flexion of the foot. Dorsiflexion is the movement at the ankle joint where the toes are brought closer to the shin, curling upwards and decreasing the angle between

the dorsum of the foot and the leg (5, 6). On the other hand, plantar flexion describes the extension of the ankle so that the foot points down and away from the leg (7). A normal ankle moves from approximately 20° dorsiflexion to 50° plantar flexion in the sagittal plane and total range of motion in the frontal plane is approximately 35° (23° inversion; 12° eversion) (5, 8, 9). Adequate ankle mobility allows the lower limb to interact with the ground being a fundamental requirement for walking, body stability and activities of daily living. On the other hand, a limited ankle ROM is associated with a greater dynamic knee valgus and medial knee displacement, as well as a reduced activation of the quadriceps and increased activation of the soleus (5, 8, 9).

Gender difference has shown that females exhibit a greater ankle ROM than males. Possibly, because muscle stiffness is lower in females, since contribute with higher tolerance to muscle stretch being responsible for increased ROM (10). Besides, the females show high geometrical parameters of plantar flexor's muscle–tendon complex active parts of the series elastic component (11). Another important factor observed in the scientific literature was the greater ROM at the talocrural and subtalar joints in females (12). Thus, ROM of the ankles plantar flexion for females is greater than males; but ROM of ankle-dorsiflexion no significant gender difference (13).

On the other hand, few studies have examined the link between the ankles ROM, muscle strength and functional performance in the gender difference. Accordingly, the aim of this study was to compare the ankle ROM, ankle isometric muscle strength and ankle functional performance between males and females practitioners of RE.

METHODS

Study design

This is a randomized comparative study. The sample size was determined by including all participants that complied with the eligibility criteria. All participants (male and female) were practitioners of resistance exercise and underwent three tests to assess ROM, strength, and functionality. All tests were performed in a single assessment session in the following order: anthropometric measurements; ankle range of motion (dorsiflexion and plantar flexion); ankle muscle strength; and ankle functional performance testing. All assessment were taken in a temperature-controlled environment (temperature 21 ºC, 65% relative humidity) by a Hygro-Thermometer with Humidity Alert (Extech Instruments, Massachusetts, EUA). All assessments occurred between 2:00 and 4:00 P.M.

Participants

Forty participants were recruited and separated into two groups: male (33.5 ± 7.8 years; 176.1 ± 7.6 cm; 79.6 ± 7.8 kg; 22.5 ± 2.8 kg/m^2, n = 20) and female (29.5 ± 7.1 years, 164.3 ± 8.7 cm, 67.1 ± 8.9 Kg; 20.5 ± 2.5 kg/m^2, n = 20). All subjects regularly practiced resistance exercise 5.2 ± 0.4 days week^{-1} and low aerobic training of 1.3 ± 0.6-day week^{-1} with a total volume of 252.7 ± 8.3 minutes per week. Subjects with at least one year of resistance exercise experience were included to participate in the current study. Exclusion criteria included: 1) use of anabolic steroids, drugs, or medication with potential impact in physical performance (self-reported); 2) presence of musculoskeletal injury in the past 6 months and 3) previous hip, knee, and/or ankle surgery.

All participants performed a routine of RE that engaged the whole body with resistance bands, free-weights, and medicine balls. All participants completed the Physical Activity Readiness Questionnaire (PAR-Q). This study was approved by the Ethical Committee for Human Experiments of the Augusto Motta University Center, Rio de Janeiro, Brazil (CAAE: 32033420.1.0000.5235). The present study was conducted at the Rehabilitation Science Center, Augusto Motta University Center, Rio de Janeiro, Brazil meeting the ethical standards of the jornal (14). The study was performed in accordance with ethical standards in sport and exercise science research. All participants were informed of the experimental procedures and gave written informed consent prior to participation. No clinical problems occurred during the study.

Anthropometric measurements

Body composition was measured following an 8-h overnight fast by bioelectrical impedance analysis using a device with built-in hand and foot electrodes (BIO 720, Avanutri, Rio de Janeiro, Brasil). The participants wore their normal indoor clothing and were instructed to stand barefoot in an upright position with both feet on separate electrodes on the device's surface and with their arms abducted and both hands gripping two separate electrodes on each handle of the device. All biometric measurements were carried out in an air-conditioned room (21 °C). No clinical problems occurred during the study.

Ankle dorsiflexion and plantar flexion range of motion

Measurements were taken in both ankles with a digital goniometer (Global Medical Devices; Maharashtra, India). Ankle-dorsiflexion and plantar flexion ROM were

measured with the subjects lying supine with an extended knee on a standard treatment table. This position was selected because both hip and knee joints are extended simultaneously, simulating the stance phase of gait just before heel-off (15). During assessments, all subjects wore shorts to provide adequate exposure to the ankle-foot-leg complex and were instructed to actively do a dorsiflexion and plantar flexion of the ankle joint (*i.e.*, as far as comfortable without pain). The rotational axis of the goniometer was placed just distally to the lateral malleolus, and the goniometer arms were aligned with the head of the fibula and parallel to the fifth metatarsal, respectively. The convention was followed of neutral position being 0° and ankle motion being the number of degrees of angular movement from that position in either a dorsal or plantar direction. Measurements were recorded in degrees, and the mean score of three measurements was computed. This testing method has shown intra-rater reliability measures greater than 0.93 for the assessment of the active ankles ROM.

Ankle Isometric Muscle Strength Testing

Ankle isometric muscle strength was measured using a commercially available load cells (E-lastic, E-sports Soluções Esportivas, Brasilia, Brazil). During the assessments, the left and right forefoot were individually secured by bands and fixed in the load cells. Participants performed three times (dorsiflexion and plantar flexion) for both ankles and the highest value obtained from the three trials was used for statistical analysis (**figure 1**). All assessments were performed with the subjects lying supine with an extended knee on a standard treatment table. Force values were registered during 5 seconds of isometric contraction and rest interval of 60 secs between the trials. Isometric force data of load cell were simultaneously transferred via Bluetooth to a mobile cellphone (sample rate = 10 Hz). Verbal encouragement was always provided, and no subjects were excluded through injury during the experimental procedure. This testing method has shown intra-rater reliability measures greater than 0.94.

Figure 1. Ankle isometric muscle strength testing.

Ankle Functional Performance Testing

Ankle functional performance was assessed with the single leg hop test performed bilaterally (right and left). Subjects, with footwear, positioned themselves single leg 30 cm behind of the first photocell beam (Brower Timing System, Salt Lake City, 174 UT, USA; accuracy of 0.01 sec) (**figure 2**). For the time record, subjects covered as fast as possible a 6-m distance that was timed by the second photocell beam. The test was repeated three times for both legs and a mean score of the three trials was then calculated. The subjects rested for 30 secs between the trials. Verbal encouragement was always provided, and no subjects were excluded through injury during the experimental procedure. This test it was valid when it exhibits reliability that is higher than 0.90. Prior to functional performance testing, participants conducted a 10-min mobility and stability exercise.

Figure 2. Schematic diagram of the single-leg hops for time test.

Statistical analysis

All data are presented as mean ± standard deviation. Statistical analysis was initially performed using the Shapiro–Wilk normality tests and the homoscedasticity test (Bartlett criterion). To test the reproducibility between the tests, the intraclass correlation coefficient (ICC) was used. Two-way analysis of variance (ANOVA) was used to test for main and interaction effects of the group (males *vs* females) and timing of measurement for each outcome variable independently (right *vs* left) and the post hoc Bonferroni was used to possibility a statistically significant. Correlations between variables were assessed using Pearson correlation coefficients and their corresponding 95% confidence intervals. The level of statistical significance was set at an alpha

level of P < 0.05 using GraphPad Prism® software (Prism 6.0, San Diego, CA, USA).

RESULTS

The two-way ANOVA yielded main effects for group ($F_{(1,37)}$ = 10.70, p < .002), such that Bonferroni post- hoc showed significant differences in plantar flexion ROM between males *vs* females group for both ankles (**table I**). No significant difference (p > .05) was found between the groups (male *vs* female) to dorsiflexion ROM. Absolute isometric muscle strength during dorsiflexion ($F_{(1,37)}$ = 9.06, p < .004) and plantar flexion ($F_{(1,37)}$ = 11.46, p < .001) showed main effects for groups demonstrating that it was significantly lower in the females when compared to males for both ankles (p < .01) (**table I**).

Table II compares the ankle functional performance during single leg hop test between males *vs* females. Single leg hop test showed main effects for groups (Second: $F_{(1,37)}$ = 69.77, p < .0001; m/s: $F_{(1,37)}$ = 74.58, p < .0001; km/h: $F_{(1,37)}$ = 74.50, p < .0001) demonstrating better functional performance in the males when compared to females for both ankles (p < .001) (**table II**). In addition, number of jumps ratio also showed main effects for groups ($F_{(1,37)}$ = 59.00, p < .0001), such that Bonferroni post- hoc showed significant differences (p < .001) between males *vs* females group for both ankles (**table II**).

Table III showed no correlation significant between single leg hop test and ankle range of motion. On the other hand, Pear-

Table I. Performance of ankle range of motion, absolute and relative isometric muscle strength between males *vs* females practitioners of resistance exercise (n = 40).

		Male	Female	Confidence interval (95% CI)	P <
Dorsiflexion (º)	Right	21.9 ± 2.9	20.7 ± 2.6	- 1.14 (- 3.36 to 1.07)	> .05
	Left	21.6 ± 4.1	20.8 ± 2.3	0.78 (- 2.99 to 1.43)	> .05
Dorsiflexion Absolute isometric muscle strength (kg)	Right	14.6 ± 4.1	11.5 ± 1.9	- 3.13 (- 5.54 to - 0.71)	< .01
	Left	14.4 ± 4.4	11.4 ± 2.0	- 2.99 (- 5.41 to -0.58)	< .01
Dorsiflexion Relative isometric muscle strength (kg/kg)	Right	5.6 ± 1.2	5.9 ± 1.3	0.30 (- 0.59 to 1.20)	> .05
	Left	5.7 ± 1.0	6.0 ± 1.3	0.26 (- 0.63 to 1.17)	> .05
Plantar flexion (°)	Right	42.1 ± 5.0	45.7 ± 3.0	3.64 (0.64 to 6.64)	< . 01
	Left	41.6 ± 4.2	45.5 ± 3.9	3.91 (0.91 to 6.91)	< . 01
Plantar flexion Absolute isometric muscle strength (kg)	Right	29.6 ± 6.4	24.2 ± 3.3	- 5.36 (- 9.01 to - 1.71)	< .01
	Left	29.1 ± 6.4	23.8 ± 2.6	- 5.16 (- 8.81 to - 1.51)	<. 01
Plantar flexion Relative isometric muscle strength (kg/kg)	Right	2.7 ± 0.5	2.8 ± 0.4	0.02 (- 0.32 to 0.38)	> .05
	Left	2.8 ± 0.5	2.8 ± 0.4	0.00 (- 0.35 to 0.35)	> .05

Table II. Performance during single leg hop test between male *vs* female practitioners of resistance exercise (n = 40).

		Male	Female	Confidence interval (95% CI)	P <
Single Leg Hop test (sec.)	Right	1.8 ± 0.2	2.6 ± 0.4	0.78 (0.54 to 1.02)	< .001
	Left	1.9 ± 0.2	2.7 ± 0.3	0.77 (0.54 to 1.01)	< .001
Single Leg Hop test (m/s)	Right	3.2 ± 0.3	2.3 ± 0.3	- 0.92 (- 1.19 to 0.66)	< .001
	Left	3.1 ± 0.3	2.2 ± 0.3	- 0.89 (- 1.15 to - 0.63)	< .001
Single Leg Hop test (km/h)	Right	11.6 ± 1.3	8.2 ± 1.3	- 3.34 (- 4.28 to - 2.40)	< .001
	Left	11.3 ± 1.3	8.1 ± 1.1	- 3.2 (- 4.16 to - 2.28)	< .001
Number of jumps	Right	3.1 ± 0.3	4.2 ± 0.4	1.09 (0.77 to 1.41)	< .001
	Left	3.2 ± 0.4	4.1 ± 0.4	0.93 (0.62 to 1.25)	< .001

Table III. Scatterplots displaying the correlation analysis (Pearson's coefficient) between performance during single leg hop test (second) and ankle range of motion (°) in male and female practitioners of resistance exercise (n = 40).

		r	Confidence interval (95% CI)	P <
Dorsiflexion right (°)	Male	0.19	- 0.28 to 0.59	0.42
	Female	0.39	- 0.71 to 0.06	0.08
Dorsiflexion left (°)	Male	0.25	- 0.22 to 0.63	0.29
	Female	0.00	- 0.43 to 0.45	0.96
Plantar flexion right (°)	Male	0.06	- 0.40 to 0.50	0.79
	Female	- 0.27	- 0.63 to 0.19	0.24
Plantar flexion left (°)	Male	- 0.00	- 0.46 to 0.44	0.97
	Female	0.12	- 0.33 to 0.53	0.61

son's analysis showed that absolute isometric muscle strength during plantar flexion was significantly correlated with plantar flexion range of motion in males (r = 0.52; p < .02) and female (r = 0.46; p < .03) for the right ankle (**table IV**).

Pearson's analysis also illustrated that performance in the single leg hop test was negatively correlated with absolute isometric muscle strength during dorsiflexion in female for both ankles (Right: r = - 0.54; p < .01; left: r = - 0.76; p < .0001) (**table V**). Negative correlation also was observed between performance during single leg hop test and absolute isometric muscle strength during plantar flexion in female only for the left ankle (r = - 0.44; p < .04) (**table V**).

Table IV. Scatterplots displaying the correlation analysis (Pearson's coefficient) between absolute ankle isometric muscle strength (kg) and ankle range of motion (°) in male and female practitioners of resistance exercise (n = 40).

		r	Confidence interval (95% CI)	P <
Dorsiflexion right (°)	Male	0.00	- 0.45 to 0.45	0.99
	Female	0.03	- 0.46 to 0.41	0.89
Dorsiflexion left (°)	Male	0.09	- 0.37 to 0.52	0.69
	Female	- 0.25	- 0.62 to 0.20	0.27
Plantar flexion right (°)	Male	0.52	0.09 to 0.79	< .02
	Female	0.46	0.03 to 0.75	< .03
Plantar flexion left (°)	Male	0.34	- 0.13 to 0.68	0.15
	Female	- 0.18	- 0.58 to 0.28	0.43

Table V. Scatterplots displaying the correlation analysis (Pearson's coefficient) between performance during single leg hop test (second) and absolute ankle isometric muscle strength (kg) in male and female practitioners of resistance exercise (n = 40).

		r	Confidence interval (95% CI)	P <
Dorsiflexion right Absolute isometric muscle strength (kg)	Male	0.07	- 0.39 to 0.51	0.75
	Female	- 0.54	- 0.79 to - 0.13	< .01
Dorsiflexion left Absolute isometric muscle strength (kg)	Male	0.38	- 0.08 to 0.71	0.10
	Female	- 0.76	- 0.90 to - 0.48	< .0001
Plantar flexion right Absolute isometric muscle strength (kg)	Male	0.39	-0.07 to 0.72	0.09
	Female	- 0.36	- 0.69 to 0.08	0.11
Plantar flexion left Absolute isometric muscle strength (kg)	Male	0.36	-0.10 to 0.70	0.12
	Female	- 0.44	- 0.74 to - 0.007	< .04

DISCUSSION

The present study showed that male showed greater absolute isometric muscle strength during dorsiflexion and plantar flexion. However, female showed greater plantar flexion ROM. On the other hand, was observed a significant correlation between absolute isometric muscle strength during plantar flexion and plantar flexion ROM in right ankle to both groups. But the main findings showed negative correlation between functional performance and absolute isometric muscle strength during dorsiflexion in female for both ankles. In addition, females also showed negative correlation between functional performance and absolute isometric muscle strength during plantar flexion of the left ankle.

Decreased strength of the ankle musculature decreases the ability to stabilize the lower extremity, resulting in a faulty alignment of the lower extremity (such as adduction and rotation of the hip and knee valgus) (7, 8). Specifical-

ly, on the isometric muscle strength during dorsiflexion, our results showed that males had greater production of isometric strength than females (21.2% and 20.8% in the right and left ankles, respectively). Other studies observed a gender difference of the isometric muscle strength during dorsiflexion between 28% and 39% (16, 17). However, the studies did not use trained subjects (16, 17). It seems that the main factor for the gender difference in isometric dorsiflexion contraction is related to muscle cross-sectional area (CSA) of the tibials anterior (*i.e.*, type II muscle fiber diameters in males is 20% larger than females) (18, 19). In addition, the deformation of the surrounding fascicles and retinaculum bands causes the tibials anterior distal tendon to shift away from the axis of rotation when going from rest to maximum muscle tension (20).

Plantar flexion describes the extension of the ankle so that the foot points down and away from the leg (7, 21). Thus, ankle plantar flexors are important muscles to control for the mechanical work during squat exercise while also to strengthen the posterior-lateral muscles of the leg (7, 21). Our results reported that males had greater production of isometric strength than females during plantar flexion (18.2% and 18% in the right and left ankles, respectively). Some studies have shown that this gender difference in isometric strength muscle can be related to larger muscle physiological cross-sectional area (CSA) in males (CSA of type II fibers, fascicule length, and fascicule angles) being a factor that can contribute to greater isometric muscle strength (11, 22, 23). In addition, males have a larger Achilles tendon CSA and series elastic component that likely related to increased force generation capacity in the muscle fibers (11, 22, 23). On the other hand, our results showed greater ROM during plantar flexion in females when compared to males (7.8% and 8.5% in the right and left ankles, respectively). These results may be related a higher stiffness of the series elastic component and tolerance to muscle stretch (muscle stiffness of plantar flexors) in males (10, 11, 13).

The functional performance tests require agility to better represent functional movements and may be more difficult to perform with decreased strength and power of the ankle muscles (7, 24, 25). The dorsiflexors are eccentrically contracting to control the concentrically contracted plantar flexors to prepare the foot for push-off during functional performance tests (Single Leg Hop) (7, 23, 26). Thus, our results indicate that males showed greater power muscle during functional performance tests. In general, it seems that the morphological difference between genders is related to the better functional performance possibly associated with greater strength and power of the ankle muscles in males (10, 13, 18, 19).

Although our results showed a correlation between absolute ankle isometric muscle strength and plantar flexion ROM. A significant correlation was observed only in the right ankle for males and females. These results may be related to the fact that the dominant leg of the study participants was the right leg and contribute with greater pushing force that leads to more ROM at ankle plantar flexion required for the lower limb to propel the body forward towards toe-off (8). On the other hand, we observed a negative correlation between performance during the single leg hop test and the absolute ankle isometric muscle strength in females. This result makes us hypothesize the participation of other intrinsic factors (*i.e.*, balance; coordination; action of the tibials anterior and triceps surae muscles) to improve functional performance since we also did not observe a significant correlation between performance during single leg hop test and ankle ROM (11, 13, 24). The limitations of the study include the absence of measures of physiological parameters of physical exertion, which would be interesting; this, yet, does not limit the answer to the study question. In addition, longitudinal studies are needed to define a cause-and-effect relationship between gender difference, resistance training model and ankle functional performance.

CONCLUSIONS

This study showed better ankle functional performance and greater absolute isometric muscle strength during ankle dorsiflexion and plantar flexion in males. On the other hand, plantar flexion ROM was greater in females. However, both genderes showed a positive correlation between absolute ankle isometric muscle strength and plantar flexion ROM. These data contribute to the qualitative and quantitative understanding of gender differences in normal ankle function and may be useful to better understand and treat ankle joint pathologies in a gender-specific manner.

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CONFLICT OF INTERESTS

The author declare thet they have no conflict of interests.

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ORIGINAL ARTICLE

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The Comparative Effects of Cupping Massage and Exercise Training in Patients with Trapezius Myofascial Syndrome on Pain, Disability, and Fatigue. A Randomized Controlled Trial

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SUMMARY

Background. Cupping therapy is one of the treatments for myofascial pain that has not been sufficiently studied. This study aimed to compare the effects of cupping massage and exercise training on pain, disability and Fatigue severity in patients with trapezius myofascial syndrome.

Patients and methods. Forty-four patients were randomly divided into two groups of cupping massage and conventional exercise training. The outcome measure included pain intensity, disability, and fatigue. The duration of intervention was 4 weeks (3 times a week) for both groups. The intervention included moving cup with negative pressure on the trapezius muscle for 5 minutes in each session in the cupping group and strengthening and stretching exercises for trapezius muscle in the exercise group. Questionnaires were completed and analyzed immediately after the last intervention session and three months later.

Results. Immediately after the intervention and three months later, in both groups, pain, disability and fatigue severity were significantly reduced (P value < 0.01). These changes were significantly greater in the cupping group than in the exercise group (P value < 0.01).

Conclusions. Massage cupping should be considered as a feasible, safe, fast, and effective method for patients with trapezius pain syndrome, also, can be combined with other rehabilitation programs in the treatment of myofascial pain and muscle strain.

KEY WORDS

Myofascial pain syndrome; cupping therapy; exercise training; disability; fatigue.

INTRODUCTION

Myofascial pain syndrome (MPS) characterized by nonspecific pain in muscles with both motor and sensory abnormalities due to presence of trigger points (1, 2). Trigger points are excitatory points in muscle bands that cause local tenderness and pain when pressed. They exist due to overload, prolonged repetitive and mechanical stress, are one of the most important causes of myofascial pain (3).

Mechanical stimulation such as snapping-palpation, pressure, or needle insertion can elicit a local twitch response and non-dermatomal, non-myotomal referred pain (4). Trigger points activate dorsal horn neurons through sustained nociceptive input, resulting in neuronal microstructural alterations (5).

Considering sedentary lifestyle and static postures during daily work and leisure tasks, such as using cellphone and

computer, myofascial pain syndrome is one of the most common musculoskeletal disorders that reduce the function and constitutes a considerable individual and socioeconomic burden (6, 7). MPS is reported to have a prevalence ranging from 30% to 93% (8).
The trapezius muscle is one of the most common place for trigger points, causing pain and functional problems, which is associated with carrying light loads and certain postures, such as working on a computer for long periods of time (9). The symptoms include aching sensation in the upper trapezius muscle, posterior neck and headaches and shoulder regions causing difficulty with sleeping due to shoulder pain. The symptoms can be increased with activity and relieved by rest.
In addition to pharmacological treatments, there are various non-pharmacological treatments for patients with myofascial pain, including electrotherapy, massage, cupping therapy, dry needling, acupuncture and trigger point injections to allow participation in an active exercise program such as postural correction and exercise therapy (10-12). Oral medications can have side effects or interactions with other drugs the patient may be taking. A systematic review of a limited number of trials indicates that combined stretching and strengthening exercise has positive small to moderate effects on pain intensity in MPS (13).
Cupping therapy is an old method that is used by creating negative pressure through heat or suction by cups (cups) that are placed on the skin (14). The mechanism of action of cupping therapy is not yet fully understood (15). However, the most important mechanisms proposed is the effects of negative pressure and mechanical pressure of cupping massage, by increase of peripheral blood circulation and immunity, alteration of skin biomechanical properties, increase of pain threshold, inhibition of neural activity, stimulation of local mechanoreceptors, pain gate control inputs, release of local endorphins and brain enkephalins, mechanical stretching of tissues, alters gel-like tissues to softer state, release of taut bands and enhanced energy flow, improvement of local anaerobic mechanism, decrease inflammation, and modulation of cellular immune system (2, 16-21).
According to the immune modulation theory, microscopic environmental changes in the skin cause the conversion of biological signals and activation of the neuroendocrine immune system (22). From the point of view of genetic theory, mechanical stress of the skin due to negative pressure and local anaerobic mechanism causes physiological and mechanical signals that activate or inhibit gene expression (23).
In recent years, the effect of cupping therapy has been studied on various diseases, including neck pain and Low back pain (24-31). In our country, cupping therapy is more traditional and little research has been done in this regard.
In cupping massage, combining the effects of both massage and cupping therapy can increase the effectiveness of treatment in myofascial pain. Therefore, the aim of this study was to evaluate the short-term and long-term effects of cupping massage on pain, disability, and fatigue in comparison with conventional exercise training in patients with myofascial pain of trapezius muscle.

MATERIALS AND METHODS

Study design

This study was a randomized controlled clinical trial with two parallel groups which performed in Shariati hospital in Tehran University of Medical Sciences (TUMS) from October 2020 to December 2021. The subjects were diagnosed and referred an attending physiatrist according to the inclusion and exclusion criteria. After description, the study goals, assessment and intervention, the patients signed the informed consent form and entered into the study. The subjects were randomly assigned into two groups using sealed envelopes: the massage cupping and the exercise therapy groups. The study protocol was approved by the ethics committee of TUMS (IR.TUMS.MEDICINE.REC.1398.177) and registered in the Iranian Registry of Clinical Trials Database (IRCT20180804040685N2).

Sample size

The sample size was calculated to be 20 in each group based on the mean difference and standard deviation of pain intensity (2.5 ± 3.5) in a similar study, when $\alpha = 0.05$ and $\beta = 0.2$. Considering 10% of missing cases, we designated 22 cases in each group.

Participants

Forty-four patients with trapezius myofascial pain participated in the study. The inclusion criteria consisted of an established clinical diagnosis of trapezius myofascial pain syndrome by a physical medicine and rehabilitation specialist, duration of at least 3 months, age range 30 to 55 years and pain intensity at least 3 (based on a numerical scale of 0 to 10). The exclusion criteria consisted of history of trauma, disc herniation, congenital deformity of the neck and shoulders, inflammatory and systemic diseases, mental disorders that need treatment, pregnancy, a history of surgery in the past month, and participation in another

treatment or exercise program during this study. **Figure 1** displays the flowchart of the study.

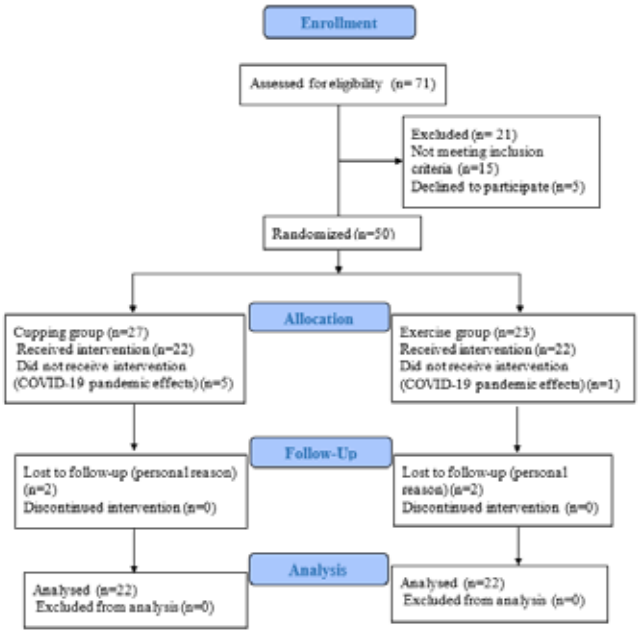

Figure 1. Flowchart of the study.

Initial evaluation

Before the intervention, demographic data and medical history, pain intensity (numerical scale 0 to 10), Dash Quick disability questionnaire (32) and Fatigue Severity Scale (Fatigue Severity Scale) (33) were completed by the patients. Dash Quick disability questionnaire consisted of 19 items with a 7-point Likert scale and the fatigue severity questionnaire consisted of 9 items with a 5-point Likert scale.

Intervention

Each group was treated for 4 weeks (three sessions per week). Both groups were the same in terms of drug treatment (Acetaminophen 500 mg). The cupping group was treated by a physiotherapist by applying negative pressure through a suction device and moving the cup with a diameter of 4 cm on the trapezius muscle. Patients were lying in prone position. Maintaining negative pressure, the cup was massaged from the occiput to the middle of the thoracic vertebrae along the upper trapezius muscle for 5 minutes using a lubricant gel. The patient was reminded that the massage site would be red and sensitive for several days. For the second group (control group), a conventional exercise therapy was performed. The exercises consisted of three strengthening exercises and five stretching exercises for the upper, middle and lower trapezius muscles. Each movement was performed 10 repetitions and 3 times a day. These exercises were taught face to face and by given an educational pamphlet to remind the patient at home and follow-up phone calls were also made to monitor their exercise program on a weekly basis during the study so that the physiotherapist confirmed the correctness of their exercise performance. The effectiveness of home-based exercise therapy was confirmed by previous studies (34).

Immediately after the last treatment session and three months later, all initial measurements were performed again for both groups.

Statistical analysis

Data were analyzed by IBM SPSS statistics 24 software with a significance level of $P < 0.05$. Shapiro-Wilk test was used to evaluate the normality of data distribution. Independent t-test was used to compare the data between the two groups and paired t-test was used to compare the data before and after the intervention in each group. If the data distribution was not normal, the Wilcoxon signed-rank test was used to compare the variables before and after the intervention in each group, and the Mann-Whitney test was used to compare the variables between the groups. The results were analyzed by an assessor blinded to the group allocation.

RESULTS

A total of 44 patients (mean age 42.73 ± 10.88) were admitted to the study after being diagnosed by a physician and examining the inclusion and exclusion criteria, and were divided into two groups of intervention (massage cupping therapy) and control (exercise therapy). Preliminary patient information is presented in **table I**. Prior to the intervention, there was no significant difference between the two groups in terms of age, body mass index, duration of illness, pain intensity, disability and fatigue severity. According to the COVID-19 pandemic effects 5 patients in cupping group and one patient in exercise group abandoned the study. There were no adverse effects for exercise group but most patients in cupping group felt some pain just the day after intervention and someone accompanied with ecchymosis at the site of the cupping technique. Almost all of these adverse effects were related to the first two weeks of treatment course.

The results of the present study showed a significant reduction of pain intensity, disability and fatigue severity immediately after the intervention and three months later in each group (**table II**). The between group comparison showed that there was a significant different between the two groups

Table I. Basic characteristics of the studied population.

Variables	Total (n = 44)	Cupping group (n = 22)	Exercise group (n = 22)	P Value
Male (%) Female (%)	9 (20.5) 35 (79.5)	5 (22.7) 17 (77.3)	4 (18.2) 18 (81.8)	0.71
Age (year)	42.73 ± 10.88	43.09 ± 10.6	42.36 ± 11.39	0.83
Body mass index (kg/m2)	26.84 ± 3.13	27.15 ± 2.99	26.53 ± 3.32	0.52
Duration of disease (month)	31.88 ± 25.22	28.64 ± 23.69	35.29 ± 26.88	0.31
Pain intensity (score)	6.55 ± 1.65	6.86 ± 1.67	6.23 ± 1.60	0.15
Disability (score)	39.75 ± 8.39	40.86 ± 5.67	38.64 ± 10.46	0.39
Fatigue severity (score)	39.32 ± 6.71	39.09 ± 7.12	39.55 ± 6.43	0.83

in terms of pain intensity, disability and fatigue severity, so that the pain intensity and disability reduced in the cupping group more than the exercise group. The reduction of the fatigue severity score in the cupping group was more than the exercise group immediately after the intervention and three months later but the difference was significant only after three months (**table III**).

DISCUSSION

This is the first study to evaluate the short-term and long-term effects of cupping massage on pain, disability, and fatigue in comparison with conventional exercise training in patients with myofascial pain of trapezius muscle.

The results of the present study showed that both cupping and exercise therapy were effective in reducing pain, disabil-

Table II. Intra-group comparison before and after the intervention.

Groups	Time	Pain intensity Mean ± SD	Disability Mean ± SD	Fatigue severity Mean ± SD
Cupping group (n = 22)	Pre-intervention	6.90 ± 1.70	41.24 ± 5.52	39.67 ± 6.76
	Post intervention	2.67 ± 1.46	27.57 ± 7.60	27.52 ± 8.21
	P Value	0.00	0.00	0.00
	3-months follow-up	2.12 ± 2.10	25.10 ± 7.05	24.52 ± 10.40
	P Value	0.00	0.00	0.00
Exercise group (n = 22)	Pre-intervention	6.15 ± 1.53	38.65 ± 8.76	39.15 ± 5.47
	Post intervention	3.95 ± 1.47	32.85 ± 7.36	34.45 ± 5.96
	P Value	0.00	0.00	0.00
	3-months follow-up	4.38 ± 2.27	31.62 ± 9.15	33.86 ± 9.98
	P Value	0.001	0.00	0.00

Table III. Between-group comparison of mean difference after the intervention.

Groups	Time	Pain intensity Mean ± SD	Disability Mean ± SD	Fatigue severity Mean ± SD
Post intervention	Cupping group	- 4.20 ± 1.87	- 13.67 ± 7.45	- 12.14 ± 5.82
	Exercise group	- 2.20 ± 0.83	- 5.80 ± 4.67	- 4.70 ± 3.71
	P. Value	0.00	0.04	0.07
3-months follow-up	Cupping group	- 4.67 ± 2.56	- 15.52 ± 8.51	- 14.33 ± 10.99
	Exercise group	- 1.90 ± 2.07	- 7.19 ± 5.93	- 5.57 ± 5.90
	P Value	0.001	0.001	0.04

ity, and fatigue severity in patients with trapezius strain in both short-term (immediately after treatment) and long-term (three months later). However, the effect of cupping therapy was greater than exercise therapy, so that immediately after treatment and three months later, a significant difference was observed between the two groups for pain intensity, disability, and fatigue.

It should be noted that a similar study in this regard has not been done before, but these findings were consistent with the results of the Saha (18) study, in which, the effect of five sessions of massage cupping on pain intensity, disability and quality of life of patients with non-specific chronic neck pain was investigated. However, the control group in that study had performed routine treatment, while in the present study, the effects of massage cupping were compared with exercise therapy and the results showed the superiority of massage cupping over the exercise therapy. The synergistic effects massage and cupping therapy for reducing pain and improving function in these patients can be related to increasing local blood circulation and reducing spasm and thus reducing the sensitivity caused by tissue ischemia (18, 19). Michalsen showed the effect of a cupping therapy session on the trapezius muscle in reducing the symptoms of carpal tunnel syndrome and improving neck pain in 52 patients. They used wet cupping technique on trapezius muscle using static mechanical suctioning. Pain intensity decreased (29). In a study by Lauche, the use of a cupping therapy session was effective for pain reduction in chronic nonspecific neck pain, compared to the control group, and the amount of pain reduction was clinically acceptable and comparable to other methods used in other studies such as dry cupping or massage (30).

In another study, the results of four randomized clinical trial studies were analyzed (31). The effect of one wet cupping session, 5 dry cupping sessions, pulsative cupping and cupping massage in patients with chronic nonspecific neck pain were compared to the control group. The pain intensity (VAS), disability (Neck Disability Index), and quality of life (SF-36) were measured before and after the intervention. Minimal clinically significant difference (MCID) and substantial clinical benefit (SCB) were also measured. MCID and SCB for pain relief were 21.3% and 66.8% respectively. MCID and SCB for disability were 9.8% and 29.8% and for quality of life were 20.5% and 43.1%, respectively. According to these results, the patient's perception of the usefulness of treatment in reducing pain is comparable to other common treatments, but in terms of disability and quality of life, this effect is not as great as other studies, which can be due to higher quality of life and lower disability score at onset of the Study (31).

A meta-analysis review study examined the effect of cupping therapy on neck pain (35). The results showed that this technique was effective in improving pain, disability, and quality of life compared to the control group. In this meta-analysis, the rate of pain reduction and disability compared to the control group without treatment was 2.42 and 4.34 units, respectively, but compared to the active control group, pain reduction was less than one unit and there was not a significant difference between groups for functional improvement. However, due to the low quality of the evaluated studies, for better and more accurate conclusions, more research is needed. In the present study, the reduction of pain and disability compared to the exercise therapy group was 2.04 and 7.87, respectively. Variation in the method of cupping technique may have played a role in the difference in effectiveness in the studies. However, the guidelines revealed a good level of evidence for therapeutic exercises in rehabilitation treatment (36, 37).

Several studies showed the influence of manual therapy on trapezius, neck, and shoulder myofascial pain reduction (38-40). An investigation showed that manual techniques on upper trapezius with latent trigger point improved the cervical range of motion and the pressure pain sensitivity and the effects persisted for one week after the intervention (41). A study by Alghadir *et al.* (42) showed that muscle energy technique plus ischemic compression technique is highly effective in dismissing myofascial trigger points pain within a very brief period of time, is cost effective, is noninvasive, and achieves relief without causing much pain. Massage and dry cupping as a manual technique are safe and effective treatment modalities for MPS. In the present study, the synergistic effects of the combined dry cupping and massage were evaluated and the results showed a significant improvement of pain, disability, and fatigue.

In this study, we aimed to investigate the role of massage cupping on treatment of a common and debilitating disease. The findings of our study revealed that this manual approach can be used as a practical method in MPSs, especially trapezius strain by reason of its safety, effectiveness, and less time and energy consumption. This method does less damage to the therapist joints because of using the negative pressure of the cup and the mechanical pressure of the cup itself, which makes it easier for the therapist to apply force. Therefore, it is applicable even by therapist with less strength to perform manual-therapy for the patients who need.

Limitations

This study had some limitations including short duration follow-up and use functional assessments to evaluate the functional effects of massage cupping technique in compression to exercise therapy.

CONCLUSIONS

According to our finding for improvement of pain, disability, and fatigue in patients with trapezius pain syndrome, massage cupping should be considered as a feasible, safe, fast, and effective method for patients with trapezius pain syndrome, also, this method could be combined with other rehabilitation programs in the treatment of myofascial pain and muscle strain. More studies need to confirm these results as well as the effectiveness of this method on improvement of the other conditions such as myofascial pain syndrome of different muscles or other musculoskeletal disorders.

ETHICS

This study was conducted with the approval of the Vice Chancellor for Research, TUMS. Informed consent form was received from all participants at the beginning of the study. Participants could withdraw the study at any stage when they did not want to continue without affecting their treatment process.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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Electroacupuncture and Transcutaneous Electrical Nerve Stimulation in Chronic Nonspecific Low Back Pain: a Blind Randomized Clinical Trial

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SUMMARY

Background. Chronic nonspecific low back pain impairs function in affected individuals. Transcutaneous Electrical Nerve Stimulation (TENS) has shown to be effective in reducing the intensity of chronic nonspecific low back pain and should be used as a complementary treatment. The same can be said for electroacupuncture (EA), which consists of the application of electrical stimulation through punctured needles in acupuncture meridians, generating physiological reactions and leading to therapeutic effects.
Objective. To compare the effects of EA and TENS in subjects with chronic nonspecific low back pain.
Methods. Blind randomized clinical trial of 48 subjects with chronic nonspecific low back pain. The patients were allocated to the following groups: Conventional Kinesiotherapy (CG), Conventional Kinesiotherapy plus Transcutaneous Electrical Nerve Stimulation (CTENSG), and Conventional Kinesiotherapy plus Electroacupuncture (CEAG). The individuals were evaluated before and after interventions and at a 30-day follow-up for the following factors: pain level, flexibility, lumbopelvic stability, and function. A total of ten interventions were performed three times a week for four weeks.
Results. All groups significantly improved pain. However, CEAG reduced pain significantly more than GTENSG and CG ($p < 0.05$). The three groups significantly improved function at endpoint ($p < 0.05$). Regarding the Roland Morris questionnaire, GCEAC scored significantly lower than CTENSG and CG ($p < 0.05$). Lumbopelvic stability improved in all tests for CEAG and CG.
Conclusions. The association between electroacupuncture and exercise improved pain, function, and lumbopelvic stability in comparison to exercise alone or in association with TENS.

KEY WORDS

Low Back Pain; electroacupuncture; Transcutaneous Electrical Nerve Stimulation; physiotherapy; Exercise Therapy.

INTRODUCTION

Low back pain is a public health problem worldwide. Its prevalence rate is 80% (1), being one of the most common and disabling musculoskeletal disorders in the world (1, 2). In some patients, initial acute pain can last for three months and eventually develop into chronic low back pain (1). The diagnosis is usually defined as pain below the costal margin and above the inferior

gluteal folds, characterizing specific or nonspecific low back pain (3).

Nonspecific low back pain is defined as a symptom for which there is currently no reliable method to identify the pathology. It affects approximately 70% to 85% of the population at some stage in their lives (4). Psychosocial factors play a role in the development and maintenance of this condition (5), where a wide variety of factors have some influence. It leads to functional disability, limited general mobility, worse self-reported health, lower quality of life, absenteeism in the workplace, and depression (5, 6). Low back pain patients often report limitations in their daily activities, which affects interpersonal relationships and socialization that are important for any individual (5).

Etiology can be subdivided into mechanical, systemic, and referred groups. The most frequent cause is mechanic (97%), generating "nonspecific low back pain" (4). This definition is used when the cause of pain cannot be precisely determined, thus excluding those cases of patients presenting with a specific cause (*e.g.*, fracture, infection, cancer) (4).

Physiotherapy aims to improve the functional capacity and quality of life of these patients (7), and exercise is an effective alternative to reduce pain (8). Transcutaneous electrical nerve stimulation (TENS) is also effective in reducing the intensity of nonspecific low back pain and should be used as a complementary treatment (9). Among its modalities of application is the acupuncture mode (TENS-AC), which uses an electrical stimulation with low frequency (< 10 Hz), high pulse width (> 150 mp), and high intensity, respecting the patient's tolerance. This mode (TENS-AC) stimulates Aδ and C nociceptive fibers, decreasing pain from the activation of endogenous opioid mechanisms known as diffuse noxious inhibitory controls (9).

Another method used is electroacupuncture (EA), which consists of the application of electrical stimulation through puncture needles in acupuncture meridians, generating physiological reactions and leading to therapeutic effects such as analgesia (10). Electroacupuncture (EA) modulates pain through significant changes in opioids, serotonin, and norepinephrine, which are stimulated to defined sites, acting in the spinal cord and supraspinal structures (11). Electroacupuncture (EA) derived from the integration of traditional acupuncture and modern electrical stimulation is generally accepted because it is a relatively direct, safe, and inexpensive therapy in comparison to other conventional therapies (12). In addition, EA has become increasingly used in clinical practice due to its repeatability and standardization of frequency, intensity, and duration. After the needles are inserted into the acupuncture points, the electrodes are connected to the pairs of needles and then a small electrical current is administered (12).

Considering that nonspecific low back pain is one of the main causes of disabilities today, the present study compared the effects of EA and TENS in subjects affected by this condition.

METHODS

Blind randomized clinical trial of patients with chronic nonspecific low back pain. Subjects of both sexes and aged between 18 and 70 years were recruited. Sample calculation considered the immediate effect of TENS on pain reduction in patients with chronic nonspecific low back pain as the primary outcome of the study. Following Ebadi *et al.* (13), for an initial sample calculation, we used a study power of 80%, a significance level of 95%, a sampling error of 5%, and a sample size ratio of 1: 1: 1 (Kinesiotherapy group – CG: Kinesiotherapy and TENS group – CTENSG: Kinesiotherapy and Electroacupuncture group – CEAG), reaching the estimated number of 14 subjects for each intervention group. Believing that losses and refusals would be around 15%, we reached the final number of 16 subjects for each study group, totaling 48 subjects.

Inclusion criteria were as follows: medical prescription for physiotherapeutic treatment of nonspecific low back pain, history of low back pain for more than three months, and no other type of concurrent treatment. Subjects with three absences, with history of previous surgery on the lumbar spine, or with imaging exams showing intervertebral degenerative process and pain radiated to the lower limbs were excluded.

This research was approved by the Ethics and Research Committee of the Lutheran University of Brazil under opinion No. 3,738,209. All participants previously signed an Informed Consent Form (ICF). The study was registered in the Brazilian Registry of Clinical Trials (REBEC) under the number RBR-9w54gd. This study meets the ethical standards of Muscles, Ligaments and Tendons Journal (14).

Data collection, evaluation and randomization

After fulfilling the eligibility criteria, the study participants received information about the research and were evaluated by a previously trained evaluator who did not know to which group the subject had been assigned (blind evaluator). Evaluations took place before and after intervention and thirty days after the intervention.

An evaluation form was used to collect sociodemographic data. The visual analog scale (VAS) was used to assess pain level. The Wells Bench was used to assess posterior chain flexibility, as described by Pitanga (15). Lumbopelvic stability was assessed using the static trunk endurance test, in which the subject seats with hips and knees flexed at 90º, arms folded and crossed at the chest, and feet fixed by the evaluator, with the trunk at an angle of 60º. In this test, the subject holds the isometric posture for as long as possible and the examiner records the posture maintenance time. The Sorensen endurance test and the side bridge test were also performed.

Function was assessed using the Roland-Morris Disability Questionnaire (RMDQ). This questionnaire consists of 24 self-answer questions, which participants complete in less than five minutes. The questions have a dichotomous answer (yes or no) and the final result corresponds to the sum of the yes answers. This result can vary between 0 and 24, with zero corresponding to a person without complaints and the maximum value corresponding to a patient with very severe limitations.

After going through the initial assessment, the subjects were assigned by an independent collaborating researcher, that is, who did not participate in the other phases of the study. Randomization took place using sealed envelopes corresponding to each of the groups to which the subject was assigned: Kinesiotherapy group (CG), Kinesiotherapy and TENS group (CTENSG), and Kinesiotherapy and Electroacupuncture group (CEAG).

Intervention program

Three interventions were performed weekly for four weeks, totaling ten interventions. Consultations lasted 50 minutes, with vital signs being checked before and after intervention. Heart rate (HR) and peripheral O_2 saturation were measured using a G-Tech® LED oximeter. Blood pressure was measured using a G-Tech® LED sphygmomanometer and a Littman® stethoscope, both previously calibrated. Finally, respiratory rate was measured using a Western® digital chronometer.

Participants from the conventional kinesiotherapy group (CG) initially performed three 20-second sets of stretches for posterior muscle chain, followed by 10 repetitions of strengthening exercises for core stabilizing muscles (held for 6" in the first and second weeks, 8" in the third week, and 10" in the fourth week). These exercises were: supine bridge, single leg supine bridge, side bridge, prone plank (**table I**).

Participants in the kinesiotherapy and TENS group (CTENSG) underwent the same kinesiotherapy program but with TENS application after kinesiotherapy. A portable two-channel transcutaneous electrostimulator (brand Ibramed®, model Neurodyn; ANVISA registration number 10360310012) was used. Before applying the electrodes, the skin of the participants was cleaned using cotton and 70% alcohol gel. Total application time was 20 minutes. The parameters were pulse width of 250 µs, pulse frequency of 10 Hz, and current intensity according to the participant's tolerance. Two channels with four 5 × 5 cm self-adhesive Valutrode electrodes were used, positioned bilaterally in the paravertebral musculature (L1 and L5 region).

In the CEAG, participants underwent the same kinesiotherapy program as the previous groups, but with the application of electroacupuncture after kinesiotherapy. For that, an acupuncture electrostimulator (brand Sikuro®, model DS100jr; ANVI-

Table I. Description of the kinesiotherapy program.

Week	Description	Repetitions/Time
1 and 2	Exercise 1: Stretching of hamstring and gastrocnemius muscles, leg elevation with extended knee.	3 × 20"
	Exercise 2: Stretching of paravertebral muscles and hip extensors, leg flexing bringing the knee closer to the chest.	3 × 20"
	Exercise 3: Supine bridge.	Hold for 6", repeat 10X
	Exercise 4: Single leg supine bridge.	Hold for 6", repeat 10X
	Exercise 5: Side bridge.	Hold for 6", repeat 10X
	Exercise 6: Prone plank.	Hold for 6", repeat 10X
3	Exercise 1: Stretching of hamstring and gastrocnemius muscles, leg elevation with extended knee.	3 × 20"
	Exercise 2: Stretching of paravertebral muscles and hip extensors, leg flexing bringing the knee closer to the chest.	3 × 20"
	Exercise 3: Supine bridge.	Hold for 8", repeat 10X
	Exercise 4: Single leg supine bridge.	Hold for 8", repeat 10X
	Exercise 5: Side bridge.	Hold for 8", repeat 10X
	Exercise 6: Prone plank.	Hold for 8", repeat 10X
4	Exercise 1: Stretching of hamstring and gastrocnemius muscles, leg elevation with extended knee.	3 × 20"
	Exercise 2: Stretching of paravertebral muscles and hip extensors, leg flexing bringing the knee closer to the chest.	3 × 20"
	Exercise 3: Supine bridge.	Hold for 10", repeat 10X
	Exercise 4: Single leg supine bridge.	Hold for 10", repeat 10X
	Exercise 5: Side bridge.	Hold for 10", repeat 10X
	Exercise 6: Prone plank.	Hold for 10", repeat 10X

SA MS registration number 80470920001) was used. Before application, the puncture area was cleaned with cotton and 70% alcohol gel. Then, 25 × 40 mm needles (brand Gold Dragon®) were used for puncture at bladder meridian points B22 (L1) and B26 (L5). The same reference points of the intervention in CTENSG were maintained. Total procedure time was 20 minutes. For this procedure, signal 1 was used: continuous pulse train, with pulse frequency of 10 Hz. Current intensity was administered according to the participant's tolerance, using E-JS18 cables ('alligator'-type electrodes) to connect the device to the puncture needles. Two channels were used with four stimulator cables connected to the needles. After intervention, the needles were immediately discarded in a Descarpack box duly appropriated for this purpose.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) version 23.0 was used for data analysis. Initially, a descriptive analysis of the study variables was performed through absolute value, frequency, mean, and standard deviation. Afterwards, the variables were statistically analyzed by parametric tests. Analysis of Variance (ANOVA) for repeated measures was used within each group from baseline to follow-up assessments, and unpaired Student t test for analysis of the variables between groups at each moment. For nonparametric variables, the Kruskal-Wallis and Mann-Whitney tests were used, respectively. The level of significance established for the statistical test was $p < 0.05$.

RESULTS

The initial evaluation included 54 subjects. Of these, 6 were excluded for not meeting the eligibility criteria. Therefore, 48 subjects were randomized, 16 for each group, as shown in **figure 1**.

Table II shows the baseline characteristics of the participants in the study groups. The groups were homogeneous for the analyzed variables (**table II**).

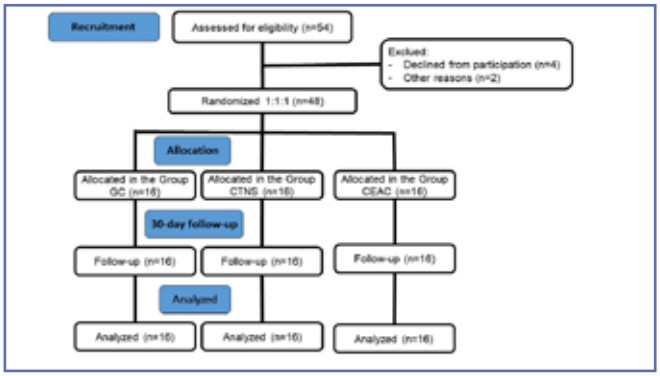

Table II. Characterization of study subjects (n = 48).

	Intervention Group			
	CTENSG (n = 16)	**CEAG (n = 16)**	**CG (n = 16)**	**p value**
Age, years (m ± sd)	52.50 ± 12.42	45.37 ± 13.51	50.81 ± 12.96	0.28*
Gender, n (%)				0.49**
Male	3 (18.75)	6 (37.5)	5 (31.25)	
Female	13 (81.25)	10 (62.5)	11 (68.75)	
Skin color, n (%)				0.76**
White	15 (93.75)	14 (87.5)	15 (93.75)	
Black	1 (6.25)	2 (12.5)	1 (6.25)	
Weight, kg (m ± sd)	76.62 ± 11.57	76.44 ± 10.49	74.19 ± 10.70	0.84*
Height, m (m ± sd)	1.62 ± 0.09	1.67 ± 0.09	1.65 ± 0.07	0.18*
BMI, n (m ± sd)	28.95 ± 4.32	27.36 ± 4.00	27.29 ± 3.11	0.39*
Occupation, n (%)				0.23**
Housekeeper	6 (37.6)	3 (18.8)	5 (31.3)	
General services	6 (37.6)	10 (62.1)	5 (31.3)	
Retired	3 (18.8)	0 (0.0)	2 (12.4)	
Other	1 (6.0)	3 (18.8)	4 (25.0)	
Time of pain, years (m ± sd)	5.56 ± 3.72	7.37 ± 5.47	5.00 ± 3.59	0.28*

Kg: kilogram; m: meter; *One-way ANOVA; **Chi-Square.

All intervention groups improved pain (VAS) from baseline to follow-up. The CEAG group had a significantly lower pain score than groups CTENSG and CG both in the final assessment and in the follow-up assessment ($p < 0.05$).
Posterior chain flexibility, assessed through the Wells Bench, improved significantly from baseline to endpoint only in CG ($p < 0.01$). When comparing groups, CEAG and CG had a higher level of flexibility than CTENSG in the final and follow-up assessments ($p < 0.05$) (**table III**).
Lumbopelvic stability tests showed an increase in posture maintenance in CEAG and CG from baseline to endpoint. The CTENSG group improved scores only in the static trunk endurance test. The comparison between groups showed that CEAG subjects maintained the correct posture significantly longer than CTENSG and CG subjects in the follow-up assessment (**table IV**).
Function assessment using the RMDQ showed that the three groups significantly improved scores from baseline to endpoint and follow-up. However, CEAG showed a significantly lower score than CTENSG and CG in the final and follow-up assessments ($p < 0.05$) (**table V**).

DISCUSSION

The present study compared the effects of EA and TENS on pain in subjects with nonspecific low back pain. Recent studies on the same pathology showed sample homogeneity (16-18). That said, the subjects of this study were homogeneous for gender, age, skin color, Body Mass Index (BMI), occupation, and time of pain.
Our research followed a kinesiotherapy protocol similar to the method described by Mendes *et al.* (19), who also obtained positive results in improving pain and function in the kinesiotherapy group. For the CTENSG intervention, the TENS parameters applied were similar to those of the study by Tousingnat-Laflamme *et al.* (9), in which pain also improved significantly. For the CEAG intervention, the applied electroacupuncture parameters were similar to those of the study by Comachio (16). In that study, pain improved significantly with the use of electroacupuncture and manual acupuncture as a complementary treatment for low back pain. These results corroborate those obtained for CEAG, with pain improvement after intervention and significant results in pain relief in three months of follow-up. Electroacupuncture (EA) modulates pain through significant changes in opioids, serotonin, and norepinephrine, which are inhibitory neurotransmitters in the dorsal horn of the spinal cord, activated by electrical stimuli at the acupuncture points to act in the spinal cord and supraspinal structures (11).
When comparing intergroup results, pain improved significantly in CEAG in comparison to CTENSG and CG in the final and follow-up assessments. Likewise, Leite *et al.* (17) conducted a randomized clinical trial to verify whether electroacupuncture is effective in reducing pain and in quantitative responses to sensory tests in patients with chronic nonspecific low back pain. The authors used an electroacupuncture treatment group and three different control groups, with a total of 69 participants. As a conclusion, the electroacupuncture group significantly reduced

Table III. Pain score (VAS) and posterior chain flexibility in the study groups.

	Intervention Group			p value
	CTENSG (n = 16)	CEAG (n = 16)	CG (n = 16)	
Pain score				
Baseline	8.88 ± 1.03	8.75 ± 0.93	9.19 ± 0.83	0.40
Endpoint	3.56 ± 2.85	1.25 ± 1.18	3.31 ± 2.85	0.02[#$]
Follow-up	4.00 ± 3.18	0.94 ± 1.06	3.81 ± 2.29	0.001[#$]
P value	0.0001[ab]	0.0001[ab]	0.0001[ab]	
Posterior chain flexibility, cm				
Baseline	14.44 ± 4.98	19.88 ± 8.23	20.38 ± 8.62	0.05
Endpoint	17.13 ± 7.35	24.13 ± 8.55	24.69 ± 7.10	0.01[#&]
Follow-up	16.06 ± 7.51	24.00 ± 9.83	24.25 ± 6.57	0.009[#&]
P value	0.17	0.29	0.001[ab]	

Cm: centimeters; [a]$p < 0.01$ when comparing baseline with endpoint. ANOVA for repeated measures; [b]$p < 0.01$ when comparing baseline with follow-up. ANOVA for repeated measures; [#]$p < 0.05$ between CTENSG and CEAG. One-Way ANOVA; [$]$p < 0.05$ between CEAG and CG. One-Way ANOVA; [&]$p < 0.05$ between CTENSG and CG. One-Way ANOVA.

Table IV. Results of lumbopelvic stability tests in the study groups.

	Intervention Group			p value
	CTENSG (n = 16)	CEAG (n = 16)	CG (n = 16)	
Static trunk endurance test, sec				
Baseline	17.94 ± 12.21	28.81 ± 31.33	24.37 ± 18.52	0.38
Endpoint	43.06 ± 30.69	83.87 ± 62.51	39.44 ± 17.77	0.006[#$]
Follow-up	39.12 ± 31.31	73.37 ± 50.15	33.75 ± 19.73	0.006[#$]
p value	0.002[ab]	0.009[ab]	0.0001[ab]	
Sorensen test, sec				
Baseline	22.62 ± 15.89	30.50 ± 20.28	37.94 ± 25.24	0.13
Endpoint	33.50 ± 29.89	60.37 ± 38.69	54.56 ± 28.19	0.06
Follow-up	30.44 ± 26.97	74.00 ± 59.76	47.00 ± 27.26	0.01[#]
p value	0.08	0.009[ab]	0.0001[abc]	
Right side bridge test, sec				
Baseline	13.19 ± 8.23	22.37 ± 15.44	22.56 ± 18.36	0.13
Endpoint	21.57 ± 14.59	39.94 ± 22.89	34.69 ± 19.53	0.03[#]
Follow-up	19.18 ± 14.58	38.44 ± 20.71	32.75 ± 20.16	0.02[#]
p value	0.009[a]	0.0001[ab]	0.0001[ab]	
Left side bridge test, sec				
Baseline	14.75 ± 11.04	19.87 ± 14.18	24.69 ± 18.21	0.18
Endpoint	22.50 ± 18.18	39.00 ± 20.80	34.87 ± 20.46	0.06
Follow-up	21.12 ± 17.59	38.25 ± 19.71	30.81 ± 19.73	0.04[#]
p value	0.09	0.0001[ab]	0.0001[abc]	

Sec: seconds; [a]p < 0.05 when comparing baseline with endpoint. ANOVA for repeated measures; [b]p < 0.05 when comparing baseline with follow-up. ANOVA for repeated measures; [c]p < 0.05 when comparing endpoint with follow-up. ANOVA for repeated measures; [#] p < 0.05 between CTENSG and CEAG. One-Way ANOVA; [$]p < 0.05 between CEAG and CG. One-Way ANOVA; [&]p < 0.05 between CTENSG and CG. One-Way ANOVA.

Table V. RMDQ scores in the study groups.

	Intervention Group			p value
	CTENSG (n = 16)	CEAG (n = 16)	CG (n = 16)	
RMDQ				
Baseline	16.63 ± 3.96	16.50 ± 4.41	19.81 ± 3.23	0.03[$]
Endpoint	10.31 ± 7.13	4.44 ± 5.67	9.88 ± 5.62	0.02[#&]
Follow-up	9.56 ± 7.57	3.56 ± 5.32	10.38 ± 5.19	0.005[#&]
P value	0.001[ab]	0.0001[ab]	0.0001[ab]	

[a]p = 0.0001 when comparing baseline with endpoint. ANOVA for repeated measures; [b]p = 0.0001 when comparing baseline with follow-up. ANOVA for repeated measures; [c]p < 0.05 when comparing endpoint with follow-up. ANOVA for repeated measures; [#]p < 0.05 between CTENSG and CEAG. One-Way ANOVA; [$]p < 0.05 between CEAG and CG. One-Way ANOVA; [&]p < 0.05 between CTENSG and CG. One-Way ANOVA.

pain at rest and on exertion. Dohnert *et al.* (20) described pain reduction after four weeks of lumbopelvic stabilization and Mckenzie exercises. The authors state that the analgesic effects generated by different types of exercises can have different explanations (20). For example, the application of controlled forces to the spine through exercise can temporarily reduce pain intensity, altering the fluid dynamics of the injured tissue (20). Another justification would be that lumbopelvic stabilization exercises can reduce the load and improve the quality of movements after improving the coor-

dination of trunk muscles (20). In addition, stabilization exercises mainly activate deep muscles, commonly affected by low back pain (20). Choopani *et al.* (21) treated 24 patients with low back pain secondary to spondylolisthesis twice a week for two months. The patients were divided into a group of conventional exercises and a group of lumbopelvic stabilization exercises. The authors observed that both programs reduced pain and disability, with no differences between groups (21).

Corroborating the analgesic effect of electroacupuncture in this research, Awad *et al.* (22) compared the effect of EA and low-level laser therapy on postpartum low back pain. The study included 50 women with complaints of low back pain, divided into a group with 25 women treated with electroacupuncture three times a week and a group with 25 women treated with low level laser therapy three times a week. The visual analog scale was used to measure pain intensity before and after treatment. The authors concluded that electroacupuncture is more effective in reducing pain than low level laser therapy.

Function improved significantly in the three groups from baseline to endpoint and follow-up. This corroborates the findings of Comachio *et al.* (16), who carried out a study to evaluate the efficacy of electroacupuncture and manual acupuncture on pain and function in patients with low back pain. These authors concluded that both treatments have similar effects, reducing pain and improving function in participants.

Therefore, electroacupuncture is effective in improving function. Following this line of thought, CEAG had a significantly better score in function in comparison to CTENSG and CG in the final and follow-up assessments. These findings corroborate with Kong *et al.* (23), who evaluated the effect of electroacupuncture and placebo electroacupuncture on pain and function in adults with low back pain. The authors found a significant improvement in function in the electroacupuncture group.

Electroacupuncture promotes positive regulation of adenosine and increases the inhibitory effects induced by adenosine in substance P, generating clinical benefits (24). The technique also improves sensory symptoms and regulates pain through neurophysiological mechanisms that activate sympathetic nervous fibers to increase endogenous opioids at the site of pain (25).

Activation of sympathetic nervous fibers increases the expression of the intracellular adhesion molecule in the blood vessels of inflamed tissue to promote the migration of polymorphonuclear leukocytes containing β-endorphin, metenkephalin, and mononuclear cells. Moreover, the activation of sympathetic neuron-derived norepinephrine stimulates adrenergic receptors in inflammatory cells to release β-endorphin, reducing pain (25) and improving function.

Only CG significantly improved posterior chain flexibility. In the comparison between groups, CEAG and CG showed higher flexibility than CTENSG in the final and follow-up assessments. Corroborating with the results of the present research, the study by Dohnert *et al.* (26) assessed 30 female participants with chronic low back pain. Study participants were divided into three groups: CORE exercises (CG), neuromuscular electrical stimulation (NMES) (NG), and CORE exercises in association with NMES (CNG). The authors obtained a significant increase in flexibility after intervention in the CORE group and in the CORE + NMES group, but only the CORE group maintained the level of flexibility in the follow-up assessment (26).

Song *et al.* (27) investigated the effect of TENS in association with static stretching on hamstring flexibility. One group of participants received three sessions of static stretching interventions, while the other group performed static stretching in association with TENS. All groups significantly improved hamstring flexibility. The study thus demonstrates that kinesiotherapy significantly improves flexibility and that adding electroacupuncture to the protocol can enhance this improvement in individuals with nonspecific low back pain.

Core stability exercises reduce the intravertebral load and improve the quality of movements by improving trunk muscle coordination, with positive effects on flexibility (25). These exercises mainly activate deep muscles, commonly affected by pain in the lumbar region. Electroacupuncture generates analgesic effects in these muscles so that they can be more quickly activated to carry out their biomechanical functions.

The results of lumbopelvic stability tests showed an increase in posture maintenance in CEAG and CG from baseline to endpoint. In the comparison between groups, CEAG participants maintained the correct posture for longer than CTENSG and CG participants in the follow-up assessment. This finding agrees with Armando *et al.* (28) in their study that compared TENS and stabilization exercises to prevent fatigue and improve muscle activation in patients with low back pain due to herniated disc. The authors concluded that although TENS relieves pain, it is not effective as a single therapy since stabilization exercises alone improved all the measured results. Regardless of the support of electrophysical agents, core stability exercises for patients with low back pain improve the size and recruitment of deep spinal muscles such as TrA, improving pain and function in the short term (20).

Dohnert *et al.* (26) also investigated lumbopelvic stability tests in a randomized, double-blind clinical trial in which intervention groups were divided into kinesiotherapy with

CORE exercises and kinesiotherapy with CORE exercises + NMES. Both groups improved posture maintenance time in all tests in the final and follow-up assessments. Kinesiotherapy exercises are thus effective in improving lumbopelvic stability, regardless of associated electrotherapy.

The proposed mechanism of action for core stability exercises improves motor control and the motor coordination of deep muscles of the spine and trunk such as the transversus abdominis, internal oblique, and rectus abdominis (28). Activation of these muscles improves stability in the points of origin of the segmental muscles and positively affects lumbopelvic stability (29). However, doubts still exist regarding the results of the association of therapeutic electrical currents in the approach for low back pain. Results are still controversial due to variations in stimulation parameters, demographic and anthropometric characteristics of participants, study design, outcome measures, and duration and planning of interventions. Laybidy *et al.* (30) are conducting a systematic review to clarify which electrical stimulation current improves pain and function in chronic nonspecific low back pain. The results can be valuable in clinical practice to optimize therapeutic planning.

Limitations

Our study has some limitations that may limit the extrapolation of results. Initially, the sample (despite having been calculated) is small. In addition, the short follow-up prevents us from analyzing these results in the long term.

We interpret that, although CEAG had presented better results in the evaluated items, kinesiotherapy may have influenced the results of CTENSG. Even considering that the results demonstrate the effectiveness of each of these techniques, we understand that new studies adding a control group and groups contemplating more parameters of regulation of electrical currents must be carried out to develop better guidelines for the treatment of nonspecific low back pain. Finally, we showed few records of recent studies using EA in low back pain, which makes it difficult to have a wide-ranging discussion about the technique.

CONCLUSIONS

Exercises proved to be the basis for the treatment of chronic nonspecific low back pain. The association between EA and exercise significantly improved pain, function, and lumbopelvic stability in comparison to exercise alone or in association with TENS. Based on the results of this clinical trial, EA can be used as an adjunct to lumbopelvic stability exercises in the clinical practice of low back pain. This would provide patients with a more effective reduction of symptoms and consequent enhancement of the proposed exercise.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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Efficacy of a 6-Week Supervised Strengthening Exercise Program with EMG-Biofeedback in Patients with Muscular Dystrophy: a Randomized Controlled Trial

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ABBREVIATIONS:
MD: Muscular Dystrophy
EMG-BFB: Electromyographic Biofeedback
CG: Control Group
BBS: Berg Balance Scale
MFM-32: Motor Function Measure-32
FSS: Fatigue Severity Scale
TUG: Timed Up Go test
SCT: Stair Climb Test
SST: Stand up from Supine position Test

SUMMARY

Background. Although previous trials investigated the effectiveness of exercise training programs in muscular dystrophy (MD) patients, we faced a lack of evidence on biofeedback. This study aimed to assess the safety and efficacy of a 6-week supervised strengthening exercise training program with electromyography-Biofeedback (EMG-BFB) on muscle strength, motor function, and balance of MD patients.

Methods. Forty MD patients were assigned randomly to EMG-BFB group (n = 20) and supervised strengthening exercise program as the control group (CG). Training strengthening program consisted of 12 sessions twice weekly for 30 minutes for 6 weeks. Patients in EMG-BFB group did exercises using biofeedback. Patients in CG also performed conventional exercises under the supervision of therapist for 6 weeks. The primary outcome was change in muscle strength. Other secondary outcomes were fatigue severity test, Vignos scale, timed up go test, stair climb test, stand up from supine position test, Berg balance scale, and quality of life. All outcomes measured pre- and post-intervention and 3 months after program.

Results. The compliance rate was 60% in this study. Significant improvements with training were observed in the hip muscles strength and Berg balance scale (BBS), motor function measure-32, and fatigue scale by week 6 and further to week 12 in both groups. Only the BBS improvement showed significant change in the intervention group in comparison to the control group (P = 0.040), which was not correlated with muscle strengths (P = 0.420).

Conclusions. This study demonstrated benefits of a supervised strengthening exercise program in MD patients. These benefits seem to be occurred without any adverse effects to cause patients to drop out of the study. According to some balance improvements, EMG-Biofeedback might be helpful in rehabilitation programs of MD patients.

KEY WORDS

Muscular dystrophy; function; strength; balance; rehabilitation; biofeedback.

INTRODUCTION

Muscular dystrophies (MDs) are a group of heterogeneous inherited diseases affecting cytoskeletal proteins causing progressive weakness (1). Muscle atrophy and dysfunction lead to significant functional impairments, including motor functions and swallowing difficulties, deformities such as scoliosis, respiratory alterations, and cardiac events (1). Their reduced physical activity contributes to deconditioning and increased fatigue perception that causes mental problems and social isolation (2). Falling is a frequent event among MD patients with catastrophic consequences like musculoskeletal injuries, fractures, and falling recurrence phobia, which aggregate their functional status (3).

Literature showed a growing body of evidence in support of exercise role in neuromuscular diseases incredibly muscular dystrophies (4). Although in ancient studies, doubts were raised about the possibility of muscle fiber damage by exercise among muscular dystrophy patients, recent studies using objective measures including serum Creatine-kinase level, Syndecans expression, ultrasonographic findings and T2-MRI muscle signal change following exercise proved its safety for this group of patients (5-8). During the literature review, we identified seven controlled trials investigating exercise effectiveness on MDs, three evaluating aerobic exercises (9-11), three strength-training (12-14), and one consisted of both exercise type (15) focusing mainly on FSHD and myotonic dystrophy subtypes. Aerobic exercises have been proved to be applicable on aerobic capacity and quality of life among MD patients (9-11). Controversies existed on strengthening exercises; however, consensus on resistive exercises with mild intensity was founded (12-14). One trial suggested combined aerobic and strengthening exercise as a more effective method than aerobic training alone (15). Most studies in this regard are with inappropriate methodology, insufficient sample size, with short-time follow-up.

The original biofeedback focusing on static muscle training and movement changed its place to novel biofeedback systems with advanced cue methods and control systems for task-oriented biofeedback (16). Although biofeedback is introduced to improve motor control through functional training among neuro-rehabilitation patients (17), its usefulness in motor control is reasonable in neuromuscular patients. Limited studies existed on the effects of biofeedback on neuromuscular diseases, and to our knowledge, there is no study on its effectiveness on MDs which approves novelty of this study.

Therefore, a randomized controlled trial was conducted to investigate the efficacy of 6-week strengthening exercises using electromyographic biofeedback (EMG-BFB) on muscular dystrophy patients evaluating muscle strength, motor function, and balance.

METHODS

Study design

This study is a randomized clinical trial with a two-arm and allocation ratio of 1:1, implemented in the physical medicine and rehabilitation (PM&R) clinic of Shariati hospital in Tehran University of Medical Sciences (TUMS) from May 2020 to February 2021. The protocol of this study is approved by the ethics committee of TUMS (code: IR.TUMS.MEDICINE.REC.1398.031) and registered in the Iranian Registry of Clinical Trials Database (IRCT20200326046864N1). This study meets the ethical standards of the Muscles, Ligaments and Tendons Journal (18). After trial commencement, low compliance of patients for follow-up has been supposed due to COVID-19 pandemic, therefore considering patient retention, we prepared some medical consultation about COVID-19 for participants.

Sample size calculation

The primary outcome measure of this study was muscle strength; so, the sample size was calculated with a statistical level of significance; 0.05, and test power of 0.8 using repeated-measures ANOVA. In conclusion, 12 participants were calculated for each group. Considering the high probability of patients numbers falling during the intervention and follow-up process, we entered 20 patients for each group.

Inclusion criteria

We recruited all subjects with ≥ 18 years of age and proximal muscles involvement with at least 10 meter walking ability without assist whose diagnosis were confirmed by a neuromuscular fellowship and genetic tests. Ethically each patient signed the informed consent form before participation in the study.

Exclusion criteria

Our exclusion criteria were recent history of trauma or surgery or acute event during the past three months, history of other neurologic or systematic disorders, use of drugs affecting muscles within three months. Participants with auditory or vision problems, cognition impairment, and cardiomyopathy were excluded. Patients were asked to inform the therapist if they tended to participate in other rehabilitation programs during the study.

Intervention

Patients were randomly assigned to two groups; the conventional strengthening exercise and EMG-BFB groups. Simple randomization, using a random number table, was applied as a randomized assignment method. The researcher (NM) selected the randomized number and assigned it to the specific group.

Before all interventions, demographic information such as age, gender, height, weight, and drug history was gathered. In the first session, muscle strength and functional tests were recorded, and further intervention sessions were set. The intervention group received strengthening exercises using EMG-BFB (Saebo MyoTrac Infiniti, threshold setting: 50% of maximum contraction) for 6 weeks, twice weekly, for 30 minutes with at least 48 hours interval between training sessions. The control group with the same schedule performed the exercises under a therapist supervision. Isometric strengthening exercises involved hip flexors, extensors, adductors, and abductors in addition to knee flexors and extensors, and EMG-BFB intensity was set for 50% of maximum voluntary contraction. Furthermore, they were also educated on lifestyle modifications and strengthening exercises, verbal and through a brochure, filling checklist, and evaluating exercise adherence for more 6 weeks after intervention until the last assessment. Muscle strength and functional tests were assessed after the 12-session intervention for 6 weeks for the second time and 3 months after the beginning of the program for the third time. Previous studies suggested low to moderate intensity of exercises for MD patients (14). We used the Borg scale for intensity evaluation during the intervention, and the program was stopped if Borg > 14. Muscular cramp or pain during or after exercise were other indications of program discontinuation.

Outcome measurements

The primary outcome measure was muscle strength which was measured by one examiner (N.S) in all patients, using a handheld dynamometer type CT 3001 (CITEC handheld dynamometer, C.I.T. Technics, Groningen, The Netherlands) (19). This tool is more sensitive than manual testing for moderate impairments (manually quoted at 4 and higher). By using verbal encouragement, forces are recorded 3 times for each muscle group, and the mean measure was used for analysis. Intra-rater variability of strength testing was below 10%.

Secondary outcome measures were the motor function measure-32 (MFM-32), fatigue severity scale (FSS), Vignos scale, timed up go test (TUG), stair climb test (SCT), stand up from supine position test (SST) and Berg balance scale (BBS). MFM-32 is a scale for evaluation of motor function of neuromuscular patients consist 32 items with 4-point Likert scoring for each item (20). FSS is used for assessment of fatigue perception and its effects on life and functions which includes 9 items with 7-point for each item (21). Lower limbs function evaluated by Vignos scale. It scores 1 to 10 in which score 1 means ability to walk and climbing up without assistance and score 10 means laying on the bed (22). Time needed for walking 10 meters in moderate speed (23) and going 3 steps up and down (24) assessed via TUG and SCT, respectively. SST specifies the time needed to stand from supine position and sit (25). BBS as a known scale used for balance evaluation and it consists 14 items with 4-point Likert scoring for each item (26). To survey quality of life, SF-36 questionnaire was used. This tool consists 36 items and scores between 0-100 including 8 subscales: physical function, physical role, bodily pain, general health, social function, emotional role, mental health, and vitality (23).

Data analysis

Statistical Analyses were performed by Stata software version 16. A P-value of less than 0.05 was considered significant. All analyses were performed on 12 patients per group. Categorical data are presented as percent, and Continuous demographic variables are presented as Mean ± Standard deviation or Median (Interquartile range). For Further analyses, Shapiro–Wilk and Levene's tests, respectively, were used for normal data distribution and variance homogeneity assessment. Two-way repeated-measures ANOVAs were performed to analyze changes between IG and CG at each time point. Bonferroni multiple comparison procedure was used for post-hoc analysis. Pearson's correlation coefficient was performed to determine relationships between outcomes.

RESULTS

A total of 76 patients are assessed from the point of inclusion criteria, and they were called for participation in this study, and 40 persons were accepted and randomized into two groups. Eleven patients left the study during training sessions for reasons such as transportation problems, COVID-19 related quarantine, being affected by COVID-19, or fear of COVID-19 infection. Five patients did not come back for the final assessment, which was after 3 months from the rehabilitation program. Finally, data of 24 patients were analyzed who were completed all training and assessment sessions (**figure 1**). Four patients had Becker muscular dystrophy, 8 persons facioscapulohumeral dystrophy, 10 participants limb-girdle muscular dystrophy, and 2 persons

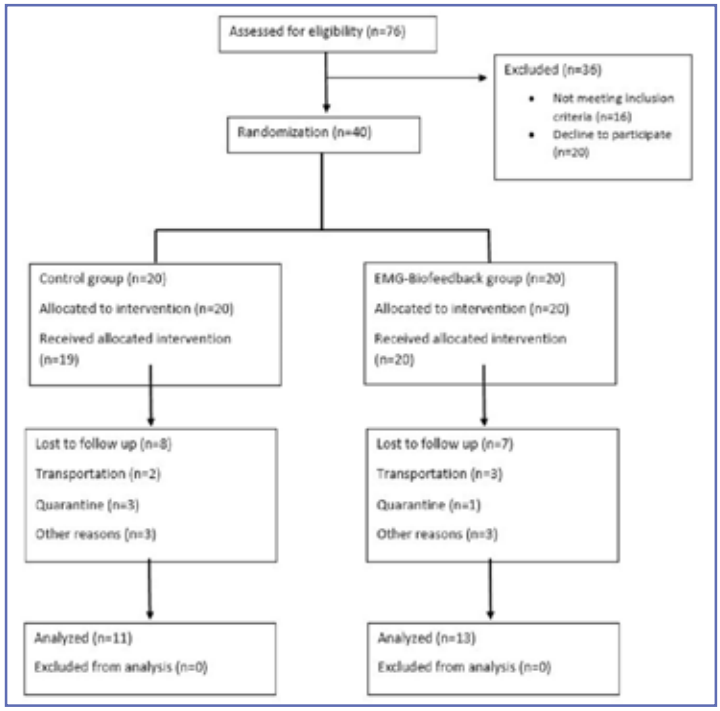

Figure 1. Trial chart.

myotonic dystrophy. Approximately 25% of participants were women, and the mean of participants' age was 35.1 ± 5.4. **Table I** shows demographic data, initial muscle strength and functional tests of participants, and comparison between two groups. There were no statistically significant differences between the two groups, and participants' characteristics were similar in both groups. No complication was reported during or after training sessions, and there was no need for exercise discontinuation for none of the participants.

Figure 2 shows changing pattern of functional tests before, after, and 3 months after the intervention. The MFM-32, FSS and BBS were improved significantly in both CG and EMG-BFB group during all 3 assessments. The SST, SCT and TUG tests showed some improvements in both groups after the intervention, but this improvement was not observed in the last evaluation after 3 months. The Vignos test was not changed significantly after training sessions in both groups. **Figure 3** reports results of functional tests comparing between two groups, CG and EMG-BFB. Based on figure, SST and BBS showed better results in the intervention group, although other tests resulted in

Table I. Characteristics of participants.

	Control group (n = 20)	EMG-B group (n = 20)	P (t test, χ^2)
Demographic variables			
Sex (Women %)	25	27.3	0.441
Age (yr)	31.2 ± 5.5	37.3 ± 1.8	0.309
Weight (Kg)	68.7 ± 4.5	72.4 ± 3.7	0.501
Height (cm)	168.7 ± 6	171 ± 5	0.641
Functional tests			
FSS	4.7 ± 0.9	4.7 ± 1.0	0.687
BSS	44.2 ± 3.5	38.8 ± 4.2	0.303
TUG	14.2 ± 2.8	15.4 ± 1.6	0.757
SCT	10 ± 3.2	11.8 ± 3.0	0.606
MFM-32	55.2 ± 6.1	56 ± 5.9	0.881
Vignos test	3.9 ± 0.6	4.5 ± 0.8	0.349
SST	9.4 ± 1.9	9.5 ± 1.9	0.898
Muscles strength			
Hip flexion (Nm)	10.4 ± 2.1	9.4 ± 2.2	0.460
Hip adduction (Nm)	6.2 ± 1.1	6.6 ± 1.0	0.768
Hip abduction (Nm)	10.2 ± 1.2	8.8 ± 2.3	0.286
Hip extension (Nm)	9.3 ± 2.3	8.7 ± 3.5	0.262
Knee flexion (Nm)	6.6 ± 1.2	5.4 ± 1.5	0.412
Knee extension (Nm)	6.5 ± 1.0	6.5 ± 1.1	0.974

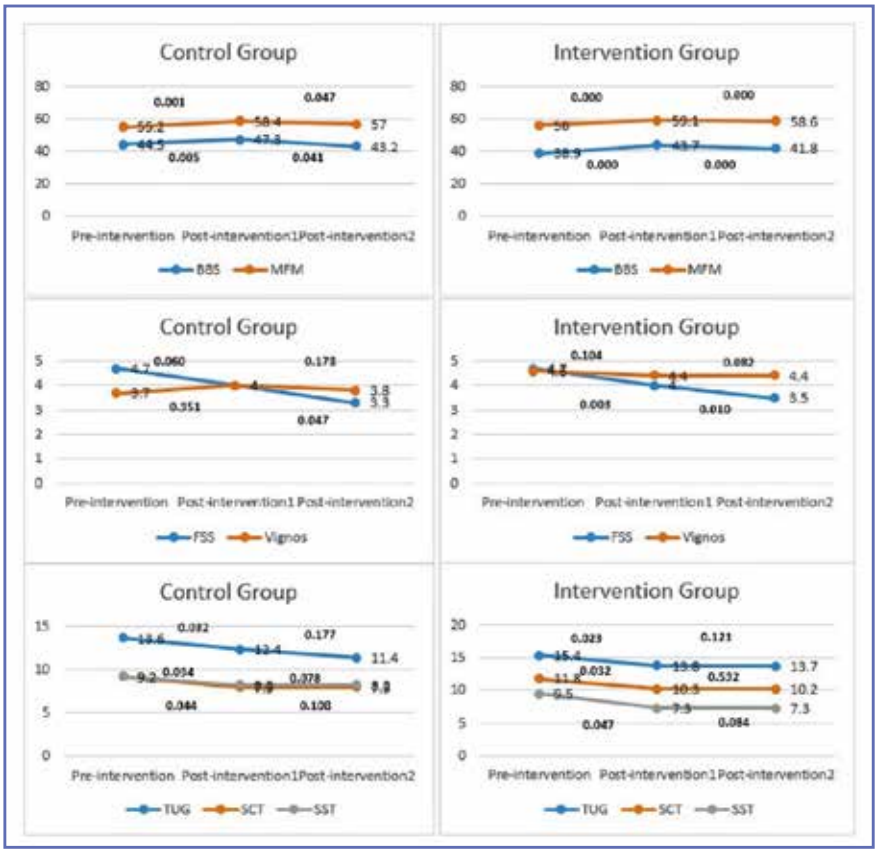

Figure 2. MFM-32, BBS, Vignos test, FSS, TUG, SCT and SST changes during three assessments in both EMG-BFB group and CG.

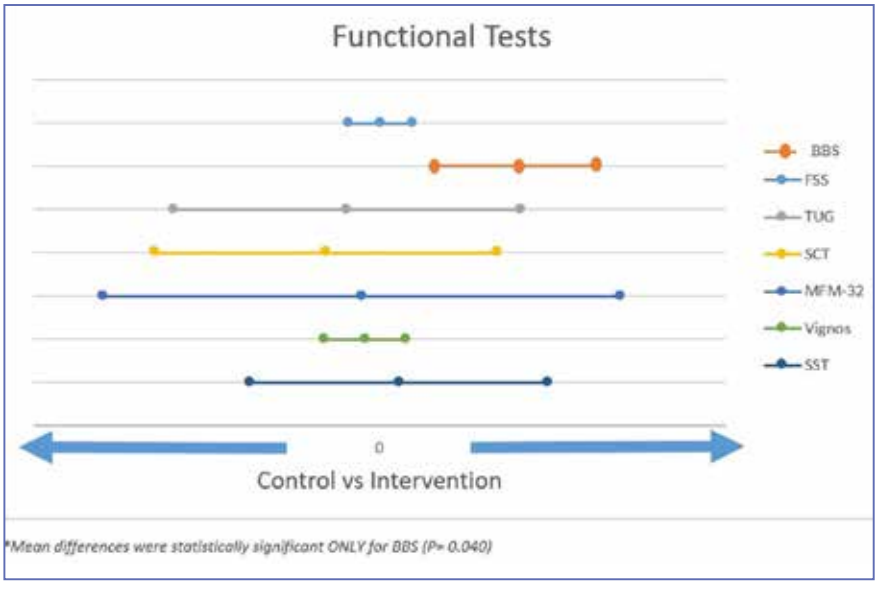

Figure 3. Functional tests mean differences between intervention *vs* control groups.

better findings in CG. Finally, only BBS showed statistically significant improvement toward the EMG-BFB (P = 0.040). While evaluating relationship between BBS and muscle strength and functional tests, significant association was found between BBS and TUG (r = - 0.733, p = 0.000), BBS and MFM-32 (r = 0.637, p = 0.001), BBS and SST (r = - 0.441, p = 0.021), but significant relationship was not detected between BBS and muscle strengths (P = 0.420).

Table II shows muscle strength changes during three assessments in both groups. In CG and EMG-BFB group, hip flexion, abduction, adduction, and extension strengths were improved after the intervention. Significant differences were not detected between the two groups comparing the EMG-BFB group and CG.

Table III shows the SF-36 results of participants. Considering table data, physical function, bodily pain, social function, emotional role, and mental health subscales improved after intervention in the EMG-BFB group, but no improvement is seen in CG. In the EMG-BFB group, physical and general health showed better results than CG (P = 0.034, 0.037).

Subgroup analysis based on dystrophy type suggested no differences between different types, neither in muscle strength improvements nor functional tests.

DISCUSSION

To the our knowledge, this study evaluated, for the first time, the safety and efficacy of strengthening exercises using EMG-biofeedback for MD patients through a randomized clinical trial on muscle strength, motor function, balance, and quality of life over 12 weeks. The main findings of this study included muscle strength, motor function, balance, and some aspects of quality of life improvement following intervention and decreased experienced fatigue.

Table II. Muscle strength change before and after intervention in EMG-BFB and CG.

Muscle group	EMG-BFB	CG	P value
Initial hip flexion strength (Nm)	9.4 ± 2.2	10.4 ± 2.1	0.460
Final hip flexion strength (Nm)	12.7 ± 2.0	13.1 ± 3.2	0.751
P	0.004	0.036	
Initial hip extension strength (Nm)	8.7 ± 3.5	9.3 ± 2.3	0.262
Final hip extension strength (Nm)	10.4 ± 2.1	11.8 ± 1.7	0.577
P	0.034	0.025	
Initial hip abduction strength (Nm)	8.8 ± 2.3	10.2 ± 1.2	0.286
Final hip abduction strength (Nm)	12.9 ± 2.2	13.5 ± 2.7	0.584
P	0.017	0.019	
Initial hip adduction strength (Nm)	6.6 ± 1.0	6.2 ± 1.1	0.768
Final hip adduction strength (Nm)	8.2 ± 2.1	8.3 ± 2.3	0.847
P	0.046	0.043	
Initial knee extension strength (Nm)	6.5 ± 1.1	6.5 ± 1.0	0.974
Final knee extension strength (Nm)	6.8 ± 1.7	6.9 ± 2.0	0.843
P	0.100	0.110	
Initial knee flexion strength (Nm)	5.4 ± 1.5	6.6 ± 1.2	0.412
Final knee flexion strength (Nm)	6.2 ± 1.9	6.5 ± 2.2	0.741
P	0.181	0.116	

Table III. Results of quality of life questionnaire and its subscales.

Subscale	Intergroup difference for Control	P value	Intergroup difference for Intervention	P value	Between groups differences	P value
Physical Function	6.4 (- 3.5, 16.3)	0.163	10 (4.6, 15.3)	0.001	3.2 (- 10.6,17.1)	0.634
Role of Physical	2.1 (- 0.3, 4.6)	0.078	1.7 (- 3.5, 5.8)	0.355	13.5 (1.1, 26.1)	0.034
Bodily Pain	4.3 (- 0.1, 8.4)	0.055	10.7 (5.7, 15.6)	0.000	2.1 (- 8.4, 12.7)	0.678
General Health	1.8 (- 2, 5.7)	0.284	1.6 (- 5.4, 13.4)	0.133	10.4 (1.7, 20.2)	0.037
Social Function	7.7 (- 3.5, 18.9)	0.145	9.4 (4.5, 13.2)	0.011	11.4 (- 1.1,23.8)	0.070
Role of Emotional	1.1 (- 2.7, 5.1)	0.482	4.7 (1.4, 8.6)	0.021	2.6 (- 10.2,15.5)	0.675
Mental Health	2.4 (- 6.8, 13.5)	0.564	6.4 (2.4, 10.4)	0.004	5.3 (- 10.9, 21.3)	0.490
Vitality	3.2 (- 0.7, 7.3)	0.093	10 (1.6, 18.2)	0.216	6.8 (- 10, 23.7)	0.429

In the same vein of this study, Alemdaroğlu *et al.* suggested mild, submaximal strengthening exercises as an appropriate choice for Duchene muscular dystrophy (DMD) patients (14). Lott *et al.* confirmed the same findings for DMD ambulatory patients after performing home-based isometric strengthening exercise program on knee muscles and detecting strength improvements in knee flexors and extensors (27). Bankolé *et al.* reported decreased fatigue perception and 6-minute walk test and increased maximum muscle contraction after combined aerobic and strengthening exercises among FSHD patients (28). Conversely, Lindeman *et al.* showed that maximum voluntary contraction was not significantly changed after the strengthening training in myotonic dystrophy patients (29). These controversies could be explained with different primary muscle strengths before intervention and different age-gender-related combinations of participants.

Hammarén *et al.*, studying balance training on myotonic dystrophy type 1 patients, found decreased falling, improved activities-specific balance confidence (ABC) scale, unchanged TUG and knee extensors strength, and increased time for the 10-meter walk. Authors interpreted findings that self-assessed balance confidence is impressed by rehabilitation program but not functional tests(30). In another study on myotonic dystrophy patients, improved BBS, fast gait speed, and muscle strength were observed; however, they did not assess balance and muscle strength relationship (31). Considering mentioned studies, using different tests with different levels of sensitivity could be responsible for detected discrepancies.

We found BBS changes more prominent in the EMG-biofeedback group in comparison to CG. Previous studies suggested that auditory or visual feedback during biofeedback endorses unused or underused synaptic pathways that cause motor learning. Additionally, EMG as a source of feedback leads to down-training of a hyperactive muscle or up-training a weak muscle, thus improving muscular control (16). These two mechanisms could explain better results in the EMG-B group regarding balance-related findings independent of muscle strengths.

Although we found some changes in some subscales of quality of life in our study after the intervention, since many potential confounding factors were not assessed in our research, it is better not to interpret these findings. Quality of life improvements are not related only to rehabilitation programs, and other factors such as family or society qualification, career or economic condition could influence their quality of life (32).

We faced some limitations. Despite acceptable sample size, patients dropped out during training sessions, and follow-ups were considerable (40%). However, considering COVID-19 pandemic effects and MD patients' disabilities, this drop-out rate is not surprising. Second, we did not use any objective tests such as imaging or laboratory tests to evaluate muscle damage. However, none of the participants left the study due to pain or progressive weakness, or injury. Third, it was better to select one type of MD for this study because of different protein involvement. Using different functional tests with a wide range of sensitivity is one of the strengths of this study.

CONCLUSIONS

This study demonstrated the benefits of a supervised strengthening exercise program in MD patients. These benefits seem to be occurred without any adverse effects to cause patients to drop out of the study. Clinicians might be encouraged to use EMG-based biofeedback in rehabilitation programs of MD patients, which could be more influential due to motor control improvements, especially on their balance, leading to decrease fallings and its catastrophic consequences. Studies with a focused evaluation of one specific type of MDs with appropriate sample size and longer follow-up is recommended.

FUNDINGS

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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CASE REPORT

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Results of Lisfranc's Surgery in Athletic Patients

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SUMMARY

Background. Early detection and treatment of Lisfranc injuries in the athletic population is paramount for successful return to sport as well as outcome. The present study evaluates surgical interventions for these types of injuries as well as return to activity (RTA), function and post-operative degenerative joint disease.

Methods. The data from nineteen patients prospectively followed from 2008 through 2016 presented. Of these patients, six (31.6%) were treated with suture-button fixation, seven (36.8%) with metallic screws and plates and six (31.6%) with bioabsorbable screws. The average RTA was 24.0 ± 10.3 weeks. The post-operative Roles and Maudsley (RM) score was 1.2 ± 0.4. 21% of the patients were noted to have joint space narrowing at the final post-operative x-ray. Hardware removal was performed in six (31.6%) patients.

Results. All of the patients were able to return to their sporting activities.

Conclusions. Based on the current study, anatomic reduction showed to be the highest predictor of surgical outcome, regardless of fixation technique, consistent with previous studies. ORIF of Lisfranc injuries in the athletic population provides safe and predictable results with good medium-term outcomes.

KEY WORDS

Athlete; Lisfranc joint; return to activity; suture-button.

INTRODUCTION

The incidence of Lisfranc injuries has increased over the years as participation in sports continues to grow. Approximately 1 in 55,000 people have had a Lisfranc injury reported in the United States on an annual basis (1). These injuries are missed in nearly 20 % initial presentation (2). Despite the lower incidence compared to other foot and ankle pathology, these fractures or dislocations can be devastating injuries due to high incidence of post-traumatic degenerative joint disease.

The anatomy of the Lisfranc joint is very complex and involves the articulations between the bases of the metatarsals with the cuneiforms and cuboid, stabilized by weak dorsal, strong plantar, and interosseous ligaments (3). Lisfranc injuries can occur as a result of a wide variety of traumatic mechanisms and are separated into two groups: high and low energy (4). High-energy Lisfranc injuries often occur due to a direct axial load to the midfoot, frequently seen as a result from a motor vehicle accident or crush injury. These are more likely associated with bony injuries and compartment syndromes (5).

Lisfranc trauma in the athletic population typically occur from indirect mechanisms and can cause more ligamentous than bony injuries (5). They are categorized a low energy trauma and typically caused by an axial longitudinal force on a hyper-plantarflexed foot (6).

Clinical assessment is crucial in early diagnosis of Lisfranc injuries. As previously mentioned, Lisfranc injuries in the athletic population are often purely ligamentous and have subtle presentations (7). Patients may or may not have generalized swelling and/or bruising over the midfoot but usually have pain with manipulation of the tarsometatarsal joints. Initial weight-bearing radiographs should be taken, with comparison contralateral films to help in diagnosis subtle Lisfranc's injury.

Nunley and Vertullo introduced a classification system for subtle, low-energy Lisfranc injuries (8). Stage 1 injuries are classified as a midfoot or Lisfranc sprain and do not show any diastasis on anteroposterior radiograph. Stage 2 injuries have a diastasis between 2 and 5 mm between the first and second metatarsal bases on anteroposterior radiograph with-

out collapse of arch height. Stage 3 injuries are represented by greater than 5 mm diastasis with collapse of arch height. Additional imaging can also be helpful with diagnosis and classification purposes. Bone scans or SPECT (Single Photon Emission Computerized Tomography) scans are used and can show some metabolic bone activity, especially in stage 1 injuries (9, 10). CT (Computerized Tomography) scans are useful in assessing fracture comminution. Magnetic Resonance Imaging (MRI) is often used to assess for ligament tears as well as bone marrow edema. Raikin *et al*. compared MRI results to intra-operative findings and determined that MRI is accurate in detecting traumatic injury to the Lisfranc ligament (11). However, it is important to keep in mind that MRI is not a weight-bearing test, so it may not provide much additional information in subtle cases (9). Many surgical fixation techniques have been described in the literature for Lisfranc fractures and dislocations (10, 12-20). In this prospective study, we analyze the clinical outcomes of athletic patients with Lisfranc injuries who required surgical intervention. Screw and/or plate, suture-button, bioabsorbable pin, and Kirschener-wire fixation were used in this study. It is acknowledged that fixation variability may obscure the results and it is not anticipated we would have definitive findings as to the best fixation type. Medium to long-term follow up is analyzed to assess clinical outcomes including return to sport, maintenance of reduction, and complications.

METHODS

Active patients under than age of 40 years who underwent open reduction or percutaneous fixation surgical treatment for acute Lisfranc injuries from January 2008- December 2016 were prospectively followed (18). An IRB was obtained from our institution (SHIRB #: 2017.081EXP IRBNet #: 1089721-8). During this time frame, 112 patients with Lisfranc injuries were treated by the primary author. Pre-operative imaging included weightbearing xrays, CT or MRI scans if needed to further assess the injury. Inclusion criteria required the patient to be athletically active, ability to document their sport, return to activity timeframe (RTA), radiographic findings (for measurement of reduction and assessment of arthritis) and Roles and Maudsley (RM) scores (21). Exclusion criteria was the inability to document the previous information and loss of follow-up prior to one-year, chronic injuries (past six weeks), as well as those undergoing primary fusion for severe intra-articular injuries.

Nineteen patients of the 112 (17%) met the inclusion criterion, six (31.6%) males and 13 (68.4%) females, average age 20.6 ± 7.9 years (r = 13-39). There were nine "Right" feet and 10 "Left" feet. Sixteen were medial Lisfranc injuries with diastasis of the Lisfranc's articulation and three involved the lateral Lisfranc's region at the level of the 4th metatarsal-cuboid region. All were active in sports, the majority in soccer (seven individuals), two each in volleyball and basketball, four in running sports such as track and field, and one each in football and swimming. The fixation type was recorded. Post-operative X-ray reductions were assessed by an individual (typically a Fellow) not involved with the index surgery, as well as the incidence of post-operative DJD, RM scores and return to activity time frames (15). Reductions were determined to be anatomic if ≤ 1 mm of offset of the metatarsal to corresponding cuneiform or cuboid or widening < 4 mm of the region of Lisfranc's ligament (first cuneiform to the second metatarsal or distance between the first and second metatarsal bases) Student-T, one-way ANOVA for post-hoc comparison and Chi-squared tests were used to analyze the data with STATA Version 14.2® (Statacorp LLC, College Station TX, USA), with P-value set at ≤ 0.05.

Surgical technique

The surgery is typically performed on an outpatient basis using general anesthesia with a regional local block. A thigh or mid-calf tourniquet may be used. The patient is then prepped and draped in the normal aseptic fashion.

Screw fixation

Three (16%) patients had "traditional" one screw fixation. Attention was directed to the medial aspect of the first metatarsal cuneiform joint. A small incision was made over the medial aspect of the first cuneiform. A large reduction clamp was then utilized to reduce the diastasis with the tips placed on the medial cuneiform and lateral aspect of the second or third metatarsal base. A 3.5 mm cortical screw was then placed across the medial cuneiform and into the base of the second metatarsal, following the trajectory of Lisfranc's ligament. After placement of this screw, the bone clamp was removed. Fluoroscopic guidance was used to confirm reduction and alignment and placement of hardware. Inter-cuneiform instability was then assessed. If instability was confirmed, proximal screw was placed from medial across the cuneiforms to reduce the diastasis. A bridge plate or distal screw was used to stabilize the first metatarsal to the first cuneiform. Plate fixation was used to stabilize lesser metatarsal fractures. The hardware was typically removed after 12 weeks. This was performed for any screw spanning a joint, bridge plating and when prominent. In one patient (5%) with open physes, K-wires were used and

removed at six weeks. Screws placed across joints (*i.e.*, first TMT) were abandoned after 2013 for concern of inducing osteochondral defects and subsequent potential for arthrosis; bridge plating was subsequently used in this situation (16) (**figures 1, 2**).

Bio-absorbable Screw

This technique was performed on two (10.5%) patients treated from 2008-10 based on prior studies showing reasonable results (14, 17). In situations where hardware is to be left remaining, *i.e.*, under a flap after an open injury, bio-screws are an option. Attention is directed to the area of the Lisfranc joint. A bone reduction clamp as above, was carefully used percutaneously to reduce and compress the joint. Alignment and reduction are assessed with fluoroscopic guidance in multiple planes. Then, a 2.5 mm drill was used across the Lisfranc joint. Subsequently, a 3.5 mm bio-absorbable screw was placed across the joint. The bone clamp was removed. Stability and alignment were once again checked with fluoroscopic guidance. If the stability was insufficient, the bioscrew was exchanged for a metallic screw.

Suture-button

A contraindication for this technique is ligamentous laxity. To date, there is a small series (seven patients) supporting this technique in dancers and high-level athletes (10, 19). Similar to bioscrews, open injuries or in cases when the hardware will remain, suture-button technique may be an option. This was performed on six (31.6%) of patients. Attention was directed to the medial aspect of the first metatarsal cuneiform joint. Layered dissection was made carefully taking great care to avoid any vital neurovascular structures. A bone reduction clamp was placed as above to reduce the diastasis. After adequate exposure for the first cuneiform, a drill bit was introduced from medial to lateral across the Lisfranc joint towards the base of the second metatarsal. Fluoroscopic guidance was used in multiple planes to assess proper alignment. A single suture-button device with braided non-absorbable suture and metallic buttons was then inserted through the pre-drilled hole across the Lisfranc joint and tied down medially with adequate compression and maintaining anatomic alignment of the Lisfranc joint. Once again, intra-operative fluoroscopy was used to assess proper seating of the metal-

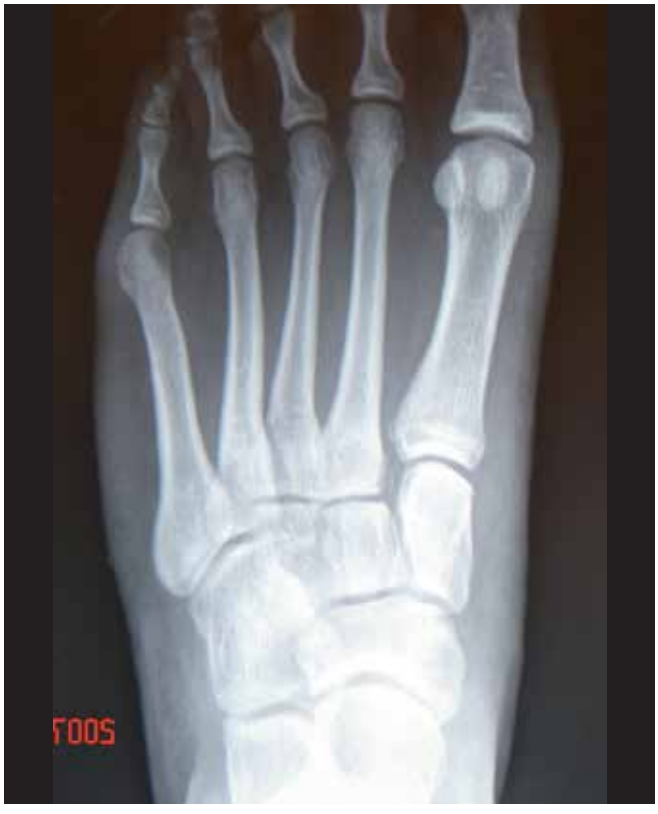

Figure 1. Pre-operative radiograph.

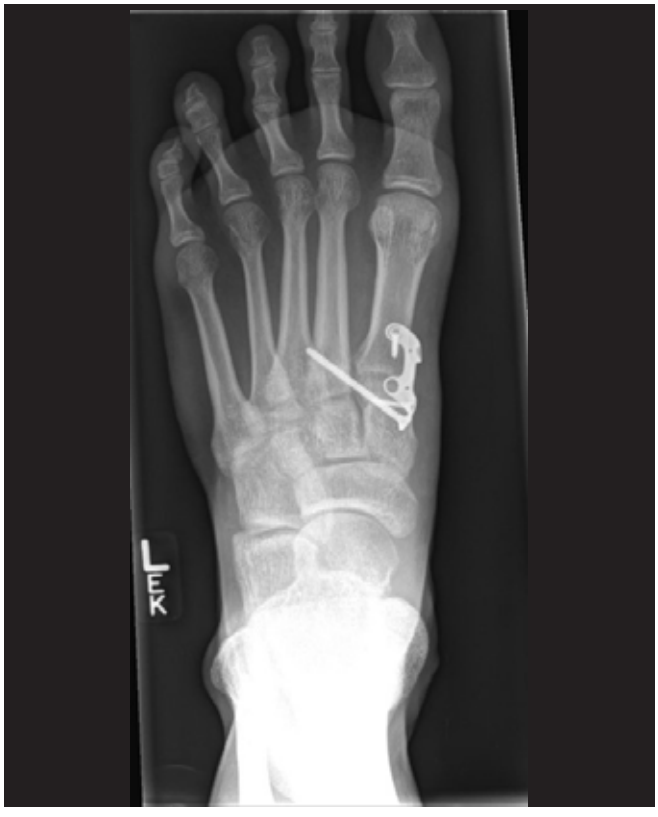

Figure 2. Post operative radiograph.

lic suture-buttons, which should be flush to the bone. The bone clamp was released. If stability is insufficient, a metallic screw was place proximal or distal to the suture-button. This is a hybrid technique.

Lateral fixation

Three patients had lateral column Lisfranc's injuries (16%) involving the fourth tarsometatarsal joint. Attention was directed to the dorsal aspect of the fourth tarsometatarsal joint. Layered dissection was made carefully to avoid any vital neurovascular structures. Sharp and blunt dissection was used to dissect through the extensor digitorum brevis muscle belly. After adequate exposure of the fourth tarsometatarsal joint, the joint was first stabilized with k-wire fixation across the fourth tarsometatarsal joint, from distal to proximal and reduction was verified using fluoroscopic guidance. Then, suture anchor in the base of the fourth metatarsal and a knotless anchor was placed into the cuboid placing the suture from the anchor under tension. The suture was used to recreate the dorsal tarsometatarsal ligament with the joint held in anatomic reduction which may have better long-term stability (21). The calcaneal cuboid joint was also assessed for instability or subluxation. The k-wire was removed at the four-week post-operative appointment.

RESULTS

Nineteen patients were seen on average 19.4 ± 30.1 months post-index procedure for follow-up and evaluated by a Fellow not involved in the index surgery. These patients had their charts were evaluated at an average of 58.9 ± 30.1 months post-index surgery, by a different Fellow, again, not involved in the index surgery. All 19 patients had anatomic reductions noted immediately post-operatively. Their final post-operative weightbearing X-rays were noted to be anatomic in all patients taken at an average of 12.5 ± 25.5 months, though four patients were noted to have joint space narrowing at the time of that imaging (21%). Of these four patients, three had medial injuries (two with screw and plate fixation, one with suture-button) and one with a lateral injury. Hardware removal was performed in six patients including one with a portion of their suture button. There were six patients with suture-button fixation, seven with metallic screws and plates, and six with bioabsorbable screws.

Average RTA of the entire cohort was 24.0 ± 10.3 weeks (range = 10-50 weeks). Medial Lisfranc's patients (16) had RTA 22.8 ± 8.9 weeks, while the three with lateral injuries RTA was 31.0 ± 16.8 weeks (P = 0.21). Four patients returned to high level sports (three collegiately and one professionally), and none had decreased desired activity level. Post-op RM score for the cohort was 1.2 ± 0.4. There were no significant differences with the RTA, RM, post-op DJD in regard to sex, medial *vs* lateral injury, nor fixation type. The results are summarized in **table I**.

DISCUSSION

Lisfranc injuries are rare, complex and vary in their presentation and mechanism of injury. Low energy, indirect impact is typically the mechanism creating Lisfranc injury seen in the athletic population resulting in more subtle soft tissue injury. There is no consensus on the appropriate treatment, let alone the best type of fixation needed when surgical correction is required. MacMahon *et al.* showed that athletic patients had decreased participation or limitations to their activity after partial arthrodesis for Lisfranc injuries (22). MacMahon *et al.* reported on a cohort of 38 patients, average age of 31.8 years at the time of surgery, who engaged in high impact activities that had a limited primary arthrodesis. They concluded that these patients had decreased participation and increased difficulty with impact sports showing that many were experiencing limitations with high demand sports. There is also a concern for long term prognosis, especially in the athletic population due to the potential adverse incidence of osteoarthritis.

Porter *et al.* recent reported on a large series of competitive athletes. In 82 patients with an average age of 21 years (similar to our study) their cohort return to sport was 7.5 months. They did not perform any fusions in their patients nor report on arthritis (23).

Our study also showed that reduction was maintained at 1 year follow up, which is the most important indicator of outcome (9, 16-18, 20). However, our series was too small to determine if loss of reduction or DJD was associated with fixation method. Despite metallic hardware having to be removed, that cohort had a faster RTA than other fixation types. The fact that the fixation type varied may be a sign the surgeon is paying attention to their results and following the patients.

Based on our current study findings, anatomic reduction, regardless of fixation used, showed to be an important predictor of outcome as suggested in several other studies (9, 17, 24). Although Lisfranc injuries have been well documented and better recognized in the athletic population, their impact on their return on play is not always well documented (25, 26). McHale *et al.* showed that NFL ("American Football") players took an average of 11 months to return to competition; about 10% could not return to that level (27). Our study also demonstrated that surgical reduction and fixation without primary fusion leads to a successful return to activity as all our patients were able to return to their sporting activities. The patients in our study

Table I. Subjects demographics and results.

Injury	Side	Age	Sex	Procedure	Sport	Fixation	PO DJD?	HWR	RTA
Fract/disloc	R	18	F	ORIF Medial	Soccer	Bioabsorbable screw	No	No	22.0
Dislocation	L	17	F	ORIF Medial	Soccer	BIoabsorbable screw	No	No	15.0
Sprain	L	39	F	ORIF Lateral	Soccer	Bio-anchor and k-wire	Yes	No	25.0
Sprain	L	15	F	ORIF Lateral	Swimming	K-wire	No	No	18.0
Sprain	L	15	F	ORIF Lateral	Volleyball	K-wire	No	No	50.0
Dislocation	R	13	F	ORIF Medial	Soccer	K-wires	No	No	18.0
Dislocation	L	16	F	ORIF Medial	Runner	Plate and screws	Yes	Yes	17.0
Dislocation	R	20	F	ORIF Medial	Softball	Plate and screws	Yes	Yes	15.0
Fracture	R	30	F	ORIF Medial	Runner	Plate and screws	No	No	30.0
Dislocation	L	19	M	ORIF Medial	Basketball	Plate and screws	No	Yes	24.0
Dislocation	R	38	M	ORIF Medial	Basketball	Screw	No	Yes	24.0
Sprain	L	17	M	ORIF Medial	Soccer	Screw	No	Yes	15.0
Dislocation	L	16	M	ORIF Medial	Football	Screw	No	Yes	23.0
Dislocation	R	24	M	ORIF Medial	Track Indoor	Endo-button	No	No	31.0
Sprain	R	30	M	ORIF Medial	Runner	Endo-button	No	No	48.0
Dislocation	R	16	F	ORIF Medial	Soccer	Endo-button	No	No	10.0
Sprain	L	15	F	ORIF Medial	Runner	Endo-button	No	No	21.0
Dislocation	L	14	F	ORIF Medial	Soccer	Endo-button	No	No	26.0
Dislocation	R	19	F	ORIF Medial	Volleyball	Endo-button	Yes	No	26.0
		20.6	6				4		22.8
		7.9	13						9.4

PO DJD?: Post-operative DJD noted at last visit; HWR: Hardware removal; RTA: Return to activity.

were able to resume their sport at an average of 24 weeks post-surgery, which was defined as beginning practice, not competition. The end point of return to competition may be highly variable and could be biased by when in the season an athlete sustained their injury. The RTA from our cohort is within similar time frames of previously reported (5, 23, 25, 26), with described time frames between 24 and 44 weeks (25, 27).

Other authors advocate primary arthrodesis for Lisfranc's injuries (22, 28, 29). We did not compare this form of treatment and in general would not consider, unless the injury involved intra-articular, comminuted injury and patients of older age. The average age of our cohort was under 20 years. In the largest study on athletic patients (n = 82), Porter *et al*. reported on a 50% incidence instance of inter-cuneiform ligament tears. Their cohort was of similar age as ours with an average return to sports of 7.5 months (30). This was a component of the injury we did not assess. Robertson *et al*. performed a systematic review and meta-analysis of 17 studies containing 366 athletes. They revealed percutaneous reduction, internal fixation (with a metal screw) had statistically better "return rates" and "mean return times" when compared to both open reduction internal fixation and primary arthrodesis (31).

As with any longitudinal clinical study, ours has weaknesses. The primary weakness was that fixation type varied. It was modified based on clinical experience and current literature at the time. For example, bioscrews were popular earlier in the series (primarily because they would not need to be removed), however over time, some of the screw heads worked their way subcutaneously and became prominent, necessitating removal. Also, the bioscrews could break across Lisfranc's junction and be symptomatic. Suture-button technique became popular around 2008 following the "success" of suture-buttons for ankle diastasis, the main benefit being the lack need of removal, however some of the buttons became prominent and also had to be removed. Regardless of the type of fixation used, our study showed a similar

osteoarthritis development rate, seen in 21% of our subjects, coinciding with previously reported rates of 25% (9).

CONCLUSIONS

We can conclude from our study that ORIF regardless of fixation type for Lisfranc injury in athlete, is a safe and predictable treatment procedure if anatomical reduction is achieved. Faster RTA was achieved in those who had screw fixation and subsequent hardware removal. Based on this experience, the senior author currently prefers this mode of fixation for medial diastasis injuries. Despite anatomic reduction, approximately 20% of athletes will have radiographic evidence of arthrosis approximately two-years post-surgery. Longer follow-up is needed to see if the arthrosis becomes limiting and requires more intervention.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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Radial *versus* Combined Shockwave Therapy in the Management of Proximal Hamstring Tendinopathy: Similar Functional Outcomes in Running Cohort

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SUMMARY

Background. Proximal hamstring tendinopathy is a common cause of gluteal pain. Extracorporeal shockwave therapy may be an effective treatment in proximal hamstring tendinopathy. However, published outcomes are primarily limited to evaluating radial shockwave, and the use of combined treatment (focus and radial treatment) and outcomes for management in runners are not well described. The purpose of this report was to characterize functional outcomes using radial and combined shockwave in the management of proximal hamstring tendinopathy in runners. We hypothesized that runners who received R-SWT or C-SWT would experience improvement in functional outcomes using the VISA-H.

Methods. This study is a quality improvement initiative evaluating clinical outcomes in a single outpatient clinic. Sixty-three runners (mean and standard deviation for age and duration of symptoms 42.8 ± 14.7 years and 16.9 ± 23.8 months, respectively), were identified as receiving treatment for management of unilateral or bilateral proximal hamstring tendinopathy. Patients were treated with either radial (n = 40) or combined shockwave therapy (n = 23) using similar post-procedure protocols, including recommendations to complete physical therapy exercises of core and lumbopelvic stabilization with gradual progression to eccentric strengthening of the hamstring complex. Victorian Institute of Sport Assessment - Proximal Hamstring Tendons (VISA-H) was used to assess treatment outcomes, evaluated as differences between treatment cohorts by mean values from baseline to follow-up after shockwave treatment. The number in both treatment groups who met minimal clinical important difference (MCID) was defined as a gain of 22 points or more on VISA-H.

Results. Patients in both radial and combined shockwave groups received a similar average number of treatments (5.0 ± 2.2 *vs* 5.2 ± 1.9; p = 0.740). The radial and combined shockwave groups' mean VISA-H scores were similar at baseline (39.4 ± 17.4 *vs* 40.7 ± 17.0) and achieved similar final scores (62.6 ± 19.7 *vs* 63.4 ± 21.3; p = 0.812), and nearly all had measured increases of VISA-H with treatment (P < 0.0001). The MCID was met in a majority of patients who received either radial (62.5%) or combined treatment (56.5%).

Conclusions. Overall findings suggest radial and combined shockwave treatment with physical therapy exercises can be effective in the management of proximal hamstring tendinopathy in runners.

KEY WORDS

Athletes; C-SWT; extracorporeal shockwave therapy; functional outcome; nonoperative treatment; proximal hamstring tendinopathy; R-SWT; sports medicine.

BACKGROUND

Proximal hamstring tendinopathy is a common overuse injury that affects athletes and the general population. The injury may present as an insidious and progressive deep buttock pain localized to the ischial tuberosity that often worsens at initiation of activity, during acceleration running/sprinting, and with prolonged sitting. Athletes who participate in distance running, sprinting, and endurance sports are frequently affected (1). Non-athletes who develop proximal hamstring tendinopathy often have an occupational or lifestyle history of movements involving repetitive hip flexion that compressively load the proximal hamstrings (2, 3). Similar to other tendinopathies, proximal hamstring tendinopathy is commonly a chronic degenerative condition that arises from mechanical overload and repetitive stretch leading to cumulative tendon microtraumas (1, 4). In most cases, the diagnosis is made clinically by obtaining a careful history and physical examination (1, 3). In cases when the diagnosis is unclear or there is concern for more advanced tendon disease, magnetic resonance imaging (MRI) may be obtained; MRI can demonstrate peritendinous edema, bone marrow edema at the ischial tuberosity, and tendon thickening or degeneration along with presence of tendon tear (5).

Initial management of proximal hamstring tendinopathy consists of a multimodal approach including activity modification, analgesics/anti-inflammatory medications, and physical therapy focusing on core and lumbopelvic stabilization with eventual progression to eccentric strengthening (6). Ultrasound-guided corticosteroid peritendinous injections, platelet rich plasma (PRP) injections, and extracorporeal shockwave therapy (ESWT) are alternative treatment options considered for proximal hamstring tendinopathy (7, 8). Image guided peritendinous corticosteroid injections have been shown to provide short-term improvement, but long-term benefits are often not sustained, and tenotoxicity may limit use (5). PRP offers better long-term pain and functional improvements with case series demonstrating 63-68% of patients having sustained relief at 6 months post-treatment (8, 9). One key limitation in PRP protocols is that they typically require six weeks or greater time away from running or impact activities to allow for initial tendon healing (8).

ESWT is a non-invasive intervention that has been evaluated in running populations with good response across lower extremity injuries (10). Shockwaves are typically produced using either radial shockwave therapy (R-SWT) using a pneumatic or ballistic device or focused shockwave therapy (F-SWT) using electromagnetic, electrohydraulic, or piezoelectric sources (11). The limited research on the topic evaluated R-SWT for proximal hamstring tendinopathy by Cacchio *et al.*, who conducted a randomized control trial in 40 professional athletes. R-SWT outperformed standard therapeutic exercise program with 80% of athletes assigned to R-SWT returning to pre-injury level of sports participation compared with no athletes in the standard treatment cohort, and benefits in the R-SWT cohort were sustained to one year (7). While most shockwave literature evaluates R-SWT or F-SWT in isolation, more recent studies have shown positive results when using both devices in a single treatment session, termed combined shockwave therapy (C-SWT) (12, 13). Studies evaluating F-SWT or C-SWT have not been reported on management of proximal hamstring tendinopathy nor have results been measured using the Victorian Institute of Sport Assessment - Proximal Hamstring Tendons (VISA-H) questionnaire.

The objective of this quality improvement study was to evaluate functional outcomes of R-SWT and C-SWT for the management of proximal hamstring tendinopathy in runners. We hypothesized that patients who received R-SWT or C-SWT would experience improvement in functional outcomes using the VISA-H.

MATERIALS AND METHODS

This report is from results of a quality improvement initiative approved by the Department of Physical Medicine and Rehabilitation with waiver of Institutional Review Board approval. SQUIRE-2 guidelines were used for reporting quality improvement data (14). Patient characteristics, treatment measures, and functional outcomes collected as standard of care were extracted using chart review from August 2017 to March 2021 by five authors (D.M.R., S.D., A.S.T., P.H.Y, C.R.) in all patients receiving R-SWT or C-SWT for the management of proximal hamstring tendinopathy. All treatments were performed at the senior authors' outpatient sports medicine clinic (A.S.T). The study was written in compliance with the international and ethical standards of Muscles, Ligaments, and Tendons Journal (15).

The diagnosis of proximal hamstring tendinopathy was determined by senior author (A.S.T.) based on history and physical examination. Prior MRIs were also reviewed, and in some cases, MRI was obtained prior to treatment as clinically determined. Inclusion criteria were the following: 1) primary diagnosis of proximal hamstring tendinopathy and 2) completed baseline and follow-up functional outcome measures VISA-H. Exclusion criteria were the following: 1) previous proximal hamstring tendinopathy surgery, 2) diagnosis of connective tissue or inflammatory disease (*e.g.*, rheumatoid arthritis), 3) concurrent treatment of a separate lower extremity injury using shockwave therapy (*e.g.*, plantar fasciitis, piriformis mediated pain, Achil-

les tendinopathy, medial tibial stress syndrome), 4) referred pain from the lumbar spine, and 5) non-runners.

Treatment procedure

Patients received R-SWT or C-SWT. The clinic performing shockwave therapy offered R-SWT from August 2017 to January 2019. C-SWT was introduced as a treatment option beginning in January 2019. Since ESWT is not covered by insurance in the United States, patients paid a one-time fee for shockwave treatment sessions that was the same for both R-SWT and C-SWT. ESWT sessions occurred once a week for a minimum of four weeks based on the prior study by Cacchio *et al.* (7). Follow-up visits were scheduled at 6-8 weeks following the initial series of four treatment sessions. At these follow-up visits, additional sessions were offered on a case-by-case basis to maximize clinical outcomes. Patients who received R-SWT initially and reported unsatisfactory outcomes were offered the option to receive C-SWT at no additional cost.

R-SWT treatments were provided using the Storz Extracorporeal pulse activation technology (EPAT®) device (Storz Medical, Tägerwilen, Switzerland). Two applicator heads were used for each treatment, with a minimum of 3000 strikes per head at 15 Hz and a minimum pressure of 2.5 Bar (range used in patient cohort: 2.5-5.0 Bar). During R-SWT, the applicator heads were positioned over the affected area using the clinical focusing technique, with careful attention to treat over the hamstring tendon origin and over other affected area/s (such as the myotendinous junction and muscles of the biceps femoris, semimembranosus and semitendinosis).

C-SWT treatments involved consecutive application of R-SWT and F-SWT in each treatment session. F-SWT sessions were conducted with the Storz Duolith device (Storz Medical, Tägerwilen, Switzerland) set to a minimum of 1000 shocks with an energy intensity minimum of 0.12 mJ (range used in patient cohort: 0.12-0.5 mJ). During C-SWT treatment, F-SWT targeted the proximal hamstring origin at the ischial tuberosity, and R-SWT was primarily applied over the myotendinous junction and muscles with both modalities using the clinical focusing technique.

No local or regional anesthetic was used. All patients reported pain while receiving shockwave therapy. Patients were instructed to avoid NSAIDs and icing the affected area until completion of the treatment course. Patients were allowed to resume or continue regular activities, including running, as tolerated with three days of initial rest recommended following first session of C-SWT. Physical therapy was prescribed to each patient with no prior treatment. Those who completed an extensive course of physical therapy were recommended to complete their home exercise program focusing on core/pelvic strengthening and control, progressive hamstring strengthening (concentric advanced to eccentric), gastrocnemius/soleus stretching, and deep tissue massage along the hamstring origin for soft tissue mobilization. Patients were encouraged to perform the prescribed exercise program in conjunction with ESWT treatment and throughout follow-up.

Outcome assessment

VISA-H questionnaires were collected on initial treatment day and repeat measures were obtained periodically including after completion of treatment series (typically treatment 4) and after follow-up visits. The VISA-H uses self-report on level of physical impairment from an eight-item questionnaire, covering three domains of pain, function, and sporting activity. The sporting activity section assesses the patient's ability to perform more difficult activities and is population-specific to athletes. The first six questions (1-6) concern pain and function, while the last two questions (7-8) concern sporting activities. Questions 1-7 use a 0-10 numerical rating scale, while question 8 is rated out of 30 points, to give a total summation of 100 points as the maximum attainable score. A higher score corresponds to greater physical ability; a symptomatic patient would score lower, with the minimum score being 0 points (16). Adverse outcomes were monitored during treatments and follow-up visits.

Statistics

Descriptive statistics were presented as mean and standard deviations for continuous data and frequencies with percentages for categorical data. Demographic data were compared using Welsh's t-tests (continuous), chi-squared tests (categorical for cell values greater than 5), or Fischer's exact tests (categorical for cell values less than or equal to 5); p-values were not specifically reported, as none were significant. Outcome values were evaluated between R-SWT and C-SWT mean differences and the number of patients who met criteria for clinical response using minimal clinical important difference (MCID) value of ≥ 22 point (16). Categorical outcomes whether a patient met MCID between R-SWT and C-SWT were compared using chi-squared tests. VISA-H data were compared using mixed-design ANOVA to compare the overall effect of ESWT and to measure differences between R-SWT and C-SWT. Data was tested for normality and homogeneity of variance and covariance prior to analysis. For those treated with R-SWT who later received C-SWT, outcomes used were based on their final VISA-H score following last R-SWT treatment. All statistical analyses used two-tailed tests, and a threshold of $p < .05$ was considered significant; calculations were performed in R (R Core Team (2021), Vienna, Austria).

RESULTS

Chart review of a single provider (A.S.T.) who performed shockwave therapy in clinic identified 90 patients who had proximal hamstring tendinopathy that were treated with either R-SWT or C-SWT during the study period (**figure 1**). Eighteen of these patients were excluded for additional lower extremity pathologies treated concurrently; one was excluded for systemic or rheumatologic diagnoses; one was excluded due to concurrent referred pain from the lumbar spine; seven were excluded due to non-runner status. This resulted in 63 patients eligible for inclusion.

Demographic and clinical characteristics demonstrate the population treated was on average 42.8 ± 14.7 years old and had symptoms for 16.9 ± 23.8 months (**table I**). Overall, 84.1% of patients completed prior formal physical therapy before initiating ESWT.

Figure 1. Patient inclusion flowchart.

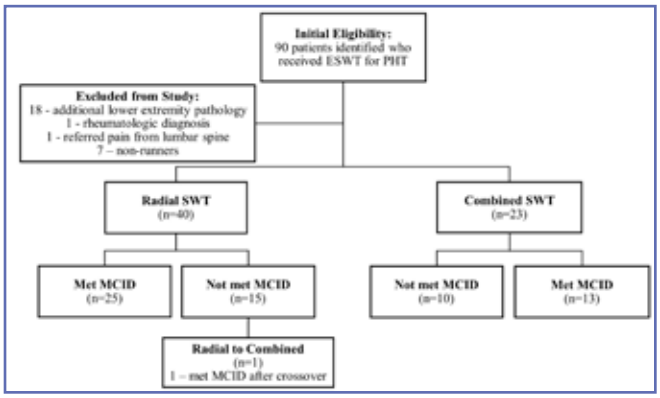


Table I. Demographic and clinical characteristics.

Continuous Variables mean ± SD	Runners All n = 63		R-SWT Met MCID [1] n = 25		R-SWT Not Met MCID[†] n = 15		C-SWT Met MCID [1] n = 13		C-SWT Not Met MCID[†] n = 10	
Age in years	42.79 ± 14.74		43.84 ± 15.53		44.73 ± 15.09		39.23 ± 16.22		41.90 ± 10.95	
BMI in kg/m^2	21.82 ± 2.82		21.80 ± 3.27		21.40 ± 2.41		23 ± 2.48		21 ± 2.48	
Duration	16.90 ± 23.78		10.96 ± 11.93		22.20 ± 37.01		16.92 ± 23.48		23.80 ± 21.27	
Total ESWT sessions[s]	5.06 ± 2.06		4.68 ± 1.90		5.53 ± 2.55		4.92 ± 1.97		5.50 ± 1.77	
Length of follow-up[i]	17.11 ± 14.82		12.48 ± 11.04		17.80 ± 17.80		25.53 ± 17.71		16.70 ± 10.69	
Baseline VISA-H	39.88 ± 17.05		39.88 ± 16.73		38.66 ± 19.09		37 ± 16.08		45.50 ± 17.17	
Final VISA-H	62.85 ± 19.51		71.64 ± 16.24		47.40 ± 15.37		71.07 ± 18.57		53.40 ± 16.84	
Change in VISA-H	22.96 ± 14.70		31.76 ± 7.84		8.73 ± 10.08		34.07 ± 10.10		7.90 ± 6.95	
Categorical Variables	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Female	41	65.07	17	68	10	66.67	7	53.84	7	70
Prior physical therapy[b]	53	84.12	20	80	14	93.37	12	92.30	7	70
Prior imaging[b]	36	57.14	12	48	10	66.67	7	53.84	7	70
Corticosteroid injection	5	7.93	1	4	1	6.67	2	15.38	1	10
Platelet-rich plasma injection	2	3.17	0	0	0	0	1	7.69	1	10
Prolotherapy	1	1.58	0	0	1	6.67	0	0	0	0
Trigger point injection[¥]	2	3.17	1	4	1	6.67	0	0	0	0
Tenotomy	2	3.17	0	0	1	6.67	0	0	1	10
Oral corticosteroids	0	0	0	0	0	0	0	0	0	0
Diabetes mellitus	0	0	0	0	0	0	0	0	0	0
Hypothyroidism	4	6.34	2	8	1	6.67	0	0	1	10
Laterality										
Left	31	49.20	11	44	7	46.67	8	61.53	5	50
Right	22	34.92	10	40	5	33.33	4	30.76	3	30
Bilateral	10	15.87	4	16	3	20	1	7.69	2	20

[1]Met MCID equals a 22-point change or greater on the VISA-H; [†]Not Met MCID equal a 21-point change or fewer on the VISA-H; [s]Total number of ESWT sessions from baseline VISA-H to final VISA-H; [i]Length of follow up in weeks from initial intake evaluation to final VISA-H; [b]Prior being before initial shockwave session; [¥]Trigger point injection performed with lidocaine; BMI Body mass index; ESWT: Extracorporeal shockwave therapy; MCID: Minimal clinically important difference; SD: Standard deviation; VISA-H: Victorian Institute of Sports Assessment – Proximal Hamstring Tendons.

Characteristics were similar between R-SWT and C-SWT cohorts (**table I**). VISA-H scores were similar at baseline between R-SWT and C-SWT groups (39.4 ± 17.4 *vs* 40.7 ± 17.0) and were not different in VISA-H scores between R-SWT and C-SWT groups following treatment (62.6 ± 19.7 *vs* 63.4 ± 21.3; p = 0.812). Within the entire cohort, VISA-H scores significantly increased from baseline to follow-up for both treatment groups (p < 0.0001, **figures 2, 3**). Most patients met MCID in both R-SWT (25 of 40, 62.5%) and C-SWT (13 of 23, 56.5%), but there was no difference between the proportion of patients that met MCID between R-SWT and C-SWT (p = 0.641). The mean number of sessions until patients first met MCID was 3.8 ± 0.9 for R-SWT and 4.5 ± 2.0 for C-SWT. For all the patients who met MCID, all who received R-SWT (n = 25) and almost all treated with C-SWT (12 of 13, 92.3%) did so within six treatment sessions (**figure 4**).

One patient who was initially treated with R-SWT but desired additional functional gains elected to complete C-SWT and met MCID after receiving C-SWT. There was no report of major adverse reactions to shockwave therapy during treatment or follow up for both groups.

DISCUSSION

The purpose of this quality improvement report was to evaluate the functional outcomes using R-SWT and C-SWT in runners with proximal hamstring tendinopathy quantified using VISA-H. We observed that most patients met criteria for clinical improvements following R-SWT or C-SWT. No major complications were observed. To our knowledge, no studies have previously compared these two forms of shockwave therapy nor reported on outcomes after C-SWT for proximal hamstring tendinopathy management in a running population. These findings suggest runners with proximal hamstring tendinopathy may achieve functional gains with either ESWT treatment method.

Our findings are consistent with the existing literature on ESWT for proximal hamstring

Figure 2. Radial Shockwave Cohort VISA-H Score Changes.

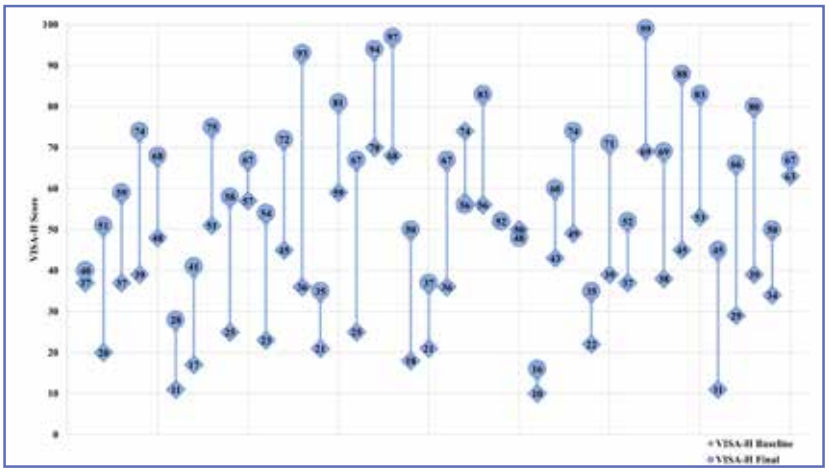


Figure 3. Combined Shockwave Cohort VISA-H Score Changes.

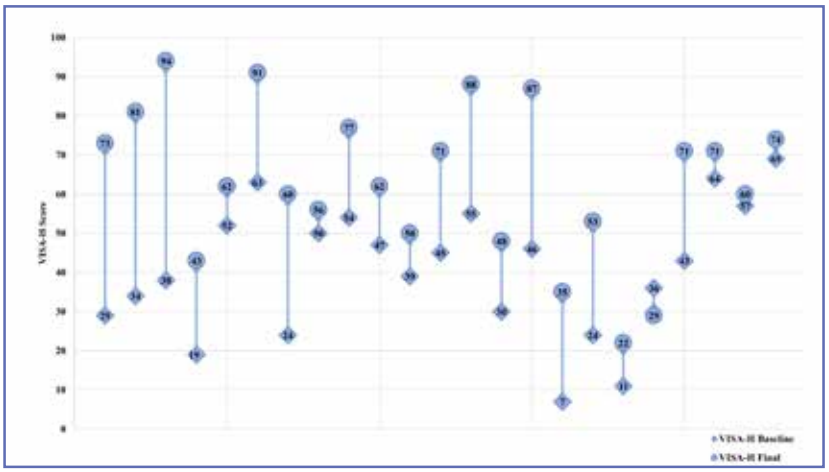
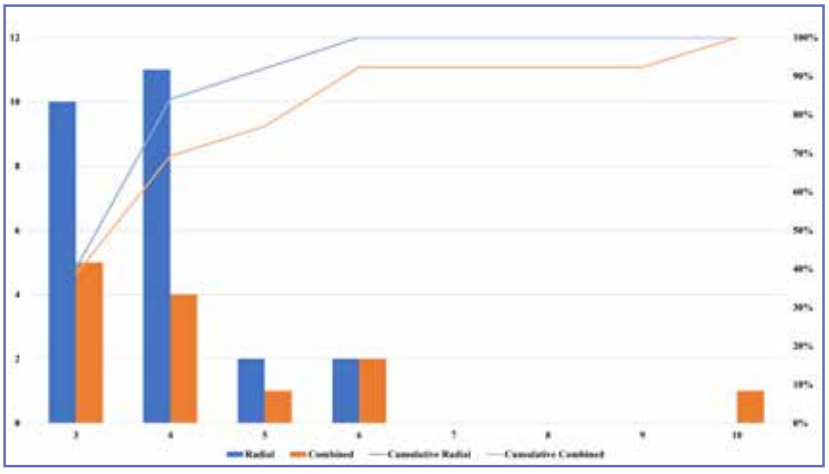


Figure 4. Number of treatments to minimal clinically important difference in radial and combined shockwave groups.



tendinopathy. Cacchio *et al.* conducted a randomized control trial of 40 professional athletes with chronic proximal hamstring tendinopathy and found R-SWT to outperform standard therapeutic exercise at three months with a primary outcome of pain scores (7). The R-SWT group received four weekly treatment sessions using 2,500 strikes at 4 Bars, which equivalates to an energy flux density (EFD) of 0.18 mJ/mm^2. Notably 80% of athletes in the R-SWT and no participants receiving therapeutic exercises were able to return to pre-injury status. We observed a majority of patients responded to R-SWT and C-SWT using a different validated outcome measure specific to athletes with the VISA-H. Our findings expand on prior overlapping cohort from a smaller case series by Mitchkash *et al.*, which noted clinically important VISA-H score improvements in 69% of runners treated with R-SWT for proximal hamstring tendinopathy (10).

The form of ESWT did not result in differences in functional outcome measures. Limited studies to date have compared C-SWT to R-SWT. One study demonstrated that treatment of Achilles tendinopathy had more favorable outcomes measured with higher number of patients meeting MCID who received C-SWT compared to R-SWT (89.7% *vs* 63.8%, p = 0.022) (17). In contrast, a separate report in the treatment of plantar fasciitis with C-SWT to R-SWT observed similar gains with nearly three-quarters of patients meeting MCID using the Foot and Ankle Ability Measure (FAAM) in both forms of treatment (13). Notably higher total EFD delivered during treatment may be more important than treatment energy intensity levels for obtaining successful outcomes (18). While we did not directly measure total EFD, the mean number of strikes (6000) with a goal to aim for 4 bars (similar to the energy level achieved by Cacchio) may contribute to a high total EFD in those treated with R-SWT. While it has not been specifically evaluated in proximal hamstring tendinopathy, it is plausible that total EFD received explains our similar high success rates between groups. In addition, we aimed to highlight the safety profile of ESWT for proximal hamstring tendinopathy. No major complications were observed in our cohort, similar to prior reports (7).

While this study is the first to compare R-SWT and C-SWT for proximal hamstring tendinopathy in a running cohort, we recognize limitations in our report. All enrolled patients were runners, which may limit generalizing findings to other athlete populations or those who are less active. The quality improvement study design limits use of randomization, and we did not have a control group to account for influence of physical therapy on functional gains. However, most patients had prior physical therapy and reported a mean duration of symptoms exceeding a year which suggests gains in VISA-H are unlikely to be primarily due to spontaneous healing. We could not control for the specific physical therapy exercises or frequency/compliance to performing this program. Furthermore, ESWT is not covered by most insurers in the United States for musculoskeletal injuries, and patients incurred out of pocket costs which may introduce potential for bias and limit generalizing findings.

CONCLUSIONS

In summary, our findings suggest most runners with proximal hamstring tendinopathy benefit from both R-SWT and C-SWT, and no major complications were observed for either group. Practical application of findings suggests both R-SWT and C-SWT may be helpful in the management of proximal hamstring tendinopathy when combined with physical therapy exercises, and six sessions of treatment may be required to optimize response. These results may aide in the development of future randomized controlled trials or prospective cohort studies evaluating the use of shockwave therapy use in management of proximal hamstring tendinopathy.

ETHICS

Approval was obtained from our institution's quality improvement advisory board; IRB approval was thereby waived by the institution. The letter of approval can be provided if requested.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

Nr 2021;11 (4):749-755

Two-Stage Flexor Pollicis Longus Tendon Reconstruction Using Pedicled Palmaris Longus Tendon Graft

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SUMMARY

Background. Free tendon grafts are frequently used in zone 2 flexor pollicis longus tendon reconstructions. However, pedicled tendon grafts have less risk of adhesion than free tendon grafts. The aim of this study was to evaluate the results of two-stage flexor pollicis longus tendon reconstruction using a pedicled palmaris longus tendon graft.

Methods. Six patients who underwent two-stage flexor pollicis longus tendon reconstruction using a pedicled palmaris longus tendon graft between 2016 and 2018 were retrospectively evaluated in this study. The mean follow-up was 17 months (range: 13-25 months).

Results. In the final follow-ups, the Buck-Gramcko score was excellent in three patients, good in two patients, and fair in one patient. Mean Disabilities of the Arm, Shoulder and Hand score was 12.9 (8.3- 26.7).

Conclusions. We conclude that good results can be achieved with two-stage flexor pollicis longus tendon reconstruction using a pedicled palmaris longus tendon graft. This method appears to be an alternative for flexor pollicis longus tendon reconstruction using free tendon graft.

KEY WORDS

Flexor pollicis longus tendon reconstruction; palmaris longus tendon graft; Paneva-Holevich; pedicled tendon graft; two-stage flexor tendon reconstruction.

BACKGROUND

The reconstruction of the zone 2 flexor tendon divisions using a tendon graft has been performed for many years. Hunter and Salisbury defined the two-stage reconstruction in 1971 (1). Since then, it has been used in cases where single-stage flexor tendon reconstruction was not appropriate (2-4). In 1972, Kessler reported the results of two-stage flexor digitorum profundus (FDP) reconstruction using a pedicled flexor digitorum superficialis tendon graft together with a silicone rod (5). This technique is now called the modified Paneva-Holevich technique and is often used in FDP reconstructions (6-10). In 1978, Foucher et al. described two-stage flexor tendon reconstruction using a pedicled palmaris longus (PL) tendon graft, which was prepared in the distal part of the forearm (11). Although this technique has not found a wide range of use for FDP reconstruction, it seems to be suitable for flexor pollicis longus (FPL) reconstruction in terms of providing a pedicled tendon graft (6, 8, 12). Currently, zone 2 FPL reconstructions are often performed with free tendon graft. However, the results obtained with this technique are not encouraging (13-15). We hypothesized that the problem with this technique is the use of free tendon graft. Because the free tendon graft needs surface neovascularization during the healing phase and this situation tends to the adhesion of the tendon graft to the surrounding tissues (16). Pedicled tendon grafts provide a great advantage in this respect.

The aim of the present study was to evaluate the results of two-stage FPL tendon reconstruction using a pedicled PL tendon graft.

MATERIALS AND METHODS

Patients and methods

Six patients who underwent two-stage FPL tendon reconstruction using pedicled PL tendon graft between April 2016 and July 2018 were included in the present study. Only the clean-cut injuries are included to the study. The mean age of the patients was 32 years (range: 19-44 years). The study included four male patients and two female patients. The injury was in the dominant hand of one patient and in the non-dominant hands of the other patients.

While three patients were employed in various jobs, two were homemakers and one was a student. None of the patients were manual workers. All patients were injured due to cutting incidents and the primary injury site of the FPL tendon was in zone 2. None of the patients had previously undergone surgery due to this injury. All patients had missed injuries. The mean duration from injury to first stage surgery was 14 months (range 5-34).

Preoperatively, the primary complaint of four patients was loss of function in the thumb, while the primary complaint of the remaining two patients was pain in the volar aspect of the thumb interphalangeal joint.

In the final follow-ups, ranges of motion of the interphalangeal and the metacarpophalangeal joint were measured clinically with a goniometer. Static pinch force was measured with key pinch grip and second finger pulp using a pinch gauge (Baseline Pinch Gauge, Alimed Corp., Dedham, MA, USA). Results regarding active range of motion were assessed by Buck-Gramcko technique. Hand function was evaluated with Disabilities of the Arm, Shoulder, and Hand (DASH) score.

Patients were asked to rate their thumb functions as excellent, good, fair, or poor in the postoperative period.

Statistical evaluation was performed using Wilcoxon signed-rank test.

All procedures performed in our study were in accordance with the ethical standards of the Declaration of Helsinki and were approved by the local Ethics Committee (2020-1472). Informed consent was obtained from all patients involved in this study.

Surgical technique

All operations were performed under regional or general anaesthesia by application of an arm tourniquet.

As in the classical two-stage FPL reconstruction, the thumb flexor system was exposed through a Bruner incision in the first stage and all the scarred tissues around the flexor system were excised carefully. Nearly 1 cm distal attachment of the FPL tendon was preserved and the dysfunctional part of the FPL tendon was excised. All preservable pulley structures were preserved. Subsequently, the proximal end of the FPL tendon was explored by approaching with a curved incision at the volar aspect of the distal side of the forearm. The distal end of the FPL tendon was pulled into the incision site on the forearm. The silicone tendon implant was passed through the carpal tunnel and advanced to the base of the thumb (**figure 1 A**). Subsequently, the distal end of the PL tendon was cut as distally as possible. The FPL tendon was shortened. The distal end of the FPL tendon was sutured to the distal end of the PL tendon with Pulvertaft sutures. The tenorrhaphy line remained outside the carpal tunnel (**figure 1 B**). The silicone tendon implant was threaded through the pulley system to the tip of the thumb. The distal end of the implant was sutured to the surrounding soft tissue. While the tendon implant was being pulled proximally, the thumb was flexed and there was no bowstring effect (**figure 1 C**). Subsequently, the wound was closed. After the third postoperative day, passive range of motion exercises for the thumb were started.

The second stage was performed 3 months after the first stage. In the second stage, the distal aspect of the forearm was explored and the proximal end of the silicone tendon implant was located. Subsequently, the distal tip of the tendon implant was located by approaching the volar side of the distal thumb. Through transverse incisions made in the forearm, the PL tendon was freed and cut from the distal end of the musculotendinous junction and the proximal end of the tendon was pulled distally (**figure 1 D**). The tip of the pedicled PL tendon graft was sutured to the proximal end of the silicone tendon implant. The implant was pulled from the distal incision of the thumb, delivering the PL tendon graft to the distal phalanx. At this stage, the tension of the tendon graft was adjusted by holding the interphalangeal joint flexed at 30 degrees while the wrist was stabilised at neutral position. The PL tendon graft was temporarily fixed to the distal side of the thumb with a hypodermic needle (**figure 1 E**). Subsequently, excess tendon graft was excised. The tendon graft was fixed to the distal phalanx with a pull-out suture using a button and direct suturing to the FPL stump (**figure 1 F**).

Postoperatively, a dorsal short arm splint was applied with the wrist in neutral position, the thumb carpometacarpal joint abducted at 30 degrees, and the metacarpophalangeal and the interphalangeal joints flexed at 30 degrees. Range of motion exercises including passive flexion and active exten-

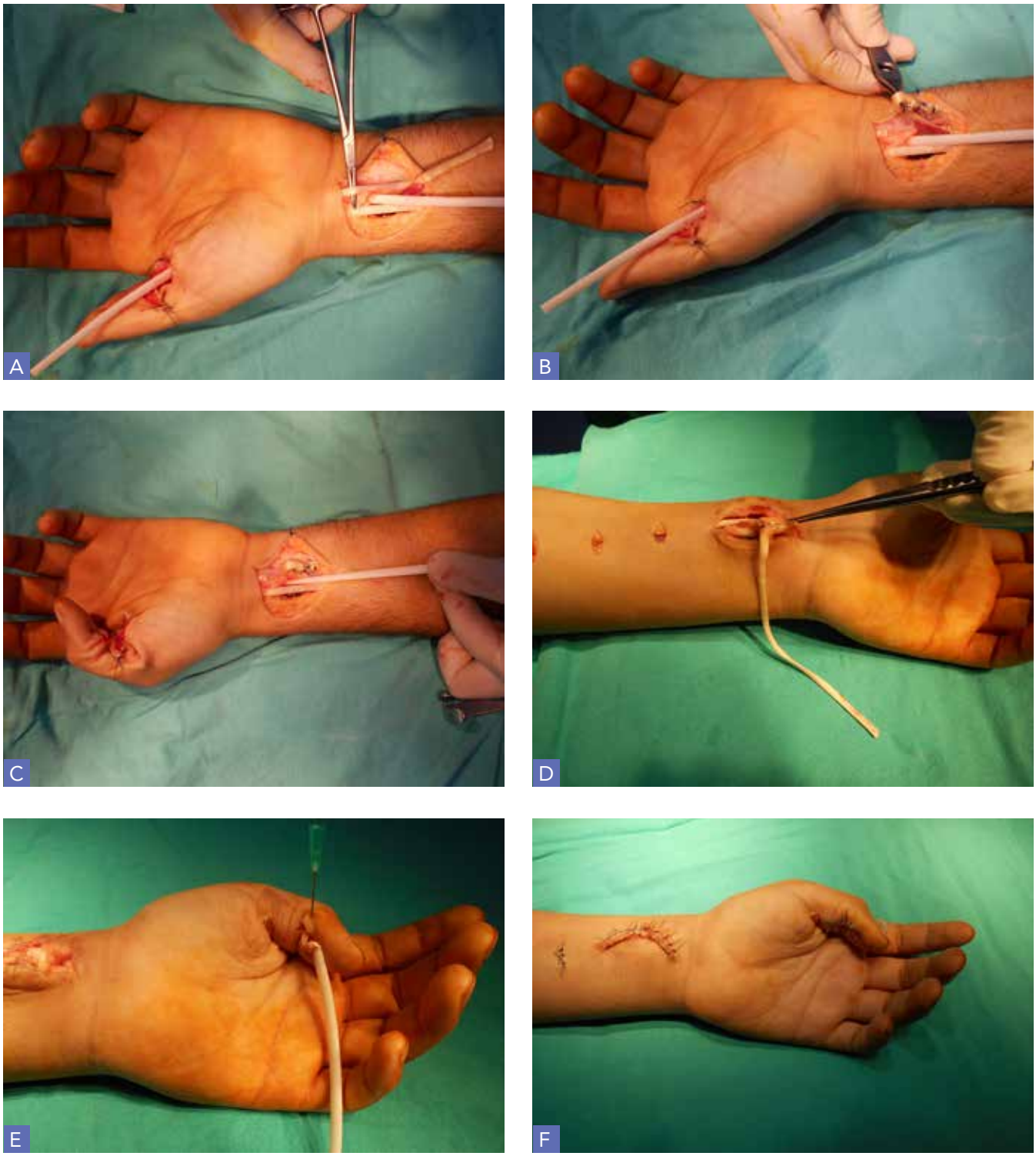

Figure 1. (A) Intraoperative photograph shows the silicone tendon implant inserted through the carpal tunnel with flexor pollicis longus (FPL) and palmaris longus (PL) tendons on its ulnar side; **(B)** FPL and PL tendons with their distal ends sutured together; **(C)** No bowstring is observed after the tendon implant is pulled proximally; **(D)** Pedicled PL tendon graft; **(E)** Adjustment of the length of the tendon graft with hypodermic needle; **(F)** Appearance of the thumb after pull-out suture.

sion were instructed to be performed for six weeks postoperatively. Active thumb flexion exercises were started with the removal of the button at the end of the sixth week.

RESULTS

The mean follow-up duration was 17 months (range: 13-25 months). None of the patients developed infection or tendon rupture. No additional surgical intervention was performed in any patient.

Results are summarised in **table I** and see **figure 2**.

After reconstruction, mean extension deficits of 14 degrees (range: 0-25 degrees) and 11 degrees (range: 0-20 degrees) were observed in the interphalangeal joint and the metacarpophalangeal joint, respectively. The range of motion of the interphalangeal and the metacarpophalangeal joint on the operated side was significantly lower than that on the contralateral side ($P < 0.05$). The values of key pinch strength and pinch grip strength for the index finger were significantly lower on the operated side than on the contralateral side ($P < 0.05$). The Buck-Gramcko score was excellent in three patients, good in two patients, and fair in one patient. Mean DASH score was 12.9 (8.3-26.7).

Patients subjectively evaluated their thumb functions postoperatively. Five patients rated their functions as good, while one patient rated the functions as fair. Two patients with main complaint of pain at admission stated that the pain was relieved in the postoperative period. Three patients who

Table I. Results of the flexor pollicis longus tendon reconstruction.

	Mean	Range	% of contralateral side mean value
Active Flexion IP	59°	35°-75°	81.9%
Active ROM IP	45°	20°-65°	62.5%
Active ROM MCP	44°	25°-60°	69.8%
Active ROM IP + MCP	89°	60°-120°	65.9%
Pinch strength to the index finger (kg)	5.5	2.3-8.8	70.5%
Key pinch strength (kg)	6.9	3.8-12.1	62.7%

IP: interphalangeal joint; MCP: metacarpophalangeal joint; ROM: range of motion.

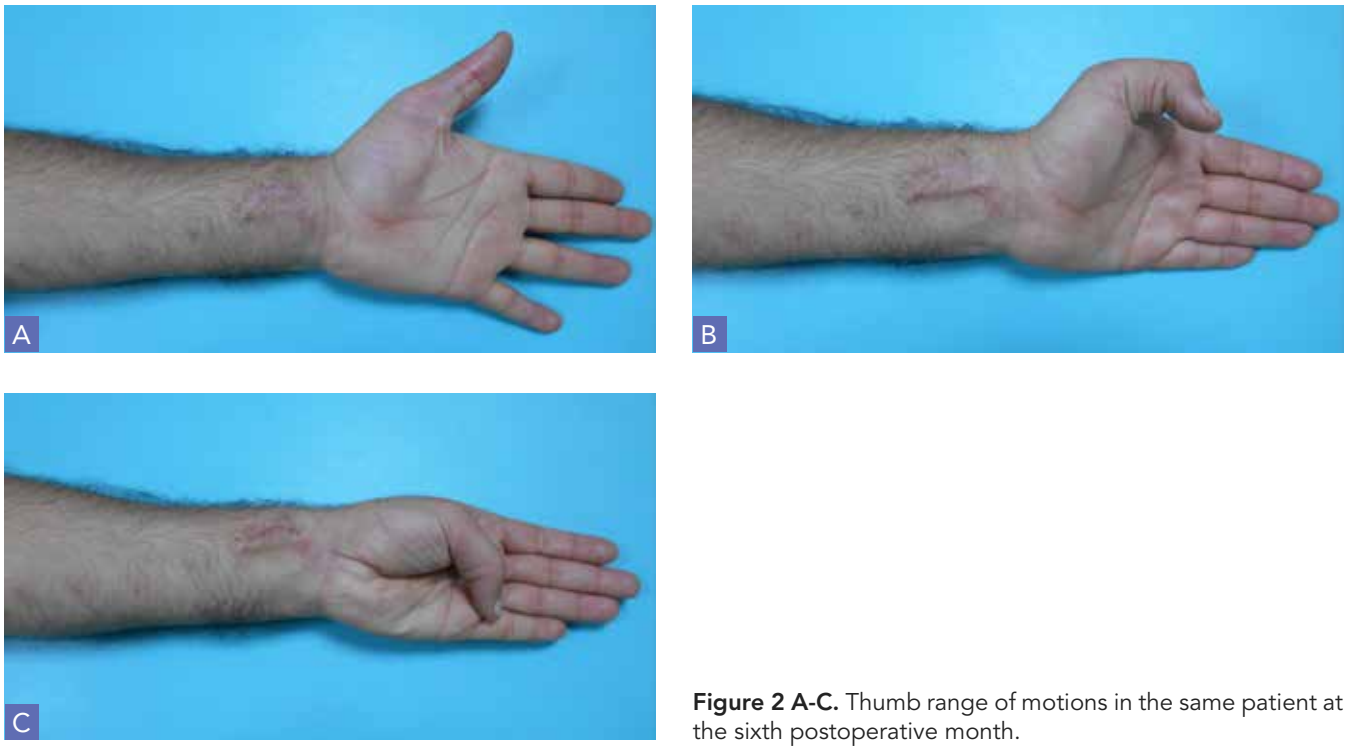

Figure 2 A-C. Thumb range of motions in the same patient at the sixth postoperative month.

were employed in jobs were able to return to their previous jobs at a mean duration of 13 weeks (range: 12–15 weeks) after the second stage.

DISCUSSION

Successful results are frequently obtained with primary repairs of zone 2 FPL divisions (17). If primary repair is not possible or fails, tendon grafting is the common treatment method. Unfortunately the results of tendon grafting are generally not as successful as primary repairs (13). According to the Buck-Gramcko score, we achieved excellent and good results in five of the six patients that we performed FPL tendon reconstruction with pedicled PL tendon graft.

The main limitation of this study was the small number of patients. Currently, most of the FPL divisions are successfully diagnosed and treated in the acute period, consequently limiting the number of patients. All the patients in the present study were injured by cutting incidents. However, FPL divisions may be associated not only with this type of injury, but also with high-energy injuries. Patients who had undergone unsuccessful repair of the FPL division previously are also the candidates for FPL reconstruction. However, all patients in the present study had missed injuries. Hence, the patients in the present study had better prognoses.

Single stage FPL reconstructions are generally considered appropriate for patients with intact tendon sheath and mobile joints (Boyes grade 1), as is the case with single stage FDP reconstructions (18-20). There is a key factor distinguishing the FPL tendon from the FDP tendon, which is the path between the distal phalanx and the forearm. There are very sharp angulations along this path that creats a suitable ground for adhesion of the free tendon graft. While the proximal tenorrhaphy can be performed to the palmar of the hand in most FDP reconstructions, in FPL reconstructions, proximal tenorrhaphy is frequently performed in the distal forearm, which causes the free tendon graft to pass through the carpal tunnel, requiring the graft to be longer. For these reasons, we believe it to be beneficial to create a pseudosheath formation with two-stage reconstruction in all FPL reconstructions. Two-stage reconstruction also gives us the chance to use a pedicled tendon graft. The waiting period between the two stages is a disadvantage of this method, but this time is not considered as a problem by patients who have learned the value of the thumb function.

Functional return may be unsatisfactory in patients undergoing a two-stage FPL reconstruction using free tendon grafts. Therefore some authors recommended considering interphalangeal joint arthrodesis instead of FPL reconstruction (14, 15, 21). However, single interphalangeal arthrodesis in patients with chronic FPL injury has been reported to cause a significant loss of pinch strength. Therefore, FPL reconstruction is critical to maintain the pinch force (22). In light of these reports, it is necessary to obtain better results in two stage reconstruction.

Free tendon grafts begin to vascularise on the twenty-first postoperative day and the number of surviving cells is limited (23). Surface neovascularisation plays a role in the healing phase of all free tendon grafts irrespective of its extent. This tends to result in adhesion to the surrounding tissues (16). However, healing process of pedicled tendon grafts differs from that of free tendon grafts. Pedicled tendon grafts that were separated from their proximal origin 5 weeks after the first stage have been shown to preserve their vascularisation (24). We did not use free tendon graft in this technique. Thus, we tried to overcome the problem of adhesion.

Pain is expected in the unrepaired zone 2 flexor tendon divisions, especially in the palm. This pain limits itself within a few months, even if the patient is not treated. In our study, two patients who suffered from pain in the volar aspect of the thumb interphalangeal joint preoperatively reported no pain at the final follow-up. We assumed that this pain was caused by the use of the volar side of the thumb interphalangeal joint to increase the grasping strength while holding the objects. Since active interphalangeal joint flexion was achieved, patients' complaints were relieved, as the load transfer was performed by the pulp of the thumb.

Irrespective of the treatment (primary repair or reconstruction), a functional pulley system is indispensable in flexor tendon surgery. Especially, A1 pulley is important for the FPL function (25-27). Least possible damage to the pulley system directly increases the functional gain. However, this never be considered as, allowing a tissue in the flexor system that will prevent tendon slipping. At the end of the first stage, the pulley system should be re-evaluated with movement of the tendon prosthesis and if necessary, pulley reconstruction combined with scar tissue excision should not be avoided.

Adjusting the length of the tendon graft in FPL and FDP tendon reconstructions is one of the most important challenges associated with the surgery. Impossibility of adjusting the length of the graft using the proximal tenorrhaphy area appears to be an important technical difficulty in this technique. Keeping the graft too tight or too loose may cause quadriga syndrome and lumbrical plus finger problems in FDP reconstructions. However, the complication interval is narrower, since these problems are not encountered in FPL reconstructions (6, 28).

In this technique, some problems have still not been overcome. One of these problems is the mismatch between the cross sectional area (CSA) of the tendon graft and the CSA of the reconstructed tendon. In flexor tendon reconstruc-

tions, the CSA of the tendon graft is always intended to be close to that of the tendon to be reconstructed (29). Therefore, the CSA of the PL tendon being considerably lower than the CSA of the FPL tendon is considered a disadvantage (29,30). The CSA of the flexor carpi radialis (FCR) tendon is very close to that of the FPL tendon (30). Therefore, it may be appropriate to use a part of the FCR tendon as a pedicled tendon graft in this technique. However, it should be taken into account that the maximum obtained length of the FCR tendon graft will be shorter the length of the PL tendon graft. It may be preferable to perform the proximal tenorrhaphy using modified Kessler technique instead of the Pulvertaft technique to provide sufficient tendon graft length. The use of pedicled FCR tendon graft may also an option for patients without a PL tendon.

Many studies have suggested that the use of intrasynovial tendons as grafts, especially in the pulley system, is more appropriate than the use of extrasynovial tendons (16, 31, 32). The use of PL tendon, which is an extrasynovial tendon, is another disadvantage of this technique.

CONCLUSIONS

Over the years, the unsatisfactory results we obtained with zone 2 FPL reconstructions using free tendon graft led us to seek a different method. As a result, we abandoned FPL reconstruction with a free tendon graft because we consider that these poor results were due to the use of an avascular tendon graft. We apply a two-stage reconstruction with pedicled tendon graft to each patient who is deemed suitable for either a single or two-stage reconstruction by classical evaluation. This method is technically more difficult and has to be done in two stages. However, the FPL function we are trying to achieve is more valuable than these disadvantages. Good results can be obtained with two-stage FPL tendon reconstruction using a pedicled PL tendon graft. This method appears to be an alternative for FPL tendon reconstruction using a free tendon graft.

ETHICS

This study was approved by the local Ethics Committee of Şişli Etfal Teaching and Research Hospital and was conducted in accordance with the ethical standards of the Declaration of Helsinki.

INFORMED CONSENT

Informed consent was obtained from all patients involved in this study.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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Location of the Flexor Carpi Radialis Myotendinous Junction: a Cadaveric Study

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LEVEL OF EVIDENCE: 5

SUMMARY

Background. The flexor carpi radialis (FCR) tendon is frequently selected for use as an interposition graft, for tendon transfers, and as a landmark for volar forearm approaches. Original surgical techniques of FCR harvest recommend incision placement 10 cm proximal to wrist crease. To date no anatomic study has been conducted to precisely define the location of the FCR myotendinous junction.

Methods. 5 fresh frozen cadavers were dissected, exposing the full length of the FCR. The radial styloid (RS) was selected as a distal anatomic landmark, the medial epicondyle (ME) was chosen as a proximal landmark. All measurements were taken along the length of the FCR tendon. As the FCR myotendinous junction is chevron shaped, we marked both the proximal myotendinous junction (PMT) and distal myotendinous junction (DMT), with the DMT indicating the beginning of purely tendinous FCR. Four measurements were taken for each arm: 1) RS to DMT, 2) RS to PMT, 3) ME to PMT, 4) ME to DMT.

Results. Pearson correlation coefficient comparing tendinous length of the FCR to the overall forearm length was 0.896 indicating a strong positive correlation ($p = 0.040$). The mean ratio of tendinous FCR length to forearm length was 0.42 ± 0.05, ranging from 0.38 to 0.50. This correlated to a distance 11.7 ± 2.3 cm proximal from the radial styloid.

Conclusions. This study demonstrates the location of the myotendinous junction of the FCR. Our results suggest incision placement 10 cm from proximal wrist crease to be unreliable in forearms too short or too long. Instead, we recommend measuring 40% of the length from RS to ME as a more consistent marker for FCR harvest.

KEY WORDS

Flexor Carpi Radialis; cadaveric study; myotendinous junction; tendon harvest; volar forearm anatomy.

INTRODUCTION

The flexor carpi radialis (FCR) is a wrist flexor and radial deviator found in the superficial compartment of the volar forearm. It originates at the common flexor origin on the medial epicondyle and inserts primarily at the base of the second metacarpal. It is a frequently selected tendon for use in carpometacarpal (CMC) arthroplasty interposition, tendon transfer, and as a landmark for volar forearm approaches (1-6, 10). To date no anatomic study has been conducted to delineate the precise location of the FCR myotendinous junction. We hypothesized that the FCR myotendinous junction has a predictable anatomic relationship to the radial styloid (RS) and medial epicondyle (ME).

MATERIALS AND METHODS

In accordance with established ethical standards, 5 fresh frozen cadavers were selected involving fully preserved upper extremities (Padulo *et al.* (11)). All cadavers were placed in full supination, 90 degrees of elbow flexion, and

secured to a table. Skin was carefully dissected out over the volar forearm exposing the full length of the FCR. The RS was selected as a distal anatomic landmark, and a line was drawn across the volar wrist perpendicular to it. Similarly, the ME was marked as a proximal landmark. All measurements were taken as a straight line along the length of the FCR tendon. Since the FCR myotendinous junction is chevron shaped, both the proximal myotendinous junction (PMT) and distal myotendinous junction (DMT) were demarcated. Four measurements were taken for each arm: 1) RS to DMT, 2) DMT to ME, 3) RS to PMT, 4) PMT to ME. In the interest of highlighting clinical relevance, the DMT indicates the location of purely tendinous FCR (**figure 1**).

Statistical methods

Statistical analysis was performed with SPSS version 25 (IBM, Armonk, NY). Pearson correlation was performed to compare the forearm length (measured from the tip of the radial styloid to medial epicondyle) with the tendinous length of the FCR (measured from the insertion to the distal most aspect of the musculotendinous junction). Descriptive statistics were then calculated.

RESULTS

The distances measured in each cadaveric specimen are shown in **table I**. Pearson correlation coefficient comparing tendinous length of the FCR to the overall forearm length was 0.896 indicating a strong positive correlation ($p = 0.040$). The mean ratio of tendinous FCR length to forearm length was 0.42 ± 0.05, ranging from 0.38 to 0.50. This correlated to a distance 11.7 ± 2.3 cm proximal from the radial styloid.

DISCUSSION

Since the original description of ligament reconstruction and tendon interposition (LRTI) for CMC arthritis by Eaton and Littler in 1973, various techniques have been described for FCR harvest (2, 7-9). Some of these techniques require at least four separate incisions, with the proximal most extent of the FCR tendon estimated to be 10 cm from the proximal wrist crease. This, however, fails to consider patients of different sizes and forearm lengths. One specimen showed the distal end of the myotendinous junction to be only 9.5 cm from the radial styloid, which would be more distal than the oft-estimated 10 cm. Also, many of the specimens

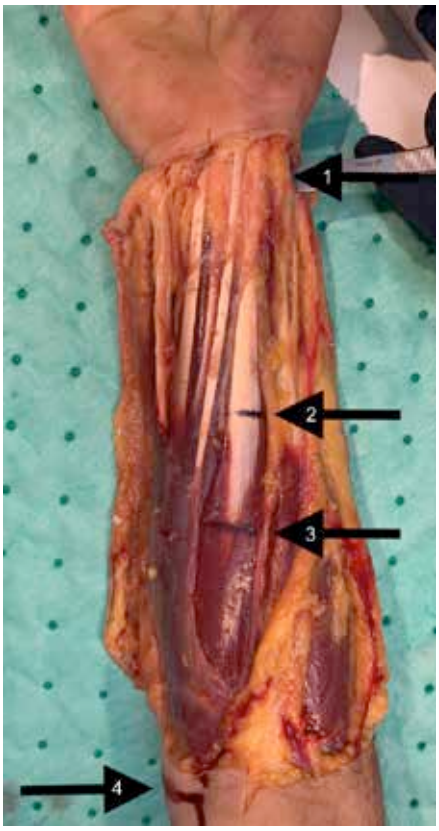

Figure 1. Volar Forearm Exposure of the Flexor Carpi Radialis Tendon.

Arrow 1 indicates the radial styloid, arrow 2 indicates the distal most portion of the musculotendinous junction, arrow 3 indicates the proximal most portion of the musculotendinous junction, arrow 4 indicates the medial epicondyle.

Table I. Measurements taken from each cadaveric specimen.

Specimen	RS to DMT (CM)	DMT to ME (CM)	RS to PMT (CM)	PMT to ME (CM)	Total Length (CM)
1	14.5	14.5	19.0	10.0	29.0
2	9.5	15.2	13.2	11.5	24.7
3	10.3	17.1	15.6	11.8	27.4
4	13.8	16.3	17.8	12.3	30.1
5	10.5	16.2	13.7	12.9	26.6

RS: radial styloid; ME: medial epicondyle; DMT: distal myotendinous junction; PMT: proximal myotendinous junction.

showed the distal end of the myotendinous junction to be much further including the largest measuring 14.5 cm. Our results demonstrate that the tendinous portion of the FCR is located 11.7 ± 2.3 cm proximal from the radial styloid. This correlates to 42% of the distance from radial styloid to medial epicondyle. As such, conventional descriptions of incision placement 10 cm from proximal wrist crease would be sufficient for most indications, but for patients with very short or very long forearms, adjustments should be made.

CONCLUSIONS

The primary limitation of the study is its sample size of 5 cadavers; however, the strong correlation found between forearm length and FCR tendon length reached statistical significance. Larger sample size cadaveric studies would prove beneficial toward further evaluation of FCR tendinous anatomy. The authors acknowledge a further limitation in that elbow flexion was controlled at 90 degrees during all measurements because one of the cadavers did not have full elbow extension. This could create a slight disparity between cadaveric measurements (taken in elbow flexion) and intra-operative measurements (performed in full extension).

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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Incidence of Accessory Brachialis Muscle, Variations in its Insertion and Relation with Surrounding Neurovascular Structures

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SUMMARY

Background. Accessory brachialis (AcBr) muscle can compromise the surrounding neurovascular structures due its variable insertion. In this context, the objective of the present study was to determine the incidence of AcBr muscle, variation of its insertion and relation with surrounding neurovascular structures.

Methods. The study was performed in 84 formalin fixed human cadaveric upper limb specimens, which were available in the department of anatomy. The insertion pattern of the AcBr muscle was divided into 5 types (type 1, type 2, type 3a, type 3b and type 4).

Results. It was observed that, AcBr muscle was present in 46 (54.8%) cases. In 31 cases (67.4%), this muscle inserted into the main tendon of brachialis muscle (type 1). It joined the tendon of biceps brachii (type 2) in 6 cases (13%). In 7 cases (15.2%), it gave a slip which joined the supinator muscle after passing deep to the radial recurrent vessels (type 3a). In a single case (2.2%), the slip merged with the supinator after passing superficial to the radial recurrent vessels (type 3b). In one case (2.2%), it gave muscular fibres which crossed the radial nerve and merged with the brachioradialis muscle (type 4).

Conclusions. The present study observed higher frequency of incidence of AcBr muscle, however, the limitation of this study is that the small number of specimens studied. The findings will be more accurate with a larger sample size. The gender-based comparison was not performed, since the study involved disarticulated upper extremities.

KEY WORDS

Anatomic variation; biceps brachialis; muscles; nerve compression syndromes; orthopedic disorders.

BACKGROUND

Accessory muscles may produce a palpable swelling or could lead to pressure effect on the adjacent neurovascular structures. Accessory muscles at the arm region may contribute to neurovasculopathy by compressing the brachial artery and median nerve (1, 2). In clinical cases, where an apparent cause for such symptoms is unknown, evaluation of accessory muscles might help in the accurate diagnosis. In the radiological setup, study of cross-sectional images can reveal the accessory muscles and help in differentiating them from the soft-tissue tumors (3). Entrapment of the vessels and nerves can happen, because these accessory muscles pass anterior to them. The accessory muscles of the arm region have clinical implications and to be considered as the etiology in median, ulnar and medial cutaneous nerve of forearm paralysis on few occasions. They can also cause symptoms of compression of brachial vessels (4). In the surgical planning, if there is an anatomical variation, it becomes hard to identify the topographical location of the nerves in the muscular compartments, which complicates the surgical approach (5). It was reported that the variations of brachialis muscle are rare in the scientific literature (6). Accessory brachialis (AcBr) muscle, if present can compress the surrounding neurovascular structures in the supracondylar region (6-8). It can also affect the movements of the elbow joint like flexion, extension, pronation and supination. The AcBr muscles are grouped together as the brachialis anticus (9). The knowledge of unusual insertion of brachialis muscle is

important during the surgical approach to the elbow joint (10). It was reported that the distal tendon of AcBr may split and enclose the median nerve. This could lead to the symptoms of median nerve entrapment (7). Careful examination of the cubital tunnel for an accessory muscle might help, which can be a causative factor in the musculoskeletal problem of the upper limb (3). Because of these clinical relevance, the present anatomical investigation was conducted to determine the incidence of AcBr muscle, variability in its insertion pattern and to study the relation to the surrounding neurovascular structures.

MATERIALS AND METHODS

This research work was executed in 84 formalin fixed human cadaveric upper extremities. Among them, 48 were right upper limbs and 36 belonged to left upper limbs. The gender of the cadavers was not taken into consideration. The extremities were from the donated cadavers, which were available in the department of anatomy. The present study was assessed and certified as approved (IEC KMC MLR 08-18/190) by the ethics committee of our medical college. We state that the present study is as per the guidelines of international ethical standards suggested by Padulo *et al.* (11). The cubital fossa and arm were meticulously dissected and the floor of cubital fossa was reached to expose the brachialis and supinator muscles. The presence of AcBr was checked next to the brachialis in the arm and cubital fossa. If AcBr is present, its distal attachment was dissected and noted. The photographs were taken after cleaning the structures. The relation of AcBr with the surrounding neurovascular structures was also studied.

The present study classified the AcBr into 5 types, depending on its insertion pattern as below (**figure 1**):

- type 1: AcBr inserting into the tendon of brachialis muscle;
- type 2: AcBr inserting into the tendon of biceps brachii;
- type 3a: AcBr inserting into the supinator muscle after passing deep to the radial recurrent vessels;
- type 3b: AcBr inserting into the supinator after passing superficial to the radial recurrent vessels;
- type 4: slip of AcBr crossing the radial nerve and inserting into the brachioradialis muscle.

RESULTS

According to observations of the present study, AcBr muscle was present in 46 (54.8%) cases. It was observed in 27 right sided and 19 left sided upper extremities. The present study revealed that the AcBr was more prevalent in

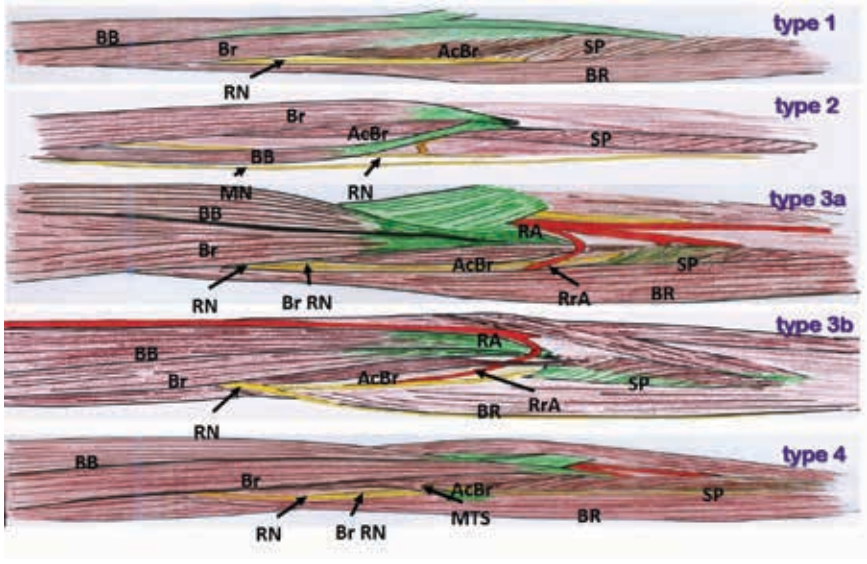

Figure 1. Various types of AcBr muscle observed in the present study, inserting into brachialis (type 1); biceps brachii (type 2); supinator and passing deep to radial recurrent vessels (type 3a); supinator and passing superficial to radial recurrent vessels (type 3b); brachioradialis (type 4).

AcBr: accessory brachialis; BB: biceps brachii; Br: brachialis; SP: supinator; BR: brachioradialis; RN: radial nerve; MN: median nerve; Br RN: branch of radial nerve; RA: radial artery; RrA: radial recurrent artery; MTS: musculo-tendinous slip.

the right upper extremity in comparison to the left side (28:18). In 31 cases (67.4%), the AcBr was inserting into the brachialis muscle (type 1, **figure 2**). Among them 16 were right sided and 15 were left sided upper extremities. AcBr joined the tendon of biceps brachii in 6 cases (13%), which included 4 right and 2 left sided upper limbs (type 2, **figure 3**). In 7 (15.2%) specimens (6 right sided and 1 left sided), AcBr gave a slip, which joined the supinator muscle after passing deep to the radial recurrent vessels (type 3a, **figure 4**). In only one case (right sided), the slip merged with the supinator (2.2%) after passing superficial to the radial recurrent vessels (type 3b, **figure 5**). In one right upper limb (2.2%), musculotendinous fibres of AcBr crossed the radial nerve and merged with the brachioradialis muscle (type 4, **figure 6**). The frequency distribution of variability in the insertion of AcBr of this study is represented in **figure 7**. It was observed that, all the cases of AcBr were innervated by the branch of radial nerve, which was originating at the distal part of the arm.

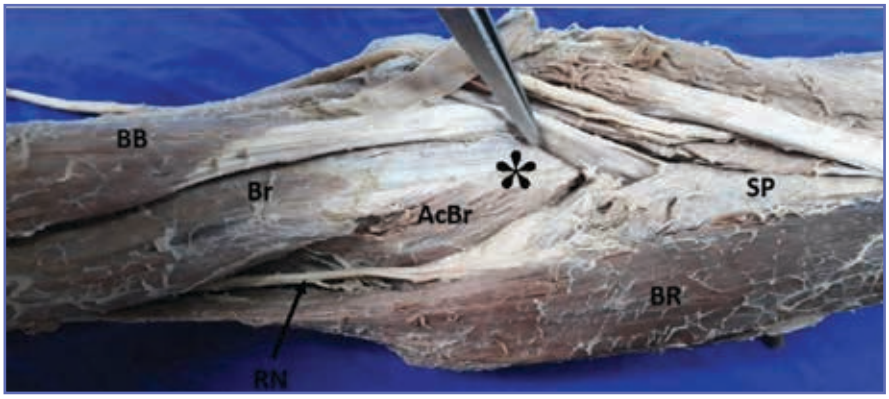

Figure 2. AcBr inserting into the tendon of Br (67.4%; type 1).

BB: biceps brachii; Br: brachialis; AcBr: accessory brachialis; BR: brachioradialis; SP: supinator; RN-radial nerve. *Insertion of AcBr into Br.

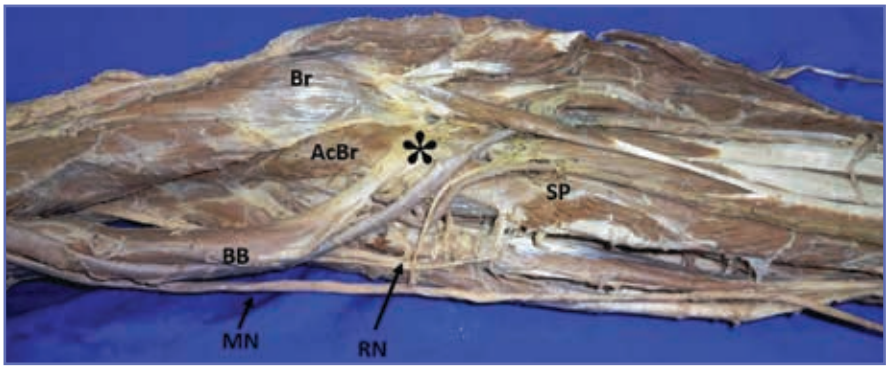

Figure 3. AcBr inserting into the tendon of BB (13%; type 2).

BB: biceps brachii; Br: brachialis; AcBr: accessory brachialis; SP: supinator; RN: radial nerve; MN: median nerve. *Insertion of AcBr into BB.

DISCUSSION

The muscular variations can be understood by studying the embryological development of the muscles. The upper limb musculature develops from the limb bud at the level of lower six cervical and upper two thoracic segments. In the fifth week of gestation, the development of forelimb muscles take place in the mesenchyme of the para-axial mesoderm. The somites will be partitioned into the sclerotome, myotome and dermatome. Muscle primordia develop from the posterolateral side of the somite cells, which migrate into the limb buds around 28th day of intrauterine life (12). The myotomes will develop as the muscles as numerous growth factors are secreted by the cells in the proximal limb

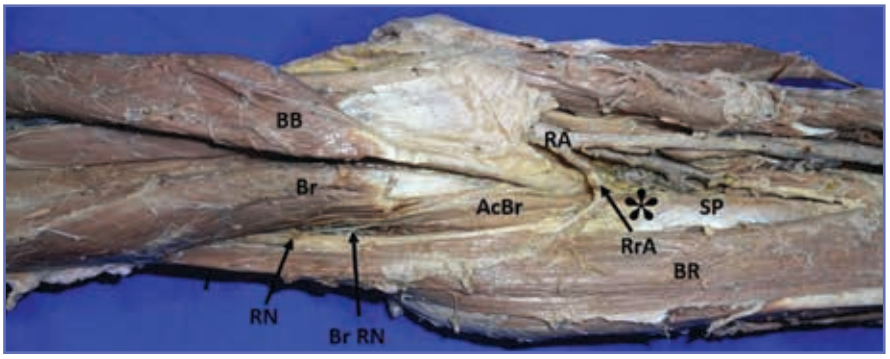

Figure 4. AcBr crossing deep to the RrA and inserting into the SP (15.2%; type 3a).

BB: biceps brachii; Br: brachialis; AcBr: accessory brachialis; BR: brachioradialis; SP: supinator; RA: radial artery; RrA: radial recurrent artery; RN: radial nerve; Br RN: branch of radial nerve supplying AcBr. *Insertion of AcBr into SP.

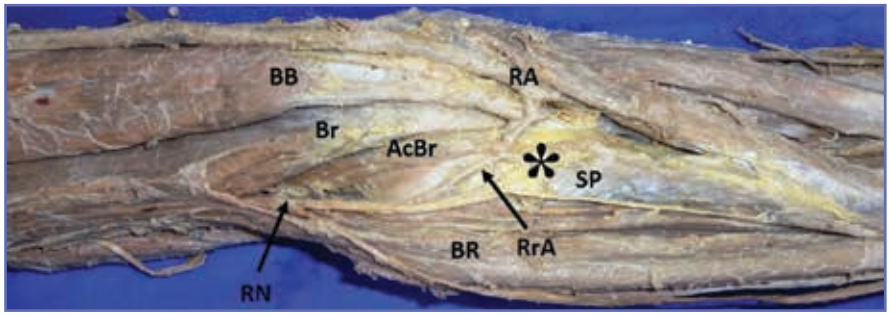

Figure 5. AcBr crossing superficial to the RrA and inserting into the SP (2.2%; type 3b).

BB: biceps brachii; Br: brachialis; AcBr: accessory brachialis; BR: brachioradialis; SP: supinator; RA: radial artery; RrA: radial recurrent artery; RN: radial nerve. *Insertion of AcBr into SP.

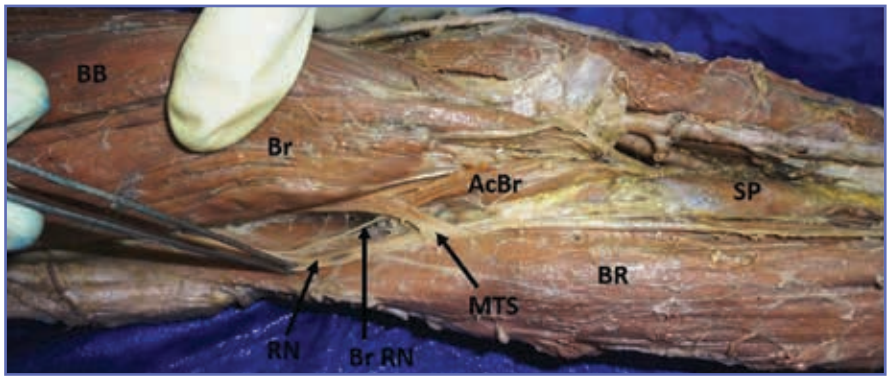

Figure 6. MTS from AcBr crossing over the RN to join the BR (2.2%; type 4).

BB: biceps brachii; Br: brachialis; AcBr: accessory brachialis; BR: brachioradialis; SP: supinator; RN: radial nerve; Br RN: branch of radial nerve supplying AcBr. MTS: musculotendinous slip.

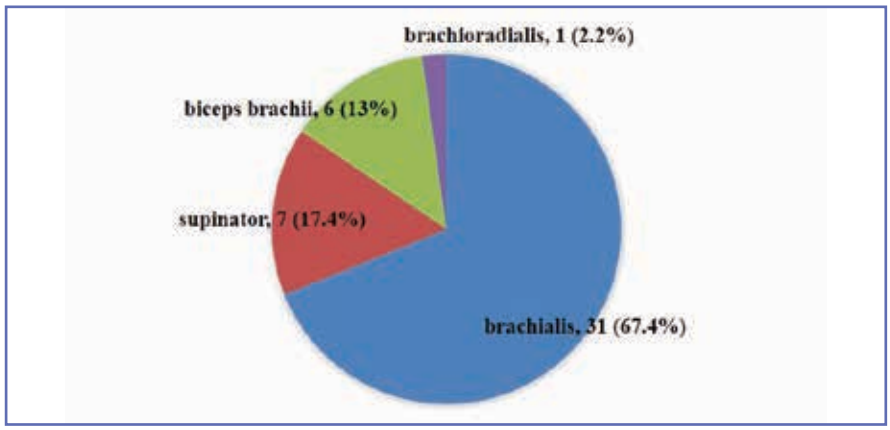

Figure 7. Frequency distribution of variability of insertion of AcBr observed in this study (n = 46).

bud (13). These growth factors will help in the migration of myoblasts into the developing limb buds. The adhesion molecules which are expressed by the myoblasts are involved in the distribution of growth factors in a proper arrangement in the limb (14). The muscle primordia later get split into muscles of flexor and extensor compartments. This division is due to signaling by the connective tissue, which is derived by the lateral plate mesoderm. Postero-anterior development is under the control of WNT7A and the antero-posterior development is due to signalling of sonic hedgehog protein, which is secreted at the zone of polarizing activity in the posterior limb bud (12). Alteration in these pathways can lead to various anatomical variations in the muscular system. The muscle precursors which are found as different layers in the arm fuse to form a single muscle at a particular stage (13, 15). However, some primordial muscle cells go for apoptosis in spite of their contents as myofilaments (13). The failure of their apoptosis may contribute to the development of accessory muscles. It was reported that any changes in the structure of myotome or the somite, or in the distribution of adhesion molecules in the myoblasts may lead to muscular variations, which includes the accessory muscles (16). This is the developmental basis of the presence of accessory muscles.

In the present study, AcBr was observed in 54.8% of cases. AcBr muscle usually originates from the lateral intermuscular septum of arm and the fascia covering deltoid and triceps brachii muscles (17). Disparities in the morphology of brachialis though unusual, but are recorded in the literature that it may be divided into two or more parts, which blend with the brachioradialis, biceps brachii or pronator teres. The distal attachment of the AcBr into the shaft of radius below the bicipital tuberosity can hinder the flexion of the

elbow joint at the ulnar component. This will further lead to difficulty in the pronation and supination of the forearm due to the variant AcBr muscle aponeurosis (9, 17). In the present study the AcBr has joined with the tendons of supinator, brachioradialis, brachialis and biceps brachii muscles. Here the AcBr may be enhancing the actions of the supinators of forearm. The AcBr reported by Pai *et al.* (6) inserted into the supinator and pronator teres after crossing the radial nerve thus contributing to the factors leading to radial tunnel syndrome. In the present study AcBr joined the supinator in 8 upper limbs (17.4%), this insertion pattern may lead to narrowing of the radial tunnel during the supination of forearm thus leading to radial tunnel syndrome. In one of the specimen in the present study, the tendon of AcBr traversed superficial to the radial recurrent artery before inserting into the supinator (2.2%), thus compressing the vessels in certain positions of the forearm. This type 3b variety, may not cause ischemia due to the very good anastomosis around the elbow joint. In one of the specimens of the present study, musculotendinous fibres arising from the AcBr bridged across the radial nerve and merged with brachioradialis (type 4, 2.2%). This bridge also narrows the radial tunnel and may contribute to the radial tunnel syndrome by compressing the radial nerve. In our previous study (18) with different anatomical specimens which was performed few years ago, it was observed that brachialis was giving muscular slips to the brachioradialis muscle. This was observed in 28.5% cases of the upper extremities. In that study (18) we classified the connecting bridges into split type, slip type and tendinous types. In all those cases, the intermuscular connection was oblique and entrapping the radial nerve. There have been instances of AcBr crossing the brachial artery and the median nerve (7, 8). This shows that the presence of AcBr could cause medial nerve entrapment in the forearm and compression of the brachial artery. We did not find any such compression pathology in our study. Nelluri *et al.* (17), has reported a case of AcBr, which was crossing the radial artery, thus having the potential to compress it. The brachialis is usually supplied by the musculocutaneous nerve and radial nerve. The AcBr in the present study were all supplied by twigs from the radial nerve. It was described that, brachialis has dual heads of origin, superficial and deep. Between these two an internervous plane can be found, which can be utilized for the anterolateral surgical approach to the cubital articulation (9).

Akhtar *et al.* (16) reported the insertion of AcBr into the pronator teres muscle. But this morphological variant was not observed in the present study. George and Nayak (19) encountered a variation in which few fibres of brachialis merged with superficial muscles of front of forearm and to the olecranon process. Loukas *et al.* (7) observed an AcBr muscle, which was splitting to envelop the median nerve and finally inserted back to the brachialis tendon. Dharap (20) reported an accessory muscle, which was forming a tunnel for median nerve and brachial artery in the arm. Paraskevas *et al.* (4) and Vadgaonkar *et al.* (21) observed an AcBr muscle, which was merging with the medial intermuscular septum. These type of morphological variants are not observed in the present study. The AcBr inserting into the biceps brachii is termed as brachiobicipitalis (14). This was observed in 6 cases (13%) in our specimens. This morphological variant can offer greater supination of the forearm and hand.

Mistry *et al.* (22) observed AcBr in only 5% of their specimens. These muscles were inserting into the brachioradialis and bicipital aponeurosis. Khandey *et al.* (9) observed AcBr in only 3.5% of their cases. The present study is not agreeing with the incidence rate of AcBr with respect to these studies. We observed higher incidence of AcBr (54.8%), which is not as per the descriptions of classical anatomical text books. The morphological knowledge about AcBr is important to understand the conditions like median nerve entrapment. The knowledge is also important during the surgical procedures around the elbow joint and brachial artery catheterization. This will also prevent misinterpretations during the radio diagnostic procedures and angiographic studies around the cubital fossa. It was reported that the tendon of AcBr muscle can be harvested for the reconstruction of annular ligament of the superior radioulnar joint, tibial collateral ligament of the knee joint and also in tendon transfer surgeries (6). The ruptured tendon of biceps brachii can be reconstructed by using the AcBr, by transferring it to the radial tuberosity (22). The fibres of AcBr are also utilized in reconstruction of ulnar collateral ligament of the elbow joint. Since the muscles of forearm are not always available for the tendon transfer, if AcBr is present, this can be used as an alternative donor for the reconstruction of the flexor digitorum profundus and flexor pollicis longus muscles during the brachial plexus injury (23).

AcBr can alter the arthrokinematics of the shoulder joint, leading to pain during the mobility of the rotator cuff. The clinical testing of the brachialis muscle can be misinterpreted due to the presence of AcBr. The accessory muscles in the arm should not be misinterpreted as tumours during the reporting of the MRI in this region (24). They can be misinterpreted as soft tissue tumours like fibroma and neuroma (14). Presence of AcBr and the variations in its insertion is definitely an important factor contributing to nerve compression syndromes like radial tunnel syndrome. The accessory muscles at the arm can be considered as the reason, whenever there is an entrapment neuropathy along with the brachial artery or brachial vein compression. Surgeries in the cubital fossa, especially elbow joint surgeries may get

complicated due to the presence of AcBr. The percutaneous brachial approach is often preferred during the carotid and vertebral angiography. The knowledge about AcBr can prevent the complication in this procedure. The ultrasound guided brachial artery puncture is suggested in these situations and it should be considered (25).

We believe that the present study has provided additional information about the AcBr muscle. However, the limitation of the present study is that, since it is a cadaveric study, the biomechanics of the elbow joint and signs and symptoms of the entrapment neuropathy could not be assessed in this anatomical investigation. Future implications of this study include studying the AcBr in vivo by using ultrasound, MRI and electromyogram methods. Elastography is a newly established North American based technique to assess the tissue stiffness (26). The AcBr can be studied in vivo by using elastography and the stiffness of the arm can be evaluated. Analan and Ozdemir (26) reported that, neurological disorders are not the only etiological factors for the muscle stiffness, few more factors like anatomical variations, posture, gender and exercise also have a role.

CONCLUSIONS

The present study offers the information about the incidence of AcBr, variability in its insertion and relation with the surrounding neurovascular structures. The preoperative knowledge about AcBr is important to the orthopedicians, anaesthesiologists and radiologists. This can avoid the misinterpretation and subsequent complications. The details are essential for the accurate diagnosis and management of the musculoskeletal disorders of the arm.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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