REVIEW

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Motor Behavior Disorders in Children with Developmental Dyslexia: a Comprehensive Narrative Review of the Literature

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SUMMARY

Background. Difficulty with literacy acquisition is only one of the symptoms of developmental dyslexia. Dyslexic children also show poor motor coordination and postural control. Several studies have shown that dyslexic children and adults perform worse than non-dyslexic children in tasks that also involve postural control.

Methods. In January 2022 the main online databases were accessed. All the articles that investigate possible concomitance between the developmental dyslexia with motor behavior disorders were considered.

Results. The association between developmental dyslexia and motility disorders would deserve investigations on larger patient cohorts. The evidence of relationships between impaired motor behavior and dyslexia is inconclusive, partly because few studies have been undertaken and partly because tests and procedures differed between studies.

Conclusions. If an association between motor behavior and dyslexia can be confirmed, tests of motor skills could be included as tools in screening batteries, thereby potentially improving diagnostic accuracy, optimizing the management of patients with this disease, and designing more effective physiotherapy programs. Such measures might also be used to identify prereaders at risk of developing dyslexia prior to the manifestation of any reading difficulties.

KEY WORDS

Developmental; dyslexia; reading; learning; motor; motility; motion; disorders.

INTRODUCTION

Developmental dyslexia is a learning disability characterized by an inability to achieve the expected literacy skills for a given age despite adequate intelligence and adequate educational opportunities (1). Individuals with dyslexia have difficulties with accurate or fluent word recognition and spelling despite adequate instruction and intelligence and intact sensory abilities (2). The ultimate goal of reading is comprehension, which is a function of both decoding ability and oral language comprehension (3). Dyslexia is defined by difficulties with decoding, whereas by comparison, listening comprehension is typically more intact. The prevalence of dyslexia varies from 2 to 17% of the school-aged population (4, 5), making dyslexia one of the most common developmental disorders, enormously impacting the educational system. A number of comprehensive theories have been suggested to explain the underlying causes of dyslexia, such as a phonological deficit caused by neurological abnormalities in the language areas of the brain (6, 7), a sensory deficit hypothesis based upon evidence of reduced sensitivity to visual and auditory stimuli (8, 9), and a hypothesis about the reduced speed of informa-

tion processing (10, 11). A cerebellar deficit hypothesis has been advocated by Nicolson *et al.* (12, 13). The latter suggests that children with dyslexia demonstrate deficits in some motor skills. A crucial role is played by central pattern generators (CPGs), *i.e.*, spinal neuronal networks that control the basic rhythms and patterns of motoneuron activation during locomotion and other rhythmic behaviors (14-16). The association between dyslexia and attention deficit hyperactivity disorder (ADHD) is frequent and widely described (17), with a reported incidence of 18-42% (18). Instead, weaker are the evidence on the correlation of dyslexia to developmental disorder of coordination (DCD) and dyspraxia (19). If an association between motor behavior and dyslexia can be confirmed, motor skills tests could be included as tools in the screening batteries, thus potentially improving diagnostic accuracy, optimizing the management of patients with this disease, and designing more effective physiotherapy programs. These measures could also have a predictive role, that is, they could be used to identify subjects at risk of developing dyslexia even before reading difficulty occurs.

MATERIALS AND METHODS

Search strategy

The literature search of the present narrative review was conducted according to this protocol:

- patients: developmental dyslexia;
- comparison: motor behavior disorders;
- outcomes: concomitance of motility disorders in dyslexic subjects.

Literature search

In January 2022 the following databases were accessed: Pubmed, Embase, Scopus, Web of Science, Google Scholar. The following keywords were used in combination: developmental, dyslexia, motion, disorder, motor, behavior, motility, problems, posture, balance, gait, poor, reading, learning. If title and abstract matched the topic, the full text was accessed. The bibliographies of the full-text articles were also screened for inclusion. Disagreements were solved by a third author (**). All the articles that investigate possible concomitance between the developmental dyslexia with motor behavior disorders were considered. According to the authors language capabilities, articles in English, French, German, Italian, and Spanish were considered.

RESULTS

Four articles were included in our database that meet the criteria (**table I**). All articles studied children with range age 8 < 16 years old for a total of 219 males and 73 females.

DISCUSSION

The relationship between developmental dyslexia and impaired motor behavior is a topic of increasing interest, justified by the potential benefits of a tailored treatment for those patients. Nevertheless, the available evidence is still meagre and conflicting, and the argument is strongly debated. Postural stability tests and walking tests are useful in assessing motor skills of dyslexic patients. Postural stability tests have already provided some evidence of a link between motor skills deficits and developmental dyslexia. Postural stability is known to be task dependent (20-22). Therefore, balance measurements during quiet standing may not be valid estimates of balance during perturbed standing. Furthermore, standing balance may not reflect balance control during walking. Common gait parameters, such as stride frequency, stride length, and walking speed, can be sensitively and reliably measured outside the laboratory environment. Therefore, they may only be suitable as part of a dyslexia screening tool to assess motor skills, after careful distinction between disabled and normal readership groups. Nicolson *et al.* (12) argued that standard motor skill batteries do not capture the range of deficits associated with cerebellar abnormalities, and that classic cerebellar tests depend to some extent on clinical judgements. To devise a more objective procedure, they devised a postural stability test in which the subjects' balance was perturbed by calibrated pushes to the small of the back. Using this method, however, postural sway ratings still depend on the clinical judgment of the experimenter, who is not always blind to the child's state. If manual perturbations (*i.e.*, thrust) are not modified relative to each person's body weight, then the validity of this test would also suffer. In the study by Moe-Nilssen *et al.* (23) it was investigated whether the previous results of a lack of balance control during the upright position could be confirmed with an objective posturography by researchers who were blind to the group membership of the subjects. The assessment of gross motor skill assessments was also expanded to include balance control during walking using established gait cycle parameters. These measures can add discriminatory power to any motor skill component of a dyslexia screening test. Their primary aim was to investigate whether continuous-scale gait and body swing parameters could provide new information on balance control during standing and walking in developmental dyslexia. Timed testing and trunk accelerometry measurements allow field data to be obtained at different locations to ensure ecological validity. It was investigated whether these parameters could

Table I. Comparison of dyslexia studies.

Author, year	Journal	Groups	Sex	Participants (n)	Purpose	Results
Brookes *et al.* 2010 (1)	*Dyslexia*	Dyslexic adults 18-26y	11 males, 9 females	20	clarify between-study heterogeneity, employing quantitative, continuous measures of balance and blindfolded balance, and using both adult and child participants without comorbid ADHD	there is a significant incidence of balance difficulties in children and adults with dyslexia, even for those without comorbid attention deficit
		Matched adult controls 20-24y	14 males, 16 females	30		
		Dyslexic children 11-14y	11 males, 5 females	16		
		Matched child controls 10-13y	11 males, 14 females	25		
Fawcett *et al.* 1999 (29)	*Journal of Motor Behaviour*	Children with dyslexia 8-16y	59 males	59	establish whether cerebellar difficulties found in laboratory research with dyslexic children are representative of the dyslexic population at large	the dyslexic children showed highly significant impairments on the cerebellar tests, with deficits on postural stability and muscle tone comparable in magnitude with their reading and spelling deficits. Furthermore, over 95% of the dyslexic children showed clear evidence of deficit on muscle tone or stability
		Controls children 8-16y	67 males	67		
Moe-Nilssen *et al.* 2003 (23)	*Exp Brain Res*	Children with dyslexia 10-12y	15 males, 7 females	22	investigate if continuous-scaled measures of standing balance and gait could discriminate between groups of impaired and normal readers when investigators were blind to group membership during testing	confirmed the previous finding of an association between deficient balance control during standing and developmental dyslexia
		Controls children 10-12y	9 males, 9 females	18		
Stoodley *et al.* 2005 (27)	*Exp Brain Res*	Children with dyslexia 8-14y	11 males, 5 females	16	investigate low-level sensory and motor deficits which have been found in dyslexic populations	dyslexic children were less stable than the control children in both eyes-open conditions (left foot, right foot). While there were no group differences during the eyes-closed conditions, the dyslexic children dropped a foot to correct balance significantly more often than control children.
		Controls children 8-11y	11 males, 8 females	19		

differentiate between two groups of children aged between 10 and 13 years with and without developmental dyslexia. They found that when subjects walked on uneven surfaces, very fast walking speeds, as well as cadence and step length at common normalized walking speeds, correctly classified 77.5% or more of the subjects into their respective group, and the effect was controlled by gender. The children with dyslexia in their study walked with shorter steps and higher cadence than the controls when compared at normalized speed and with body height controlled. When cadence was compared at the preferred speed, however, no significant differences between groups were found. This observation stresses the importance of controlling for differences in walking speed when comparing speed-dependent parameters between subjects. It is well documented that a subject walking at preferred speed will demonstrate a natural cadence, which can be explained by pendulum characteristics (24-26). Discriminatory power was generally lower for tests of walking on an even surface, and for tests of quiet standing with the eyes open. Tests of quiet and perturbed standing with the eyes closed did not discriminate significantly between groups. The same finding was supported by Stoodley *et al.* (27), but is in opposition to the results of Fawcett, Nicolson and Dean (28) and Nicolson and Fawcett (29). In the study of Stoodley *et al.* normal readers (n = 19) and children with developmental dyslexia (n = 16) were asked to perform various cognitive, literacy, and balancing tasks. Children balanced on the left or right foot, with eyes open or closed, for a period of 10 seconds during which their movements were recorded with a motion-tracking system. Dyslexic children were less stable than the control children in both eyes-open conditions (left foot $p = 0.02$, right foot $p = 0.012$). While there were no group differences during the eyes-closed conditions, the dyslexic children dropped a foot to correct balance significantly more often than control children ($p < 0.05$). Samson *et al.* (30) found that step length but not cadence decreased with age in adults (aged 19–90 years) walking at preferred speed. Shortening of step length may therefore be a compensatory strategy in balance control, and may explain why the children with dyslexia in Moe-Nilssen *et al.* study (23) demonstrated similar cadence at preferred speed, but not at normalized speed, compared to controls. Use of the same compensatory strategy would also explain why the group with reading impairments, demonstrated similar cadence as controls, but with shorter step length and consequently lower maximal speed, when instructed to walk very fast. It is interesting that controlling for gender consistently increased the statistical significance of the group differences found on the walking tests. The differences in motor skills between boys and girls found in Moe-Nilssen *et al.* study (23) are in agreement with previous findings for normal children and adolescents (31-33). Their data also indicate that the group of children with dyslexia were consistently impaired relative to controls on tests of undisturbed standing balance when they were instructed to look at a target 0.6 m away, but not on the same test when performed with eyes closed. Thus, as a group, the poor readers in their study seemed not to take adequate advantage of the visual cue. This may have resulted from some impaired readers' inability to maintain steady fixation, a visual impairment that would also be disadvantageous during reading. Fixation instability and jerking eye movements have been claimed to be associated with developmental dyslexia, but opinions differ as to whether eye movement differences are a cause or consequence of reading difficulties (34-38). They also found that the children with dyslexia were of smaller physical stature than the controls and that this could not be explained simply by a difference in gender ratios between the groups. This height difference is interesting and warrants further study. Their study confirmed the previous finding of an association between deficient balance control during standing and developmental dyslexia (29). They have further shown that gait parameters, when appropriately adjusted for differences in body size and walking speed and controlled for well-known gender differences in motor performance (31-33), may effectively discriminate between children with dyslexia and age-equivalent controls with up to 85% accuracy. We cannot rule out the possibility that factors other than reading skills, such as intelligence, have modulated the effects of the group. An overall effect of IQ differences between groups is expected to impact the performance of all psychophysical and psychometric tests to some extent. However, there is no *a priori* reason to expect intelligence to affect certain tests more than others. The finding that the dyslexic group differed from controls on specific posturography measures is therefore consistent with a more specific deficit rather than with a generalized decrease in performance resulting from differences in IQ. Previous studies have emphasized the importance of cerebellar function in discriminating between groups of impaired and normal readers and have provided a pathogenetic hypothesis that explains the connection between sensory and motor functions in developmental dyslexia (12). The literature to-date indicates that studies have shown differences (between and within experiments) according to age, balance position, method of measurement, dual/single task paradigms, blindfolded/unblindfolded conditions, type of standing surface and length of test. For example, found that balance deficits were not observed when participants were blindfolded. The reasons for these differences could be due to the specific measurement techniques employed across the studies, the measurement scales, and stance. The study by Brookes *et al.* (1) attempted to clar-

ify these issues, employing quantitative, continuous measures of balance and blindfolded balance, and using both adult and child participants. Eighty-seven individuals participated: dyslexic adults (n = 17), matched adult controls (n = 30), dyslexic children (n = 16) and matched child controls (n = 24). The study found significant balance deficits for the child dyslexic group in the eyes-open task and a result approaching significance in the blindfolded task. By contrast, the adult dyslexic group showed significant deficits in the blindfolded task only. This highlights the need for the use of age-appropriate tests, and may explain some of the heterogeneity in the literature. It is concluded that there is a significant incidence of balance difficulties in children and adults with dyslexia. Given the range of techniques and participants that were employed, it is not surprising that balance and dyslexia studies yielded different results. However, there is sufficient evidence of balance deficits in dyslexia to make this report worthy of further investigation. Clinical gait tests are easy to perform, and the evaluated parameters can be obtained without the use of expensive equipment. Currently there are no scientifically approved clinical tests available that can correlate these aspects, so the development of a screening test that can evaluate motor disorders to make early diagnosis of developmental dyslexia would be of absolute importance. These elements can therefore prove to be valuable in addition to the development of screening tests for developmental dyslexia, although further studies are needed to better define the role of the visuomotor system in controlling balance and its contribution to reading difficulties.

CONCLUSIONS

The association between developmental dyslexia and motility disorders would deserve investigations on larger patient cohorts. The evidence of relationships between impaired motor behavior and dyslexia is inconclusive, partly because few studies have been undertaken and partly because tests and procedures differed between studies. Furthermore, tests with a greater discriminatory component between the various components of motor skills should be carried out. Meanwhile, these findings may provide new insights into the motor disorders associated with developmental dyslexia, useful for optimizing the management of patients with this disease and for designing more effective physiotherapy programs. The identification of these disorders will allow to establish the subjects who can benefit from specific rehabilitation protocols. Current scientific evidence suggests the integration of adequate exercises in a multidisciplinary context to treat the young patients involved, or even prevent those most at risk. Ultimately, a tailored treatment for the individual is potentially useful for children with developmental dyslexia.

FUNDINGS

None.

DATA AVAILABILITY

Data are available under reasonable request to the corresponding author.

CONTRIBUTIONS

GV, ET: literature revision and analysis, manuscript writing. MC, JP: articles for study eligibility assessment and literature analysis. NM, FO: study supervision and manuscript revision.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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Therapeutic Effect of Aquatic Resistance Exercise on Rectus Femoris and Knee in a Model of Rheumatoid Arthritis

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SUMMARY

Background. Rheumatoid Arthritis (RA) is characterized by progressive joint destruction due to an inflammatory, degenerative condition of the joint cartilage and adjacent muscles, impairing the patient's functional status. Resistance exercise is considered a good tool in the management of RA. The objective of this study was to evaluate the effect of resistance exercise, in an aquatic environment, on strength, motor control, and morphometric parameters of the rectus femoris muscle and knee joint of rats submitted to a model of RA.

Methods. Forty male rats divided into four groups were used: CON (Control); RA (Injury Group); EX (Exercise Group) and EXRA (Injury Group treated with resistance exercise in aquatic environment). RA was induced by immunization at the base of the tail and intra-articular injection of Freund's Complete Adjuvant (CFA) in the tibio-femoral joint of the right knee. The exercise protocol was performed three times a week in a water tank with an overload of 50% of the animal's weight for 22 days, progressing the number of sets and repetitions every three treatments. Both pelvic limbs were evaluated for grip, motor control in the inclined plane, joint and muscle morphology, and their respective morphological measurements. Generalized Linear Models with LSD post-test was used for statistical analysis.

Results. There was a reduction in strength of both pelvic limbs of the injured animals and the AR and EXRA groups also had morphological changes in the joint and in the rectus femoris muscle. The resistance exercise protocol in an aquatic environment helped to maintain strength and the quality of histological tissues.

Conclusions. Resistance exercise in aquatic environment promoted functional and muscle tissue improvement in the knee joint of Wistar rats with RA with experimentally induced RA.

KEY WORDS

Autoimmune diseases; rheumatoid arthritis; musculoskeletal; physical exercise; hydrotherapy.

INTRODUCTION

Rheumatoid Arthritis (RA) is a disease characterized by inflammatory and progressive autoimmune activation that manifests itself in connective tissues, markedly in synovial joint (1). The disorders evolve symmetrically, beginning commonly in the extremities with progression to proximal joint region (2). The manifestations occur with the presence of edema, heat, paresthesia, and pain for indeterminate period (3). Chronic inflammation promotes accumulation of cytokines and immune system cells in the synovial membrane, leading to destruction of the articular cartilage

as well as adjacent structures such as the synovial membrane and muscle component (4). RA is estimated to affect 1% of the world's population and about 3 million individuals in the United States alone (5). It also represents a reason for death in 0.03% of the cases in São Paulo, Brazil, and 0.17% in Sweden (6).

In addition to structural involvement, the RA patient also develops functional limitations resulting from symptom exacerbation (7). Thus, the presence of rheumatoid cachexia due to reduced lean body mass and increased muscle catabolism induced by inflammatory cytokines, commonly affects the muscular compartments of the quadriceps femoris, promoting changes in the functional capacity of individual (8).

In general, resistance training provides physical benefits, such as muscle hypertrophy, power, and speed gain, developed through adaptive responses resulting from the training program (9). The elaboration of the treatment consists in the choice of the number of sets, repetitions, rest period and overload (10, 11). The parameters must be determined according to the proposed objectives as well as the individual's functional capacity (12). Muscle strengthening is essential for the treatment of degenerative joints in order to improve neural activation, maintenance of cartilage tissue, muscle strength, joint mobility, and optimize shock absorption (13, 14).

The aquatic environment generates low impact and represents a controlled environment for both therapist and patient. The reduction of gravitational force through buoyancy facilitates postural control (15). In addition, the hydrostatic pressure associated with the viscosity of the water promotes positive sensory and proprioceptive feedback during the execution of movements (16, 17). Exercise in orthostatic position in water is indicated for various populations, including individuals who present joint lesions (18, 19). Although swimming is the most conventional physical practice, it requires specific skills as well as high intensity to perform. Thus, exercises in vertical position in water are suggested for patients to optimize the rehabilitation process (19, 20).

Since resisted physical exercise in water presents benefits to the healthy population in physical training programs due to its benefits to musculoskeletal health, the investigation about the application in chronic rheumatic comorbidities, such as RA, becomes pertinent, even though the literature is controversial regarding the indication of resisted physical exercise for this population (21). Therefore, the present study aims to investigate the effects of resisting aquatic jump exercise on the parameters of grip strength, motor control, and morphometric analysis of the rectus femoris muscle and knee joint of Wistar rats with experimentally induced RA.

MATERIALS AND METHODS

This study is characterized as experimental, conducted in the Universidade Estadual do Oeste do Paraná (Unioeste). All experimental procedures only began after approval by the Ethics Committee on Animal Use (CEUA) of Unioeste under protocol n°. 19-19, on July 25, 2019.

Animals

The sample consisted of 40 male Wistar rats, three months old and weighing 347.31 ± 25.1, obtained from the Central Animal Facility of Universidade Estadual do Oeste do Paraná, during the experimentation, the animals were kept in the animal cages, obeying a 12-hour light-dark period, temperature of 21 ± 1 °C, and were treated with water and feed *ad libitum*. After one week of adaptation, the animals were randomly distributed into 4 groups, with 10 rats each:

- CON (Control Group): received no intervention;
- RA (Injury Group): induction of RA in the knee joint of the right pelvic limb (RPL);
- EX (Exercise Group): aquatic jumping protocol on alternate days;
- EXRA (Injury + Exercise Group): induction of RA in the knee joint of the RPL and subjected to a water jumping protocol.

RA induction injury protocol

For RA and EXRA, the method described by Gomes *et al*. (22, 23), which consists of Freund's Complete Adjuvant (CFA) with *Mycobacterium butyricum* (0.5 mg/ml, Difco®); isotonic sodium chloride solution (0.9%, Aster®) and iodized alcohol (1%, Rialcool®).

The animals were previously submitted to immunization by intradermal injection at the base of the tail, after trichotomy and asepsis of the injection site with iodized alcohol (1%). Subsequently, a 1 ml syringe and a 13 × 4.5 mm needle were used and inserted approximately 1 centimeter into the subcutaneous region at the base of the tail. The RA and EXRA groups received pre-sensitization with 50 µl (microliters) of CFA (0.5 mg/ml, *Mycobacterium butirycum*). In the RA and EX groups, a saline solution (sodium chloride 0.9%) was injected in the same volume.

Seven days after sensitization, the animals were manually restrained so that the knee region of the RPL was trichotomized and, subsequently, asepsis was performed with iodized alcohol (1%). With the help of a 1 ml syringe and a 13 × 4.5 mm needle, the groups that received the RA induction were submitted to a new application in the tibio-femoral joint of 50 µl (0.5 mg/ml) of CFA *Mycobacterium butirycum*, as well as the other groups, received the application with saline solution (0.9% sodium chloride).

Treatment protocol with aquatic jumping

A 220-liter capacity tank was used, with water at a temperature of 33 °C, where a cylindrical-shaped tube 55 cm high, 30 cm in diameter, and a water level of 45 cm was placed. The EX and EXRA animals received an overload of 50% of body weight, with lead weights positioned in the abdominal region by means of a Velcro strap. Each animal was individually placed in the tube and with the overload, they were submerged to the bottom of the tank, with each impulse to reach the surface counted as a jump (24) (**figure 1**).

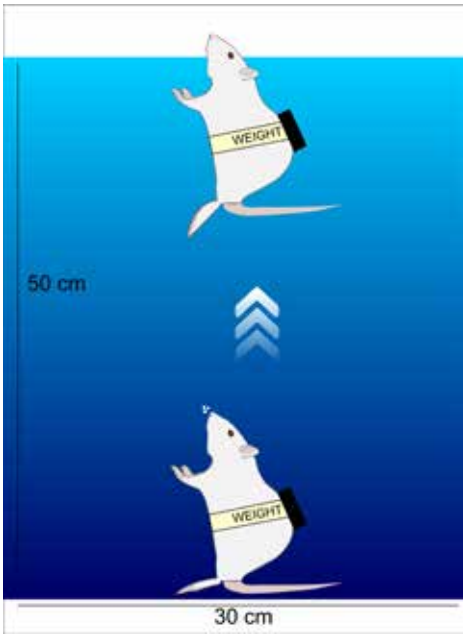

Figure 1. Representation of the jump exercise in aquatic environment, with body overload.

The animals were previously trained, before the induction of RA, with the weight attached to their bodies. The treatment was carried out on alternate days, starting 24 hours after the intra-articular injection, and the last treatment was performed on the 28th day. In the first week, the jumps were made in two series of 10 repetitions; from the second week on, there was a progression in the number of series, being made three series of ten jumps; in the third week, the progression in the number of series followed, being made four series of ten repetitions, with a one-minute interval between series, as can be seen in **figure 1** (24). Prior to the beginning of the experiment, all animals were trained and adapted to the equipment that was used in the evaluations. Seven evaluations were performed during the 30 days of the experiment (**figure 2**).

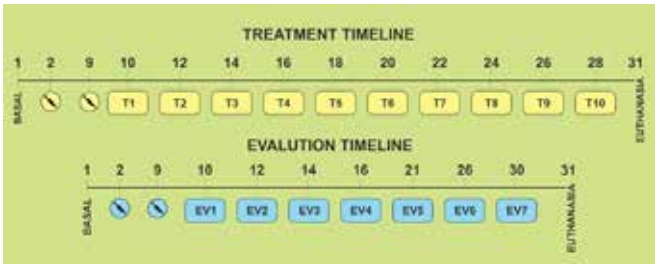

Figure 2. Timeline of treatments and assessments.

Day 1 (Basal), day 2 (Sensitization at the base of the CFA tail), day 9 (Intra-articular CFA Injection), T1-T12 (Treatment Day with jumping in an aquatic environment), EV1-EV7 (Functional Evaluation Day) and day 31 (Euthanasia).

Functional assessments

Muscle strength assessment – Grip strength

To assess the muscle strength of both pelvic limbs, a grip strength device (Insight®) was used, in which the animal was immobilized by the evaluator, leaving only the limb to be evaluated free. The animal was positioned so that its plantar region could grip the transducer grid and then the evaluator pulled the animal with increasing force until it was released. Three measurements were made for each pelvic limb and the mean value was considered (25).

Motor function evaluation

In the evaluation of motor function, the animal's ability to maintain itself at different angles according to its joint proprioception and motor activation was tested. We used an inclined plane equipment (Insight®) consisting of an acrylic ramp with a manual mechanism for angular variation (0°-90°) and non-slip surface. The animal was positioned in two directions at the top of the ramp: the first with the cephalic region up and the second with the cephalic region to the side (vertical position), and the RPL under evaluation was positioned at the lowest point of the ramp. The evaluation started at 45°, and if the animal remained there for 5 seconds, the ramp was raised another 5° until it showed any reaction of instability or imbalance. Three measurements of the maximum angle were made, and the average of the values was considered (26).

Analysis of the rectus femoris muscle

On the twenty-second day, the animals were anesthetized with intraperitoneal injection of ketamine hydrochloride (95 mg/Kg) and xylazine (12 mg/Kg) and subjected to euthanasia by anesthetic overdose, the rectus femoris muscle of both pelvic limbs was collected and fixed in methacarn (70% methanol, 20% chloroform, 10% glacial

acetic acid) for 24 hours and stored in 70% alcohol. The muscle tissues were then subjected to an alcoholic increasing series dehydration process, diaphanization in xylol, and paraffin embedding. Cross sections of 5 µm thickness were obtained with a microtome (Olympus CUT 4055) and stained with Hematoxylin and Eosin (HE) for general analysis of the muscle tissue.

Measurements of cross-sectional area and the smallest diameter of muscle fiber were performed, as well as evaluation of muscle fiber morphology. The slides were analyzed under a light microscope (Olympus BX60) and for the measurement of the cross-sectional area and smallest diameter of the muscle fiber, 10 images were obtained using a 40x objective. We considered 10 fibers from each image to perform the measurements using the Image-Pro-Plus 6.0 program (Media Cybernetics), totaling 100 measurements per animal (27).

Morphological analysis of the knee joint

After euthanasia, the right and left knee joints were collected and dissected, and then fixed in Metacarn (70% methanol, 20% chloroform, 10% glacial acetic acid) for 48 hours, and after that period the material was fixed in 70% alcohol for 15 days. After washing for 24 hours in running water, the material started the decalcification process in 5% trichloroacetic acid where it remained for 7 days. Afterwards, the material was washed again for 24 hours, following the routine histological processing for paraffin embedding. To make the slides, the joints were cleaved using a microtome (Olympus CUT 4055) at a thickness of 7 µm. In the staining protocol, Hematoxylin and Eosin was used to identify the structures in the morphological analysis. The slides were analyzed using a light microscope and photomicrographed in a photomicroscope (Olympus DP71).

The measurement of cartilage thickness and the counting of the number of chondrocytes were performed in three distinct areas of the femur and tibia: P1, anterior articular extremity; P2, middle region of the joint; and P3, posterior articular extremity. These areas were photomicrographed at 200x magnification and later analyzed using Image Pro Plus 6.0 software. The thickness was measured from the end of the cartilage to the osteochondral junction. For the chondrocyte count, a rectangle 100 µm deep by 200 µm long was used, superimposed on the three photomicrographed points (P1, P2, and P3), disregarding the upper margin and the deep margin closest to the subchondral bone region. The average of the three regions was considered for both cartilage thickness and the number of chondrocytes in the femur and tibia (28).

Statistical analysis

The SPSS 20.0 program was used for statistical analysis. The data were presented as mean and standard deviation by means of illustrated figures in the GraphPad Prism 8.0.2 program. The inferential analysis was performed with Generalized Mixed Linear Models for the functional data, for comparison of total leukocytes, Generalized Linear Models was used, in all cases, the post-test was LSD, with an accepted significance level of 5%.

RESULTS

In the assessment of muscle strength for RPL, there was a statistical difference between the groups (WaldX2(3;254) = 61.931, $p < 0.001$) and in the interaction between the groups and assessments (WaldX2(21;254) = 2.252, $p < 0.05$). A statistical difference was also observed for the left pelvic limb (LPL) between the groups (WaldX2(3;253) = 8.951, $p < 0.001$).

With the induction of injury, the RA and EXRA groups showed a reduction in the grip strength of the RLP ($p < 0.05$), however the group treated with exercise obtained a restoration of strength from the AV5, and in the AV7 it was similar to the group EX. In the intragroup analysis, RA and EXRA showed a difference ($p < 0.05$) in the reduction of strength from AV2, but an increase in values was observed in AV7, where the means returned to baseline values (**table I**).

In LPL, there was a statistical difference for RA and EXRA with reduced grip strength in relation to CON and EX ($p < 0.05$) (**table II**).

In the evaluation of motor function on the inclined plane, there was a statistical difference between groups in the vertical position (WaldX2(3;256) = 6.560, $p < 0.001$) and interaction between groups and evaluations (WaldX2(21;256) = 1.654, $p < 0.005$). In the horizontal layout, there was a statistical difference only for the groups (WaldX2(3;256) = 11.017, $p < 0.001$).

In the vertical plane, there was no difference between the groups in the Baseline evaluation. There was a statistical difference ($p < 0.05$) in EV1 compared to the Baseline evaluation for the RA and EXRA groups for CON and EX, with a reduction in the inclination angles. Throughout the evaluations, both injured groups showed recovery of the averages, similar to the CON group. The same was observed in the intragroup evaluation, where there was a statistical difference ($p < 0.05$) from baseline to EV1 in the RA and EXRA groups, with a reduction in the mean. Throughout the evaluations, values similar to baseline were obtained in the groups with RA induction (**table III**).

In the horizontal arrangement, there was a statistical difference ($p < 0.05$) in the CON group, which obtained a higher mean compared to the other groups (**table IV**).

Table I. Grip analysis of right pelvic limb (RPL).

MUSCLE STRENGTH PREHENSION - RPL

	BASAL	EV1	EV2	EV3	EV4	EV5	EV6	EV7
CON	68.525 ± 4.72^{Aa}	95.92 ± 18.02^{Ab}	104.60 ± 22.99^{Ab}	97 ± 16.86^{Ab}	96.11 ± 12.87^{Ab}	95.1 ± 17.99^{Ab}	99.76 ± 16.44^{Ab}	95.72 ± 15.69^{Ab}
RA	74.53 ± 12.80^{Aa}	68.93 ± 19.86^{Bac}	40.06 ± 23.57^{Bbd}	57.28 ± 28.45^{Bade}	46.55 ± 20.45^{Bbef}	55.96 ± 33.06^{Bbceg}	61.56 ± 28.72^{Bafg}	61.12 ± 30.36^{Bafg}
EX	80.03 ± 5.67^{Aa}	94.63 ± 21.50^{Aa}	95.95 ± 17.81^{Aa}	89.83 ± 9.70^{Aa}	92.14 ± 17.89^{Aa}	87.58 ± 11.42^{Aa}	94.88 ± 12.32^{Aa}	84.25 ± 13.69^{ACa}
EXRA	76.37 ± 5.28^{Aa}	65.76 ± 20.56^{Bac}	54.22 ± 30.58^{Bbcd}	55.96 ± 23.17^{Bbce}	61.58 ± 16.77^{Badef}	52.34 ± 23.34^{Bbcfg}	58.69 ± 18.16^{Bbcfh}	68.15 ± 19.26^{BCadegh}

The values were obtained in grams and expressed as mean and standard deviation. CON: Control Group; RA: Lesion Group; EXE: Exercise Control Group; EXRA: Exercise Lesion Group; EV1: Assessment 1; EV2: Assessment 2; EV3: Assessment 3; EV4: Assessment 4; EV5: Assessment 5; EV6: Assessment 6; EV7: Assessment 7. Capital letters denote statistical differences between groups and lower-case letters between assessments.

Table II. Grip analysis of left pelvic limb (LPL).

MUSCLE STRENGTH PREHENSION - LPL

	BASAL	EV1	EV2	EV3	EV4	EV5	EV6	EV7
CON^{A}	86.04 ± 13.39	87.60 ± 12.88	96.41 ± 12.63	89.48 ± 6.67	90.85 ± 9.84	84.60 ± 15.83	87.83 ± 9.38	83.45 ± 9.38
RA^{B}	92.67 ± 14.93^{Aa}	85.75 ± 16.24	74.82 ± 9.22	79.41 ± 7.46	81.04 ± 8.93	74.73 ± 9.85	80.29 ± 11.41	79.08 ± 11.50
EX^{A}	73.92 ± 11.11^{Aa}	87.14 ± 11.24	81.78 ± 8.98	79.72 ± 7.42	85.87 ± 8.64	86.03 ± 10.18	88.97 ± 6.74	82.81 ± 15.84
EXRA^{B}	76.50 ± 3.86^{Aa}	75.06 ± 12.01	70.03 ± 16.83	74.03 ± 18.37	83.62 ± 14.08	78.26 ± 11.27	79.28 ± 18.73	73.76 ± 14.01

The values were obtained in grams and expressed as mean and standard deviation. CON: Control Group; RA: Lesion Group; EXE: Exercise Control Group; EXRA: Exercise Lesion Group; EV1: Assessment 1; EV2: Assessment 2; EV3: Assessment 3; EV4: Assessment 4; EV5: Assessment 5; EV6: Assessment 6; EV7: Assessment 7. Capital letters denote statistical differences between groups and lower-case letters between assessments.

Table III. Motor funcion analysis of right pelvic limb (RPL).

MOTOR FUNCTION - VERTICAL POSITION

	BASAL	EV1	EV2	EV3	EV4	EV5	EV6	EV7
CON^{A}	67.46 ± 2.16^{Aa}	63.51 ± 1.08	64.56 ± 2.65	64.15 ± 4.28	62.67 ± 3.21	64.16 ± 1.78	62.46 ± 3.57	62.07 ± 4.93
RA^{B}	66.07 ± 5.07^{Aa}	59.77 ± 3.66	60.88 ± 6.68	60.34 ± 4.49	60.53 ± 4.57	62.18 ± 3.12	60.51 ± 3.53	58.86 ± 3.02
EX^{B}	63.85 ± 1.67^{Aa}	64.41 ± 2.67	63.64 ± 2.74	63.11 ± 3.27	61.82 ± 4.42	63.12 ± 3.04	61.84 ± 3.18	61.45 ± 4.87
EXRA^{A}	63.14 ± 1.66^{Aa}	62.3 ± 3.16	59.96 ± 3.50	58.97 ± 3.17	61.13 ± 4.16	59.64 ± 3.58	60.48 ± 3.86	61.47 ± 4.04

The values were obtained in grams and expressed as mean and standard deviation. CON: Control Group; RA: Lesion Group; EXE: Exercise Control Group; EXRA: Exercise Lesion Group; EV1: Assessment 1; EV2: Assessment 2; EV3: Assessment 3; EV4: Assessment 4; EV5: Assessment 5; EV6: Assessment 6; EV7: Assessment 7. Capital letters denote statistical differences between groups and lower-case letters between assessments.

Table IV. Motor funcion analysis of left pelvic limb (LPL).

Motor Function - Horizontal Position								
	BASAL	**EV1**	**EV2**	**EV3**	**EV4**	**EV5**	**EV6**	**EV7**
CON	71.95 ± 2.04^{Aa}	70.32 ± 1.83^{ABa}	70.25 ± 1.75^{Aa}	70.96 ± 2.06^{Aa}	70.93 ± 1.96^{Aa}	71.92 ± 1.42^{Aa}	71.76 ± 3.42^{ABa}	72.22 ± 1.28^{Aa}
RA	72.75 ± 2.05^{Aa}	68.87 ± 3.10^{Ab}	71.46 ± 3.17^{Aa}	70.53 ± 3.83^{Aa}	70.16 ± 3.86^{Aa}	71.83 ± 3.48^{Aa}	70.54 ± 2.76^{Aa}	70.32 ± 3.36^{Aa}
EX	71.45 ± 2.28^{Aa}	72.01 ± 2.61^{ABa}	70.68 ± 3.61^{Ba}	70.9 ± 2.22^{Aa}	69.24 ± 3.24^{Aa}	71.44 ± 3.17^{Aa}	70.92 ± 1.88^{ABa}	69.77 ± 2.95^{Ba}
EXRA	71.64 ± 2.23^{Aa}	69.81 ± 2.15^{ABa}	68.15 ± 4.21^{Bb}	70.15 ± 3.09^{Aa}	71.64 ± 2.94^{Aa}	70.47 ± 3.43^{Ba}	73.31 ± 2.09^{Bc}	72.48 ± 3.07^{Aac}

The values were obtained in grams and expressed as mean and standard deviation. CON: Control Group; RA: Lesion Group; EXE: Exercise Control Group; EXRA: Exercise Lesion Group; EV1: Assessment 1; EV2: Assessment 2; EV3: Assessment 3; EV4: Assessment 4; EV5: Assessment 5; EV6: Assessment 6; EV7: Assessment 7. Capital letters denote statistical differences between groups and lower-case letters between assessments.

In the analysis of the morphology of the rectus femoris muscle, the CON showed normal tissue appearance, with polygonal, multinucleated fibers, with myonuclear in subsarcolemmal position, loose joint tissue with a characteristic arrangement involving the fibers and fascicles composing the endomysium and perimysium (**figure 3 A**). On the other hand, the RA the RPL muscle demonstrated discrete fascicular disorganization, some amorphous fibers with reduced area, congested blood vessels with the presence of mononuclear and polymorphonuclear cells both in the intravascular region and in the interstitium between the muscle fibers (**figure 3 B**). The rectus femoris of the LPL also exhibited inflammatory cells in its morphology, but with better tissue appearance than the lesion side.

The EX showed muscle fibers with morphology like the CON without changes (**figure 3 C, G**). The EXRA, on the other hand, showed a morphological aspect with good organization of the muscle fibers, presence of connective tissue for the formation of the endomysium and perimysium, medium blood vessels with reduced vascular congestion and the decrease of inflammatory infiltrate in isolated muscle areas. Compared to RA, better cellular organization, and morphological appearance of the rectus femoris muscle of the exercise-treated group can be observed (**figure 3 D**). Organizational parameters like CON were found in the LPL of the EXRA (**figure 3 H**).

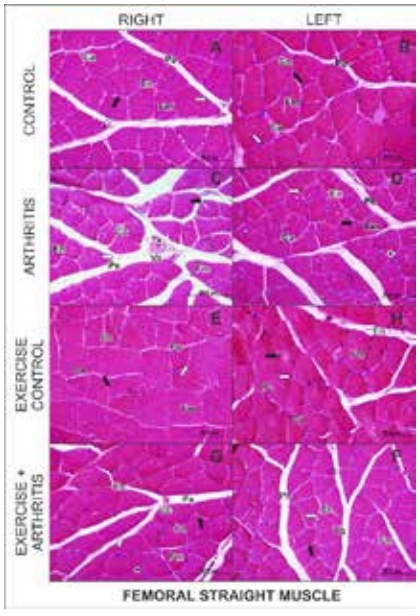

Figure 3. Photomicrographs of the right and left rectus femoris muscle of Wistar rats, cross section, hematoxylin and eosin stained.

For all groups of RPL **(A, B, C, E)** and LPL **(D, E, F, G)**, polygonal muscle fibers (Fm), blood capillaries (Ca), Perimysium (Pe), Endomysium (En), peripheral nuclei (hollow arrow), satellite cells (black arrow) and the presence of fibroblasts are visualized. Also, for RA **(B, F)** and EXRA **(D, H)**, there is presence of inflammatory cells (asterisk), congested Blood Vessels (Vs).

For the cross-sectional area of the rectus femoris muscle, there was a statistical difference between the right and left sides (WaldX2(1;28) = 15.860, $p < 0.001$) and interaction between groups and sides (WaldX2(3;28) = 5.381, $p < 0.05$).

The RPM of the RA group had a statistically significant reduction in the mean ($p < 0.001$) compared to the other groups. In the LPL, the EXRA showed decreased area ($p < 0.05$) compared to the COM and RA groups, but similar to the EX (**figure 4 A**). In the interaction between groups and right and left sides, only RA showed a statistical difference ($p < 0.001$), as can be seen in **figure 4 B**.

Regarding the smallest muscle fiber diameter, the statistics showed a significant difference between the right and left sides (WaldX2(1;32) = 14.645, $p < 0.001$) and in the interaction between groups and sides (WaldX2(3;32) = 5.474, $p < 0.001$).

The RA of the RPL obtained a reduction in the mean ($p < .05$) compared to the other groups on their respective sides. As for the LPL, the EXRA was similar to the EX; however, it was statistically different from the other groups ($p < .05$) as shown in **figure 7 A**. In the data corresponding to the interaction between groups and sides, the animals from the CON ($p < .05$) and RA ($p < .001$) showed statistical difference (**figure 4 B**).

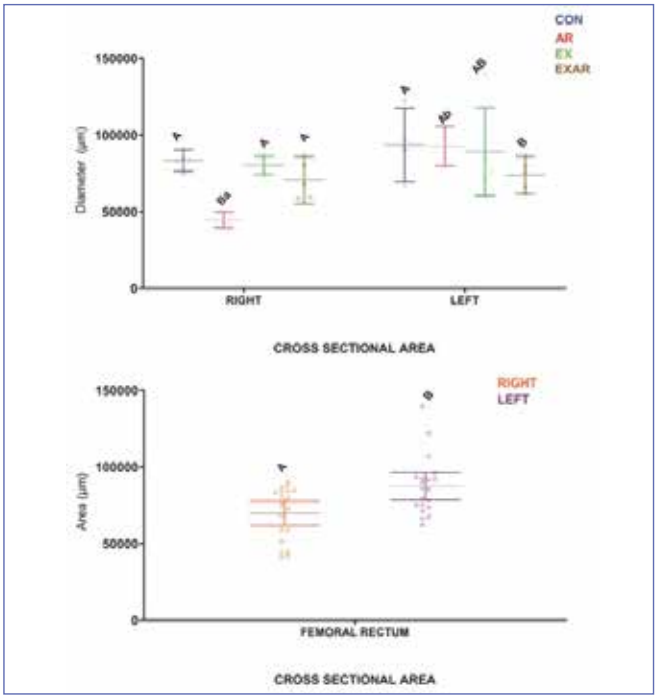

Figure 4. Analysis of the cross-sectional area of the rectus femoris muscle.

Data expressed as mean and standard deviation. CON: Control Group; RA: Lesion Group; EXE: Exercise Control Group; EXRA: Exercise Lesion Group. **(A)** Capital letters denote statistical differences between groups and lower-case letters the interactions. **(B)** Capital letters denote statistical differences between sides.

In the morphological analysis of the knee joints, the tibial femur cartilage of the CON and EX groups presented normal aspects with a smooth and organized surface composed of four cellular layers. In the superficial zone, there was a greater density of flattened chondrocytes arranged in horizontal clusters. In the intermediate zone the cells assume a rounded morphology, isolated or in isogenic groups. In the deep zone, there is the presence of chondrocytes organized in lacunae, divided from the calcified zone by the tidemark and the presence of vascularization (**figure 5 A**, **E, C G**; **figure 6 A**, **E, C, G**).

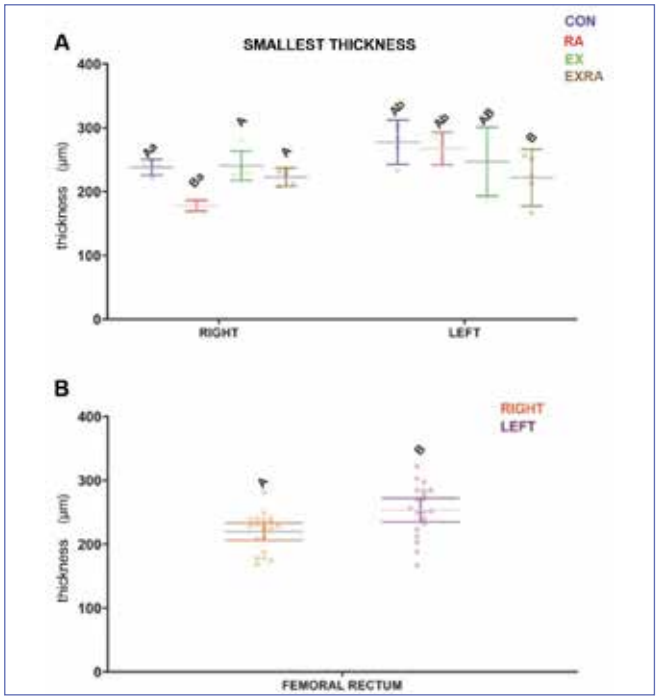

Figure 5. Analysis of the smallest diameter per fiber of the rectus femoris muscle. Data expressed as mean and standard deviation.

CON: Control Group; RA: Lesion Group; EXE: Exercise Control Group; EXRA: Exercise Lesion Group. **(A)** Capital letters denote statistical differences between groups and lower-case letters the interactions. **(B)** Capital letters denote statistical differences between sides.

In the RA group, both for the femur and the tibia, deleterious aspects were observed in the articular cartilage with some areas of less thickness and presence of flocculations on the surface, discontinuity of the tidemark, irregularly arranged chondrocytes, and subchondral bone invagination (**figure 5 B**, **F**; **figure 6 B**, **F**). Regarding the EXRA group, morphological changes were also observed in the femur and tibia of the injured and exercise-treated animals, showing areas of discontinuity of the tidemark and invaginations of the subchondral bone, however, with a better organization of the chondrocytes and an apparent recovery of the cartilage thickness similar to the CON group, demonstrating a recovery of the morphology of the right and left knees of the animals with rheumatoid arthritis (**figure 5 D**, **H**; **figure 6 D**, **H**).

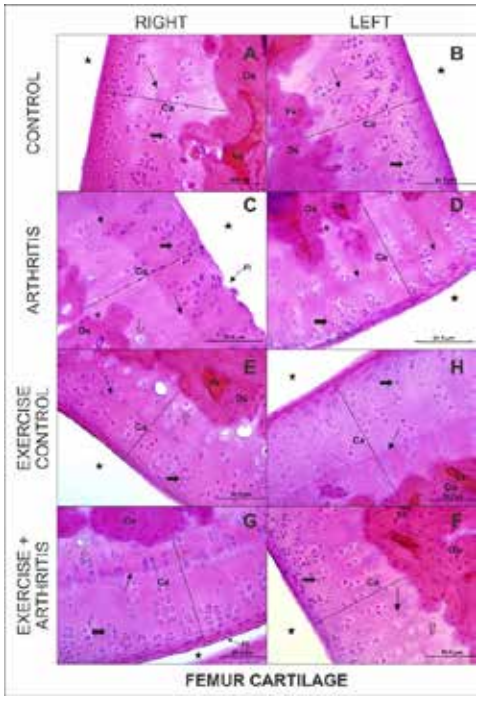

Figure 6. Morphological analysis of the femur bone in the periarticular region of the right and left pelvic limbs. Photomicrographs of the right and left femurs of Wistar rats in longitudinal section, hematoxylin and eosin stained.

The Control Group **(A, E)** and the Exercise Control **(C, G)** showed normal aspects of the articular cartilage **(Ca)**, presence of the continuous tidemark (continuous arrow), normal arrangement of the chondrocytes (filled arrow) with greater density in the superficial zone, subchondral bone (Os), blood vessels (Vs) and articular cavity (star) with normal aspects for both limbs. The Arthritis Group **(B, F)** presented morphological alterations of the articular cartilage (Ca) such as flocculations in the peripheral region (Fl) and cellular disorganization (filled arrow), regions of discontinuity of the tidemark (dotted arrow), and invagination of the subchondral bone (asterisk). The Arthritis + Exercise Group **(D, H)** demonstrated degenerative aspects such as subchondral bone invaginations (asterisk) and areas of tidemark discontinuity, but with less pronounced flocculations (Fl) present and with better organization of cell layers (black arrow).

In the analysis of the synovial membrane, the CON and EX groups denoted the expected organizational aspects, with synovial organization in two cell layers, synovial intima composed of synoviocytes and the subintima composed mostly of fat cells, without alterations in either the right or left limbs (**figure 7 A**, **C**, **E**, **G**). The RA group showed an intense inflammatory process of both the synovial intima, with consequent thickening, and the subintima with a reduction of fat cells and extensive synovitis (**figure 7 B**, **F**).

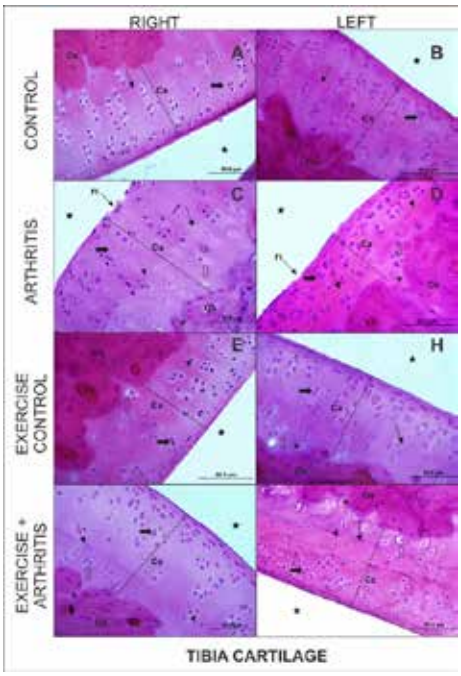

Figure 7. Morphological analysis of the tibia bone in the periarticular region of the right and left pelvic limbs. Photomicrographs of Wistar rats' right and left tibias in longitudinal section, hematoxylin and eosin staining.

The Control Group **(A, E)** and Exercise Control **(C, G)** show normal aspects of the articular cartilage (Ca), presence of continuous tidemark (continuous arrow), normality of the chondrocyte arrangement (filled arrow) with higher cell density in the superficial zone, extracellular matrix and subchondral bone (Os), blood vessels (Vs) and articular cavity (star) with normal aspects. The Arthritis Group **(B, F)** presented morphological alterations of the articular cartilage (Ca) such as flocculations in the peripheral region (Fl) and cellular disorganization (filled arrow) points of discontinuity of the tidemark (dotted arrow), invagination of the subchondral bone (asterisk). The Arthritis + Exercise Group **(D, H)** showed degenerative aspects such as presence of flocculation area (Fl) in the cartilage, subchondral bone invaginations (asterisk), however with greater cellular organization (black arrow) and better integrity of the tidemark (continuous arrow).

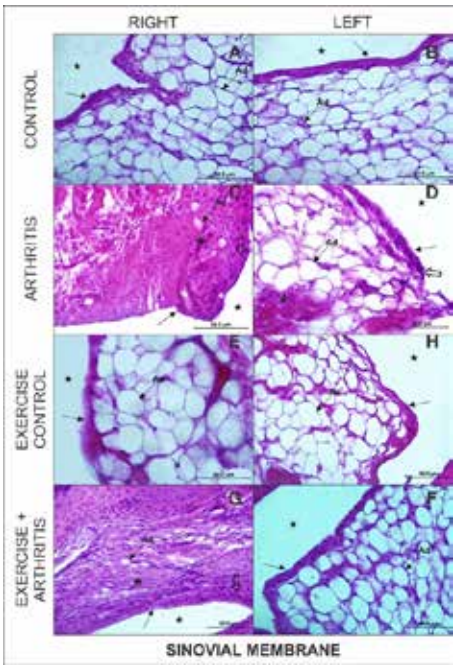

Figure 8. Morphological analysis of the synovial membrane of the right and left pelvic limbs. Photomicrographs of the synovial membrane of the right and left knee joint, longitudinal section, hematoxylin and eosin stained.

The Control **(A, E)** and Exercise Control **(C, G)** groups showed normal morphological aspects of the synovial membrane, with the presence of a synovial intima with synoviocytes (arrow), subintima composed of adipocytes (Ad), and a joint cavity (star). For the Arthritis **(B, F)** and Arthritis + Exercise **(D)** groups, the presence of an intense inflammatory process in the subintima (asterisk) with thickening of the synovial intima (arrow) denoting synovitis. The EXRA showed a slight reorganization of adipocytes in the RPL and control of the inflammatory infiltrate in the left pelvic limb with recovery of the synovial subintima.

For the RPL of the EXRA group, it is possible to observe a large concentration of inflammatory cells in the synovial subintima; however, a subtle presence of fat cells can also be identified, denoting a subtle tissue repair (**figure 8 D**). In the contralateral limb analysis, the synovial membrane presented an aspect similar to the CON and EX groups, with a normal morphological aspect (**figure 8 H**).

In the analysis of cartilage thickness, there was a statistical difference between the groups (WaldX2(3;32) = 7.839, $p < 0.001$). The RA group showed reduced mean ($p < 0.001$) compared to the other groups, while the EXRA was statistically similar to the CON, differentiating from RA and EX as can be seen in **figure 9**. There was no statistical difference between the sides.

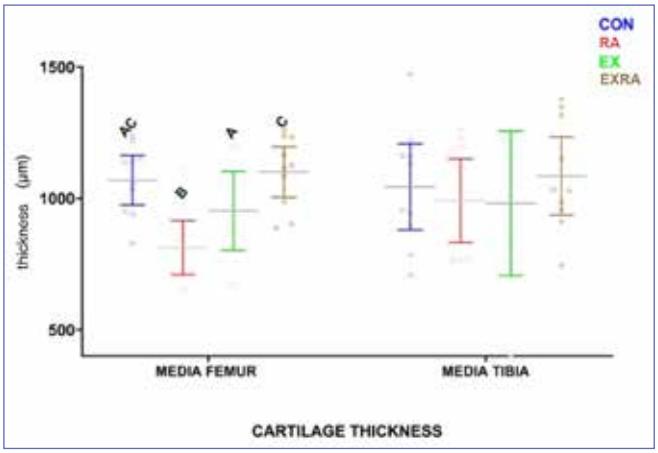

Figure 9. Articular cartilage thickness.

Data expressed as mean and standard deviation. CON: Control Group; RA: Lesion Group; EXE: Exercise Control Group; EXRA: Exercise Lesion Group. Capital letters denote statistical differences between the groups.

For the chondrocyte count in the femur cartilage, statistical difference was observed between groups (WaldX2(3;28) = 7.297, $p < 0.001$) and between right and left sides (WaldX2(1;28) = 4.301, $p < 0.05$). The RA, EX, and EXRA animals reduced the number of chondrocytes compared to CON ($p < 0.05$). Between sides, the LPL had fewer chondrocytes compared to the RPL ($p < 0.05$). No statistical differences were observed for the tibia as can be seen in **figure 10**.

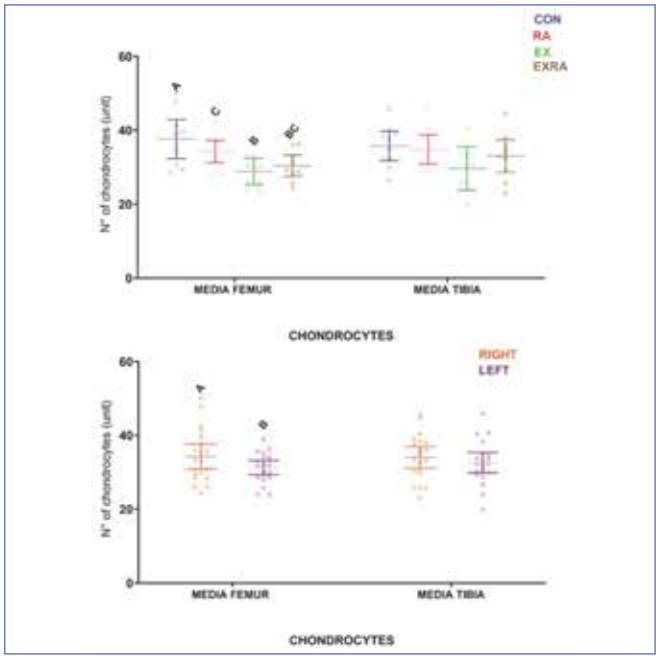

Figure 10. Number of chondrocytes in the femur and tibia cartilage.

Data expressed as mean and standard deviation. CON: Control Group; RA: Lesion Group; EXE: Exercise Control Group; EXRA: Exercise Lesion Group. (A) Capital letters denote statistical differences between groups and lower-case letters the interactions. (B) Capital letters denote statistical differences between sides.

DISCUSSION

In the present study, we observed the repercussions of resisting exercise in an aquatic environment in Wistar rats with RA, considering grip strength, motor control, morphological and morphometric parameters induced in the knee joint by CFA. Both the groups RA and EXRA injured groups presented arthritic symptoms as described by Micheli *et al.* (29), which were analyzed by means of functional evaluation. Gomes *et al.* (23) compared two models of RA, with 2 injections of CFA, one containing *Mycobacterium tuberculosis* and the other *Mycobacterium butiricum*, report that the most suitable model for exercise analysis in a model of RA is with *Mycobacterium butiricum*.

In the functional analysis of strength, the EX and EXRA showed a deficit of the averages after CFA injury. The literature points out that contractile function can be impaired by what is called skeletal muscle disease in RA, characterized by reduced strength and endurance parameters, as well as the presence of sarcopenic obesity, defined by increased fat percentage and reduced contractile tissue in the intramuscular region (30, 31).

In the Uutela *et al.* (31), an investigation was carried out with 199 patients with RA, regarding the muscular performance of the upper and lower limbs and trunk, associated with several behavioral factors. The findings showed that 47% of the sample showed a high reduction in muscle strength, concluding that strength capacity is associated with the level of disease activity and that this risk factor may be modifiable through the practice of physical exercises. In the present study, we also observed a reduction in strength in the injured animals after the induction of arthritis, which was more pronounced immediately after the injury. However, the EXRA obtained a better recovery of strength, similar to the EX, demonstrating the effectiveness of the exercise protocol proposed for the preservation of muscle strength.

A lower limb aquatic exercise program, 3 times a week for 16 weeks, in 82 women with RA can promote beneficial effects in controlling disease activity and improving functional parameters (32). In the present study, the evaluation of the inclined plane in the horizontal position showed that the RA and EXRA groups had a reduction in angular tolerance after the injury; however, both groups recovered, exhibiting an average similar to the baseline parameters at the end of the experiment. Among the main comorbidities associated with RA, functional disability and significant loss of postural balance due to musculoskeletal changes and that in the long term also affect the vestibular and ocular systems, this being a relevant topic for investigation (33).

However, the model of RA induction used in the present experiment is predictive of the promotion of an accentuated inflammatory state during the first seven days after its application, justifying the result of motor control recovery of both injured groups during the experiment. Thus, it is suggested that new investigations be done contemplating specific evaluations for motor control.

Regarding the tissue analyses, it can be verified that the RA model promoted alterations in the rectus femoris muscle, with reduced cross-sectional area and smaller diameter of the injured limb in the RA group, besides the presence of inflammatory cells among the fibers and in the connective tissue region and higher concentration of red blood cells in medium caliber blood vessels. An adaptive response found in arthritic individuals is the presence of hypoxia resulting from synovial inflammation by hypoxia-induced

factors (HIFs) that alter the local oxygen supply (34) and the increase of proinflammatory cytokines such as TNFα that has a catabolic effect by altering protein synthesis and degradation (35).

Baker *et al.* (36) demonstrated by means of an evaluation of the calf muscle density using the computed tomography technique and force dynamometry in 50 individuals with RA and found an association of reduced strength in the presence of a smaller fiber area. Furthermore, the study concluded that lean mass deficits are associated with greater joint destruction.

In addition, local joint inflammation promotes endocrine repercussions that alter extra-articular tissues such as skeletal striated muscle (37). This hypermetabolic inflammatory state associated with physical inactivity, adiposity level, insulin resistance and endothelial dysfunction in RA, are determining factors for the development of long-term rheumatoid cachexia (30, 38).

The EXRA also showed a reduction in cross-sectional area and the smallest fiber diameter, but in the LPL, which can be explained by a possible biomechanical imbalance that may have generated a greater energy demand due to the intense inflammatory process in the RPL (39). Moreover, the presence of this contralateral atrophy may have occurred due to the increase in cortisol hormone, since pain promotes high levels of stress and has already been associated with the aquatic treatment protocol (40). However, for the injured limb, the proposed exercise protocol provided benefits in muscle morphometric parameters.

As for the repercussions on the knee joint, morphologic signs of degeneration of the articular cartilage and synovial membrane found on RA and EXRA were observed. The migration of synoviocytes, similar to fibroblasts, is the main pannus-forming event, which implies a severe inflammation of the synovial tissue, followed by the formation of numerous chemokines, cytokines, matrix metalloproteases that favor joint destruction (41, 42). These synoviocytes present in synovial inflammation produce enzymes involved in cartilage destruction and also the receptor activator ligand nuclear factor kappa B (RANKL) that regulates the differentiation of osteoclasts that carry out bone destruction and blood vessel neoformation (34).

The RA group also showed reduced cartilage thickness and amount of chondrocytes in the femur. Bone erosion associated with cartilage destruction are common radiological findings in patients with chronic RA, although cartilage damage seems to be more associated with physical disability (43). The aquatic resistance exercise protocol preserved the integrity of the tibial and femoral cartilage and promoted the permanence of fat cells in the synovial subintima, evidencing the need for exercise for joint maintenance.

In the study of Vieira *et al.* (44), the effect of resistance exercise in stair climbing (five days a week, three weeks, and overload of 100 g) on morphological parameters of the ankle joint of Wistar rats submitted to a model of sciatic pain was observed. The groups that suffered nerve damage showed an increase in the number of chondrocytes, which was justified by a possible hypertrophy due to exercise or due to joint kinematics, since the weight load on the pelvic limb was altered. These data differ from those presented in the present study, being similar in the use of resistance training for treatment, but performed in an aquatic environment, 3 times a week with 50% of the animals' weight as overload. Both RA and EXRA obtained a reduction in the number of chondrocytes in the femur, although the exercised groups presented similarities and lower statistical means.

It is hypothesized that the physical properties of the water may have interfered with the proliferative demand of chondrocytes, as well as the reduced weight load characteristic of aquatic exercise. However, this reduced mean number of chondrocytes was not related to the findings regarding the femur cartilage thickness of the animals in the EX group. These were similar to the CON and EXRA groups, with no influence on the cartilage thickness, reinforcing the evidence that exercise had a chondroprotective effect.

Finally, the present study showed increased chondrocyte numbers in RPL compared to LPL. Chondrocyte proliferation correlates with proinflammatory regulation. In a stress situation, the production of cytokines is of greater relevance to the immune response than that of type II collagen and glycosaminoglycans (GAGs). This stress may be associated with systemic inflammatory conditions, physical activity, or even the presence of physical properties such as hydrostatic pressure that act in modulating the number of cartilage chondrocytes (41, 45).

In the present research, it was possible to observe repercussions of the RA induced by CFA, in relation to the parameters of strength, motor control, and symmetrical joint manifestations, and it was demonstrated that resisting physical exercise performed in an aquatic environment improved muscle strength and the morphological characteristics of both the rectus femoris muscle and the knee joint of Wistar rats with rheumatoid arthritis. Based on the results obtained, future investigation of inflammatory markers, such as tissue cytokines, is suggested, in addition to this, which is also a limitation of the study, an exercise protocol with only one water temperature was performed, since this characteristic can generate different therapeutic effects, it is suggested that exercises with different protocols at different water temperatures should also be performed. Obviously, the extrapolation of these results to humans is a limitation, but they allow us to suppose that resistance exercise in aquatic environment can also promote, besides muscle strength

gains, a greater joint preservation in individuals with RA, which should be explored in future clinical research.

CONCLUSIONS

Resistance jumping exercise in an aquatic environment promoted benefits in muscle strength and preserved morphometric aspects of the rectus femoris muscle and knee joint components of Wistar rats with RA experimentally induced model.

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DATA AVAILABILITY

Data are available under reasonable request to the corresponding author.

CONTRIBUITIONS

ALFT: funding, conception, data collection, data analysis, manuscript writing. MN, TCS, LAP: conception, data collection, manuscript reviewing. TSSL, LFCR, GRFB: funding, conception, data analysis, manuscript reviewing.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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The Shoulder Activity Level: an Italian Translation, Cross-Cultural Adaptation and Validation

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SUMMARY

Introduction. In this study we aimed to cross-culturally translate the Shoulder Activity Level (SAL) into Italian language and assess its clinimetric properties including reliability, validity, and responsiveness. The objective was to evaluate activity level in patients with shoulder disorders.

Methods. Italian version of the SAL was obtained after forward-backward translation. Three questionnaires were completed by the participants: SAL, SST and SPADI. Fifty patients completed the SAL again, 1 week after the first administration to evaluate the test–retest reliability. Then was evaluated construct validity using Spearman's rank correlation, test–retest reliability and internal consistency were assessed using Intraclass Correlation Coefficient (ICC) and Cronbach's alpha, respectively.

Results. No language difficulties were reported during translation process. Test–retest reliability of the SAL was good with an ICC of 0.896 and a Cronbach's alpha level of 0.739 was also obtained. The correlation between the SAL and the SPADI was moderate, proving divergent validity (rs = - 0.235), even the correlations between the SAL and the SST were moderate proving convergent validity (rs = 0.247).

Conclusions. The study provides statistically significant results of test–retest reliability, internal consistency, construct validity, and responsiveness of the Italian version of the SAL in patients with shoulder disorders. Therefore, it seems that this instrument is a useful measure of shoulder activity level in research setting and clinical practice.

KEY WORDS

Shoulder; questionnaire; physiotherapy; activity; disorders.

INTRODUCTION

Shoulder joint disorders are the second most common musculoskeletal disorders following the low back pain (1) and it can cause significant difficulty in performing a majority of activities daily living (2). Nowadays the use of outcome measure that assess the patient's condition is very common in orthopaedics (3); however, most of them are designed to quantify only symptoms and/or functional disability (4). Although closely correlated, function and activity level must be assessed separately. In fact, function reflects how well a patient does certain task, while activity level measures how much a patient does (5). Therefore, the measurement of activity level should play a key role in patient evaluation.

After the same injury, two individuals with different levels of activity may have similar limitations and pain conditions. However, after a period of rehabilitation, their results may be different for various reasons. The patient's activity level before injury could influence the success of the therapeutic intervention and patient's perception of treatment success. If a treatment relieves night pain, it may be considered satisfactory by a patient with a low level of activity. In reverse, a more active individual expects the return to pre-injury activity level to achieve a similar degree of satisfaction.

Thus, the activity level could be an important prognostic factor related to outcomes of rehabilitation treatment and it should be measured in addition to outcome measures of symptoms and functions in patients with different musculoskeletal disorders including shoulder joint disorders (5).
In recent years the use of instruments capable of measuring the level of activity has increased and many of them are already used in subjects with problems affecting different body districts such as shoulder, knee (6) and ankle (7).
In Italy no study has yet investigated the outcome measure related to activity level in this specific patient population.
After a revision of the literature we found that the only two previous papers that evaluated the psychometric properties of the scale were the original article (Brophy 2005) (5) and Persian adaptation (Negahban 2015) (17).
In the original article the scale was developed using: item generation, item reduction, pretesting, and reliability and validity testing (5). Test-retest reliability was excellence with an ICC of 0.92. The scale was also significantly correlated with self-reported activity score (r = 0.52), Simple Shoulder Test (r = 0.46) and the Knee Activity Rating Scale (r = 0.66).
In the Persian study was assessed reliability, validity and responsiveness with an ICC of 0.98, a Cronbach's Alpha level of 0.64. The scale was significantly correlated with SF-36 (r = 0.21). Moreover, low negative correlations were found between SAL and SPADI (r = - 0.09).
Therefore the aim of this study is to cross-culturally translate the Shoulder Activity Level (SAL) into italian language and to determine its clinimetric properties including reliability, validity and responsiveness in a group of patients with different shoulder disorders.
The SAL was developed by Prof. Brophy *et al.* and it is a rating scale that measures how much patients suffering from the most common shoulder pathologies, have been able to use shoulder joint in their healthiest and most active state in the past year, during activities of daily living and sport[5]. This choice is justified by the conciseness of the scale (made up of only seven items) completed within 1 minute, its schematic structure and the simplicity of understanding which make it a useful and practical measurement tool to use by clinicians.

MATERIALS AND METHODS

The research group who conducted the study was composed of rehabilitation experts from University of Rome "La Sapienza" and experts from the Department of Italian Air Force Aerospace Medicine. Authors certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during the course of this research. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. Informed consent was obtained from all participants for being included in the study. Ethics Committee Sapienza approval was not required because the administration of these tool was part of the usual process of assessment of these individuals in clinical practice, the research involved the analysis of data collected such that individual subjects cannot be identified in any way.

Translation and cultural adaptation process

Cross-cultural translation of the SAL was performed following the "Guideliness for the process of cross-cultural adaptation of self-report measures" by Beaton *et al.* (8) and "Principles of good practise for the translation and cultural adaptation process for the patientreported outcomes (PRO) measures: report of the ISPOR Task Force for Translation and Cultural Adaptation" by Wild *et al.* (9).
Permission for translating the questionnaire has been obtained from the developer.
The translation and cultural adaptation of the original version of the SAL into the Italian version was done using a forward and backward method as summarized in **figure 1**. In the first step two independent Italian-language translators with good knowledge of English language and not familiar with the SAL have translated the original version of SAL producing two different independent versions in Italian. In the second step, an Italian native speaker and out-of-work translator, who had not been involved in any of the previous translations, optimized the two translations and produced a single one in Ital-

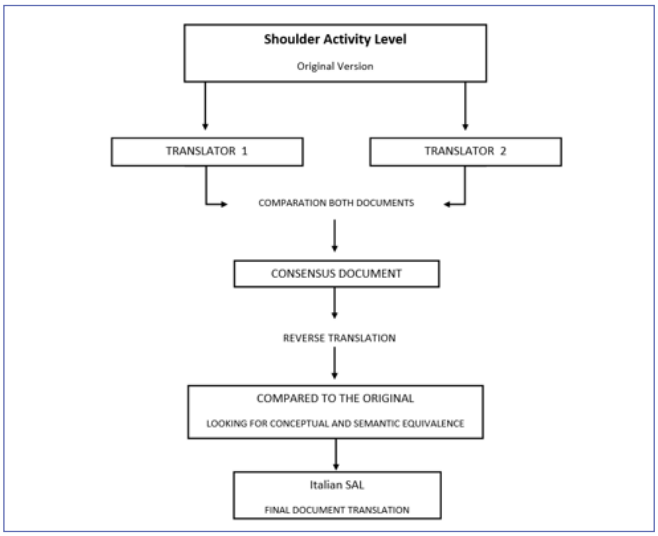

Figure 1. Translation and cultural adaptation process.

ian. In the third phase, the version obtained from the optimization process was then independently translated into the original language by two bilingual translators who were not aware of the original version of the questionnaire. In the last phase, the two backward translated versions of the questionnaire were compared with the original by a focus group composed of three physiotherapists who corrected some spelling, grammar or other errors to minimize the differences from the original version by creating a single version, in order to reach a consensus on the semantic, idiomatic, and conceptual equivalence between the Italian version and the original version of the SAL.

Patients

During a 4-month period, a sample of 100 Italian patients was recruited to participate in this project. If they agreed to participate in this study, they were informed about it and signed an informed consent form. All questionnaires were administered using Google Forms platform due to the Covid-19 pandemic which did not allow the questionnaire to be administer in person.

A link was sent to all participants that included informed consent form and three rating scales that is SAL, SST and SPADI.

The patients included in this study had different diagnosis including rotator cuff tendonitis, impingement syndrome, frozen shoulder, bursitis, and shoulder instabilities. The most common diagnosis was rotator cuff tendonitis (n = 47). Patients were excluded if they had: shoulder pain that caused by impairment in a region other than shoulder complex such as cervical joints dysfunction; radicular pain from cervical disc herniation; involvement of other joints affecting upper extremity such as elbow and wrist; systemic inflammatory rheumatic diseases, neurological, or psychiatric disorders; history of shoulder fracture or surgery (5).

All patients completed the Shoulder Activity Level (SAL), the Shoulder Pain And Disbility Index (SPADI) and the Simple Shoulder Test (SST), during test session. The patients were asked to complete the SAL, 1 week after the first visit to evaluate test–retest reliability. Between test and retest intervals, no treatments were permitted for their shoulder problems. In the retest session, patients were asked to answer whether their shoulder function has changed since the test session. Reliability was analyzed on those patients who stated that their health status has not changed between test and retest sessions, *i.e.*, 50 patients.

To assess responsiveness, data were collected in the first visit and then again after 4-weeks (10 sessions) of physiotherapy intervention or home exercises program. The sample recruited for the responsiveness study was a separate sample of test–retest reliability. Physiotherapy program consisted of shoulder mobilization techniques, shoulder stabilization, strengthening exercises with theraband and instrumental physical therapies. Moreover, the SAL was completed by the patients at 4 weeks and served as a reference standard of perceived magnitude of change in shoulder function from the 50 patient's perspective.

Instruments

The SAL score is a numerical sum of scores for five activities rated on a five point frequency scale from never performed (0 points) to daily performed (4 points). Patients were scored on the following criteria: carrying objects by hands, handling objects overhead, weight lifting or training with arms, swinging motion (*i.e.*, hitting tennis or golf ball), and lifting heavy objects. Two additional multiple-choice questions provide a score assessing participation in contact and overhead sports. This instrument evaluates the activity level of participants at their most active state over the previous 12 months. Each activity has a scoring range of 0–4: 0 point indicates performing the activity never or less than once a month, 1 point indicates activity for once a month, 2 points indicate activity for once a week, 3 points indicate activity for more than once a week, and 4 points indicate daily activity. The total score ranges from 0 (least active) to 20 (most active). Moreover, for descriptive evaluation of activity level, two questions regarding the participation in contact sports and overhead throwing sports were included in a multiple choice format. The possible answers are A) no; B) yes, without organized officiating; C) yes, with organized officiating; or D) yes, at a professional level. It can be completed quickly and used to assess activity level as a prognostic factor in patients with shoulder disorders.

The SPADI was developed to measure pain and disability related to shoulder disorders. This instrument is a self-reported questionnaire that consists of two dimensions (pain and disability), required 5–10 min to complete. All items were scored based on a visual analogue scale from 0 (no pain and disability) to 10 (most pain and disability). The range of possible scores for the pain and disability dimensions lies between 0 and 100, with higher scores indicate greater amount of pain and disability. The total score of the SPADI was achieved by averaging the pain and disability scores and the score ranges from 0 to 100 (10).

The Simple Shoulder Test (SST) consists of 12 questions about physical function with dichotomous (yes or no) response options. For each item, patients indicate whether or not they are able to carry out specific activities and if the shoulder problem allows them to sleep peacefully and to carry out their work full time. The scores ranges are reported as the percentage of items that a person answers in the affirmative (10, 11).

Assessment of clinimetric properties

Reliability

Test–retest reliability was measured using the two-way random effect's model of Intraclass Correlation Coefficient (ICC) with 95% confidence intervals (CI) (12). An ICC equal to or greater than 0.75 was considered as good test–retest reliability and a value greater than 0.90 indicates excellent reliability so that the using an instrument is reasonable for clinical implications (5). The internal consistency is a measure of item homogeneity of a scale and measures the extent to which items are inter-correlated (13). The internal consistency of the SAL was evaluated by calculating Cronbach's alpha using the data obtained from the entire group of 100 patients in the test session. An acceptable internal consistenc y will be obtained when Cronbach's alpha is higher than 0.90 (13).

Validity

Due to the absence of gold standard to determine activity level, the validity of the SAS was evaluated from the aspect of construct validity. Construct validity means how well an instrument evaluate the construct it is intended to measure (5). Construct validity in this study was evaluated by examining the associations between the scores on the SAS and the scores obtained from the SST and SPADI. The SAL has a positive correlation with the SST and negative correlation with the SPADI. The latter is because of the fact that patients with higher shoulder activity levels were expected to have lower shoulder pain/disability. However, due to the conceptual differences between activity and function, we expected waek correlation between the SAL and the SPADI. Due to the ordinal data, Spearman's rank correlation was used for all correlations. Spearman's correlation coefficients less than 0.20, 0.20–0.60, and greater than 0.60 were considered low, moderate, and strong, respectively.

Dimensionality

Dimensionality of the Italian SAL was assessed through the factor analysis with principle component analysis and varimax rotation (14). If the SAS is a uni-dimensional measure of shoulder activity level, it was expected that all items should be loaded on only one factor. To decide how many factors to select, an eigenvalue greater than 1 was used as a conventional method for factor extraction. Only factor loadings ≥ 0.40 were considered as indicative of item loading (**figure 2**).

RESULTS

Because sports participation questions do not alter the overall score of the SAL, we report the descriptive results

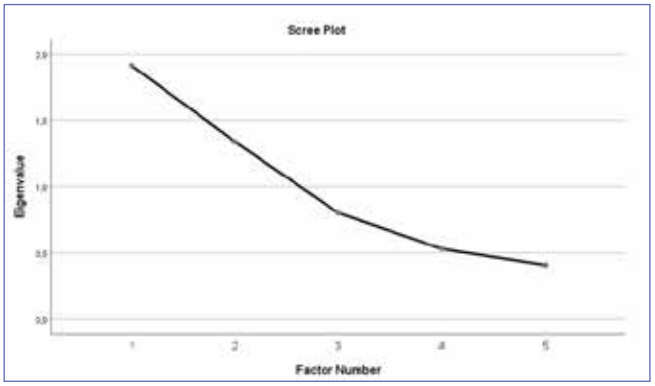

Figure 2. Scree plot.

of these questions. For question 1 (participation in contact sports), 71% answered A, 24% answered B, 4% answered C, and 1% answered D. For question 2 (participation in overhead throwing sports), 82% answered A, 13% answered B, 5% answered C and 0% answered D. Therefore, the present study showed that the patients participated in this study had low sports participation.

Translation process and cultural adaptation

Forward and backward translations of the SAS showed no problem or language difficulties. Cultural adaptations were made because the units of measurement reported in the questionnaire, *i.e.*, pounds for mass and gallons for volume, are not used in Italy; therefore, they have been replaced, respectively, by kilograms and liters by making the appropriate equivalences: 1 lb = 0.45 kg; 1 gal = 3.79 L.

Statistical analysis

Following the checklist "Consensus-Based Standards for the Selection of Health Status Measurement Instrument" (COSMIN), the reliability and construct validity of the culturally adapted scale were evaluated (15, 16).

The descriptive analysis was used to analyze the data obtained from the sample and the administration of the scales: the average and the standard deviation (DS) of the variables were calculated (**table I**).

Table I. Descriptive analysis.

	Mean	Stdandard Deviation	N
Total SAL	7.8400	3.88000	100
Total SST	7.12	2.851	100
Total SPADI	60.65	25.839	100

Mean and Standard Deviation of SAL and scales used for comparison.

Reliability

The results of test–retest reliability of the SAL (**table II**) was excellent with an ICC of 0.896 (95% CI), the Cronbach's alpha level of 0.739 was also obtained in this study (**table III**).

Validity

The SAL was significantly correlated with the SST (rs = 0.247, $p < 0.05$). Also, the SAL had a significant moderate negative correlation with the SPADI (rs = - 0.235, $p < 0.05$) (**table IV**).

DISCUSSION

The results of this study provided evidence for the test–retest reliability of the SAL based on the data obtained from 50 patients (ICC = 0.896). Internal consistency was acceptable, with Cronbach's alpha of 0.739. The findings provided

Table II. Reliability.

	Intraclass Correlation[b]	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.812[a]	.692	.888	9.494	50	50	.000
Average Measures	.896[c]	.818	.941	9.494	50	50	.000

Intraclass Correlation Coefficient (ICC) between the test and the retest of 50 participants. Two-way mixed effects model where people effects are random and measures effects are fixed. [a]The estimator is the same, whether the interaction effect is present or not; [b]Type A intraclass correlation coefficients using an absolute agreement definition; [c]This estimate is computed assuming the interaction effect is absent, because it is not estimable otherwise.

Table III. Internal consistency.

Cronbach's Alpha	N of Items
.739	6

	Scale Mean if Item Deleted	Scale Variance if Item Deleted	Corrected Item-Total Correlation	Cronbach's Alpha if Item Deleted
Item 1	12.8700	51.872	.495	.715
Item 2	14.0600	49.491	.477	.709
Item 3	14.5600	46.916	.614	.681
Item 4	14.9600	51.150	.515	.711
Item 5	14.1100	52.038	.373	.730
Total SAL	7.8400	15.206	1.000	.579

Cronbach's α, Mean and Variance if the item is deleted and for each individual item.

Table IV. Validity.

			Total SAL	Total SST	Total SPADI
Spearman's rho	Total SAL	Correlation Coefficient	1.000	.247*	- .235*
		Sig. (2-tailed)	.	.013	.019
		N	101	100	100
	Total SST	Correlation Coefficient	.247*	1.000	- .703**
		Sig. (2-tailed)	.013	.	.000
		N	100	100	100
	Total SPADI	Correlation Coefficient	- .235*	- .703**	1.000
		Sig. (2-tailed)	.019	.000	.
		N	100	100	100

Spearman Correlation Coefficient between SAL SST and SPADI. *Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed).

some evidence to support the construct validity of the SAL. Factor analysis indicated that all items strongly loaded on one factor, and this supports that the SAL measures a single construct known as the shoulder activity level. Finally, the SAL was, to some extent, responsive to changes following physiotherapy intervention. Therefore, the Italian version of the SAL is an appropriate instrument for assessing activity level of patients with different shoulder disorders.

The questions of this instrument are short, easy to understand, and requires two minutes to complete. This allows clinicians to evaluate other outcome measurements related to patients with shoulder disorders. Including a reliable, valid, and responsive measure of shoulder activity would improve the quality of clinical outcome studies performed on patients with different shoulder disorders (5).

Matching the outcomes of our work, the values are in line with previous reports. The reliability of the Italian version of the SAL (ICC = 0.896) was similar to that of the original English version (ICC = 0.92) (5) and to the Persian version (ICC = 0.98) by Negahban *et al.* (17). The results of validity provide some support for our hypotheses related to convergent and divergent validities of the SAL. As expected, because of differences in symptoms and activity constructs, the correlation between the SAL and the SPADI was negative moderate.

Our study is not without limitation. First, the responsiveness study was performed on small sample size, and this may explain the marginal level of AUC index achieved in this study. Second, the results of the present study may be more generalized to rotator cuff tendonitis that constituted a significant proportion of participants (n = 47).

CONCLUSIONS

In conclusion, the current study provides some evidences to support the test–retest reliability, internal consistency, construct validity, and responsiveness of the Persian version of the SAS in a group of patients with different shoulder disorders. Therefore, it seems that this instrument is a useful measure of shoulder activity level in research setting and clinical practice.

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None.

DATA AVAILABILITY

Data are available under reasonable request to the corresponding author.

CONTRIBUTIONS

All authors contributed equally to the manuscript and read and approved the final version of the manuscript.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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The Critical Shoulder Angle in a Middle Eastern Cohort: is There an Association with Rotator Cuff Tear?

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SUMMARY

Background. The critical shoulder angle (CSA) has been used as a tool for identifying patients with high risk of developing a rotator cuff tear (RCT). A CSA angle larger than 35 degrees has been shown to be associated with increased risk of RCT. The aim of this study was to determine if this concept is applicable to a Middle Eastern cohort of patients.

Methods. This retrospective observational study included 44 patients who underwent rotator cuff repair between 2016 and 2021 in KFUH was compared to 45 patients with normal shoulders. The CSA was measured by two independent observers on anterior-posterior radiographs. The collected data was analyzed. P-values of < 0.05 were considered statistically significant.

Results. The mean ± standard deviation (SD) CSA measured on pre-operative radiographs was significantly higher in patients with RCT (36.66° ± 4.62°) compared to patients with normal shoulder (31.97° ± 3.37°), P-value < 0.033.

Conclusions. Our current study confirms that the association of high CSA with risk of rotator cuff tears is applicable in a cohort of Middle Eastern patients, as the CSA was higher in patients who underwent RCT repair when compared to patients with normal shoulders.

KEY WORDS

Critical shoulder angle; Rotator Cuff Tear; shoulder; radiographs; MRI.

INTRODUCTION

Rotator cuff tears (RCT) are common shoulder injuries seen by orthopedic surgeons in their practice. These injuries can lead to significant disability and loss of function if not managed properly (1, 2). Recent attention has been directed towards identifying factors that may predispose patient to these types of injuries. These include increasing age, smoking status and commonly a history of shoulder trauma (3, 4). Interest has also increased in identifying radiographic risk factors for RCTs, one of which is the critical shoulder angle (CSA). This angle is formed by a line extending between the superior and inferior borders of the glenoid and a line connecting the glenoid to the inferolateral aspect of the acromion on a standard anterior-posterior radiograph (AP) of the shoulder (5-9). A CSA angle larger than 35 degrees has been shown to be associated with increased risk of

RCTs, while an angle less than 30 degrees was found to be a risk factor for osteoarthritis (5, 10).

Kim *et al*. assessed the association of degree of CSA with risk of sustaining RCTs by subdividing patients into high (> 38°), middle (33-38°) and low (< 33°) CSA groups. They found in their study that the high group had a significantly higher risk of RCTs (84.6%), while this was lower in the middle and low group (60.3% and 68.3%, respectively) (11).

The aim of our current study is to assess the association of CSA with RCTs in a Middle Eastern patient cohort. As this is a newly introduced radiographic concept, we believe that the results of this previously unreported patient cohort will add to the current literature.

METHODS

We performed a retrospective observational study to evaluate patients who underwent RCT repair at King Fahd Hospital of the University, Al-Khobar, Saudi Arabia between February 2016 to November 2019. Institutional review board approval was obtained prior to the onset of the study (Imam Abdulrahman Bin Faisal University. IRB: UGS - 2019-01-320).

Inclusion criteria were all middle eastern patients who underwent RCT repair at our institute and had adequate shoulder AP radiographs. Exclusion criteria included non-middle eastern patients, patients with history of previous shoulder surgery and patients with incomplete records or radiographs. A cohort of patients presenting to clinic with normal shoulders, confirmed by both clinical exam and magnetic resonance imaging (MRI) were included as a comparison group. Demographic data, medical history and operative records were collected retrospectively from the patient's files.

The CSA was then measured on patient's pre-operative AP shoulder radiograph in the RCT group and in the normal shoulder group by two observers (**figures 1, 2**). This was performed by them twice, with a two-week interval. Inter-observer and intra-observer reliability was analyzed and reported. In addition, the size of the tear and tendon retraction were measured by a fellowship trained musculoskeletal radiologist on the RCT group patients' pre-operative MRI (**figures 1, 2**).

A power analysis was performed using the data from Cherchi *et al*. (5). We calculated that a sample size of 64 was required to obtain a power of 80% and $\alpha = 0.05$.

Data was collected and descriptive statistics were analyzed using SPSS, version 26 (Armonk, NY: IBM Corp, USA). For data analysis the Independent-Samples t test was performed to compare the variables. Interclass Correlation Coefficients (ICC) was used to measure the interobserver and intra-observer reliability. P-value < 0.05 was considered to be significant.

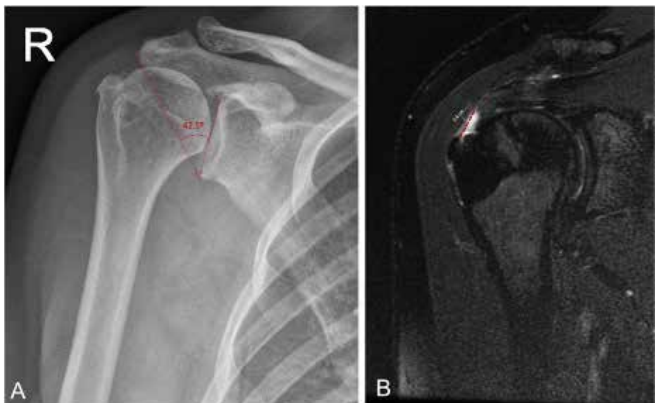

Figure 1. (A) Anterior-posterior radiograph of the right shoulder demonstrating a Critical Shoulder Angle (CSA) of 42.5 degrees. **(B)** Coronal PD fat saturated MRI image of the same patient demonstrating a large full thickness tear of the supraspinatus tendon. The tendon tear measured about 2.5 cm in anterior to posterior dimension and retraction of the torn tendon stump by about 0.8 cm.

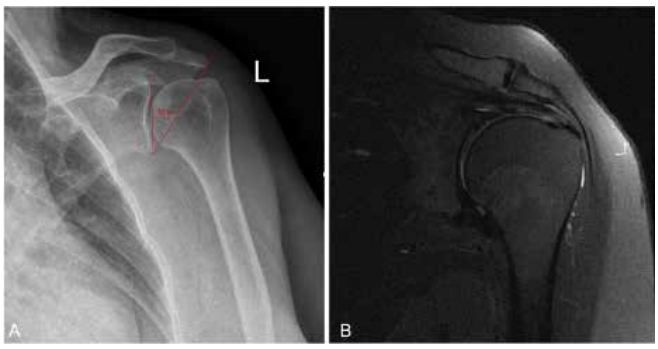

Figure 2. (A) Anterior-posterior radiograph of the left shoulder demonstrating a Critical Shoulder Angle (CSA) of 33.5 degrees. **(B)** Coronal PD fat saturated MRI image of the same patient demonstrating normal appearance of the rotator cuff tendons without a tear.

RESULTS

A total of 89 patients were included in the study, 44 patients in the RCT group and 45 patients in the normal shoulder group. The mean age of patients in the RCT group was 54.8 years ± 8.2, while in the normal shoulder group was 53.7 years ± 7.7, with no significant difference between the two groups (**table I**). The majority of patients were males in the RCT group (54.5%) and females in the normal shoulder group (66.7%), but this was not statistically significant (**table I**). All patients in the RCT group had an arthroscopic repair of RCT, with 17 of them requiring a subacromial decompression and one patient requiring an acromioplasty during the initial operation.

Table I. Demographic data of patients included in the study.

Demographics		RCT group	Normal Shoulder Group	P-value
Age (years), mean ± SD		54.8 ± 8.2	53.7 ± 7.7	0.776
CSA (mean ± SD)		36.7° ± 4.6°	31.9° ± 3.4°	0.033
Gender, n (%)	Female	20 (45.5%)	30 (66.7%)	0.044
	Male	24 (54.5%)	15 (33.3%)	

RCT: rotator cuff tear; CSA: critical shoulder angle; SD: standard deviation; n: number of shoulders.

The CSA measurement showed excellent reliability. The interobserver reliability of CSA measurement was 0.815 and the intra-observer reliability was 0.903.

The mean CSA measured on pre-operative radiographs was 36.66° ± 4.62° in the RCT group *versus* 31.97° ± 3.37° in the normal shoulder group. This difference was statistically significant (P-value < 0.05). Of the RCT group 63.6% had a CSA of 35° or more, while in the normal shoulder group it was only 13.3%.

We further subclassified the CSA into high (> 40°), middle (38-40°) and low (< 38) risk groups. Of the total 44 patients, 12 patients were in the high-risk group (27.3%), 6 patients (13.6%) were in the middle-risk group and 25 patients (56.8%) were in the low-risk group in the RCT group. While in the normal shoulder group only 1 patient was high risk (2.2%), 2 as middle risk (4.4%) and 42 with a low-risk CSA (93.3%).

DISCUSSION

Our current study evaluated the association between high CSA and risk of sustaining a RCT in a middle eastern population cohort. The mean CSA of patients with RCT included in our cohort was 31.97° ± 3.37°, this is similar to previously reported literature in other populations (3, 5, 7, 10-13). We also found that the majority of patients with RCT had a CSA higher than 35 (63.6%). This also aligns with preceding studies showing a significant association between higher CSA measurement and risk of RCT (3, 5, 11, 12). In addition, we have also confirmed the association of high CSA with RCT, as the CSA was significantly higher in patients with RCT compared to patients with normal shoulders. In our current study, we found no statistical differences in age and gender between normal and RCT groups. Gumina *et al*. studied the association of age and gender with CSA and risk of RCT in the general population and found that increasing age was associated with higher CSA and thus risk of RCT (14).

When CSA was classified into high-risk, middle-risk and low-risk groups, a large proportion of patients with RCT were among with high risk (CSA > 40) group (27.3%). This is consistent with Kim *et al*. findings that patients in the higher CSA group had a higher incidence of RCT compared to patients in the middle CSA group (11, 15, 16).

Several studies have confirmed good reliability of CSA measurement on AP radiograph (3, 5, 11). Kim *et al*. reported an inter-observer reliability of 0.897 and intra-observer reliability of 0.993 (11). We also demonstrated an excellent inter-observer and intra-observer reliability, which were 0.948 and 0.993, respectively.

A possible limitation of the study is that the shoulder radiographs obtained for CSA measurement can be affected by several factors such as projection and patient position. At our institution we perform these radiographs under a standardized protocol, thus minimizing the variations in studied images. Also, the CSA angle measurement has been shown to have excellent reliability in previous studies (5, 11). Another possible limitation is the small sample size, but we performed a power analysis using previously published literature prior to the study (32 patient per group).

CONCLUSIONS

We have shown that in a Middle Eastern population cohort, high CSA was associated with developing RCTs, thus confirming that this angle can be applied as a predictor of patients' risk of sustaining a RCT. Additional studies are encouraged to assess this association in different populations and patient cohorts.

FUNDINGS

None.

DATA AVAILABILITY

Data are available under reasonable request to the corresponding author.

CONTRIBUTIONS

SMA, AAA. TMH: conceptualization, design, study execution, manuscript writing and review. FA, ZA, NA, WA, FA,

SA, AAA, MMA: study execution, manuscript writing and review. SSA: statistical analysis, manuscript writing and review.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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Patient Body Mass Index Has No Direct Effect on The Characteristics of Primary Tenocytes Derived from Torn Rotator Cuffs

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ABBREVIATIONS
ALP: Alkaline phosphatase
BMI: Body mass index
DMEM/ F-12: Dulbecco's modified Eagle's Medium: Nutrient Mixture F-12
ECM: Extracellular matrix
FBS: Fetal bovine serum
IGF-1: Insulin-like growth factor
PDGF: platelet-derived growth factor
TGF- β1: transforming growth factor-β1

SUMMARY

Background. Obesity is associated with an increased risk of rotator cuff tears and impaired tendon healing after surgery. This study aimed: 1) to investigate the influence of patient body mass index (BMI) on cellular function in tenocytes derived from diseased torn human rotator cuff; 2) to determine if BMI altered the response of tenocytes to tenogenic growth factors.

Methods. Tenocytes were isolated from torn supraspinatus tendons of patients undergoing rotator cuff surgery. Tenocyte growth and collagen production were determined by alamarBlue and Sirius red assays, respectively, at baseline and in the presence of IGF-1, TGF- β and PDGF after 72 hours. Changes in the relative expression of genes important in tenocyte, chondrocyte and osteoblast biology were determined using real-time PCR. Correlation analyses were performed between patient BMI and tenocyte function and gene expression.

Results. BMI had no direct effect on tenocytes with no significant correlations between patient BMI and cellular behaviour, gene expression or response to growth factor treatment. Higher cellular growth and collagen production were observed in response to PDGF and TGFβ treatment, while IGF-1 had minimal effect. TGF-β was associated with higher expression of tenocyte-related genes, collagen Iα and scleraxis, while PDGF resulted in higher expression of adipose marker, *PPAR*-γ, suggesting it may be promoting de-differentiation from a tenocytic phenotype.

Conclusions. In summary, BMI does not influence tenocyte growth, collagen synthesis or gene expression profile *ex vivo*. These findings have significant clinical implications, as they suggest that growth factor treatment will be effective in patients independent of BMI.

KEY WORDS
Obesity; tendon; growth factors; healing; rotator cuff.

INTRODUCTION

Rotator cuff tendon tears are a common cause of shoulder pain and disability. With an increasingly active, ageing population, the demand for rotator cuff surgeries is rising (1–3). Despite recent advances in surgical technique, rates of post-operative re-tear remain high, and failed repairs result in persistent pain and loss of function (4–6). Surgical failure occurs mainly as a consequence of inadequate healing at the tendon-bone interface (7, 8).

Recently, obesity has been investigated as a risk factor for impaired rotator cuff healing. Several clinical studies have identified obesity as an independent negative risk factor for the occurrence and severity of rotator cuff tears (9–12) and reported a negative correlation between obesity and impaired tendon healing response (13–15). Body mass index (BMI) is associated with higher re-tear rates after rotator cuff surgery and lower post-operative functional scores (16, 17). Despite obesity emerging as a key and a potentially modifiable risk factor for poor tendon healing, few studies have investigated its effects on tendon at the cellular level (9, 13, 16, 17).

Over the past decades, orthopaedic research has focused on using growth factors to biologically augment tendon healing with promising results (18–20). Several growth factors have shown success in pre-clinical studies by promoting rotator cuff healing by increasing cellular recruitment, proliferation, differentiation, extracellular matrix (ECM) synthesis, and remodelling (21–23). In obese patients, high levels of pro-inflammatory cytokines and high mechanical load promote chronic low-grade inflammation that influences the activities of various mesenchymal cells, including tenocytes (13, 14, 24–26). For this reason, tenocytes derived from obese patients may exhibit differential cellular phenotypes and respond differently to growth factor therapies. A better understanding of these mechanisms may help to develop more effective therapeutic interventions that could reduce the musculoskeletal sequelae of obesity.

We hypothesised that 1) patient BMI would be correlated with negatively altered tenocyte phenotype; 2) patient BMI would reduce the effects of growth factor treatments on tenocyte function and gene expression. The aims of this study were: 1) to investigate how patient BMI influences cellular phenotype in tenocytes derived from diseased torn human rotator cuff; 2) to determine if BMI altered the response of tenocytes to growth factors insulin-like growth factor (IGF-1), transforming growth factor-β1 (TGF-β1) and platelet-derived growth factor (PDGF).

METHODS

Reagents and ethical approval

Dulbecco's modified Eagle's Medium: Nutrient Mixture F-12 (DMEM/F-12), penicillin-streptomycin mixture (10,000 U/mL), and fetal bovine serum (FBS) were obtained from Gibco (Thermo Fisher Scientific, Waltham, MA, USA). Bovine serum albumin (BSA) was obtained from Immuno-Chemical Products Ltd. (Auckland, New Zealand). IGF-1, PDGF and TGF-β1 were sourced from Sigma Aldrich (St Louis, MO, USA).

For human tenocytes, human sample collection was approved by the New Zealand Ministry of Health Northern Regional Ethics Committee (NTX/05/06/058/AM14 - Date of approval: March 11, 2020). All participants provided written informed consent for collection of samples. All participants were undergoing arthroscopic supraspinatus rotator cuff repair, had supraspinatus tendon tears and were symptomatic prior to surgery. Demographic details on age, gender and ethnicity and biomorphic measurements on height (cm), weight (kg) and BMI (kg/m^2) were collected. Tendon samples were divided into samples from healthy-weight and overweight patients (BMI < 30 g/m^2), and those from obese patients with a high BMI (≥ 30 kg/m^2) for a sub-group analysis (27). Because tear size has been correlated with progression of pathology with reduced likelihood of repair with chronic disease, tear sizes were classified as follows: small (≤ 1 cm), medium (> 1 and ≤ 3 cm), large (> 3 and ≤ 5 cm) and massive (> 5 cm in anterior-posterior length) (28, 29). Patients were excluded from the study if they were: < 18 years; had previous shoulder surgery; or had other shoulder pathology, rheumatoid arthritis or other systemic inflammatory diseases.

Primary tenocyte cell culture

Primary tenocyte cell culture was performed, as previously described (30). Briefly, excess tissue from patients undergoing rotator cuff repair surgery was kept hydrated at 4 °C until use. Supraspinatus tendon tissue was roughly cut into pieces smaller than 0.5 cm^2 and digested in 0.5 mg/ml of dispase (Sigma-Aldrich) and 0.5 mg/mL of collagenase (Sigma-Aldrich) in DMEM/F-12 with 10% FBS at 37 °C for up to 18 hours until all ECM had been digested. The cell suspension was then passed through a cell strainer, washed, and re-suspended in enzyme-free media. Cells were cultured in DMEM/F-12 with 10% FBS in 75 cm^2 flasks (Corning, Corning, NY, USA) and incubated at 37 °C with 5% carbon dioxide until confluent. Cell cultures were frozen down in liquid nitrogen before being used for these experiments.

Cell viability assays

As described previously (30), primary human tenocytes were seeded in 24-well plates (Greiner BioOne, Kremsmünster, Upper Austria), at a density of 2.5 × 10^4 cells/well and cultured in DMEM: F-12 with 5% FBS for 24 hours. After this, the cell media was changed to growth arresting media DMEM: F-12 with 1% FBS. Following 24 hours of incuba-

tion, initial cell growth was measured by adding alamarBlue (Life Technologies, ThermoFisher Scientific) at 5% of final concentration into each well for 4 hours at 37 °C. Then, 200 µl of the alamarBlue conditioned medium was transferred to a 96-well plate (Greiner Bio-One), and fluorescence (excitation 540 nm; emission 630 nm) was read using a Synergy 2 multi-detection microplate reader (BioTek Instruments Inc., Winooski, VT, USA). Cells were then incubated with fresh growth arresting media with treatment compounds PDFG (20 ng/mL), TGF-β (20 ng/mL), and IGF-1 (100 ng/mL). These concentrations of growth factors have previously been demonstrated to have tenogenic effects (31). Cell growth was then re-measured using alamarBlue after 72 hours, as above. To report baseline function, the change in alamarBlue was expressed as a ratio of the untreated baseline measurement after 24 hours in growth arresting media and the repeated readings after 72 hours exposure to the treatment growth factor. There were four wells in each treatment group. To report function in response to growth factors, the change in alamarBlue in the treatment group was expressed as a ratio of the untreated control fluorescence readings.

Collagen deposition assays

Tenocytes were seeded in 24-well plates, at a density of 7.5 × 10^4 cells/well, and cultured in DMEM/F-12 with 5% FBS for 24 hours, as previously described (30). Cells were then incubated in fresh DMEM/F-12 1% FBS and 50 µg/ml AA2P with the same treatment compounds as above. After 72 hours, cells were fixed with Bouin's solution (71% saturated picric acid, 24% pure formalin, 5% 0.5 M acetic acid) for 30 minutes and then stained with 0.1% Sirius red dissolved in saturated picric acid for 1 hour. At the end of this incubation, cells were washed with 0.01 M hydrochloric acid five times and left to air dry. The dye was released using 0.1 M sodium hydroxide, and 200 µl of the released dye was transferred to a 96-well plate. Absorbance was measured at 570 nm using a Synergy 2 multi-detection microplate reader. There were four wells in each treatment group.

Gene expression analysis

Gene expression analysis was performed at two experimental time points: firstly following the initial culture and isolation of tenocytes for baseline comparisons, and secondly, after cells were cultured treated as described above under cell viability assays. Analysis of gene expression was performed using a previously established protocol (31). Total cellular RNA was extracted from cultured cells and purified using the RNeasy minikit (Qiagen, Venlo, The Netherlands). Genomic DNA was removed using RNase-free DNase set (Qiagen). The quality and concentration of the extracted RNA were measured using NanoDrop Lite Spectrometer (Thermo-Fisher, Victoria, Australia). Complementary-DNA was prepared by using 500 ng of RNA with super-script-III (Life Technologies, Carlsbad, CA, USA). Primer-probe sets were purchased as TaqMan Gene Expression Assays (Life technologies). The multiplex polymerase chain reaction was performed with FAM specific for genes of interest (alkaline phosphatase (*ALP*), collagen Iα1, scleraxis, *SOX-9*, *RUNX-2, PPAR*-γ) and VIC-labelled 18S endogenous ribosomal RNA probes, according to the manufacturer's instructions, using an ABI PRISM 7900HT sequence detection system (Applied Biosystems, Foster City, CA, USA). Samples were assayed in triplicate. The ΔΔCt calculation method (32) was used to determine the relative level of messenger RNA expression, normalised to the values of the non-treated cells (control).

Statistical analysis

All results are shown as mean ± standard error of the mean (SEM). Statistical analysis was performed using a one-way analysis of variance (ANOVA) test with Dunnett's *post-hoc* analysis. Pearson r test was used for analysing the correlation between patient BMI with tenocyte cell growth, collagen deposition and relative gene expression. A Mann-Whitney U test was used to compare patients with BMI < 30 kg/m^2 with BMI ≥ 30 kg/m^2. A P-value of < 0.05 was considered statistically significant. A false discovery rate (FDR)-adjusted P-value for correlation analyses between BMI and relative gene expressions level was calculated, as previously described (33), to control for the expected proportion of falsely rejected hypotheses. All statistical analysis was performed using the GraphPad Prism 8 software (GraphPad Software, San Diego, California).

RESULTS

Patient Demographics and Biomorphic Measurements

The study group consisted of 13 supraspinatus tendon specimens from 13 patients, 5 women and 8 men, with a mean age of 59.1 ± 2.81 years (37 to 74) (**table I**). The mean BMI was 31.05 ± 1.57 (range 20.69 to 41.98). Five patients were healthy body weight or overweight (BMI < 30 kg/m^2) and eight patients were obese (BMI ≥ 30 kg/m^2). Individual patient details are shown in **appendix 1**.

Correlation analysis between tenocyte cell growth and gene expression of baseline tenocytes

There were no significant correlations between patient BMI and the tenocyte cell growth, as measured by alamarBlue assay,

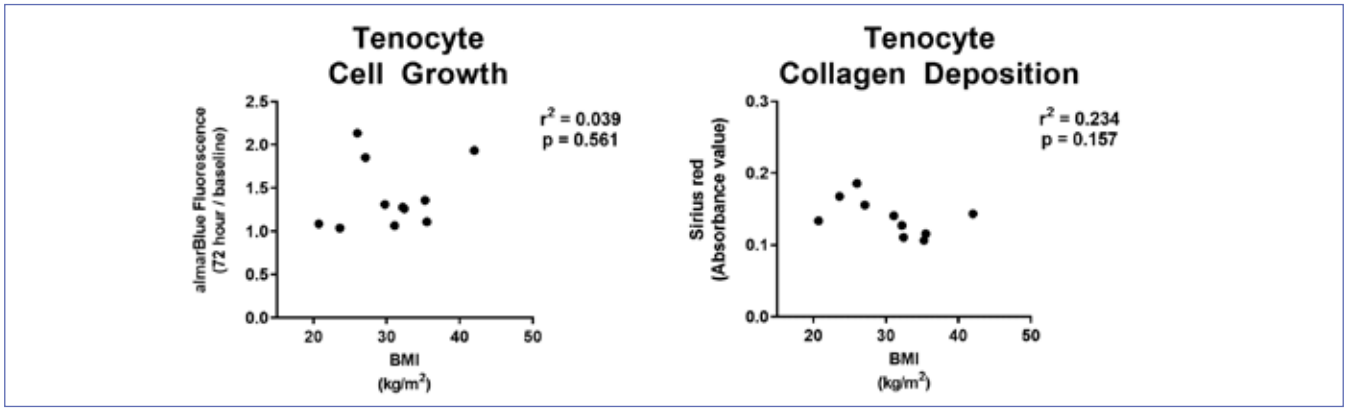

Figure 1. The correlation between BMI with [A] tenocyte cell growth and [B] tenocyte collagen deposition of baseline tenocytes over 72 hours.

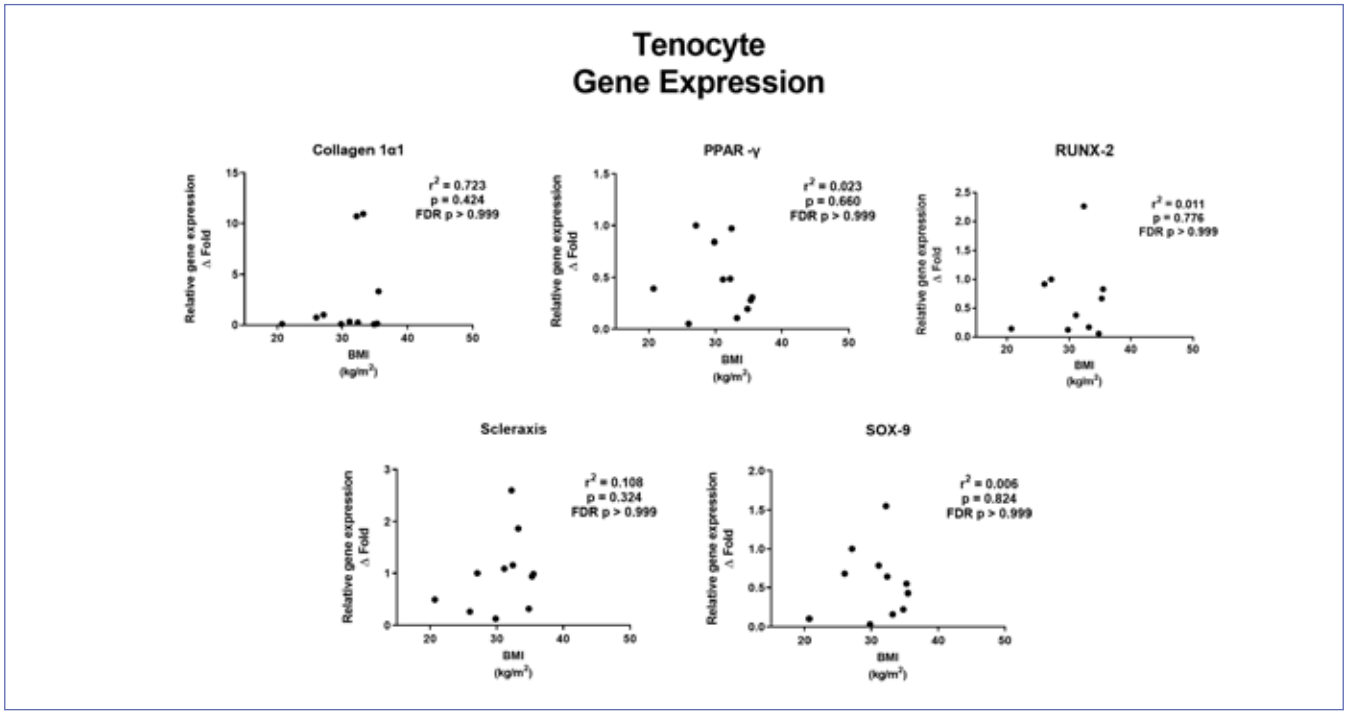

Figure 2. The correlation between BMI with tenocyte gene expression markers (collagen Iα1, *PPAR-γ*, *RUNX-2*, scleraxis and *SOX-9*).

Table I. Patient demographics, biomorphic measurements, and supraspinatus tear size.

Characteristic	Frequency
Sex	8 male; 5 female
Age	
Mean ± SEM	59.08 ± 2.80
Range	37 – 74
BMI (kg/m²)	
Mean ± SEM	31.05 ± 1.57

Characteristic	Frequency
Range	20.69 – 41.98
Ethnicity	
European	9
Māori	2
Asian	1
Pacific Peoples	1
Supraspinatus tear size	
Small	1
Medium	6
Large	5
Massive	1

Data are presented as mean ± SEM.

and collagen deposition, as measured by Sirius red assay, of the baseline tenocyte cells over 72 hours (**figure 1**). Correlation analyses did not demonstrate a significant correlation between patient BMI and baseline tenocyte gene expression markers (collagen Iα1, *PPAR-γ*, *RUNX-2*, scleraxis and *SOX-9*) (**figure 2**). In addition, there were no statistical differences between patients with BMI < 30 kg/m^2 and patients with BMI ≥ 30 kg/m^2 (**appendix 2**).

Correlation analysis between patient BMI and tenocyte cell growth, collagen deposition and gene expression in response to IGF-1, TGF-β and PDGF

Following 72 hours of treatment, tenocytes exposed to PDGF and TGF-β had significantly higher tenocyte growth, by 83.4% and 30.4% ($p < 0.05$), respectively. There was also higher collagen deposition with PDGF and TGF-β by 139.8% and 164.7% ($p < 0.05$), respectively (**appnedix 3**). However, IGF-1 did not affect cell growth or collagen deposition rate. There were no significant correlations between patient BMI and the tenocyte cell growth and collagen deposition in response to PDGF, TGF-β or IGF-1 (**figure 3**, **table II**). Furthermore, there was no statistical difference in tenocyte cell function in response to these tenogenic growth factors when comparing obese patients (BMI ≥ 30 kg/m^2) with healthy and overweight patients (BMI < 30 kg/m^2) (**appendix 4**).

PDGF treatment led to a 3-fold lower expression of the osteoblastic marker *ALP* and 4-fold higher expression of key adipose marker *PPAR-γ* ($p < 0.05$) compared to the untreated control. Expression of tenocyte-related mark-

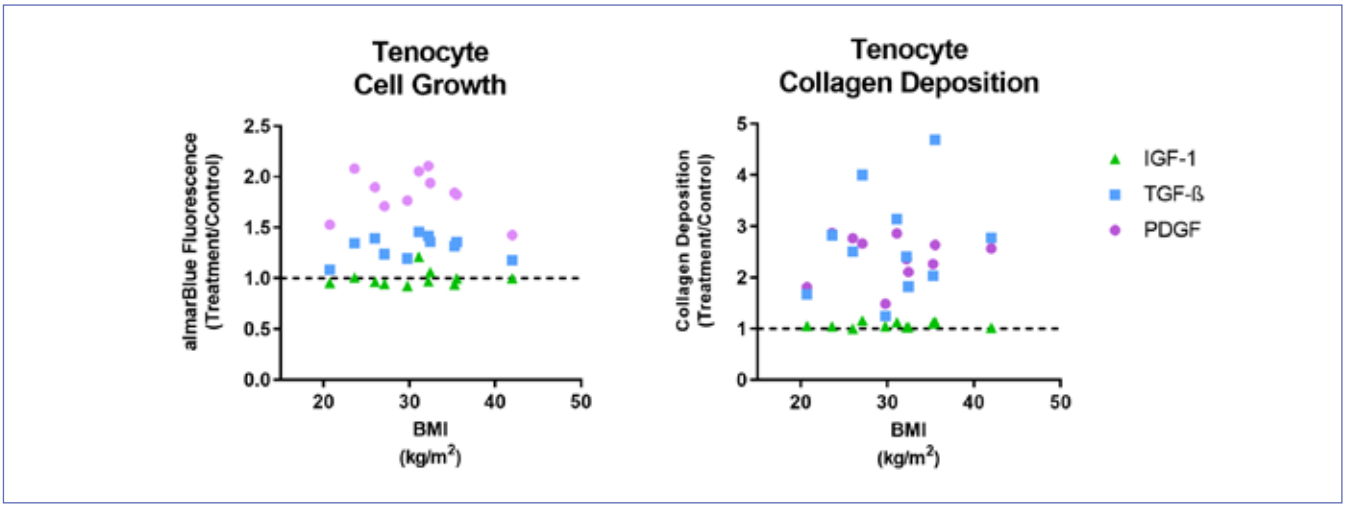

Figure 3. The correlation between BMI with tenocyte cell growth and tenocyte collagen deposition of tenocytes treated with IGF-1, TGF-β and PDGF tenocytes over 72 hours.

Table II. The correlation analyses between BMI with tenocyte cell growth and tenocyte collagen deposition of tenocytes in response to IGF-1, TGF-β and PDGF over 72 hours.

	R Squared	P-value
Tenocyte Cell Growth		
IGF-1	0.020	0.68
TGF-β	0.023	0.66
PDGF	0.034	0.59
Tenocyte Collagen Deposition		
IGF-1	0.002	0.91
TGF-β	0.041	0.55
PDGF	0.011	0.76

ers scleraxis, collagen Iα1 and chrondrocytic marker *SOX-9* were not significantly different. Tenocytes treated with TGF-β had 3-fold higher expression levels of collagen Iα, 50-fold higher expression of scleraxis, and 4-fold high expression of chondrocyte-related *SOX-9* compared to untreated cells ($p < 0.05$). There was also 5-fold lower expression levels of the osteoblast-related *ALP* ($p < 0.05$). IGF-1 treatment did not result in any significant changes in gene expression levels (**appendix 5**). Correlation analyses were also performed between patient BMI and tenocyte gene expression markers in response to each growth factor. No significant correlations were found after a FDR adjustment of P-values was performed (**figure 4**, **table III**). There were no statistical differences in gene expression between patients with BMI < 30 kg/m² and with BMI ≥ 30 kg/m² (**appendix 6**).

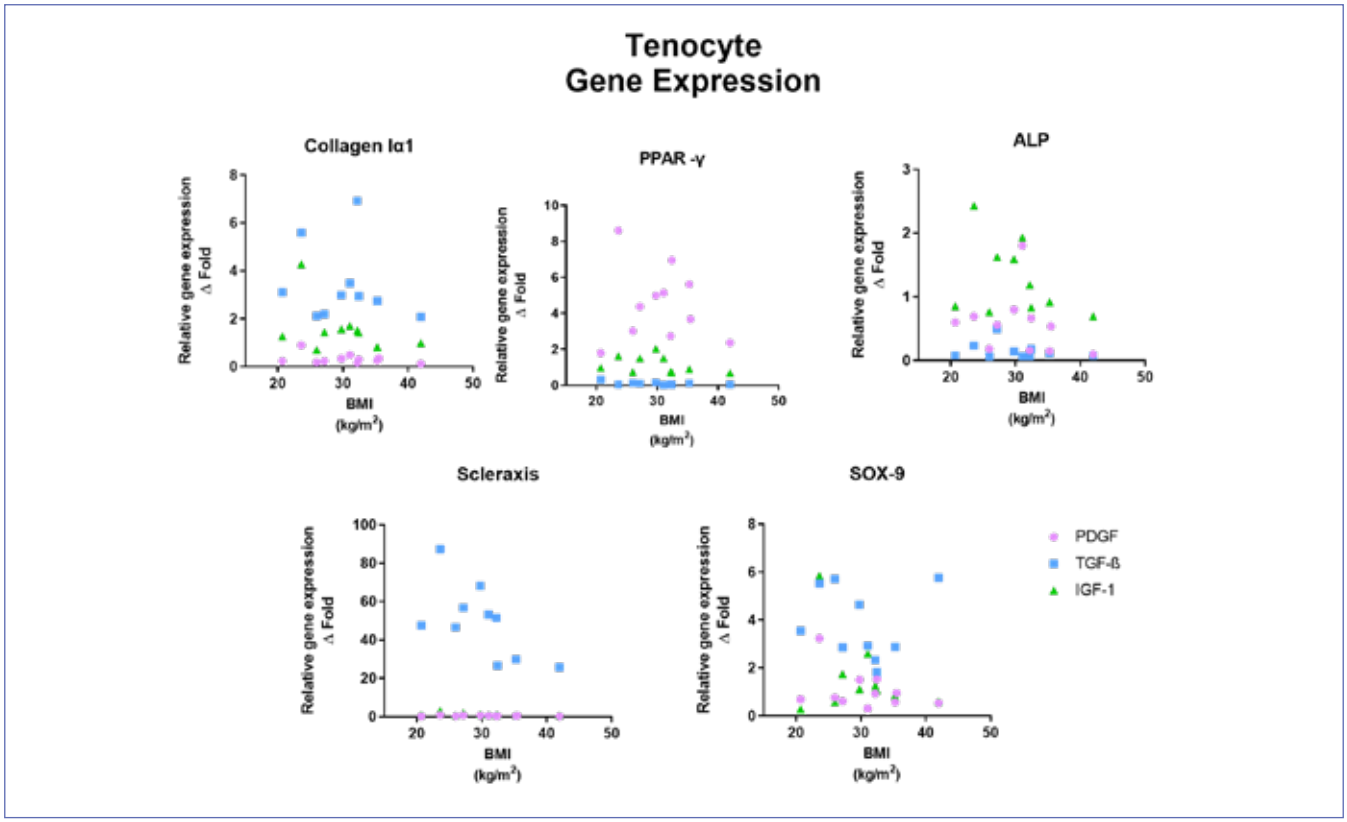

Figure 4. The correlation between BMI with gene expression markers (collagen Iα1, *PPAR-γ*, *ALP*, scleraxis and *SOX-9*) in response to IGF-1, TGF-β and PDGF over 72 hours.

Table III. The correlation analysis between BMI with gene expression markers (collagen Iα1, *PPAR-γ*, *ALP*, scleraxis and *SOX-9*) in response to IGF-1, TGF-β and PDGF over 72 hours.

Gene expression	R Squared	P-value	FDR-adjusted P-values
Collagen Iα1			
IGF-1	0.156	0.26	0.55
TGF-β	0.030	0.63	0.73
PDGF	0.141	0.26	0.55
PPAR-γ			
IGF-1	0.127	0.31	0.55
TGF-β	0.309	0.10	0.55
PDGF	0.018	0.70	0.75
ALP			
IGF-1	0.136	0.29	0.55
TGF-β	0.061	0.49	0.62
PDGF	0.057	0.48	0.62
SCX			
IGF-1	0.222	0.17	0.55
TGF-β	0.404	0.05	0.55
PDGF	0.105	0.33	0.55
SOX-9			
IGF-1	0.098	0.38	0.57
TGF-β	0.003	0.89	0.89
PDGF	0.126	0.28	0.55

DISCUSSION

Contrary to our hypothesis, here we have demonstrated that patient BMI has no direct effect on the characteristics of tenocytes derived from torn supraspinatus tendons, with no significant changes in cellular behaviour, gene expression or response to growth factor treatment. These findings have significant clinical implications, as it implies that growth factor treatment will be effective in patients regardless of their BMI.

Globally, there is an increasing trend in obesity prevalence that poses high healthcare costs, morbidity and societal burden (34). Overweight and obese individuals represent over a third of the worldwide population and are projected to make up over 60% by 2030 (35, 36). Recently a systematic review found that obese patients were at a 2-fold greater risk of having rotator cuff tears and 3-fold greater risk of re-tear after rotator cuff surgery (13).

The cellular mechanisms that underlie the relationship between obesity and rotator cuff tendinopathy are not well characterised (13). One explanation is that adipose tissue releases pro-inflammatory cytokines and hormones that influence activities in tenocytes, which directly modify the tendon healing response (37). Because many of these bioactive peptides secreted by adipose tissue increase in a near-linear fashion to body fat (38), we speculated that patient BMI may be a significant factor in altering tenocyte behaviour and gene expression. High circulating concentrations of adipokines may be acting as a prolonged disruptor of tendon healing in obese patients (14, 15, 25, 39). A recent animal study reported leptin levels, an adipokine, were negatively correlated with load to failure and worse histological structure of the repaired tendon-bone interface after rotator cuff surgery (40). However, in this current study, no significant correlation was found between patient BMI and tenocyte growth, collagen production or gene expression profile. Previous studies have demonstrated that tenocytes isolated from distinct tissue have a distinct cellular profile and decreased capacity to produce ECM components compared to healthy cells (41–43). They also respond differently than healthy cells to growth factor treatments, including TGF-β1 and BMP-2 (41, 44). We hypothesise that the disease stage-specific expression of fibrotic mediators may have a greater effect on cellular function than the comparatively minor effects of obesity, which may have masked any influence of BMI on tenocyte function and gene expression in this study. Further studies should investigate the effects of

BMI in tenocytes derived from healthy tendon and earlier stages of tendon disease.

Growth factors are an area of active research, and several different growth factors have shown success *in vitro* improving tendon healing including, IGF-1 (45), TGFβ (46, 47) and PDGF (48–50). Tendon healing involves the production and release of multiple growth factors at various phases of healing. The roles of specific growth factors typically work synergistically with other signalling molecules to increase cellularity and promote regeneration (21, 51, 52).

PDGF has been widely studied for improving tendon healing. Previous *in vitro* studies have yielded positive results with potent increases in tenocyte proliferation and collagen production (50,53). The effects of PDGF in animals models of tendon healing have been varied and inconsistent (48–50, 53, 54). In this present study, treatment of tenocytes with PDGF resulted in lower the expression of the osteoblastic marker *ALP* and higher expression of adipose marker *PPAR*-γ. Similar changes have also been reported in previous studies (31, 50). In diseased tendons, PDGF expression is increased and associated with hypercellularity (55). These findings suggest that PDGF may be promoting de-differentiation away from a tenocytic phenotype, and PDGF may be less suitable for improving tendon healing outcomes.

In previous *in vitro* studies, TGF-β has been demonstrated to promote the formation of fibrous tissue through the stimulation of collagen synthesis, regulation of matrix metalloproteinases and proliferation of fibroblasts (56–58). In this study, we found higher tenocyte growth and collagen deposition with TGF-β that was associated with higher expression of tenocyte-related genes, collagen Iα and scleraxis. These findings suggest that TGF-β may be tilting the balance of gene expression, favouring collagen and ECM synthesis. Interestingly, there was also a higher expression of chondrocyte-related *SOX-9*, suggesting TGF- β treatment may be favouring a chondrocytic response, which has been observed in previous studies (31, 59, 60).

IGF-1 did not influence tenocyte growth, collagen synthesis or gene expression. Previous *in vitro* and *in vivo* studies have shown IGF-1 increases cell proliferation and collagen synthesis in tendon (61–64). IGF-1 has structural similarities to insulin, allowing it to bind to insulin receptors and is involved in the anabolic response of tendons to loading (65). These differences between our research findings and those previously published could be the result of variations in culture conditions that can affect cell responses and that tenocytes were derived from different tendon types.

This study does have several limitations. Although we used clinically relevant samples harvested from supraspinatus tendons of patients undergoing rotator cuff surgery, there are inevitably confounding variables related to age, the severity of rotator cuff pathology and environmental exposures. In addition, these findings are limited in scope to only adult patients and patients were excluded if they had previous shoulder surgery; or had rheumatoid arthritis or other systemic inflammatory diseases.The sample size was also relatively small and did not allow the adjustment for these confounders, which may add to the impact of subjective variation. In this study, we also only had five participants with BMI under 30 kg/m^2 making it possible that we have underestimated the effect of obesity as most participants were obese (BMI ≥ 30 kg/m^2) (27).

CONCLUSIONS

This is the first study to investigate the effects of patient BMI on human tenocyte behaviour and response to growth factor treatment. In summary, we found no direct correlation between BMI and tenocyte growth, collagen synthesis or gene expression profile. These findings have significant clinical implications. They suggest that growth factor treatment for improving tendon-bone healing will be equally effective in non-obese and obese patients and can potentially overcome any deleterious effects occurring from obesity.

FUNDINGS

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DATA AVAILABILITY

Data are available under reasonable request to the corresponding author.

CONTRIBUTIONS

SMB, JTM, DSM: study conceptualization and design, data interpretation, article drafting. SMB, SK, YEP, DF, BC: data acquisition. ND: ethics required for the acquisition of data. All authors: revision of the manuscript and approval of the submitted manuscript.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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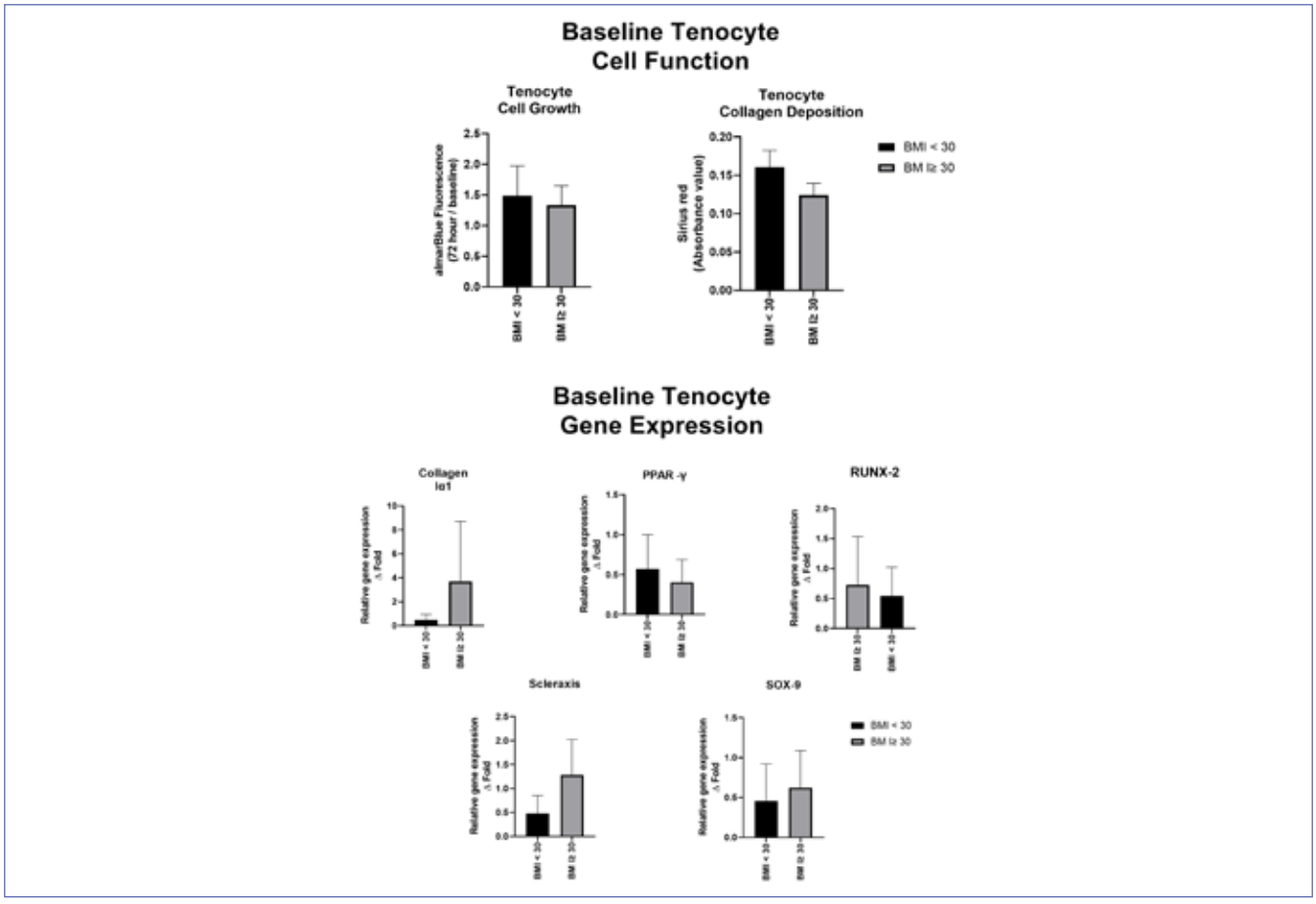
SUPPLEMENTS

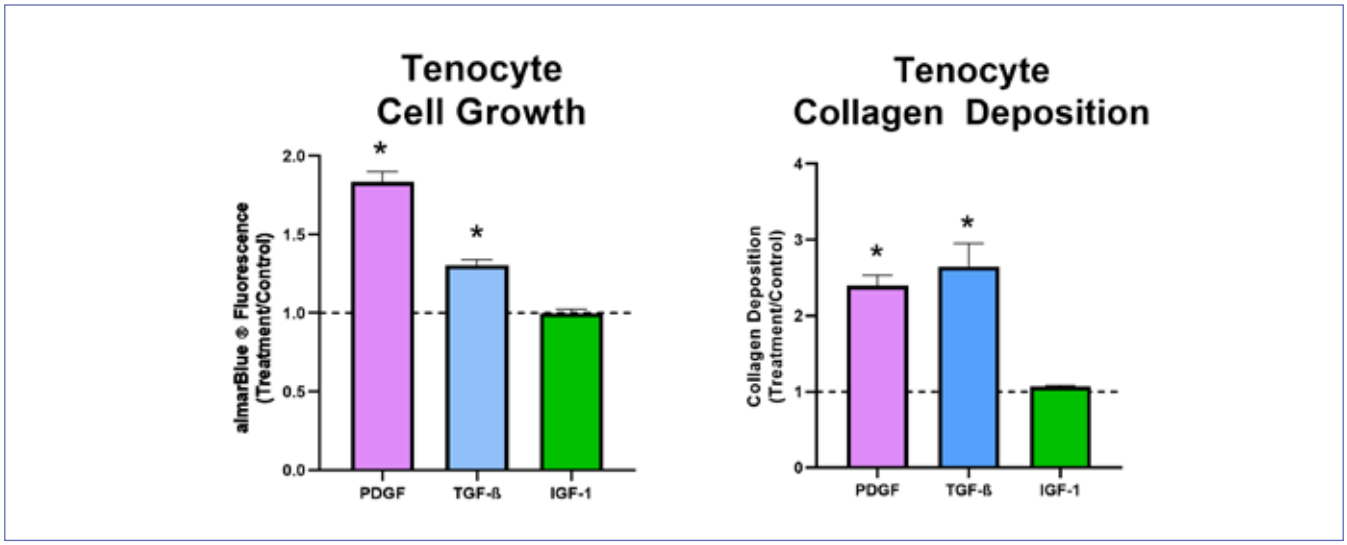
Appendix 1. Individual patient demographics, biomorphic measurements, and supraspinatus tear size.

Gender	Age	BMI (kg/m²)	Ethnicity	Tear size
Female	73	20.7	European	Medium
Female	64	23.6	Asian	Medium
Male	63	26	European	Medium
Female	74	27.1	European	Small
Male	54	29.7	Māori	Medium
Male	63	31.1	European	Medium
Male	37	32.2	Pacific Island	Small
Male	64	32.4	European	Medium
Female	55	33.2	European	Large
Male	46	34.8	European	Large
Female	61	35.3	European	Large
Male	60	35.5	European	Large
Male	54	42.0	Māori	Massive

Appendix 2. Comparison in baseline tenocyte cell function and gene expression between patients with BMI under 30 and BMI over 30.

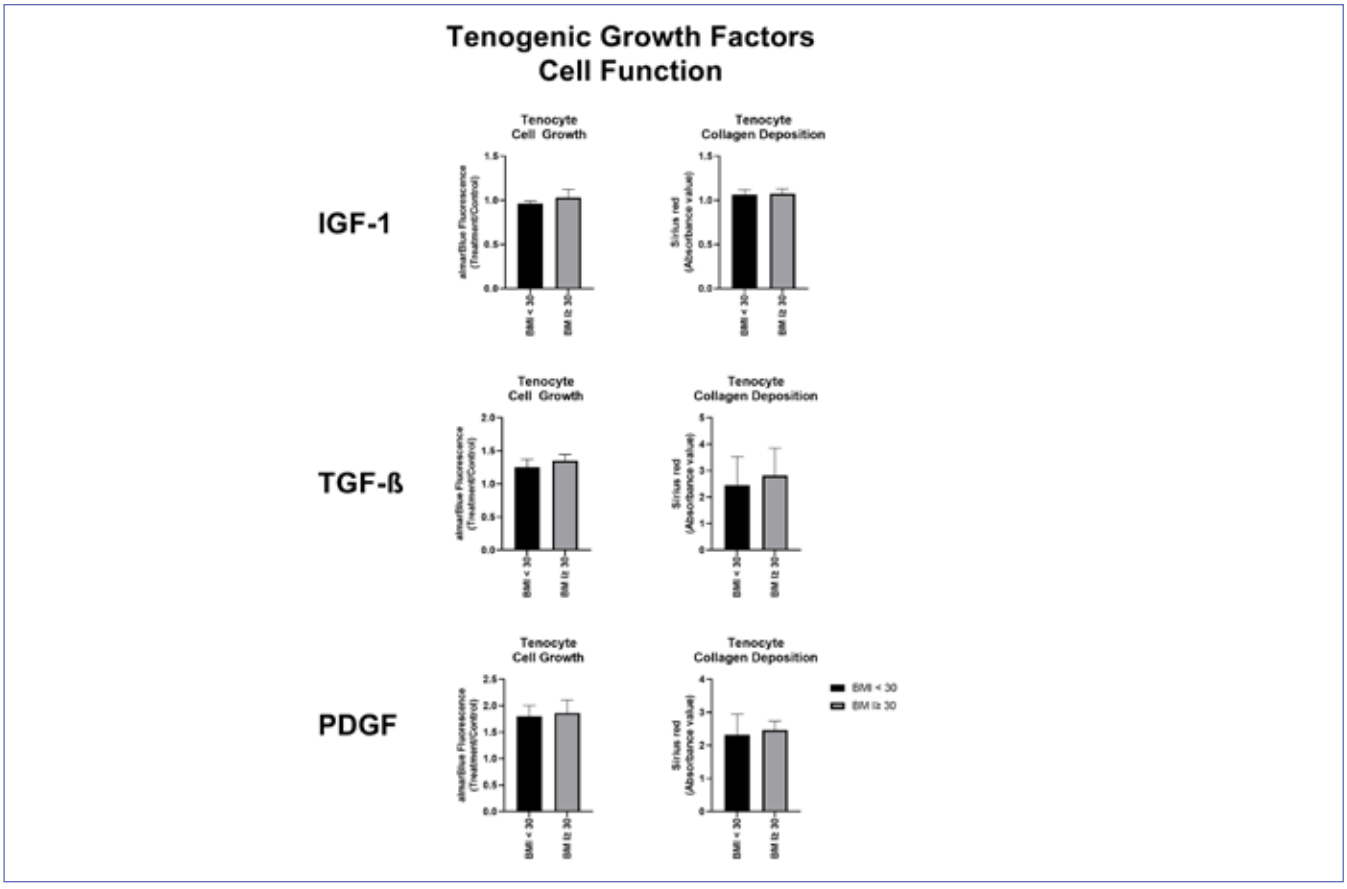


Appendix 3. Effects of the growth factors PDGF, TGF-β on IGF-1 tenocyte cell growth, determined by alamarBlue assay, and tenocyte collagen production, determined by Sirius red assay.

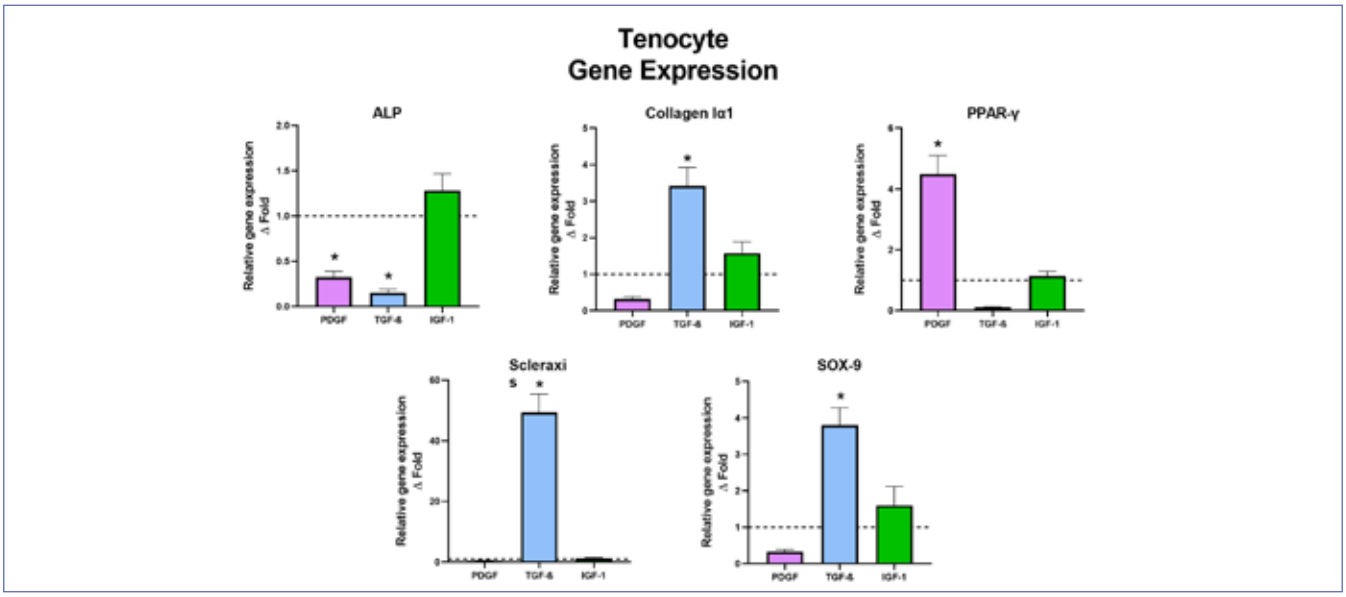


Data are presented as the mean ratio of control ± SEM. *: significantly different from control ($p < 0.05$).

Appendix 4. Comparison in tenocyte cell function in response to TGF-β on IGF-1 tenocyte cell growth and collagen production between patients with BMI under 30 and BMI over 30.

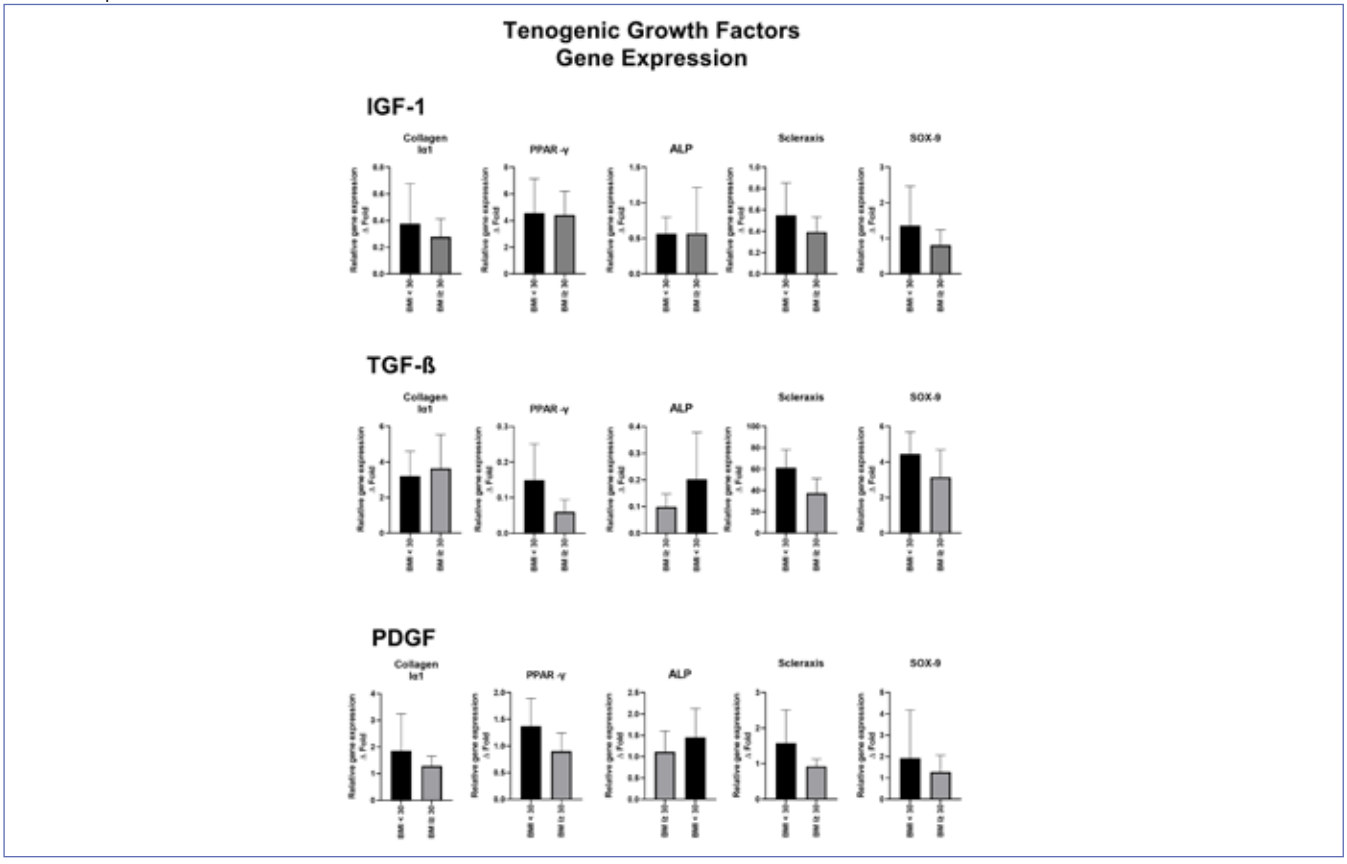


Appendix 5. Effects of growth factors PDGF, TGF-β and IGF-1 on tenocyte gene expression.



Data are presented as means ± SEM. *: significantly different from control ($p < 0.05$).

Appendix 6. Comparison in gene expression in response to TGF-β on IGF-1 tenocyte cell growth and collagen production between patients with BMI under 30 and BMI over 30.



ORIGINAL ARTICLE

Nr 2022;12 (3):303-307

Identifying the Upper Subscapular Nerve as a Target for Chronic Shoulder Pain

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LEVEL OF EVIDENCE: 5

SUMMARY

Introduction. Shoulder pain is one of the most common etiologies of chronic pain representing roughly 16% of all presenting patients (1). The branches of the subscapular nerve have been suggested as contributing to the sensory innervation of the shoulder and may have clinical value as a therapeutic target for nerve block, ablation, and stimulation.

Methods. Six formalin embalmed willed body donors were used, reflecting twelve shoulders for study. Careful dissection was performed to identify the upper and lower subscapular nerves and respective branches as they approached the shoulder joint. After placing the cadaver in anatomical position, measurements were taken from two landmarks: 1) just proximal to the immediate branch from the upper subscapular nerve (USN); and 2) the medial aspect of the coracoid process (CP). The distance from the medial aspect of the CP directly to USN just proximal to its first branch was taken using a digital caliper in millimeters (**table I**).

Results. The USN sends articular branches that directly innervate the anterior glenohumeral joint (GHJ). The average distance from the medial aspect of the CP to the USN just prior to its branches was 3.76 cm ± 0.62 cm. The average distance from the medial aspect of the CP to an intersecting perpendicular line drawn directly superior from the USN just proximal to its first branch was 0.94 cm ± 0.03 cm.

Conclusions. Our findings of the USN and LSN add to prior quantitative neuroanatomical relationships of these nerves and potential targets for therapeutic intervention.

KEY WORDS

Pain; subscapular nerve; intervention; chronic pain; therapy.

INTRODUCTION

Of the patients presenting with chronic musculoskeletal pain, chronic shoulder pain represents roughly 16% of the patient population (1). The direct and indirect costs of chronic shoulder pain have been estimated to be roughly 7 billion dollars and include the direct cost of medical management in addition to the estimated opportunity cost of reduced functionality (2, 3). Etiologies of chronic shoulder pain are numerous and diverse. These include rotator cuff tendinopathy or tear, sub-acromial bursitis, osteoarthritis, adhesive capsulitis, and nerve impingement (1, 3, 4). Many nerves have been found to innervate the shoulder joint and include the upper and lower subscapular nerves as well as the supra-scapular, axillary, and lateral pectoral nerves (7). Numerous techniques have been employed for the management of chronic shoulder pain including nerve radiofrequency ablation (RFA), intra-articular joint injection therapy, and bursal injection therapy. Understanding

the location of articular nerves which supply the gleno-humeral joint (GHJ) could have therapeutic benefit and allow for more approximate interventional targeting (7).

The upper and lower subscapular nerves most commonly originate from the posterior cord of the brachial plexus. The upper subscapular nerve (USN) and lower subscapular nerve (LSN) insert proximal to the myo-tendinous junction of the subscapularis muscle to provide motor innervation (9), after which sub-muscular (10) articular branches emerge. The USN has been found to produce multiple articular branches (USNAb) that traverse or pierce the superior border of the subscapularis muscle beneath the coracoid process, then dive deep to the muscle to innervate the anterior-superior GHJ capsule (10, 11).

Although the use of regional anesthesia for shoulder pain has been reported for use during the peri-operative period, there has been limited data discussing the use of such techniques in the chronic shoulder pain population. In the article, "Suprascapular nerve block in chronic shoulder pain: are the radiologists better," authors Shanahan and Ahern discuss the use of a single shot injection of methylprednisolone ± the inclusion of bupivacaine around the supra-scapular nerve (13). Although their results demonstrate significant pain improvement scores in all of their selected participants, a supra-scapular block does not include the articular branches from the anterior-medial portion of the shoulder and thus the block may not be as comprehensive in its nerve inclusion. That being said, nerve blocks targeting both the subscapular nerve as well as the suprascapular nerve in combination have already begun to be implemented in the clinical setting. Within the article, "Effectiveness of new nerve blocks method on the articular branches of the suprascapular and subscapular nerve to treat shoulder pain" authors Lee *et al*. performed a study whereby 52 patients with chronic shoulder pain underwent a combined suprascapular and subscapular nerve block. All of the included patients reported reduced disability index scores which persisted for greater than 6 months in much of the patient population. This demonstrates that inclusion of articular branches from the anterior aspect of the shoulder play a significant role in patient improvement and should be included during interventional management of chronic shoulder pain (12).

The USN and LSN innervate the superior and inferior portions of the subscapularis muscle, respectively; therefore damage to the nerve can have debilitating effects during medial rotation of the shoulder joint (9). Interventional methods such as RFA require understanding of the likely motor portion of the USN and LSN as well as other nearby critical structures. We aimed to quantify the location of the USN and LSN relative to anatomic landmarks to advance locational understanding of these nerves as a potential targets for regional anesthesia and interventional shoulder pain management.

METHODS

Cadavers

Six formalin embalmed willed body donors were used, reflecting twelve shoulders for study. Three of the cadavers were men and three were women. Specimens were embalmed using a 2.5% Formaldehyde, 5% Phenol, and 35% alcohol embalming fluid. The care, handling, storage, dissection, and imaging studies of all body donors were performed in compliance with the rules and regulations of the State Anatomical Board of the State of Texas and the policies, procedures, and ethical guidelines of the University of Texas Health Science Center at San Antonio's Body Donation Program.

Dissection

Each shoulder was carefully removed from the preservative fluid. The skin, adipose, and connective tissue of the antecubital fossa was removed with dissection proceeding proximally toward the posterior cord of the brachial plexus. The anterior-medial aspect of the deltoid and the lateral aspect of the pectoralis major and minor were reflected in opposing directions to expose the axilla and its underlying structures. Relevant vascular structures such as the brachial artery, axillary artery, and subclavian artery were

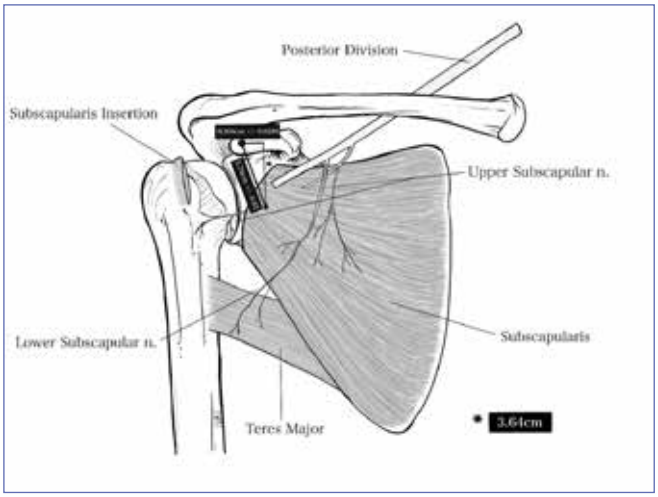

Figure 1. Visual representation of the cadaveric dissection with labeling of the USN, LSN, and relevant musculoskeletal structures. The USN sends glenohumeral articular branches laterally distal and deep to its insertion into the subscapularis muscle. On average, this point was 3.64 cm inferior to the medial border of the coracoid process.

dissected from adipose while the individual components of the brachial plexus were identified. Once the posterior cord was found, the three most immediate branches were traced, namely the LSN (**figure 1 B**), thoracodorsal nerve, and USN (**figure 1 A**). Occasionally the LSN branch branched from the axillary nerve. Care was taken to identify the end targets of the individual branches of the posterior cord to confirm their identity. The nerves were traversed towards their expected location as a form of confirmation.

Once the nerves were identified, they were individually tagged. A metal pin was used to tag the USN immediately proximal to the first glenohumeral articular branch. After placing the cadaver in anatomical position, measurements were taken from two landmarks: 1) just proximal to the immediate branch of the USN; and 2) the medial aspect of the coracoid process (CP). The distance from the medial aspect of the CP directly to USN just proximal to its first branch was taken using digital caliper in millimeters (**table I**). Measurements from the medial aspect of the CP were taken in the transverse plane to an intersecting perpendicular line drawn directly superior from the USN just proximal to its first branch (**table II**). Fluoroscopic imaging was additionally employed to evaluate the relationship of the tagged upper subscapular nerve, just before its bifurcation into the shoulder (metal pin), to bony landmarks (**figure 2**).

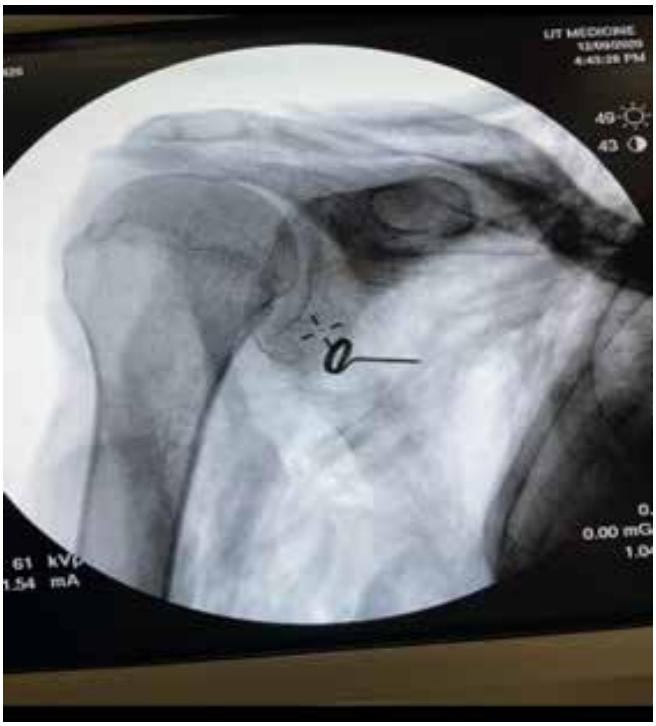

Figure 2. C-arm X-ray Image of the right shoulder with pinpoint identification of the upper subscapular nerve. A total of 12 shoulders (6 of the right shoulder, 6 of the left) were taken to provide radiographic insight into the location of the upper subscapular nerve.

Table I. Measurements from medial aspect of the coracoid process to the Upper subscapular nerve just proximal to glenohumeral articular branches.

	Right	Left
Cadaver 1	27 mm	45 mm
Cadaver 2	23 mm	39 mm
Cadaver 3	35 mm	62 mm
Cadaver 4	46 mm	37 mm
Cadaver 5	35 mm	25 mm
Cadaver 6	49 mm	28 mm

Table II. Measurement from the medial aspect of the coracoid process to an intersecting perpendicular line drawn directly superior from the Upper subscapular nerve just proximal to its first branch.

	Right	Left
Cadaver 1	8 mm	7 mm
Cadaver 2	10 mm	6 mm
Cadaver 3	20 mm	18 mm
Cadaver 4	5 mm	6 mm
Cadaver 5	0 mm	10 mm
Cadaver 6	10 mm	13 mm

Figure 3. USN Identification.

Figure 4. The lower subscapular nerve identified proximally to the bifurcation of the axillary and radial nerve.

RESULTS

Relationships are summarized in **figure 3**. The USN send articular branches that directly innervate the anterior glenohumeral joint (GHJ). The average distance from the medial aspect of the CP to the USN just prior to its branches was 3.76 cm ± 0.62 cm. The average distance from the medial aspect of the CP to an intersecting perpendicular line drawn directly superior from the USN just proximal to its first branch was 0.94 cm ± 0.03 cm. In our specimens we did not find articular contribution to the GHJ from the LSN (**figure 4)**.

DISCUSSION

Identification of anatomic landmarks to be used as neuroanatomical targets for shoulder pain is continuing to improve. Our findings of the USN and LSN add to prior quantitative neuroanatomical relationships of these nerves (6, 9). The USN appears to be more promising than the LSN as an interventional target for shoulder pain as it consistently innervates the GHJ while the LSN does not. A recent review (7) postulated that the USN articular branches may be targeted in a zone near the base of the coracoid process at anterior, superior portion of the glenoid neck based on analysis of limited available prior anatomic studies. However, we found the point of origin of articular branches of the USN to be over 3.5 cm inferior to the coracoid process, and generally overlying the inferior one-half of the glenoid. Based on our findings, fibers of the USN may not be present near the base of the coracoid process nor superior glenoid, suggesting a more inferior nerve branch target than was suspected prior. While the proposed anterior upper glenoid zone may have clinical utility, further study is needed to identify a safe zone that better incorporates articular branches of the USN specifically to better comprehensively incorporate nerves from the anterior shoulder which may be contributing to the patients' symptomatology.

We would propose, for the purposes of nerve block, a location just medial to the glenohumeral joint line, approximately 3.5 cm inferior to the base of the coracoid process, as a theoretical target for USN articular branches. Subscapular nerve blocks in this region have potential clinical benefit for acute analgesia of the anterior shoulder (9). This can supplement other known peripheral nerve blocks (suprascapular, axillary) for providing analgesia of the shoulder with less risk of phrenic nerve block than interscalene plexus block. Ultrasound guidance affords the identification of critical neurovascular structures during block performance. Nerve ablation may present potential safety challenges, namely that targeting the USN may require trajectories and end-points

in very close proximity to the brachial plexus and to the axillary artery. Further anatomic and clinical studies are needed to validate the concept of USN as a safe and effective target for chronic shoulder pain and for perioperative shoulder analgesia.

CONCLUSIONS

Shoulder pain is one of the most common etiologies of chronic pain and represents roughly 16% of all presenting patients. Indirect costs of chronic shoulder pain are significant and play a major role in reduced quality of life for patients. Moreover, direct costs are also substantial and have been measured on a scale of billions of dollars in medical expenses and lost compensation and productivity. The upper subscapular nerve has been postulated to contribute sensory branches to anterior-superior portion of the glenoid neck. Many cadaveric studies have been performed to better understand the articular branches of the shoulder joint, however our cadaveric studies suggest that the articular branch targets of the anterior shoulder may be more inferior-medial than previously described in the literature.

Through our anatomic dissections, it was evident that the articular branches of the upper subscapular nerve were in actuality inferior to the coracoid process and overlaying the inferior one-half of the glenoid. The newly identified location of the articular branches of the upper subscapular nerve will help re-direct physicians performing interventions on the shoulder for chronic shoulder pain

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None.

DATA AVAILABILITY

Data are available under reasonable request to the corresponding author.

CONTRIBUTIONS

KB: study design. KB, JF: anatomic dissection, writing manuscript. SP, OR: anatomic dissection. KH: anatomic artistry. MMcC: study design. ME: study design, anatomic dissection, writing manuscript, primary investigator.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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REVIEW

Nr 2022;12 (3):308-320

Snapping of the Upper Limb: a Clinical Overview

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LEVEL OF EVIDENCE: 5

SUMMARY
Snapping results from an abrupt displacement of an anatomic or pathologic structure during the movement of a closely related joint. Snaps are audible, palpable, and often, visible. Snapping phenomena are common within the general population and, in most cases, are associated with mild symptoms. However, especially in athletes, snapping could determine pain and functional impairment that may severely affect their sport activities. We focus on three major types of snapping occurring at specific joints of the upper limbs: the shoulder, the elbow, and the wrist. Here, we provide a comprehensive overview of major advances in the aetiology, pathophysiology, diagnostic imaging, and treatments of these specific snapping phenomena.

KEY WORDS
Snapping; upper limb; shoulder; elbow; wrist; pathophysiology; diagnosis; treatment.

INTRODUCTION

Snapping is an audible, palpable and often, a visible phenomenon, resulting from the sudden and abnormal displacement of an anatomic or pathologic structure during the movement of a neighboring joint (1-4). Usually, snapping causes mild symptoms and may not require medical treatment. When it is associated with severe pain and functional impairments, leading to significant reduction in sports and professional activities, surgical management may be necessary (4-6). Snapping phenomena have been reported in various regions of the body, especially in close proximity to joints characterized by a wide range of motion, which allows an anatomical or heterotypic structure to interact with its surrounding environment (1, 5, 6). Snapping phenomena may affect bony structures resulting in the so-called "joint instability". Repeated snaps in the field of joint instability may result from torn ligaments, a situation that is not uncommon in the knee and the wrist (1, 2). In other cases, snaps involve a large range of soft tissue structures, which can be ligamentous, tendinous or fibrocartilaginous (1, 4). Therefore, snaps can occur at intra-articular or extra-articular locations (1, 4). Snapping in the shoulder region has previously been reported secondary to "grating" of the scapula caused by impingement between the medial border of the scapula and the adjacent ribs (3, 5). Extra-articular forms of elbow snapping are commonly related to anterior dislocation of the ulnar nerve or the distal end of the medial triceps above the medial epicondyle. When intra-articular, snapping of the elbow may be related to the synovial fringe capsule-synovial layer at the junction between the radial collateral ligament and the annular ligament (4).

Snapping sensation in the wrist has been traditionally thought to be due to underlying carpal instability or tendon instability such as snapping of the extensor carpi ulnaris secondary to a subsheath tear (2, 6).

Identifying the structures responsible for the snap and their underlying pathology could be clinically challenging considering that different sites and multiple causes may contribute to the snapping phenomenon. Diagnosis

is based on both clinical assessment and imaging. As we shall see later, imaging may require different exams, due to the functional characteristics of this condition. Indeed, advanced imaging techniques can provide a better understanding of the snapping mechanism together with an image-guided diagnostic and therapeutic algorithm to improve its management.

SNAPPING OF THE SHOULDER

Definition and clinical signs

Chronic shoulder instability is often accompanied by joint noises. The snapping shoulder includes intra- and extra-articular forms. The intra-articular forms associated with loose bodies are poorly documented while several different extra-articular causes of snapping are reported. In particular, the definition "snapping scapula" is extremely frequent. This condition is related to the scapula-thoracic joint, following the impingement between the medial edge of the scapula and the poster-medial region of the rib cage (3).
The snapping scapula syndrome is usually characterized by a loud popping or cracking sound, known as crepitus, which occurs when the arm is raised up overhead. The sound is due to the rubbing of soft tissue between the scapula and the thoracic wall. The tissue caught between these two structures could be a bursa, tendon, or muscle (3). Medial scapular border tenderness is also a commonly reported clinical sign (7). Patients may or may not experience pain while moving the arm (5). Asymptomatic patients commonly reporting scapulothoracic crepitus alone do not necessitate any treatment at all (8).

Aetiology

The most common causes are related to the process of exostoses (**figure 1**), soft tissue sarcomas, chronic bursitis of the scapula-thoracic joint, congenital bone anomalies (*i.e.*, Sprengel deformity or congenital high scapula), or the presence of the Luschka tubercle (pathological hypertrophy of the super-medial angle of the scapula) (9). An anatomical variation, which is one of the main causes of the syndrome, is an anomalous anterior curvature of the superomedial angle of the scapula. The measurement of the scapula superomedial angle ranges from 124° to 162° (mean 144.34° ± 9.09) in normal anatomical specimens. When this angle is lower than 142°, the chance of developing scapular snapping increases (10, 11).
Scapular snapping can also be associated with scapular dyskinesia, which can have articular, musculoskeletal, and neurological causes (12). Regardless of the original condition causing dyskinesia, the snapping of the scapula is generated when abnormal movement brings the extremities of the scapula into closer proximity to the rib cage, leading to a scapula in pronation, which is not conducive to optimal shoulder function and results in subacromial space reduction with symptoms of impingement (9).
Other common causes include incorrect posture and incorrect training techniques during sport activities (overtraining or training before strengthening). Recurrent fractures and injuries of the scapula and rib cage can also cause bone deformities, which can increase the friction among the structures of the scapulothoracic joints (13).

Diagnosis

Clinical diagnosis heavily relies on physical examination, which is also crucial in informing following steps in the diagnostic process. Physical examination is supplemented by advanced imaging such as magnetic resonance imaging (MRI) and/or computed tomography (CT) to assess for potential bony or soft tissue aetiologies of snapping scapula (9, 14).
Physical examination should evaluate for spinal deformities, palpable crepitus, point tenderness, and scapular winging. It is worth mentioning that kyphoscoliosis, which decreases scapulothoracic congruity, can cause snapping scapula. Symmetry should be assessed to rule out periscapular muscle atrophy. Neurological assessment is also essential to rule out referred pain. Scapular winging is a common presentation in patients with scapulothoracic bursitis or snapping scapula, which can occur from long thoracic nerve injury and dysfunction of the serratus anterior muscles (5, 8, 15, 16).
Although the diagnosis of snapping scapula based on appropriate clinical assessment is reliable, the determination of the underlying aetiology may require further imaging. While plain film radiographs are the traditional first choice because of their ease of access and low associated morbid-

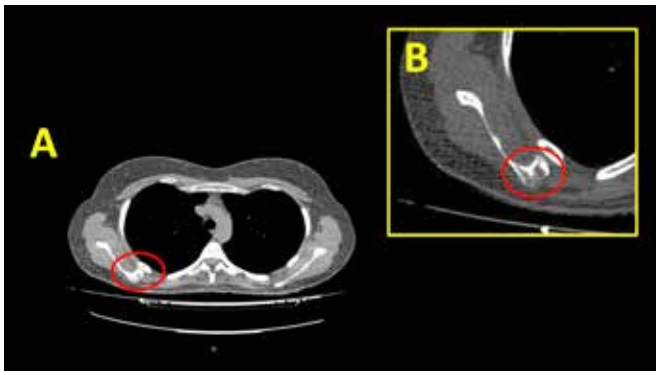

Figure 1. Right shoulder snapping in patient with scapula exostosis. **(A)** An axial CT scan of the right shoulder joint showing the exostosis circled in red. **(B)** Enlarged detail of the exostosis.

ity, Mozes *et al*. (11) reported them unreliable for definite diagnosis with only 26.9% detection of scapular bony incongruity compared with 70% detection using CT and 100% detection achieved by 3D-CT. CT, with or without 3D optimization, appears to be beneficial in further characterizing space-occupying skeletal incongruity after plain film detection. However, 3D-CT has demonstrated poor correlation to clinical findings in the setting of non-skeletal aetiologies of snapping scapula, such as scapulothoracic bursitis (8). This, together with additional limitations such as radiation exposure and costs, make CT imaging unsuitable for routine diagnoses of snapping scapula.

MRI, by providing an accurate outline of the nature and heterogeneity of soft tissue lesions, remains the most useful diagnostic method in detecting soft tissue aetiologies of snapping scapula (5). In particular, the use of MRI is recommended in investigating scapulothoracic soft tissue and space-occupying lesions as potential aetiologies of snapping scapula when nonoperative treatment fails after clinical diagnosis (see below).

Management

Treatment of patients with this syndrome begins with nonoperative methods and when nonoperative treatments fail, several surgical options exist. Nonoperative treatments include rehabilitation exercises, activity modification and pain management. Physiotherapy and rehabilitation are recommended when the offending cause of snapping scapula are altered posture, scapular winging, or scapulothoracic dyskinesis (17). Scapular malposition can lead to abnormal force distribution throughout the shoulder joint resulting in abnormal shoulder kinematics and problems with motion (18). Controlled scapular position on the thorax is essential for optimal shoulder function, providing maximum force to the rotator cuff muscles while contracting (19). The direction of the rehabilitation plan will depend on factors causing the snapping scapula. Several studies reported improvements in clinical parameters and improved rotator cuff muscle strength after restoration of scapular muscle balance (5).

Corticosteroid injections (CSI) have also proven to be effective as an initial nonoperative treatment, which can be particularly useful as a diagnostic tool differentiating between scapular superomedial angle pathology and scapulothoracic bursitis in patients with superomedial angle pain (20). Extracorporeal shockwave therapy (ESWT) is another nonoperative modality, which has been successfully used in the treatment of snapping scapula bursitis (21). Both ESWT and CSI can be utilized as adjuncts to the rehabilitation program (21).

If nonoperative management is proven ineffective, open or arthroscopic scapular superomedial resection and scapulothoracic bursectomy are the most frequently performed surgical procedures. One study managed snapping scapula with pectoralis minor tendon release, though the long-term effect of such treatment remains to be established (22). Previous studies suggest that surgical treatment should be reserved to patients with symptomatic snapping scapula after a 3-to-6-month period of unsuccessful nonoperative management (22-24).

A recent study compared the effectiveness of open superomedial scapular resection to nonoperative management for milder snapping scapula presentations (25). No significant difference in the management of snapping scapula outcomes between operative and nonoperative modalities was reported; with patients subjected to the operative intervention presenting with more pain at baseline (25). However, this study failed to provide any conclusive evidence regarding a potential superiority of nonoperative *versus* operative management because of its non-randomized nature and possible pre-operative differences in symptom severity between the two groups (25).

Arthroscopic or open scapulothoracic bursectomy is recommended for refractory patients who are symptomatic but do not present scapular skeletal abnormalities on imaging (20). Although arthroscopy offers improved cosmesis and earlier rehabilitation, potential risks associated with this approach include the possibility of injury to neurovascular structures when penetrating the rhomboids, the intraoperative swelling, and the inability to evaluate the potentially pathologic trapezoid bursa (26). The choice of arthroscopic *versus* open or combined procedures largely depends on the surgeon experience and so far, there are no studies directly comparing the two procedures.

In summary, patients presenting with medial scapular border tenderness, palpable crepitus, and audible snapping should prompt high clinical suspicion of snapping scapula. Focused history and physical examination in conjunction with imaging procedures to assess structural aetiologies, when nonoperative management fails, are essential initial steps towards the diagnosis. Nonoperative management of snapping scapula in the form of analgesia, physiotherapy, local CSI and/or ESWT should be initiated for 3 to 6 months before considering surgical management. Open or arthroscopic bursectomy with or without superomedial angle resection, can then be carried out for refractory patients depending on the musculoskeletal pathology presented.

SNAPPING OF THE ELBOW

Definition and classification

Snapping elbow is a rare condition, which is largely associated with more frequent pathologies such as epicondylitis or intra-

cellular free bodies (4, 27). Patients usually seek attention when the snapping becomes associated with pain and/or limited function (4). In some patients, the condition may be triggered by athletic performances (28). However, it is important to emphasize that for most cases, the condition can be unrelated to any sport activities (4). Snapping of the elbow joint is rightly divided into lateral (intra-articular) and medial (extra-articular) snapping as pathology, diagnostic strategy, and treatment of these two conditions are different (**table I**) (4, 29).

Briefly, snapping over the medial humeral epicondyle is caused by dislocation of the ulnar nerve or a part of the triceps tendon and is demonstrated by dynamic ultrasound and by an accurate physical examination (4, 27, 28, 29). The treatment is based on open surgery (30). Lateral snapping over the radial head has an intra-articular pathology: a synovial plica, a torn annular ligament, intra-articular tumor pathologies or a meniscus-like remnant from the fetal elbow (4, 31, 32). Pathology can be visualized by conventional arthrography, magnetic resonance arthrography, MRI and arthroscopy while conventional MRI and radiographs often appear normal (33, 34). Treatments for lateral snapping usually consist in arthroscopic or eventual open resection (4, 35, 36) (**table I**).

Extra-articular (medial) snapping

Cases of snapping more frequently involve extra-articular structures, in particular the ulnar nerve that displaces anteriorly or the tricipital tendon that snaps ventrally to the medial epicondyle during complete elbow flexion. The ulnar nerve usually snaps in the 70°- 90° flexion range, while the triceps tendon at 115° of flexion. Usually, the shot is easily audible and visible. It is not uncommon for the two conditions to coexist as the snap of the triceps favors that of the nerve, producing a double snap on the medial side of the elbow (27-29). Watts and Bain (37) found that out of 17 patients with compression of the ulnar nerve at the elbow, 14 had a pathology associated with snapping of the triceps tendon. Triceps snapping has various causes and is generally attributed to a morphologically anomaly at the distal attachment of the medial head of triceps brachii (38). Some authors (38) attribute this medial dislocation to cubitus varus, either congenital or post-traumatic (39) or an abnormal insertion of the medial head of the triceps brachii (40), or a fourth muscle belly of the triceps brachii that inserts on the medial portion of the olecranon, more distally than the standard triceps brachii insertion (41). Finally, hypertrophied medial head due to working or sport activities can also cause triceps snapping (28).

The diagnosis starts with physical examination. Triceps dislocation can be palpated during elbow flexion-extension movements and, in some cases, can even be visible. The clinical diagnosis is confirmed by a careful selection of imaging modalities. X-ray images are usually normal unless there is a previous trauma or a congenital osseous anomaly. Conventional MRI has limited diagnostic value when performed with extended elbow as in routine investigations. MRI of the elbow in various flexion positions may effectively demonstrate the dislocation of the anatomical structures involved in snapping. Dynamic ultrasound is often used as it allows real-time visualization of the underlying pathology (42).

Conservative treatment is initiated at first and consists in stopping at risk-activities, a course of non-steroidal anti-inflammatory drugs, a brace limiting elbow flexion to 70° and

Table I. Snapping elbow subtypes (medial and lateral) are caused by different pathologies and are clinically different entities.

Snapping of the Elbow	Snapping sites	Diagnosis	Treatment
Medial	Ulnar nerve skipping Medial displacement of the distal triceps Snapping of the brachial muscle	Clinical evaluation Ultrasonography	Reduction of activity Transposition/stabilization of the nerve Resection/suture of tendon
Lateral	Postero-lateral rotator instability Lateral displacement of the distal triceps Snapping annular ligament over the radial head Radio-humeral menisci interposition Synovial plica impingement Inflammatory processes Tumour	Arthrography High resolution MRI Arthroscopy	Arthroscopic/open resection

corticosteroid injections. If the patient continues to experience symptoms despite the treatment, then a surgical solution is attempted (30). Surgical treatment consists of laterally transposing the dislocated medial head of the triceps brachii or, if small, in its removal. If the snapping triceps is due to cubitus varus, a rather rare event, humeral osteotomy must also be performed to realign the elbow (30). Once the snapping triceps has been corrected and depending on the ulnar nerve intraoperative stability and on the results of the electrophysiology examination, possible interventions on this nerve may be necessary. They consist in neurolysis and in subcutaneous or submuscular transposition of the nerve (28). After the surgery, the elbow is immobilized in 90° flexion for one month to control pain while passive motion between 0° and 90° is allowed. After 1 month, active and passive movements are allowed. Outcomes are reported to be very good with a satisfactory return to physical activities (28).

Recently, a fixing and redirection tendon technique has been used to treat a case of snapping lateral triceps. This technique, previously described for repairing rotator cuff tears, is the two-strand-overhand locking (TSOL) knot, which in combination with the double pulley technique provides promising results in the treatment of this type of snapping (43).

Isolated ulnar nerve instability is not a common condition and is usually accompanied by the snapping of the medial head of the triceps muscle over the medial epicondyle. Dislocation usually occurs in the dominant extremity (27). Because of recurring events in the ulnar nerve, instability during elbow flexion and extension is felt like a snap. Repeated trauma can lead to ulnar neuritis (29). The isolated snapping ulnar nerve may be palpated. MRI in the flexion position can show that the ulnar nerve has come out of its groove (44). Dynamic ultrasound can reveal the snapping of the ulnar nerve during elbow movements (44). A recent study using dynamic ultrasound visualization identified a significant prevalence of ulnar nerve displacement in young baseball players, although some forms of nerve subluxation may also be observed in asymptomatic patients (45). In case of ulnar neuropathy, nerve conduction studies and electromyography (EMG) can assess nerve damage but are not useful to identify the underlying aetiology.

Patients with mild pain, without recurrent snapping and not much discomfort in every day-life, may receive conservative treatment (NSAIDs, posterior elbow splints, rest, and physical therapy). However, we should emphasize that non-surgical treatments reduce symptoms but do not eliminate the underlying snapping (46, 47). If there are recurrent snapping and ulnar neuropathy, surgical treatment should be considered. The surgical procedure usually involves ulnar nerve dissection and transposition to the anterior medial epicondyle. Although the nerve can be transferred to either the submuscular or the subcutaneous fixation, submuscular fixation makes the surgery safer (46).

Dincer *et al.* (29) transposed the ulnar nerve from its natural location posterior to the medial epicondyle to an anterior site, under the fascia of the flexor carpi ulnaris muscle. The medial head of the triceps muscle was also elevated and moved laterally. Intraoperative examination revealed the disappearance of the pathology. Surgical procedures such as excision of the triceps medial head, lateral lubrication of the triceps and partial medial epicondylectomy may be necessary if there is also snapping of the triceps (30, 48-52). Dincer *et al.* (29) advised to accompany the transposition of the ulnar nerve with the release or excision of the medial head of the triceps independently of the involvement of the triceps muscle in the snapping. In some cases, after isolated ulnar nerve procedures, snapping persisted and a secondary surgery was made necessary to intervene on the triceps muscle (39, 48, 53). An adequate intraoperative examination may avoid these repeated surgeries.

Rare causes of extra articular pathology include snapping of the triceps on the lateral epicondyle and snapping of the brachialis muscle, a powerful forearm flexor. Snapping brachialis is a rare condition and only a handful number of cases have been reported in the literature (54). Patients with this condition, usually, present with anteromedial elbow swelling, pain and snapping on elbow extension and/or supination (54). Normally, the very medial portion of the brachialis muscle is located medial to the trochlear border during elbow flexion and extension. Snapping brachialis occurs if the medial portion of the muscle dislocates anterolaterally, to lie outside the medial border of the trochlea during elbow flexion. On elbow extension, the tendon returns to its normal position often with a visible and/or audible snap (54). Surgical release or excision of the medial portion of the brachialis muscle responsible for the snapping results in resolution of symptoms (54).

Another, rather rare, occurrence of medial snapping at the elbow joint concerns the medial cutaneous nerve of the forearm, which runs medially to the medial epicondyle. In a case series by Cesmebasi *et al.* (51) dynamic ultrasound demonstrated snapping of the medial cutaneous nerve of the forearm in all four patients included. Patients were surgically treated with nerve transposition, with prompt resolution of symptoms.

Intra-articular (lateral) snapping

The forms of intra-articular snapping are mainly due to the presence of intra-articular loose bodies (**figure 2**), as in synovial chondromatosis or in post-traumatic capsular calcifications (4). Snapping of the annular ligament is an uncommon cause of lateral elbow pain. Slipping of the annular ligament

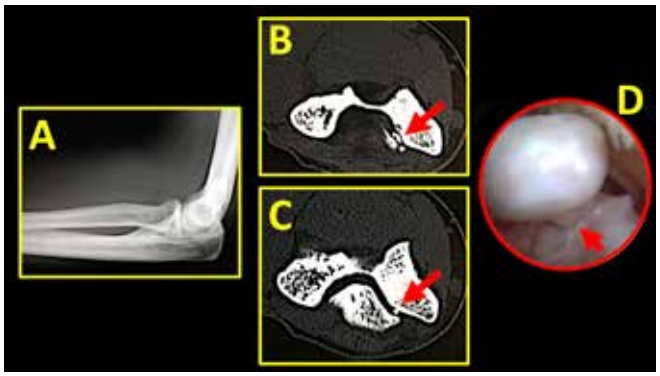

Figure 2. Intra-articular snapping of the elbow due to intra-articular free bodies. Lateral **(A)** elbow radiographs and CT axial images in **(B)** and **(C)** respectively, showing intra-articular free bodies. **(D)** Arthroscopic image of intra-articular free bodies.

into the radiohumeral joint, while flexing and extending the elbow can cause lateral snapping and pain in the elbow (31). A positive history of fractures of the distal humerus or proximal radius or a dislocation of the radial head are possible risk factors for snapping annular ligament (31, 33, 34, 55, 56). Aoki *et al.* (55) also suggested that multiple microtrauma could cause degeneration of the ligament. However, in most patients, elbow symptoms start after an acute event; suggesting that, besides chronic degeneration, snapping annular ligament can be caused by trauma (35). Diagnosis of snapping annular ligament is usually confirmed by MRI or dynamic ultrasound of the elbow (33, 34). Treatments have included arthroscopy or an open procedure with removal of the interpositioning part of the annular ligament (31, 33, 34, 55, 56).

Being snapping annular ligament an unusual condition, only a limited number of cases have been reported in the literature. In these studies, symptoms and treatments are limited to one or several patients making difficult to determine the most common clinical features associated with diagnosis and to evaluate effectiveness of treatment options (31, 34, 55, 57, 58). However, a recent paper reported a case series study in which patients with annular ligament snapping were treated with either resection (through arthroscopic or open procedure) or open annular ligament reconstruction (54). Interestingly, both surgical procedures led to a significant improvement in pain and daily functioning. Hence, these data indicate that either resection or reconstruction of the annular ligament can be performed in patients affected by this type of snapping with a substantial and clinically relevant amelioration of symptoms (35).

Painful synovial plicae in the postero-lateral corner of the radiohumeral joint may generate snapping (59). Plicae are meniscus-like remnants of fetal development of the elbow joint (32, 60). They are found in 86% -100% of cases, but symptomatic plicae are much less common representing only 7.2%-8.7% of all elbow arthroscopies (59). Plicae undergo significant modifications from homogenous structures intermingled with the annular ligament in fetuses to a more heterogenous appearance in adults (61). It has been suggested that they may function as load dispersers and may provide cushioning during the process of elbow flexion and extension (61, 62). Over the lifetime, these plicae may become hypertrophic because of repetitive strain and trauma (36, 63). This hypertrophy may then cause the plica to impinge between the radial head and the humeral capitellum, causing pain and snapping (36).

The diagnosis starts with a clinical evaluation. Elbow plica syndrome is mostly manifested with lateral-sided elbow pain commonly accompanied by local tenderness, painful limitation of movements and snapping. The general consensus is that this condition should be initially treated with conservative therapy. Patients are advised to decrease the amount of physical activity, restore a range of motion with a guided physiotherapy and use non-steroidal anti-inflammatory drugs (36, 64, 65). In case of symptomatic impinging plica, confirmed by dynamic ultrasonography and no resolution after conservative measures, arthroscopic removal should be considered without any delay to avoid possible secondary cartilage degenerative changes (36, 64, 66).

In a recent case series study, 64 patients with a history of elbow pain, snapping and unsuccessful non-operative treatment were subjected to arthroscopic resection of the synovial plicae in the radiohumeral joint (64). At final follow-up, 47% of these patients reported to have a satisfactory joint function while only 19% had a function similar to that of the healthy population (64). In striking contrast with these results, Kim *et al.* (36), reported that arthroscopic resection of symptomatic synovial plicae on a cohort of 12 throwing athletes led 75% of the patients to have an excellent outcome and 17% to have a good outcome. Reasons underlying these differences remain to be seen. However, it has been postulated that the different composition of the two study groups could explain the different outcomes. The study by Kim *et al.* (36) included healthy and young athletes (mean age: 22 years) while the cohort of patients in the study by Pedersen *et al.* (64) were nonathletic and had a mean age of 44 years.

Concisely, snapping of the ulnar nerve is extremely rare and an associated snapping triceps tendon should always be suspected. The primary diagnostic method is dynamic ultrasonography. Surgical treatment is the elective method for both a final diagnosis and a definitive treatment. Since snapping of the ulnar nerve and of the triceps are frequently associated, it is important to evaluate intraoperatively the possibility to intervene on both to avoid repeated surgeries.

Symptomatic plicae, at the elbow, causing pain and snapping are at first clinically evaluated. Clinical suspicion may be supported by using MRI or ultrasound scan. If symptoms persist despite initially non-operative management, surgical treatment with arthroscopic resection appears to be effective and safe.

SNAPPING OF THE WRIST

Definition and clinical signs

Snapping wrist is a relatively rare condition with few reported cases in the literature. The terms "snapping wrist" and "trigger wrist" are interchangeably used to define a pathology presenting with painful sensation and a clicking sound during finger and wrist movements (2, 6). The definition of trigger wrist remains controversial. Historically, the snapping wrist has been considered a condition in which movement of the wrist or fingers leads to the triggering of the wrist. However, in 1986, Desai *et al.* (67) proposed to use the term trigger wrist to indicate triggering which occurs following the movement of the wrist and not that of the fingers (68). This is true trigger wrist while the condition in which triggering at the wrist is associated with finger movements would be more appropriately described as trigger finger at the wrist (6). Recently, it has been proposed that both true trigger wrist and the trigger finger at the wrist are combined under the unifying denomination of trigger wrist (6). These differences are dictated by the causative pathology and its location.

Aetiology

Trigger wrist is caused by various conditions (**table II**). The pathology may generate from volar structures, such as the flexor tendons, from dorsal structures such as the extensor tendons and carpal bone, and from tumors (6). Suematsu *et al.* (69) proposed to classify trigger wrist into three different categories. In class A, several causative factors have been identified. The most common causes are benign tumors affecting the tendon, synovial tissue, and nerve sheath, namely ganglion, fibroma, lipoma, fibro lipoma, angiolipofibroma, leiomyoma, tenosynovitis and pigmented villonodular synovitis (PVNS) (70-79).

Class B in Suematsu's classification is due to anomalous muscle belly including an abnormal lumbrical muscle or abnormal muscle belly of the flexor digitorum superficialis (70). In class C, there is a combination of both pathologies. For instance, anomalous muscle belly of the lumbrical muscle with tenosynovitis and anomalous muscle belly of flexor digitorum superficialis with fibroma (74).

In the true trigger wrist, the pathology is related to the extensor compartment and intracarpal pathology (68). Lemon and

Table II. Leading causes of triggering at wrist at specific sites.

Snapping of the wrist	Flexor tendons	Extensor tendons	Bones	Soft tissue
Leading causes	Giant cell tumour of the tendon	Acute partial rupture of ECRB	Subluxation	Ganglion
	Flexor tenosynovitis	ECRL nodule	Snapping lunate syndrome	Lipoma
	Lexor Carpi Radialis tendinitis	De Quervain stenosing tenosynovitis	Scapholunate subluxation	Adhesion
	Anomalous muscle belly of lumbrical	Intersection syndrome		Schwannoma
	Anomalous muscle belly of FDS	EPL tenosynovitis		Fibromatosis
	Intramuscular lipoma	Fourth compartment tenosynovitis (EDC)		Neurofibroma
		Fifth compartment tenosynovitis (EDM)		Leiomyoma
		ECU tenosynovitis/ subluxation		Desmoid tumour
				PVNS (pigmented villonodular synovitis)
				Fibrous Histiocytoma
				Extensor retinacular thickening

Engber (80) reported a case presented with true trigger wrist due to a nodule in the extensor carpi radialis longus tendon. The triggering occurred each time the nodule passed through the second compartment. The triggering was treated by reduction tenoplasty and releasing the second compartment. Koob and Steffens (81) reported a synovial mass around the extensor carpi radialis brevis and extensor pollicis long tendon at the level of extensor retinacular. True trigger wrist can also be due to recurrent subluxation of extensor carpi ulnas tendon (82).

However, some of these patients may also present with carpal tunnel syndrome (83). This is due to the mass effect within the carpal tunnel that compresses the medial nerve and needs to be addressed during carpal tunnel release (72). Leading causes of trigger wrist accompanied by carpal tunnel syndrome reported so far, include soft tissue tumors such as giant cell tumor (**figure 3**) and intramuscular lipoma (70), flexor tenosynovitis and tendon adhesions, wrist ganglion cysts, anomalies of the flexor tendons, localized amyloidosis and gouty tophus deposit (6, 84). Recently, Enayati *et al.* (84) reported muscle belly hypertrophy and extension to the carpal tunnel as a common underlying cause of both trigger wrist and carpal tunnel syndrome at the volar side of the wrist.

More particularly, snapping wrist can be secondary to ligament injuries, a condition known as mid-carpal instability, due to the rupture of the radiotriquetal ligament and the ulnar bundle of the arched palmar ligament (85). Additional causes include navicular-lunate instability, capitate-lunate instability and cartilaginous free bodies (85). Swann *et al.* (86) reported two cases of young patients with dorsal snapping wrist after traumatic injury. In both cases, the patients had hypertrophy of the articular surface of the radio-triquetal ligament, with an abundance of scar tissue, without associated inflammatory reaction. One of the most frequent sites of snapping is the ulnar extensor of the carpus as its partial rupture can lead to its medial dislocation during wrist motion (87).

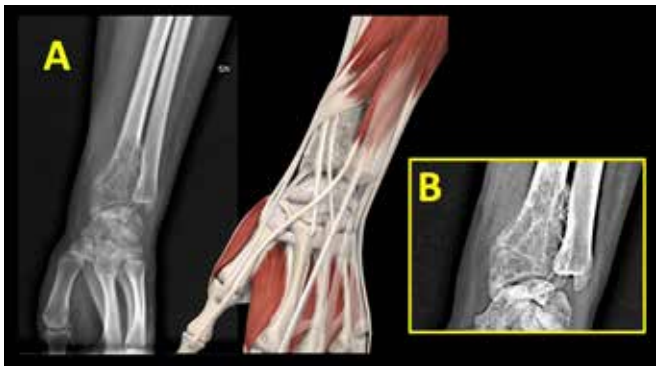

Figure 3. Snapping of the wrist flexor tendons in patient with giant cell tumour. Radiographic **(A)** and zoomed-in **(B)** images of the left wrist giant cell tumour.

As previously stated, snapping wrist can be caused by tumor formation near the carpus. In particular, fibromas or other less frequent neoformations (lipomas, lipofibromas, leiomyomas, giant cell tumor and hemangiomas) could originate from the superficial or deep flexor tendon at the crossing with flexor retinaculum (83). Unfrequently, neoformations could originate from the lumbrical muscle or from the respective tendon: fibromas are the most common cause, followed by lipomas, giant cell tumor, leiomyoma and hamartoma. Abnormalities of the muscle belly affecting the superficial flexor of the fingers, the deep flexor of the fingers, the extensor indicis proprius and the lumbrical muscles are described. In these patients, the enlarged muscle belly has been identified as the cause of the snapping (88). A case report described a patient with trigger at the wrist due to the median nerve (89). The patient had a lipofibromatous hamartoma affecting the carpal nerve and carpal tunnel syndrome (89).

Nodular neoformations of extensor tendons could determine snapping by impingement with the capsule or retinaculum of the extensors. Usually, it is secondary to partial rupture of the tendon that generates mucoid cysts, synovial cysts or masses in soft tissues. The most affected tendons are the long radial extensor of the carpus and the short extensor of the carpus, both with the long extensor of the thumb or isolated lesions of the superficial flexor of the fingers. Chronic tenosynovitis could also be associated with exudatives nodules that could generate the impingement with relative click, as in rheumatoid arthritis or in gout (90, 91).

De Quervain's stenosing tenosynovitis can cause snapping on the radial side of the wrist (92) while tendonitis with the formation of calcific bodies similar to rice grains is common in LES (79). Idiopathic synovial cysts, ganglions and iatrogenic cases were also described. Itsubo *et al.* (93) reported a case of snapping elicited by the release of the flexor tendon of the fifth finger causing its subluxation to the hook of the hook bone. Iwasaki *et al.* (94) reported a case of a patient, in which harvesting of the palmaris longus tendon produced a snapping wrist, possibly due to fibrous scar tissue between the flexor longus of the thumb and the superficial flexor of the fingers. Recently, Subramanyiam *et al.* (95) described a case of snapping wrist due to an accessory tendon in the first extensor channel.

Another cause of snapping involves the common extensor of the fingers, near the methacarpo-phalangeal joint. Typical phenomenon in boxers, it is due to repeated trauma on the knuckles of the 3rd and 4th ray, with rupture of the reti-

naculum. Ultrasound easily shows the tendon to displace ulnarly during the handshake (96). A non-traumatic form of snapping of the common extensor, affects the 5th ray and it is a condition due to the presence of a supernumerary fibrous band that causes snap (97). De Quervain's tenosynovitis is rarely associated to tendon click in the first extensor compartment. Impingement between the flexor tendon of the fingers and the flexor retinaculum is a less frequent cause of snapping wrist. This variant may be distinguished from that due to carpal instability as in this case the snapping occurs with the movement of the fingers, regardless of the movement of the radiocarpic. Trigger wrist is a relatively uncommon condition and there are no major studies reporting on its incidence. Sometimes is also underreported as patients may have been diagnosed at first with carpal tunnel syndrome or with trigger finger (2).

The stenosing tenosynovitis, commonly known as trigger finger, is thought to be caused by inflammation and is characterized by the painful popping or clicking sound elicited by flexion and extension of the affected digit (98). Inflammation and hypertrophy of the retinacular sheath progressively of the flexor tendons restricts the motion of the flexor tendons. This sheath normally forms a pulley system in each digit that serves to maximize the flexor tendon's force production and efficiency of the motion. Possible causes include local trauma and overuse but also pathologies such as arthritis rheumatoid, diabetes and gout may act as contributing factors (98). Diagnosis may require clinical and ultrasound evaluation. Initial management of trigger finger is conservative and involves non-steroidal anti-inflammatory drugs, metacarpophalangeal joint immobilization, and corticosteroid injections (99). If non-operative treatments are unsuccessful, then surgery is recommended (99).

Physical examination and diagnosis

A detailed patient history may help in the diagnosis. Patients may complain about triggering, snapping, clicking, or catching sensation at the wrist and some may even experience pain in the palm on gripping objects. Triggering at wrist is caused by finger movements, wrist movements, or forearm supination or pronation. It is necessary to differentiate whether it is a trigger finger, true trigger wrist or trigger finger at wrist. For this, an accurate clinical examination is performed to avoid unnecessary surgery, releasing of A1 pulley, or steroid injections (6).

Information about the precise nature of the triggering must be obtained from patients. If a patient does experience triggering with the movement of the fingers, and discomfort is perceived around the wrist, especially during flexion or extension of the fingers, this is highly suggestive of a trigger finger at wrist. However, it should be taken into account that triggering of the fingers may occur several weeks before the appearance of the trigger wrist phenomenon (6). In addition, this analysis may be made difficult by the fact that patients may present with discomfort or pain caused by finger movements and not by wrist movements (2). Finally, paresthesia may be brought about by the flexion of the fingers (6).

Patients can present with trigger wrist occurring during movements of the fingers or with wrist movements. Presence of tenderness around A1 pulley is suggestive of trigger finger while its absence may suggest trigger wrist. For trigger wrist, any swelling at the wrist or malunion around the wrist joint should also be examined. Palpation for any bony prominence, clicking or crepitus during wrist movements is also indicative of trigger wrist. Finally, examination for the presence of the carpal tunnel syndrome should be performed (6).

A simple radiograph of the wrist joint is needed to see any possible bony pathology such as malunion, instability or arthritis of the carpal bone (6, 89). For soft tissue assessment, ultrasound under dynamic modality would allow the assessment of the triggering pathophysiology (78). MRI or ultrasound may be necessary to further assess the space-occupying lesion within the carpal tunnel and determine characteristics of the mass such as its extension and its possible origin. Nerve conduction studies are indicated for patients with median nerve compression symptoms. Once this information is acquired, surgical intervention can be planned and successfully executed through adequate and complete excision (78).

Management

Conservative management is initially advised. The patient will be advised for activity modification to reduce the wrist and finger movements. If the pain during triggering is troublesome, a wrist splint is prescribed together with analgesics, as necessary. Patients with symptoms of carpal tunnel syndrome will need the routine conservative management before definitive surgical intervention.

Surgical treatment will depend on the causative factor. After radiological investigation to delineate the mass, surgery will be planned under general anesthesia (78). However, the advantage of doing the surgery under local anesthesia is that, during the surgery the patient can be asked to actively move the fingers or the wrist to confirm the resolution of the symptoms (100). This is advisable for patients with trigger wrist and carpal tunnel syndrome without definitive lesion detected by MRI or even CT scan. Inappropriate management may lead to worsening of the symptoms such as severe tenosynovitis, flexor tendon adhesion, or advanced carpal tunnel syndrome with thenar muscle atrophy, which requires a more extensive exploration and reconstructive surgery (2). The prognosis is good if it is done at the early stages of the disease. In chronic

cases, with irreversible pathology to the surrounding tendon or even the median nerve, the prognosis is guarded.
Briefly, due to its uncommon presentation and various possible pathologies, diagnosis of trigger wrist requires detailed history taking and examination. Cardinal symptoms of trigger wrist are more than two fingers triggering at the wrist with mild to moderate carpal tunnel syndrome and palpable mass or crepitus felt over the wrist. Subsequent investigation such as plain radiograph or ultrasound will guide detailed radiological investigation such as MRI or CT scan to delineate the extension and origin of the lesion before definitive surgery.

CONCLUSIONS

Symptomatic snapping syndrome may severely affect the every-day life of individuals and in athletes, may force them to quit the agonistic activity. Most clinicians have limited experience with the snapping syndrome, as it is a rare condition. Therefore, it is important to raise awareness about this condition to avoid the risk of misdiagnosis, delays in relevant treatment and even, inappropriate surgical procedures. Here, we summarize current evidence related to the aetiology, diagnosis and treatments of snapping phenomena occurring at the level of the shoulder, the elbow and the wrist.
Multiple and various in nature can be the causes of snapping, making the diagnosis of this condition quite challenging, at times. Although the diagnosis relies, at first, on focused history and physical examination, advanced imaging technologies may provide an excellent assist in the diagnostic process and even the treatment of these diseases. Nonoperative management consisting in reduced activity, physiotherapy, local CSI and analgesia is attempted at first; however surgical intervention still appears to be, in many cases, the most effective and safe treatment.

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DATA AVAILABILITY

N/A.

CONTRIBUTIONS

Each author has made substantial contributions to the conception, design of the work, acquisition, analysis and interpretation of data. All authors read and approved the final submitted version of the manuscript

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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Local, Distal, Proximal, and Contralateral Effects of Low-Load Blood Flow Restriction Training on Upper Extremity Neuromuscular Performance of Healthy Women: a Randomized Placebo-Controlled Trial Protocol

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SUMMARY

Introduction. Low-load blood flow restriction (BFR) training may induce positive neuromuscular adaptations, but proximal BFR effects are unclear. This study aims to investigate chronic effects of low-load resistance training (LLRT) with BFR on upper extremity neuromuscular performance of healthy women.

Methods. This protocol for clinical trial will include 78 volunteers randomized into three groups of 26 participants: LLRT (LLRT without BFR); LLRT + placebo blood flow restriction (20% BFR); and LLRT + 60% BFR. All groups will perform four sets of 15 repetitions at 20% of one-repetition maximum for each of the following muscles: serratus anterior, lateral shoulder rotators, and lower trapezius. Participants will be assessed before protocol, after completing eight weeks of protocol, and after a four-week follow-up. Primary outcome will be muscle strength, and secondary outcomes will be muscle excitation, perimetry, pain, subjective perceived exertion, affective valence with exercise, and power of upper extremity muscles.

Discussion. Exercises are often used to prevent and treat upper limb disorders. However, only two studies analyzed the effects of these exercises associated with BFR. Therefore, this protocol aims to fill the gaps in these studies and propose more reliable results on the subject.

Trial registration. EnsaiosClinicos.gov.br (Identifier: RBR-3pd52f).

KEY WORDS

Electromyography; muscle strength; resistance training; shoulder joint; vascular occlusion.

INTRODUCTION

Resistance training is recommended to increase muscle strength and muscle cross-sectional area (*i.e.*, muscle hypertrophy) and can benefit morphological and neuromuscular (*e.g.*, power and endurance) components (1). The American College of Sports Medicine recommends eight to 12 repetitions of 60-70% of one-repetition maximum (1RM) for increasing muscle strength in beginner or intermediate healthy individuals (1). In the 2000s, a new resistance training modality using blood flow restriction (BFR) was suggested as alternative to conventional resistance training (*e.g.*, 60-70% of 1RM) (2). BFR combines a pressure cuff with low-load (20-40% of 1RM) resistance exercises and high number of repetitions (*i.e.*, > 12 repetitions) (2, 3).

Some mechanisms acting simultaneously are proposed to explain the effects of exercise with BFR, among them are the metabolic stress, that increases under ischemia/hypoxia conditions and activates other mechanisms such as systemic hormonal release of growth hormone and insulin-like growth factor type 1. Exercise with BFR can also increase the production of reactive oxygen species such as nitric oxide, which can stimulate satellite cells and protein synthesis (4, 5). High-load training and low-load BFR training may induce similar hypertrophy levels in healthy people (6). However, literature regarding muscle strength gains in this population is conflicting, with previous studies showing superiority (6, 7) or similarity (8) of high-load resistance training *versus* low-load BFR training. Furthermore, it is suggested that the occurrence of fatigue during low-load resistance training is similar between the conditions of restricted blood flow and free blood flow (9).

Low-load BFR training increases strength and muscle mass of healthy adults compared with low-load training alone (10). However, while most studies reported neuromuscular adaptations on muscles distal to BFR, other investigations observed effects contralateral, distal, and proximal to BFR (11-18). Two studies reported effects of BFR training on gains in strength and muscle mass in muscles moving glenohumeral and scapulothoracic joints (19, 20). Despite this, no study used placebo, blinded participants, or included training of essential muscles for shoulder rehabilitation, such as serratus anterior and lower trapezius (21).

Thus, this study aims to investigate local, proximal, and distal chronic effects of low-load BFR training on neuromuscular performance of upper extremity muscles in healthy women. We hypothesize that BFR resistance training will increase strength of muscles proximal, contralateral, and distal to BFR and improve upper limb power and muscle excitation compared with low load resistance training alone and placebo BFR training.

METHODS

Design

This study proposes a randomized and blinded clinical trial protocol that will be conducted at the Faculty of Health Sciences of Trairi. The study was prospectively registered in the Brazilian Clinical Trials Registration Platform (Identifier: RBR-3pd52f) and was approved by the local Research Ethics Committee (No: 4.216.594 – on August 17, 2020). All procedures will be performed according to the Declaration of Helsinki.

Participants will be randomly assigned to three groups (26 per group): low-load resistance training (LLRT), low-load resistance training with 20% of BFR (LLRT + placebo application [pBFR]), and low-load resistance training with 60% of BFR (LLRT + BFR). The study will follow recommendations of the Template for Intervention Description and Replication (TiDieR) checklist (22) and the Standard Protocol Items: Recommendations for International Trials (SPIRIT) (23). The study will be reported according to the Consolidated Standards of Reporting Trials (CONSORT) guidelines (24).

Participants

Participants will be recruited using social media, announcements in local media, university community newsletters, banners, or flyers posted in strategic locations around town. Eligibility screening of those interested in participating will be performed by phone. Eligible participants will receive oral and written instructions about study aims and procedures and sign a consent form.

Personal information (name, address, and telephone number), anthropometric (age, height, and body mass), sociodemographic (profession, race, and educational level), and clinical data will be assessed. Personal data will be numerically coded, and information will be stored in a database, accessed only by the researcher responsible for randomization and blinding. Steps of the study are shown in **figure 1**.

Inclusion criteria

- Women aged between 18 and 35;
- irregularly active or sedentary (International Physical Activity Questionnaire) (25);
- body mass index between 18.5 and 30 kg/m^2;
- no previous experience with BFR training;
- no diabetes mellitus or high blood pressure;
- non-smoker (26);
- no regular use of vasoactive medications or nutritional supplements (27);
- no upper limb injuries in the last six months (26);
- no rheumatological, severe cardiovascular, or severe pulmonary inflammatory conditions that would prevent performing assessments and exercise protocol (27);

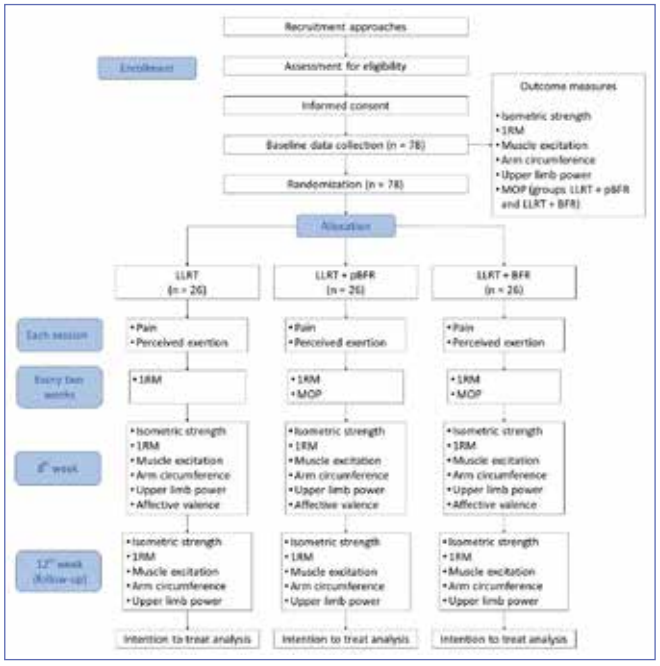

Figure 1. Study flow diagram.

- no psychiatric illness or malignant tumor and no previous zika or chikungunya infection in the last year (27);
- no regular upper limb physical training in the last six months (28).

Exclusion criteria

- Refuse to remain in the study (27);
- presence of incapacitating health conditions precluding participants from continuing the protocol (27);
- use of analgesic or anti-inflammatory medicines or both that may interfere with any outcome (27);
- unusual or strenuous physical activities during the study (27);
- missing two consecutive training days (26);
- leave the study before completing 70% of the training program (29).

Research team

This study will involve four researchers: one responsible for randomizing participants, one to conduct assessments, one to perform interventions, and one to perform statistical analyses.

Randomization and blinding

Included participants will be randomly assigned to one of three groups: LLRT, LLRT + pBFR, and LLRT + BFR. Randomization will be via www.randomization.com. Concealed allocation using individual, opaque, sealed envelopes will be conducted to avoid selection bias. Randomization will be performed by a researcher not involved in other procedures. Allocation of participants will be revealed before the first intervention, ensuring confidentiality. In addition, data collected during evaluations will not be revealed to researchers responsible for interventions. After study completion, the statistician will receive a worksheet with blinded data.

Interventions

LLRT protocol

Three exercises will be performed individually during each training session: shoulder external rotation (30), supine serratus punch (31), and prone horizontal abduction with shoulder externally rotated (32). For the first exercise, participants will be positioned in lateral decubitus, with shoulder adducted and internally rotated, 90° elbow flexion, and a towel positioned between elbow and trunk (**figure 2 A**). Participants will be encouraged to perform lateral rotation against resistance, avoiding compensations. In the second exercise, participants will be positioned supine, with 90° shoulder flexion, and elbow fully extended (**figure 2 B**). The exercise will initiate with shoulder retraction, and the participant will be asked to perform shoulder protraction against resistance with elbow fully extended. For the third exercise, participants will be positioned prone with elbow fully extended and with approximately 140° of shoulder abduction (**figure 2 C**). Participants will be instructed to lift the arm against resistance. Elbow will also remain extended throughout the exercise. Resistance for all exercises will be applied using a dumbbell. These exercises were chosen because they primarily recruit lateral rotators (30), serratus anterior (31), and lower trapezius (32), which are frequently included in rehabilitation protocols for individuals with shoulder pain (30, 33-35). Exercises will be performed in a predetermined random sequence for each participant; this sequence will be maintained throughout protocol. Cadence will be 1.5 seconds in each movement phase (concentric and eccentric) and controlled by a metronome, totaling three seconds per repetition (36).

1RM for all exercises will be predicted using Brzycki's formula (44): $predicted\ 1RM = 100 \times Load / [102.78 - (2.78 \times Repetitions)]$, in which load of 20% of predicted 1RM will be used for training (36). Predicted 1RM will be calculated every two weeks to follow physiological adaptations to exercise.

All groups will perform four sets of 15 repetitions for each exercise on each side, with a 30-second interval between sets and 1 minute between exercises, changing only the percentage of occlusion in LLRT + pBFR and LLRT + BFR groups.

Figure 2. Exercises will be performed individually during each training session.

Training sessions will be held twice a week, 48 hours apart (37), for eight weeks, totaling 16 sessions. To correct possible compensations and keep participants safe during exercises, a trained therapist will monitor the training program.

BFR protocol

Initially, maximum occlusive pressure (MOP) will be calculated. Blood pressure will be assessed after ten minutes of rest in supine position and with arms relaxed. An adapted sphygmomanometer (P.A. MED, São Paulo, Brazil), 52 cm length and 7 cm wide will be used. MOP will be determined with participants in supine position, upper limbs extended at body side and supported on the stretcher, with hand palms, face, and eyes facing forward, and lower limbs parallel with fingers facing forward (38). From this position, a portable vascular doppler (Dv 2001 – MedPej, São Paulo, Brazil) will be placed near wrist and over the radial artery, and the cuff will be slowly inflated to the point where blood pulse is abolished. The value displayed on manometer at this point will represent MOP (39). For LLRT + pBFR, the value corresponding to 20% of MOP will be considered for the exercise protocol (40). Regarding LLRT + BFR, 60% of MOP will be used (41). BFR will be performed in only one limb, which will be randomly chosen. The cuff will be deflated between exercises (26), and MOP will be reevaluated every two weeks to adjust pressure if necessary (42). Strengthening with BFR is considered safe (43), and participants will be encouraged to complete all sessions. Any adverse effects (*e.g.*, signs of deep vein thrombosis or rhabdomyolysis, persistent numbness, signs of venous injury, or any other harmful effect) (43) will be recorded. The researcher responsible for the intervention can also interrupt the intervention, if necessary.

Assessments

Muscle strength, muscle excitation, power, and arm circumference will be assessed at three time points: before training (T_0), after eight weeks of training (T_8), and after a four-week follow-up (T_{12}). Participants will be questioned at each session regarding subjective pain and perceived exertion. Participants will be contacted by phone and reminded to attend assessment days (**table I**).

Table I. Primary and secondary outcomes and assessments during the study.

	Enrolment	**Baseline (T_0)**	**Intervention**	**Post-intervention (T_8)**	**Follow-up (T_{12})**
Study Phase	**Three weeks before training**	**Day 0**	**Week 1 to 8 (2x/week)**	**Week 8 (2-3 days after the last intervention)**	**Week 12**
Enrolment					
Eligibility screening	X				
Informed consent		X			
Allocation		X			
Interventions					
LLRT			X		

	Enrolment	Baseline (T_0)	Intervention	Post-intervention (T_8)	Follow-up (T_{12})
Study Phase	**Three weeks before training**	**Day 0**	**Week 1 to 8 (2x/week)**	**Week 8 (2-3 days after the last intervention)**	**Week 12**
LLRT + pBFR			X		
LLRT + BFR			X		
Assessments					
Primary outcome					
Isometric strength		X		X	X
Secondary outcomes					
Muscle excitation		X		X	X
Upper limb power		X		X	X
Arm circumference		X		X	X
Pain			X		
Perceived exertion			X		
Affective valence				X	

LLRT: low-load resistance training; pBFR: placebo blood flow restriction; BFR: blood flow restriction.

Primary outcome

Isometric strength

A digital hand-held dynamometer (Lafayette Instrument Company, Lafayette, IN, US) will be used to evaluate isometric muscle strength of elbow flexors, lateral rotators, shoulder abductors (scapular plane), serratus anterior, and lower trapezius. Handgrip strength will be quantified using a hydraulic hand dynamometer (JAMAR, Hydraulic Hand Dynamometer® - Model PC-5030J1, Fred Sammons, Inc., Burr Ridge, IL, US). Procedures will be performed bilaterally and separately for each limb, and participants will perform up to two submaximal contractions for familiarization. Three five-second repetitions of maximal isometric contraction and 30-second rest between repetitions will be performed (44). For handgrip strength, participants will perform three grips of six seconds each, interspersed by one-minute rest (45). Quick and emphatic verbal commands (*i.e.*, "go! go! go!") will be used to stimulate maximum force production during the test (46). A universal goniometer (Fibra cirúrgica, Santa Catarina, Brazil) will be used to verify joint positioning. Coefficients of variation between repetitions will be calculated and, if necessary, additional measurements will be taken to guarantee a variability lower than 10% (47). Mean values (in kilogram-force) obtained in each limb will be included in data analysis and normalized by body mass. Positioning, fixation of apparatus, and execution of each test are shown in **table II**.

Table II. Evaluation of upper limb isometric muscle strength.

Test	Positioning	Dynamometer setting	Execution
Elbow flexors (44, 45)	Sitting, arm beside trunk, supine forearm, and elbow at 90°.	Dynamometer will be placed in the anterior forearm region, between radial styloid process and ulna, fixed around chair by an inelastic band.	Participants will perform maximum force by flexing elbow against resistance imposed by belt.
Sloulder lateral rotators (46)	Sitting, arm in adduction and neutral rotation, elbow flexed at 90° degrees with towel roll between elbow and torso, and forearm in neutral position.	Dynamometer will be attached to a bracket fixed to the wall and positioned on the posterior region of participant's forearm, three centimeters proximal to radial styloid process.	Participants will perform shoulder lateral rotation, applying maximum force against resistance imposed by the wall.

Test	Positioning	Dynamometer setting	Execution
Shoulder abductors (scapular plane) (47)	Shoulder elevated 90° in scapular plane, thumb pointing up, and elbow extended.	Dynamometer will be attached to an inelastic strip attached to the ground, positioned one centimeter proximal to radiocarpal joint.	Participants will perform maximum force toward ceiling, trying to raise the arm against resistance imposed by the band.
Anterior serratus (48, 49)	Supine with shoulder and elbow flexed at 90°. To find the initial position, participants will perform maximum protraction and retraction of scapula. Midpoint between these two movements will be considered for initial position of the test.	Hand-held dynamometer will be positioned on olecranon, perpendicular to the stretcher. An inelastic belt will be positioned around the stretcher to fixate the device.	Participants will perform maximum force toward ceiling against resistance imposed by the belt.
Lower trapezius (50)	Participants in prone, 145° shoulder abduction, thumb toward ceiling, and contralateral hand under forehead.	Hand-held dynamometer will be fixed by a belt attached to the ground, positioned laterally to distal portion of radius, just above radial styloid process.	Participants will be instructed to raise arm toward ceiling against resistance imposed by belt.
Handgrip (41, 51-53)	Sitting in a chair without armrests, feet flat on floor, shoulder adducted, elbow flexed at 90°, forearm in neutral position, and wrist between 0° and 30° of extension.	Participants will hold a hand hydraulic dynamometer with hands.	Participants will be instructed to perform maximum grip by bringing two rods together with maximum force.

Source: original from authors.

Secondary outcomes

Muscle excitation

Muscle excitation will be assessed simultaneously with muscle strength assessment. Acquisition and processing of electromyographic signals will be performed using a four-channel signal conditioning module (MCS 1000) (EMG System do Brasil®, Brazil) with analog-to-digital converter - A/D (CAD, 12/36-60K) of 12-bit resolution.

The equipment has a common-mode rejection ratio of > 80 Db, sampling frequency of 2000 Hz, and signals will be filtered between 20 and 500 Hz. Signals will be amplified 1000 times (50 times in converter and 20 times in electrodes). The electromyograph will be connected to a computer, which will receive signals that will be analyzed using EMGLab software (EMG System do Brasil®, Brazil).

Muscle excitation of serratus anterior, lower trapezius, and anterior deltoid muscles will be acquired after skin preparation (*i.e.*, trichotomy and cleaning with 70% alcohol). Simple differential surface electrodes, composed of Ag/AgCl associated with conductive gel (bipolar configuration, 4 cm × 2.2 cm of adhesive area, and 1 cm of conductive area) and separated by an interelectrode distance of 2 cm (Noraxon®, US), will be used. A monopolar reference electrode will also be used (Ag/AgCl with conductive gel, diameter of 3.8 cm of adhesive area, and 1 cm conductive area) (Noraxon®, US).

According to SENIAM recommendations (48), signal of serratus anterior muscle will be acquired with electrode positioned longitudinally anterior to latissimus dorsi and posterior to pectoralis major between sixth and eighth ribs. For lower trapezius, the electrode will be positioned 2/3 from scapular spine to eighth thoracic vertebra. For anterior deltoid, electrode will be positioned two centimeters distal and anterior to acromion. Reference electrode will be placed on the prominence of seventh cervical vertebra.

Root mean square (RMS) during muscle strength assessment will be normalized using peak RMS obtained during isometric contraction (49). Electromyographic signals corresponding to greatest isometric torque contraction using dynamometer will be included in data analysis.

Upper limb power

Unilateral seated shot-put test will assess upper limb power (50). Participants will sit on floor with torso, scapula, and

head against the wall, knees flexed at approximately 90°, and feet flat on the floor. Participants will hold a medicine ball (~ 3 kg) at shoulder height and will be instructed to push the ball forward as far as possible, keeping head and scapula on the opposite side in contact with wall and contralateral arm close to the body. The ball will be covered with chalk to facilitate measurements. After familiarization with two submaximal repetitions, participants will perform three maximum repetitions on each side with 30-second intervals between repetitions. Distance between wall and ball after first touch on the ground will be measured in centimeters and mean of three measurements will be used for analysis.

Arm circumference
Circumference of both arms will be measured using perimetry, adapted from Chapman *et al.* (51). Participants will be standing with arms at body side and ventral face of hands facing thighs. A non-elastic flexible measuring tape and semi-permanent marker will be used to measure arm circumferences (in centimeters) at three, six, nine, 12, and 15 centimeters above elbow crease line. Mean of all measurements will be considered for analysis.

Pain
Muscle pain will be monitored before and after each training exercise of all protocols and throughout the intervention program using the numerical pain rating scale. Participants will rate pain intensity with exercises by selecting a number from zero to ten, in which zero represents "no pain" and ten represents "worst possible pain" (52). At the end of intervention period, a pain diary will be created for each exercise and represented as a graph.

Perceived exertion
OMNI-RES Scale (53) will assess subjective perceived exertion at the end of each training session. This scale ranges from zero to ten, in which zero represents an "extremely easy" degree of exertion and ten an "extremely hard" degree of exertion.

Affective Valence
Affect will be determined after the last week of training using the Feeling Scale (54). This is an 11-point scale, with items ranging from + 5 (very good) to - 5 (very bad). Instructions for participants will be as follows: use numbers on this scale to indicate how you felt while performing this activity; if you felt exercise was very good (pleasant or comfortable), then the corresponding number will be "+ 5"; if you felt exercise was very bad (unpleasant or uncomfortable), then the corresponding number will be "- 5". If you feel neutral (between pleasure and displeasure /comfort and discomfort), then the corresponding number will be "0". **Table III** shows time points of assessments.

Researcher training

Researchers will be trained before initiating the study to standardize the application of exercise protocols, BFR, and assessment tools. Test-retest and intra-rater reliability for muscle strength, power, and upper limb circumference will be performed before initiating the study.

Sample size calculation

Sample size was calculated based on a previous study (55). Total sample size was estimated as 71 participants, considering mean difference of relative isometric strength (primary outcome) between groups of 0.15 Nm/cm^2, standard

Table III. Evaluation of primary and secondary outcomes.

Outcomes	Baseline (T_0)	After each intervention	After 8 weeks of intervention (T_8)	Four weeks after intervention (T_{12})
Primary				
Isometric strength	√		√	√
Secondary				
Muscle excitation	√		√	√
Upper limb power	√		√	√
Arm circumference	√		√	√
Pain		√		
Perceived exertion		√		
Affective valence			√	

deviation of 0.33 Nm/cm², 80% statistical power, and 5% significance level. Including a 10% dropout rate, 78 participants will be needed (26 per group).

Statistical analysis

Kolmogorov-Smirnov and Levene tests will be used to verify data normality and homogeneity of variance, respectively. Quantitative results will be represented as mean ± SD and qualitative results as absolute values, percentages, and 95% confidence intervals (95% CI).

Intergroup comparisons will be evaluated using linear mixed model (mixed ANOVA) or Kruskal-Wallis test, depending on data normality. When a significant F-value is found, the Bonferroni *post-hoc* test will be applied to identify the differences. Significance level at 5% ($p < 0.05$) and 95% CI will be adopted for all statistical analyses. All participants will be evaluated using intention-to-treat analysis. For missing data, results will be imputed by repeating the value of the last assessment.

DISCUSSION

Exercises for glenohumeral and scapulothoracic regions are frequently used to increase strength (35), improve function (33), and prevent (56, 57) and rehabilitate shoulder dysfunction (58-61) in different populations. Regarding upper limbs, only two studies investigated BFR effects for muscles of shoulder complex (55, 20). Methodological limitations, such as lack of placebo group and conflicting results, hinder recommending BFR for improving upper limb function.

The present protocol proposes a similar training volume between groups and exercises commonly used to prevent and treat shoulder dysfunctions (*e.g.*, rotator cuff tears (58), glenohumeral instability (59), or subacromial pain (61)). Another relevant aspect of our study is the inclusion of control and placebo groups. In particular, placebo group will receive a minimal percentage of BFR that is unable to change results significantly (40, 41). Finally, upper limb power will also be investigated and a four-week follow-up period will indicate whether training results are maintained.

As limitations, we highlight the impossibility of controlling hormonal changes of participants and measuring trophism changes of target muscles because of lack of adequate equipment, such as MRI or ultrasound.

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DATA AVAILABILITY

Data are available under reasonable request to the corresponding author.

CONTRIBUTIONS

All authors contributed significantly to the construction of the study. MTMJ, WSLJ, YTP: writing, approval of the final manuscript. GMB, MCS: assistance in protocol development. CAAL: contextualization and final review of the study.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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REVIEW

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Lower-Limb Connective Tissue Morphologic Characteristics in Runners. How Do They Relate with Running Biomechanics and Tendon Pathology? A Systematic Review

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SUMMARY

Although the role of connective tissue in running injuries and biomechanics has been widely investigated, systematic reviews on this issue were rarely reported.

The aim of this study is to systematically review the current literature regarding the morphological characteristics (*i.e.*, cross-sectional area and thickness) of the main connective tissue of the lower limb in runners and its relationship with running biomechanics and tendon pathology.

The main keywords used were: Achilles and patellar tendons, plantar fascia, ultrasound, thickness, cross-sectional area and running. Observational design English-written studies published between January 2000 and September 2020 were included.

After exclusion criteria were applied, 34 studies remained. 16 studies analysed connective tissue related to sample characteristics where mainly the differences between runners and controls were studied along other factors such as gender, weight and nationality. Regarding running biomechanics, 10 studies assessed connective tissue on running biomechanics with focusing on foot strike pattern and footwear characteristics. Regarding tendon pathology, 8 studies analysed the connective tissue assessing, mainly, whether the pathological tendons are thicker.

Runners show higher tendons, in terms of thickness and CSA, than control subjects and athletes from other disciplines whose tendons are subjected to lighter loads. Adaptation to load leads to better performance, what seems to explain these morphological differences.

Study registration. None.

KEY WORDS

Connective tissue; morphology; performance; review; running; tendinopathy.

INTRODUCTION

Tendons are connective tissue structures composed mainly by collagen (65-80%) and elastin embedded in a proteoglycan-water matrix (1). The most typical analysed morphological characteristics of the connective tissue are the cross sectional area (CSA) and thickness (2, 3). To assess these morphological characteristics, the main imaging techniques used are ultrasounds and nuclear magnetic resonance, which have proven to be useful and reliable for such use (4, 5).

Connective tissue, specifically tendons, plays an essential role in the locomotor system function. Activities such as walking, running or jumping need a correct tendon functioning as these structures are responsible for loading transmission that allows physiological movement and joint stabilization (6). In relation with its function, tendons are classified as load transmitters or movement transmitter, being the main lower limb tendons (*i.e.*, Achilles tendon [AT] and patellar tendon [PT]) load transmitters (6). Although the plantar fascia (PF) is not properly considered a tendon, some studies refer to the PF such as an extension of the AT due to the common insertion at the calcaneus bone aiding in the function of this tendon (7).

Given the elongation and shortening of these lower limb structures (*i.e.*, AT, PT and PF), they made a huge contribution to the energy storage and release during movements such as running (8). It is suggested that during running the lower limb behaves like a spring that continually compresses and decompresses allowing the movement (9). The main responsible for such compressions and decompressions are the muscle-tendon units through the stretch-shortening cycle (10). Considering that, the characteristics and condition of the aforementioned connective tissues might play a key role in both athletic performance and injury management contexts.

It has been proposed that the AT plays an essential role in running economy (11). Fletcher *et al.* (11) proposed that the AT stiffness is one of the main mechanism behind an enhanced running economy. Tendon stiffness allows the muscle acts at the appropriate length and velocity, being essential for running economy. Tendons optimize the relation between muscle's force, length, and velocity by minimising length change during muscle contraction (11). During running, the AT accommodates much of that muscle-tendon unit length change (12). Seemingly, the AT mechanical properties are optimal to accommodate the mentioned length change, and any change in those properties might result in an increase in running energy cost (11). Supporting this importance of AT in running economy, several studies show the relationship between longer AT length and better running economy (13, 14). For its part, the PT has also been shown to be an influential element in running economy. Thus, a lower PT stiffness is related to a better running economy (11). Depending on the circumstances, a higher stiffness tendon or a lower stiffness tendon can be more beneficial in running economy (11). So, when power is more important, a lower stiffness tendon helps the muscle to shorten at the velocity associated with peak-power output (15). This fact could explain why lower PT stiffness correlates with a better running economy given that it would contribute more effectively to power generation (11).

It is known that during running the main lower limb tendons are exposed to repetitive loadings. In response to these repetitive loads, tendons need to be adapted. These adaptations occur both at the morphological level (*i.e.*, higher CSA and thickness) (16, 17) and in the percentage of the type of collagen fibers (18). Thus, these adaptations would seek for a tendon better prepared for the demanding activity and therefore with a better performance such as with a better velocity (19). However, when the tendon is unable to adapt to these loads, the result is an overloaded tendon that can lead to the development of tendinopathy (20). Thus, for running, when there is not a good adjustment of the load in relation to volume and frequency, the excess load might lead to tendon injury (20). Although some studies show higher CSA and thickness in Achilles tendinopathy (21), these morphological changes are present depending on the phase of tendinopathy in which it is found (20). The most typical injuries associated to running are Achilles tendinopathy and plantar fasciitis (22). A previous study found that shorter races, where runners use more a forefoot strike pattern which demands more the AT, increase the risk of suffering an AT tendinopathy (23). In relation with the PT, patellar tendinopathy has been frequently reported among amateur runners reporting a weekly mileage between 20 and 50 km (24). However, this pathology did not show an important prevalence between marathon runners (24), so that running experience with a correct tissue adaptation could result in a protector factor for patellar tendinopathy (22). Finally, master runners seem to be more affected by plantar fasciitis (24). One of the main functions of the PF during running is to absorb the elastic tension (22). With aging, this capacity might decrease, which could explain why master runners show a higher prevalence of plantar fasciitis (22).

Actually, both the relation between the tendon injuries and running and the influence of the tendon on running biomechanics have been widely investigated (25-28).

Despite the aforementioned information, there still exist gaps that need to be bridged in regards with connective tissue morphologic characteristics and running. Furthermore, how these characteristics are related to running biomechanics and tendon pathology is not well understood. In this way, to the best of the authors' knowledge, a systematic review that analyses the literature carried out on the morphological characteristics of connective tissue in runners and how these characteristics are related to running biomechanics and tendon pathology variables has not been done yet.

Therefore, the main purpose of the present systematic review was to analyse the current literature regarding the morphological characteristics (*i.e.*, CSA and thickness) of the main connective tissue of the lower limb in runners and its relationship with running biomechanics and tendon pathology variables.

METHODS

A systematic review was completed following the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (29). Findings were also reported accordingly.

Eligibility criteria

The following inclusion criteria were considered: 1) studies published in English; 2) only human studies. The following exclusion criteria were taken into account: 1) Clinical trials, 2) case reports, 3) case series, 4) systematic reviews and 5) editor letters.

All manuscripts related to lower limb connective tissue morphology (*i.e.*, AT, PT and PF thickness and CSA) and running were included.

Information sources

A systematic search was conducted in the electronic databases Pubmed, Web of science, SPORTDiscus and Scopus for relevant studies from 1 January 2000 to 30 September 2020. Keywords were collected through experts' opinion, a systematic literature review, and controlled vocabulary (*e.g.*, Medical Subject Headings: MeSH). Boolean search syntax using the operators "AND" and "OR" was applied. Following an example of a PubMed search is shown: (Achilles OR gastroc* OR triceps surae OR patella* OR quadriceps OR rotulien OR plantar fascia) AND (Tendon* OR Tendin*) AND (Ultrasonograph* or Sonograph* or Ultrasound or US or MSUS OR cross-sectional area OR thickness) AND (Run* or Sprint* or Jog* or Interval or Long Distance or Marathon OR running OR runner). Filters: Publication date from 2000/01/01; Humans; English.

After an initial search, accounts were created in the mentioned databases. In this way, the search was updated until the initiation of manuscript preparation on September 30, 2020. Following the formal systematic searches, additional hand-searches were conducted. In addition, the reference lists of included studies and previous reviews and meta-analyses were examined to detect studies potentially eligible for inclusion.

Study selection

During the selection of studies, a filter was initially made by title, in which those articles that were not considered relevant were excluded. After this, the same procedure was carried out after evaluating the abstract. Finally, the full text of the remaining studies was assessed excluding those that did not meet the review criteria.

Methodological quality in individual studies

Selected studies were evaluated for methodological quality using the modified version of the Quality Index developed by Downs and Black (30). A good test-retest (r = 0.88) and inter-rater (r = 0.75) of the original scale was reported. Furthermore, a good reliability and high internal consistency (Kuder-Richardson Formula 20 (KR-20) = 0.89) were shown. The modified version of the Downs and Black Quality Index is scored from 1 to 14 where higher scores indicate higher-quality studies. Two independent reviewers (ARP-FGP) performed this quality assessment, and, in the event of a disagreement, a third reviewer (LERS) analysed the quality and made the final decision. Agreement between reviewers was assessed using a Kappa correlation for methodological quality. The agreement rate between reviewers was k = 0.91.

RESULTS

Study selection

The study selection process is shown in a flow diagram (**figure 1**). A total of 978 studies were found after the systematic search. 275 from Pubmed, 456 from Web of Science, 187 from Scopus and 60 from SPORDiscus. Additionally, one study was identified from a different resource. After all the selection criteria mentioned above, 34 studies were included. Regarding the methodological quality, results derived from Modified Downs and Black scale are shown in **table I**. Scores for this scale ranged from 8 to 13 out of 14.

The results of the selected studies are shown separately according to next different aspects: sample characteristics (**table II**), running biomechanics variables (**table III**) and tendon pathology (**table IV**).

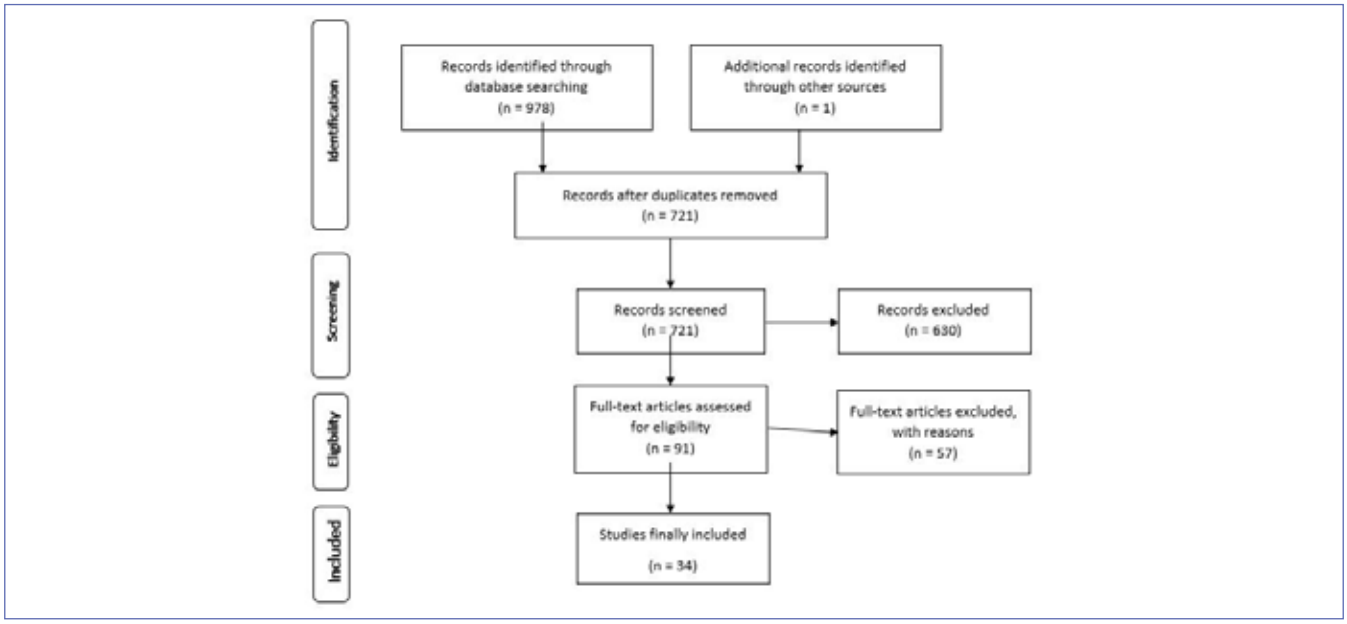

Figure 1. PRISMA flow diagram.

Table I. Modified Downs and Black scale.

Study	Item 1	Item 2	Item 3	Item 6	Item 7	Item 10	Item 12	Item 15	Item 16	Item 18	Item 20	Item 22	Item 23	Item 25	Total (Out of 14)
Chen *et al.* (2019) (50)	1	1	1	1	1	1	0	1	1	1	1	1	0	0	**11**
Dar *et al.* (2019) (38)	1	1	1	1	1	1	0	1	1	1	1	0	0	1	**11**
Devaprakash *et al.* (2020) (31)	1	1	1	1	1	1	0	0	1	1	1	0	0	0	**9**
Farris *et al.* (2012) (43)	1	1	1	1	1	1	0	0	1	1	1	1	0	0	**10**
Freund *et al.* (2012) (51)	1	1	1	1	1	1	1	1	1	1	1	1	1	0	**13**
Hagan *et al.* (2018) (52)	1	1	1	1	1	1	0	1	1	1	1	1	0	0	**11**
Hall *et al.* (2015) (58)	1	1	1	1	1	1	0	0	1	1	1	1	0	0	**10**
Hirschmuller *et al.* (2012) (53)	1	1	1	1	1	1	0	1	1	1	1	1	0	1	**12**
Histen *et al.* (2016) (44)	1	1	1	1	1	1	0	1	1	1	1	0	0	0	**10**
Hullfish *et al.* (2018) (32)	1	1	1	1	1	1	0	1	1	1	1	1	0	1	**12**
Kernozek *et al.* (2018) (45)	1	1	1	1	1	1	0	0	1	1	1	0	0	0	**9**
Kongsgaard *et al.* (2005) (37)	1	1	1	1	1	1	0	0	1	1	1	0	0	1	**10**
Kubo *et al.* (2010) (17)	1	1	0	1	1	1	0	0	1	1	1	0	0	1	**9**
Kubo *et al.* (2011) (2)	1	1	0	1	1	1	0	0	1	1	1	0	0	0	**8**
Kubo *et al.* (2015a) (39)	1	1	0	1	1	1	0	0	1	1	1	0	0	1	**9**
Kubo *et al.* (2015b) (46)	1	1	0	1	1	1	0	0	1	1	1	0	0	0	**8**
Kubo *et al.* (2015c) (16)	1	1	0	1	1	1	0	0	1	1	1	0	0	1	**9**
Kubo *et al.* (2017) (3)	1	1	0	1	1	1	0	0	1	1	1	0	0	0	**8**
Kudron *et al.* (2020) (40)	0	1	1	1	1	1	1	0	1	1	1	0	0	1	**10**
Kunimasa *et al.* (2014) (41)	1	1	0	1	1	1	0	0	1	1	1	0	0	0	**8**
Lieberthal *et al.* (2019) (54)	1	1	1	1	1	1	0	0	1	1	1	1	0	0	**10**

Study	Item 1	Item 2	Item 3	Item 6	Item 7	Item 10	Item 12	Item 15	Item 16	Item 18	Item 20	Item 22	Item 23	Item 25	Total (Out of 14)
Magnusson *et al.* (2003) (33)	1	1	1	1	0	1	0	0	1	1	1	0	0	0	**8**
Monte *et al.* (2020) (19)	1	1	0	1	1	1	0	0	1	1	1	0	0	0	**8**
Neves *et al.* (2014) (47)	1	1	1	1	1	1	0	0	1	1	1	0	0	0	**9**
Ooi *et al.* (2015) (55)	1	1	1	1	1	1	0	1	1	1	1	0	0	0	**10**
Rosager *et al.* (2002) (34)	1	1	1	1	1	1	0	0	1	1	1	0	0	0	**9**
Salinero *et al.* (2020) (35)	1	1	0	1	1	1	0	0	1	1	1	0	0	0	**8**
Shaikh *et al.* (2012) (56)	1	0	1	1	1	1	0	1	1	1	1	0	0	0	**9**
Shiotani *et al.* (2020) (42)	1	1	1	1	1	1	0	0	1	1	1	0	0	0	**9**
Sponbeck *et al.* (2017) (48)	1	1	1	1	1	1	0	1	1	1	1	1	0	0	**11**
Tillander *et al.* (2019) (57)	1	0	1	1	1	1	0	0	1	1	1	1	0	1	**10**
Ueno *et al.* (2018) (14)	1	1	1	1	1	1	0	0	1	1	1	0	0	0	**9**
Wiesinger *et al.* (2016) (36)	1	1	1	1	1	1	0	0	1	1	1	0	0	1	**10**
Zhang *et al.* (2018) (49)	1	1	1	1	1	1	0	1	1	1	1	0	0	0	**10**

0 = no / unable to determine; 1 = yes. Item 1: clear aim/hypothesis; Item 2: outcome measures clearly described; Item 3: patient characteristics clearly described; Item 6: main findings clearly described; Item 7: measures of random variability provided; Item 10: actual probability values reported; Item 12: participants prepared to participate representative of entire population; Item 15: Blinding of outcome measures; Item 16: analysis completed was planned; Item 18: appropriate statistics; Item 20: valid and reliable outcome measures; Item 22: participants recruited over same period; Item 23: Randomised; Item 25: adjustment made for confounding variables.

Table II. Sample characteristics and morphological characteristics of the connective tissue.

Study	Subject Description	Study design	Connective tissue morphologic measures	Other outcome measures	Results
Dar *et al.* (2019)	**Amateur road LDR** n = 26 (15M, 11F) 42.2 ± 9.1 years 168.6 ± 8.2 cm 62.4 ± 8.0 Kg BMI: 21.9 ±1.9 Kg/m^2 Km/wk: 37.5 ± 20.6 **Amateur trail runners** n = 17 (9M, 8F) 41.0 ± 9.5 years 171.2 ± 8.4 cm 67.1 ± 12.1 Kg BMI: 22.7 ±2.8 Kg/m^2 Km/wk: 38.2 ± 16.3	Unilateral crossover Laboratory conditions	- **AT-Thickness** **- AT-CSA** - AT UTC	- Proprioceptive ability (AMEDA) - Dynamic postural balance (Y balance Test) - Triple hop distance test - Hip abduction isometric test	- LDR AT-Thickness (cm) 0.6 ± 0.1 - Trail AT-Thickness (cm): 0.5 ± 0.1 - LDR AT-CSA (cm^2) 0.5 (0.4-0.6) - Trail AT-CSA (cm^2) 0.5 (0.4-0.6) - No significant differences for thickness and CSA

Study	Subject Description	Study design	Connective tissue morphologic measures	Other outcome measures	Results
Devaprakash *et al.* (2020)	**Trained elite LDR** n = 16 (10 M, 6 F) 25.2 ± 5.0 years 175.5 ± 7.3 cm 64.4 ± 8.4 kg BMI: 20.9 ± 1.8 Kg/m^2 **Healthy controls** n = 16 (11 M, 5 F) 30.3 ± 4.9 years 172.4 ± 10.5 cm 71 ± 16.8 kg BMI: 23.8 ± 4.5 Kg/m^2	Unilateral crossover Laboratory conditions	***- AT-CSA*** - AT lenght - AT strain - AT stress - AT elongation - AT stiffness and Young's modulus - AT force - AT torque **Measured by RNM		- AT-CSA significantly larger in LDR
Hulfish *et al.* (2018)	**Competitive runners** n = 22(12 M, 10 F) 19 ± 1.5 years BMI: 20.3 ± 1.6 Kg/m^2 **Untrained subjects** n = 12(5 M, 7 F) 25 ± 2 years BMI: 23.8 ± 2.4 Kg/m^2	Unilateral crossover Laboratory conditions	***- AT-Thickness*** - AT structure - Collagen organization - Echogenicity	- VISA-A - Foot strike pattern - Neovascularity	- AT-Thickness 48% greater in competitive runners
Kongsgaard *et al.* (2005)	**Elite LDR** n = 8 M 28.6 ± 2.2 years 182.4 ± 2.7 cm 69.5 ± 2.9 kg **Elite volley players** n = 8 M 25.3 ± 0.8 years 193.8 ± 2.8 cm 89.5 ± 2.6 kg **Elite Kayak players** n = 9 M 20.5 ± 1.1 years 181.9 ± 1.3 cm 79.9 ± 1.9 kg **AT rupture patients** n = 6 M 31.0 ± 0.7 years 181.5 ± 2.8 cm 86.0 ± 6.3 kg	Unilateral crossover Laboratory conditions	***- AT-CSA (maximal, narrowest and Nrm to mass)*** - AT moment arm AT peak tendon force - AT length - Maximal AT force - Peak AT stress	- Leg length - MVIC plantar flexion - Triceps Surae CSA	- LDR AT-CSA (mm$^{2)}$ Maximal (106 ± 8.6), Narrowest (55.1 ± 2.1) - LDR larger Nrm AT - CSA than kayak and rupture subjects - LDR narrowest Nrm AT-CSA higher than kayak and rupture subjects - The narrowest AT-CSA correlated to maximal AT force
Kubo *et al.* (2010)	**LDR** n = 12 M 20.3 ± 1.1 year 171.0 ± 4.7 cm 57.3 ± 3.5 Kg **Untrained subjects** n = 21 M 21.2 ± 1.8 years 172.2 ± 5.6 cm 64.5 ± 5.9 Kg	Unilateral crossover Laboratory conditions	***- AT-Thickness*** (Abs and Nrm to mass) ***- PT-Thickness*** (Abs and Nrm to mass) - AT and PT elongation - AT and PT stiffness	- Muscle thickness (knee extensors and plantar flexors) - MVIC (knee extensors and plantar flexors) - Resting twitch properties - Neural activation level	- LDR thickness (mm): PT: 3.24 ±0.37, AT: 4.65 ± 0.57 - Untrained thickness (mm): PT: 3.36 ± 0.36, AT 4.67 ± 0.56 - Nrm AT and PT thickness greater in LDR No differences in Abs both tendon thickness.

Study	Subject Description	Study design	Connective tissue morphologic measures	Other outcome measures	Results
Kubo *et al.* (2011)	**100-m sprinters** n = 15 M 20.8 ± 1.0 years 174.5 ± 4.3 cm 67.6 ± 5.3 Kg **Untrained subjects** n = 15 M 20.7 ± 1. 8 years 173.1 ± 4.2 cm 70.2 ± 7.7 Kg	Unilateral crossover Laboratory conditions	***- AT-Thickness*** ***- PT-Thickness*** - AT and PT elongation - AT and PT stiffness	- Muscle thickness (knee extensors and plantar flexors) - MVIC (knee extensors and plantar flexors) - Running performance	- Runners thickness (mm): PT: 3.15 ± 0.47, AT: 4.28 ±0.54 - Untrained thickness (mm): PT: 3.27 ± 0.50, AT: 4.34 ± 0.63 -No differences in tendon thickness -No significant correlations between best 100-m record and tendon thickness
Kubo *et al.* (2015a)	**Faster LDR** n = 32 M 20.4 ± 1.2 years 171.1 ± 4.5 cm 56.7 ± 3.5 Kg Experience 7.7 ± 1.9 years **Slower LDR** n = 32 M 20.3 ± 1.0 years 170.5 ± 5.6 cm 58.3 ± 4.8 Kg Experience: 6.8 ± 2.9 years	Unilateral crossover Laboratory conditions	***- AT-Thickness*** ***- PT-Thickness*** - AT and PT elongation - AT and PT stiffness - At and PT strain	- Muscle thickness (knee extensors and plantar flexors) - MVIC (knee extensors and plantar flexors) - Running performance	- Faster Thickness (mm): PT: 4.9 ± 0.5, AT: 3.2 ± 0.4 - Slower Thickness (mm): PT: 5.1 ± 0.6, AT: 3.1 ± 0.4 -No differences in tendon thickness -No significant correlations between the best 5000m record and tendon thickness
Kubo *et al.* (2015c)	**LDR** n = 20 M 20.4 ± 1.0 years 171.2 ± 4.8 cm 57.4 ± 4.6 Kg **Untrained subjects** n = 24 M 22.2 ± 3.6 years 172.3 ± 5.5 cm 66.4 ± 8.1 Kg	Unilateral crossover Laboratory conditions	***- AT-CSA*** (Abs and Nrm to mass) - AT stiffness - AT elongation	- Muscle thickness plantar flexors - Passive and Active muscle stiffness - MVIC plantar flexors - EMG plantar flexors	- LDR AT-CSA (mm^2). Abs: 74.4 ± 10.7, Nrm: 4.99 ± 0.72 - Untrained AT-CSA (mm^2). Abs: 73.5 ± 11.6, Nrm: 4.48 ± 0.63 - Nrm AT-CSA higher in LDR
Kubo *et al.* (2017)	**Sprinters** n = 14 M 20.7 ± 1.1 years 173.2 ± 5.5 cm 65.4 ± 4.3 Kg **Untrained subjects** n = 24 M 22.2 ± 3.6 years 172.3 ± 5.5 cm 66.4 ± 8.1 Kg	Unilateral crossover Laboratory conditions	***- AT-CSA*** - AT stiffness - AT elongation	- Muscle thickness plantar flexors - Muscle fascicle length - Pennation angle - Active muscle stiffness - MVIC plantar flexors - Twitch properties	- Sprinters AT-CSA (mm^2): 74.0 ± 12.0 - Untrained AT-CSA (mm^2): 73.5 ± 11.6 - AT-CSA no differences

Study	Subject Description	Study design	Connective tissue morphologic measures	Other outcome measures	Results
Kudron *et al.* (2020)	**Pro CCR** n = 27 (11 M, 16 F) 19.5 ± 1.4 years 67.2 ± 3.4 in 127.6 ± 18.1 lb BMI: 19.8 ±1.5 kg/m^2 Miles/wk: 64.1 ± 21.6	Unilateral crossover Laboratory conditions	**- AT-Thickness** **AT-CSA** - AT structure	- Dominant *vs* Non dominant side - Demographic characteristics	- Non dominant AT-CSA (cm^2): 0.71 ± 0.17 - Dominant AT-CSA (cm^2): 0.74 ± 0.19 - Non dominant AT-Thickness (cm): 0.56 ± 0.1 - Dominant AT- thickness (cm): 0.53 ± 0.09 - Positive correlation height and weight with AT-CSA - Positive correlation Miles/wk with AT-CSA and thickness - Males larger AT-CSA - Underweight subjects smaller AT-CSA
Kunimasa *et al.* (2014)	**Kenyan LDR** n = 22 M 21.9 ± 4.5 years 1.74 ± 0.06 m 57.2 ± 4.8 Kg BMI 18.9 ± 1.5 Kg/m^2 **Japanese LDR** n = 22 M 20.2 ± 2.2 years 1.73 ± 0.05 m 56.9 ± 4.6 Kg BMI 19.0 ± 0.9 Kg/m^2	Unilateral crossover Laboratory conditions	***- AT-CSA*** - AT moment arm	- Running performance - Thigh length - Leg length - Forefoot length - Gastrocnemius-AT length -Soleus-AT length	- Kenyan AT-CSA (mm^2): 60.5 ± 9.3 - Japanese AT-CSA (mm^2): 53.6 ± 9.8 - AT-CSA higher in Kenyan
Magnusson *et al.* (2003)	**Runners** n = 6 M 36 ± 7 years 1.84 ± 0.05 m 70.9 ± 4.4 Kg **Control no runners** n = 6 M 34 ± 3 years 1.81 ± 0.02 m 81.2 ± 8.7 Kg	Unilateral crossover Laboratory conditions	***- AT-CSA:*** 7 sites from 10 mm to 70 mm to calcaneus		- Runners greater AT-CSA at 10, 20, 30 and 40 mm sites
Rosager *et al.* (2002)	**LDR** n = 5 M 34 ± 6 years 1.82 ± 0.09 m 72.1 ± 4.6 kg **Control no runners** n = 5 M 33 ± 8 years 1.80 ± 0.03 m 82.2 ± 4.2 kg	Unilateral crossover Laboratory conditions	***- AT-CSA*** - AT length - AT strain - AT stress - AT displacement - AT stiffness and Young's modulus - AT moment arm	- EMG flexor and extensor ankle muscles - Angular ankle joint motion - Maximal dorsiflexion moment	- AT-CSA larger in LDR

Study	Subject Description	Study design	Connective tissue morphologic measures	Other outcome measures	Results
Salinero *et al.* 2020	**Marathon runners** n = 96 M 42.0 ± 9.6 years 175 ± 6 cm 73.7 ± 8.6 kg **Non-active people** n = 47 39.9 ± 11.6 years 176 ± 7 cm 79.6 ± 16.1 kg	Unilateral crossover Laboratory conditions	***- AT-CSA*** ***- AT-Thickness***	- Ankle dorsal flexion angle - Marathon performance - Running experience	- Runners AT-CSA (mm^2) 60.74 ± 14.41 - Control AT-CSA (mm^2): 53.62 ± 9.90 - AT-CSA significant greater in Runners - Runners AT-thickness (mm) 4.85 ± 0.75 - Control AT-thickness (mm): 4.60 ± 0.66 - AT-Thickness significant greater in Runners - AT-CSA and thickness correlates with body mass only in controls -AT-CSA and thickness correlates with height -AT-thickness correlates with years of running experience.
Shiotani *et al.* (2020)	**Amateur LDR** n = 10 M 22.0 ± 0.7 years 1.68 ± 0.04 m 55.5 ± 4.2 Kg BMI: 19.6 ± 1.2 Kg/m^2 Experience: 11.0 ± 2.2 years Km/wk: 43.7 ± 35.4 **Untrained subjects** n = 10 M 22.5 ± 1.4. year 1.70 ± 0.05 m 58.4 ± 5.6 Kg BMI: 20.3 ± 1.7 Kg/m^2	Unilateral crossover Laboratory conditions **4 measurements during 10Km test: pre, 30',60', post*	***- PF-thickness:*** 3 sites: proximal (in the proximity to the calcaneus), middle (level of navicular tuberosity) and distal (proximity to the second metatarsal head) - PF stiffness	- Foot length - Dorsal height - Navicular height - Arch height ratio - Navicular drop - FSP	- PF-thickness higher in runners at proximal site but not significant

Study	Subject Description	Study design	Connective tissue morphologic measures	Other outcome measures	Results
Wiesinger *et al.* (2016)	**Pro ski jumpers** n = 10 M 22.2 ± 2.9 years 176.3 ± 4.5 cm 64.3 ± 3.9 Kg BMI: 20.7 ± 1.0 Kg/m² **Pro LDR** n = 10 M 31.5 ± 4.6 years 180.9 ± 8.2 cm 72.8 ± 7.6 Kg BMI: 22.2 ± 1.7 Kg/m² **elite water polo players** n = 10 M 24.2 ± 3.2 years 182.4 ± 6.5 cm 84.3 ± 10.8 Kg BMI: 25.3 ± 2.8 Kg/m² **sedentary individuals** n = 10 M 31.0 ± 5.1 years 182.9 ± 7.2 cm 83.9 ± 12.3 Kg BMI: 25.0 ± 2.8 Kg/m²	Unilateral crossover Laboratory conditions	***- AT and PT CSA*** - AT and PT length - AT and PT stiffness-AT and PT stress - AT and PT strain -AT and PT Young's module	- MVIC knee flexors and extensors - MVIC ankle flexors and extensors	- LDR CSA (cm²): PT: 1.1 ± 0.1, AT: 0.7 ± 0.1 - PT-CSA (Nrm to mass and abs) higher in LDR than water polo and sedentary - AT-CSA higher in LDR than in water polo - Nrm to mass AT-CSA higher in LDR than sedentary

LDR: Long distance runners; BMI: Body mass Index; Wk: Week; AT: Achilles Tendon; CSA: Cross-sectional area; UTC: Ultrasonographic tissue characterization NMR: Nuclear Magnetic Resonance; Nrm: Normalized; Abs: Absolute; MVIC: Maximal voluntary isometric contraction; PT: Patellar tendon; CCR: Cross country runners; EMG: Electromyography; PF: Plantar fascia; FSP: Foot strike pattern.

Table III. Running biomechanics variables and morphological characteristics of the connective tissue.

Study	Subject Description	Study design	Connective tissue morphologic measures	Other outcome measures	Results
Chen *et al.* (2019)	**Amateur LDR RFS** n = 21 25.14 ± 4.,74 years BMI: 22.32 ± 2.31 Kg/m² Km/wk: 44.58 ± 24.53 **Amateur LDR FFS** N =14 26.85 ± 4.50 years BMI: 21.42 ± 1.29 Kg/m² Km/wk: 43.95 ± 25.73	Unilateral crossover Laboratory conditions	**- PF-thickness** - PF shear wave velocity - Hypoechogenicity	- FSP	- RFS PF-thickness (mm): 3.08 ± 0.35 - FFS PF-Thickness (mm): 3.41 ± 0.89 - PF-Thickness no significant differences
Farris *et al.* (2012)	**Amateur LDR** n = 12 M 27 ± 5 years 1.79 ± 0.06 m 78.6 ± 8.4 kg	Repeated Measures Laboratory conditions * Run 30´ at 12 km/h	**- AT-CSA** (pre and post 30´run) - AT stiffness - AT force -AT length and strain: T_1 (1´), T_2 (15´) and T_3 (30´)	- Ankle ROM - Running Kinematics - Hoping Kinematics	Pre running AT-CSA (mm²) 43 ± 8 -Post running AT-CSA (mm²) 41±8 - AT-CSA no significant differences

Study	Subject Description	Study design	Connective tissue morphologic measures	Other outcome measures	Results
Histen *et al.* (2016)	**Traditionally shod LDR** n = 17 (11 M, 6 F) 25.3 ± 6.5 years 165.1 ± 8.9 cm 67.3 ± 11.9 kg Miles/wk: 18.6 ± 6.7 **Minimalist LDR** n = 14 (12 M, 2 F) 30.1 ± 9.1 years 166.6 ± 8.5 cm 73.1± 7.8 kg Miles/wk: 24.1 ± 9.9	Unilateral crossover Laboratory conditions	**- AT CSA** - AT length - Tendon force - Tendon stress - Tendon elongation - Tendon strain - Tendon stiffness - Young's modulus	Traditionally/ Minimalist Shod	- Traditional AT-CSA (mm^2): 67.4 ± 8.5 - Minimalist AT-CSA (mm^2): 76.6 ± 7.7 - Minimalist runners significant greater AT CSA
Kernozek *et al.* (2018)	**RFS Runners** n = 17 F 21.9 ± 1.5 years 1.70 ± 0.03 m 59.2 ± 6.5 Kg **No RFS Runners** n = 18 F 21.6 ± 1.2 years 1.69 ± 0.07 m 61.4 ± 6.9 Kg	Unilateral crossover Laboratory conditions	**- AT-CSA** - AT stress	- RFS / No RFS - Cadence - Step length	- RFS AT-CSA (cm^2): 0.355 ± 0.02 - No RFS AT-CSA (cm^2): 0.359 ± 0.02 - No significant differences
Kubo *et al.* (2015b)	**41 trained LDR** **FFP** n = 12 M 20.4 ± 1.3 years 169.8 ± 5.2 cm 57.1 ± 4.7 Kg Experience: 6.5 ± 1.9 years **MSP** n = 12 M 20.6 ± 1.3 years 170.7 ± 4.9 cm 56.7 ± 4.0 Kg Experience: 8.4 ± 2.0 years **RFS** n = 17 M 20.6 ± 1.0 years 170.9 ± 5.6 cm 58.5 ± 4.2 Kg Experience: 7.3 ± 3.3 years	Unilateral crossover Laboratory conditions	**- AT-CSA** - AT elongation - AT stiffness - AT strain	- FSP - MVIC plantar flexors - Athletic level	- FSP AT-CSA (mm^2): 74.8 ± 10.6 - MSP AT-CSA (mm^2): 75.7 ± 11.7 - RFS AT-CSA (mm^2): 76.2 ± 12.6 - AT-CSA no significant differences

Study	Subject Description	Study design	Connective tissue morphologic measures	Other outcome measures	Results
Monte *et al.* (2020)	**LDR** n = 32 M 37.9 ± 13.0 years 70.7 ± 7.8 Kg 1.76 ± 0.06 m Experience: 9.5±8.9 years Workout: 3.7±1.8 days/week Km/wk: 43.7 ± 21.4	Unilateral crossover Laboratory conditions	**- AT-CSA** - AT length	**- Kinematic parameters:** CT, FT, SF, SL, RV, - S**pring mass model parameters:** vertical force, trochanter vertical displacement, leg spring compression, Kleg, Kvert **- Metabolic parameters** pulmonary ventilation; Vo2, HR, respiratory exchange ratio, energy cost of running - **Half marathon pace**	- AT-CSA (cm^2) 0.65 ± 0.15 - AT-CSA positive correlation with Kvert and RV
Neves *et al.* (2014)	**LDR** n = 20 F 20.7 ± 1.8 years 1.65 ± 0.06 m 60.5 ± 7.2 Kg Km/wk: 26.8 ± 12.1	Repeated Measures Laboratory conditions *Pre/Post 10´: 7 mph at 0%, 5.5 mph at + 6% and 5 mph at - 6% grade	**- AT-CSA**	- 3D Kinematics	- Pre-run average AT-CSA: 0.39 cm^2 -Post-run average AT-CSA: 0.36 cm^2 - AT-CSA significant decrease
Sponbeck *et al.* (2017)	**Pro CCR** n = 24 (8 M, 16 F) 19.88 ± 2.12 years 168.92 ± 17.16 cm 61.32 ± 20.15 kg Experience: 6.44 ± 5.54 years Run collegiately: 2.56 ± 1.54 years	Repeated Measures Laboratory conditions *4 measures: Before season -3weeks - 6weeks - End season (3-5 weeks)	**- AT-CSA**	- AT-Pain	- AT CSA significantly increased in the 3 and 6 weeks - AT-CSA no difference between the post and pre-season - Sex and mass were significant covariates
Ueno *et al.* (2017)	**Japan well-trained LDR** n = 30 M 20.2 ± 2.9 years 169 ± 4.7 cm 54.1 ± 3.4 Kg BMI: 18.7 ± 1.1 Kg/m^2	Unilateral crossover Laboratory conditions	**- AT-CSA** (Abs and nrm to mass) - AT length (Abs and Nrm to leg length)	-Energy cost at 14, 16 and 18 km/h - Best 5000 m record	- AT-CSA (mm^2): 111.2 ± 17.0 - No correlation AT-CSA and best 5000-m - No correlation AT-CSA and energy cost

Study	Subject Description	Study design	Connective tissue morphologic measures	Other outcome measures	Results
Zhang *et al.* (2018)	**Amateur neutral LDR** n = 11 (7 M, 4 F) 24.6 ± 6.0 years BMI: 22.7 ± 2.3 kg/m² Km/wk: 23.6 ± 10.5 **Amateur MCS LDR** n = 10 (6 M. 4 F) 27.9 ± 8.5 years BMI: 21.4 ± 2.0 kg/m² Km/wk: 26.4 ± 17.3 **Amateur minimalistic LDR** n = 7 (5 M, 2 F) 28.3 ± 8.4 years BMI: 22.2 ± 2.3 kg/m² Km/wk: 25.4 ± 15.0 **Amateur shoe insole LDR** n = 7 (3 M, 4 F) 25.1 ± 5.2 years BMI: 21.7 ± 1.9 kg/m² Km/wk: 26.2 ± 9.9	Unilateral crossover Laboratory conditions	**- PF-thickness:** (proximal, middle and distal) **- AT-Thickness**	- FPI - Arch height - Shoe properties - CSA and Thickness of foot muscles - Heel pad thickness	- Neutral AT- thickness (mm): 4.2 ± 0.5 - MCS AT-thickness (mm): 4.5 ± 0.4 - Minimalistic AT-thickness (mm): 4.6 ± 0.4 - Insole AT-thickness (mm): 4.2 ± 0.2 - Neutral Proximal PF (mm): 3.2 ± 0.4 - MCS Proximal PF (mm): 3.3 ± 0.2 - Minimalistic Proximal PF (mm): 2.9 ± 0.1 - Insole Proximal PF (mm): 3.0 ± 0.2 - Neutral Middle PF (mm): 1.7 ± 0.2 - MCS Middle PF (mm): 1.9 ± 0.3 - Minimalistic Middle PF (mm): 1.7 ± 0.2 - Insole Middle PF (mm)1.7 ± 0.2 - Neutral Distal PF (mm): 0.9±0.1 - MCS Distal PF (mm): 0.9 ± 0.1 - Minimalistic Distal PF (mm): 0.9 ± 0.1 - Insole Distal PF (mm): 0.8 ± 0.1 - Minimalistic 10% thicker AT compared to neutral shoe and insole - Minimalistic 9% and 12% thinner proximal PF than neutral and MCS respectively

LDR: Long distance runners; FSP: Foot strike pattern; RFS: Rear-foot strike pattern; FFS: Forefoot strike pattern; MSP: Midfoot strike pattern; Wk: Week; PF: Plantar fascia; BMI: Body mass Index; AT: Achilles Tendon; CSA: Cross-sectional area; CCR: Cross country runners; Nrm: Normalized; Abs: Absolute; CT (contact time); FT (flight time); SF (Step frequency); SL (step length); RV (running velocity); Kleg (leg stiffness); Kvert (vertical stiffness); HR (Heart Rate); MCS: Motion control shod; FPI: Foot posture index; MVIC: Maximal voluntary isometric contraction

Table IV. Tendon pathology and morphological characteristics of the connective tissue.

Study	Subject Description	Study design	Connective tissue morphologic measures	Other outcome measures	Results
Freund *et al.* (2019)	**LDR** n = 22 (20 M, 2 F) 49.1 ± 11.5 years 1.74 ± 0.09 m 70.9 ± 11.3 Kg (Trans Europe Foot Race 4487Km).	Repeated measures Laboratory conditions *Run 4487 Km. Each 1000 Km or after abortion of the run	- **AT-thickness**	- SI at AT insertion, mid-portion and at site of lesion - Distance lesion from AT insertion - Number of new lesions - Highest intraosseous SI in any foot bone - Nº of bone bruises /subchondral or osseous lesions - SI of FP was rated	- AT-thickness baseline (mm) 6.8 ± 0.37 - Larger differences on the right side: SI of the AT insertion, the SI of the plantar aponeurosis - Finishers and no finishers showed significant differences at the beginning only in the SI of the PF
Hagan *et al.* (2018)	**Collegiate CCR** n = 22 (13 M, 9 F) 19 ± 1.5years 172 ± 7 cm 60.4 ± 8 Kg **Healthy controls** n = 11 (1M, 10 F) 24 ± 5.1 years 163 ±7.32 cm 59.1 ± 11.6 Kg	Repeated Measures Laboratory conditions *(S1)1 wk before start trainings (S2) 1 wk after the season conclusion (S3) 1 wk before season conclusion	**- AT-Thickness** **- PT-Thickness** - Collagen alignment - Tendon structure	- VISA-A - Neovascularity	- AT TS not confirmed by ultrasound and VISA-A. - CCR lower tendon alignment. - Tendon thickness no changes in the CCR -Tendon collagen alignment improves at S3 compared with S2 CCR underwent remodelling of both the AT and PT without TS
Hall *et al.* (2015)	**Asym runners** n = 39 (20M, 19 F) 39.3 (20–67) years 171.2 (152.4– 190.5) cm 66.0 (52.2–88.2) Kg BMI: 22.2 (18.7–26.4) Kg/m^2	Unilateral crossover Laboratory conditions	**- PF-thickness** - Plantar heel pad thickness and compressibility - PF appearance	- Shoe type preference - Neovascularity	- PF-thicknesses (mm): Right 3.78 (2.4–7.0), Left 3.87 (2.3–6.7). - Neovascularity in 31% PF, and 44% runners. - 35% of heels among 41% of runners showed a thickener PF (> 4 mm). 52% of PF normal texture.

Study	Subject Description	Study design	Connective tissue morphologic measures	Other outcome measures	Results
Hirschmuller *et al.* (2012)	**Asym runners** n = 634 (425 M, 209 F) 41 ± 11.2 years 175.8 ± 8.7 cm 71.3 ± 11.1 Kg BMI: 23 ± 2.4 Kg/m^2 Km/wk: 39 ± 2.4	Repeated Measures Laboratory conditions * 1 year prospective	**- AT-thickness** - Spindle shaped thickening - Echogenicity	- Neovascularity - Training volume - Medical history - AT symptoms	- AT-Thickness (mm): 5.6 ± 1.1 - Echogenicity: 15% Hypo, 2% Hyper. - Neovascularity: 40% - 61 subjects new AT symptoms: 29 mid-portion tendinopathy, 3 insertion tendinopathy, 29 unspecific pain. - AT thickness significantly greater in mid-portion tendinopathy - Neovascularization significant increased risk of developing mid-portion tendinopathy - Neovascularization greatest positive predictive value for mid -portion tendinopathy
Lieberthal *et al.* (2019)	**LDR** n = 37 M 36.0 (32.0-42.0) years 180.0 (174.0-183.5) cm 77.4 (73.8-83.4) Kg	Unilateral crossover Laboratory conditions	- **AT-Thickness** - AT normal / AT abnormal (US findings)	- Standing Lunge Test - Straight Knee dorsal flexion test - Running years - Mileage - Sessions/wk - Nº Marathons or Half Marathons	- 48 Normal AT, 26 abnormal AT. - AT-Thickness (mm): Normal AT 5.4 ± 0.8, Abnormal AT 4.7 ± 0.5 - Abnormal AT significantly more years of running
Ooi *et al.* (2015)	**Marathon runners** n = 21 (13 M, 8F) 37.1 ± 11.3 years 1.75 ± 0.08 m 70.0 ± 11.6 Kg BMI: 22.6 ± 2.4 Kg/m^2 **Control** n = 20 (12 M, 8 F) 37.5 ± 12.3 years 1.73 ± 0.09 m 72.4 ± 11.8 Kg BMI: 23.9 ± 1.9 Kg/m^2	Repeated Measures Laboratory conditions *Pre and post marathon	**- AT-Thickness** **- AT-CSA** - Echogenicity - Elastography	- VISA-A - Neovascularity	- Pre AT-thickness (mm): Runners 0.46 ± 0.05 / Control 0.52 ± 0.07 - Pre AT-CSA (mm^2): Runners 0.50 ± 0.09 Control 0.57 ± 0.11 - Pre AT-CSA and AT -Thickness significant higher in runners - Hypoechogenicity significant higher in runners - No significant changes in AT-thickness and CSA post marathon - Marathon induced a significant increase in intratendinous neovascularity - 75% of neovascularity go normal after 4-6 wk.

Study	Subject Description	Study design	Connective tissue morphologic measures	Other outcome measures	Results
Shaikh *et al.* (2012)	**Pro LDR** n = 25 (19 M, 6 F) 34.2 ± 13.0 years 1.74 ± 0.08 m 69.68 ± 9.39 Kg BMI: 22.82 ± 1.71 Kg/m^2 **Controls** n = 25 (19 M, 6 F) 31.3 ± 15.1 years 1.68 ± 0.08 m 66.16 ± 15.39 Kg BMI: 23.36 ± 4.1 Kg/m^2	Unilateral crossover Laboratory conditions	- **AT-Thickness**: 3 sites: CI, MT, MTJ	- Neovascularity - AT symptoms	- LDR MTJ AT -Thickness significant higher - LDR CI and MT AT-Thickness higher but no significant - LDR AT more symptoms - Signs of tendinopathy: 36% LDR AT and 4% control AT
Tillander *et al.* (2019)	**Amateur LDR** N = 21 (15 M, 6 F) 47.5 ± 6.3 years BMI: 23.3 ± 2.0 Kg/m^2 Km/wk: 28.8 ± 13.5	Unilateral crossover Laboratory conditions	- **AT-Thickness** (Max and 30 mm to calcaneus) - AT structure	- Neovascularity - Tendinosis and bursitis: Yes/No - VISA-A	- Max AT-Thickness (mm): Asym: 5.7 ± 1.0, Sym 6.7 ± 0.8 - 30mm AT-Thickness (mm): Asym 4.5 ± 0.8, Sym 5.9 ± 0.8 - Sym AT were thicker than Asym

LDR: Long distance runners; AT: Achilles Tendon; PT: Patellar tendon; PF: Plantar fascia; SI: Signal intensity; CCR: Cross country runners; Wk: week; TS: Tendinopathy symptoms; BMI: Body mass Index; Asym: Asymptomatic; Sym: Symptomatic; US: Ultrasounds; CSA: Cross-sectional area; CI: Calcaneus insertion; MT: Midtendon; MTJ: Musculotendinous junction.

Sample characteristics

Regarding sample characteristics, 16 studies were included. In relation with morphologic characteristics of the AT, 10 studies analysed the differences between runners and healthy untrained subjects (2, 3, 16, 17, 31-36). Regarding AT, Wiesinger *et al.* (36) and Kongsgaard *et al.* (37) studied the differences of the AT morphologic characteristics between runners and other sport athletes. Differences in AT morphologic characteristics were also analysed in relation to other aspects: road runners and trail runners (38), running performance (39), leg dominant side (40) and nationality (Kenyan *versus* Japanese runners) (41). Finally, other subject characteristics such as sex, body mass, height, body mass index and running experience (35, 40) were also studied in relation to the AT morphologic characteristics.

The PT morphologic characteristics were analysed by four studies (2, 17, 36, 39). Kubo *et al.* (2, 17) and Wiesinger *et al.* (36) studied the differences between runners and healthy untrained subjects. Kubo *et al.* (39) analysed the PT differences in relation the running performance. In relation with other sports, PT-CSA was analysed between long-distance runners, water-polo players and ski jumpers (36).

Finally, regarding the PF, the morphological characteristics between runners and untrained subjects were analysed only by Shiotani *et al.* (42).

Running biomechanics variables

In relation to biomechanics variables, 10 studies were included. The morphologic characteristics of the AT were analysed by 9 studies (14, 19, 43-49). The AT morphologic characteristics were assessed in relation to foot strike pattern (45, 46) and the influence of the footwear (*i.e.*, traditional *versus* minimalist shod) (44, 49). Farris *et al.* (43) and Neves *et al.* (47) studied the AT differences pre and post 10-min and 30-min running bouts, respectively. Other aspects that have been studied in relation with AT morphologic characteristics are: lower limb stiffness (19), cross country season (48), foot characteristics (49), running energy cost and running performance (14).

Finally, two studies assessed the relation of the PF-Thickness with running variables. Chen *et al.* (50) analysed the relation between PF-Thickness and foot strike pattern, while Zhang *et al.* (49) assessed the relation between footwear characteristics and PF-Thickness.

In terms of running biomechanics variables any study assessed the morphologic characteristics of the PT.

Tendon Pathology

In relation with tendon pathology, 8 studies were included. The AT morphologic characteristics and tendon pathology variables were analysed by 7 studies (51-57).

Morphologic characteristics of the PT (52) and PF (58) were studied in relation with tendon pathology variables.

DISCUSSION AND IMPLICATIONS

The purpose of this systematic review was to critically analyse the literature that has considered the morphologic characteristics of the lower limb connective tissue (*i.e.*, thickness and CSA) and its influence on running biomechanics and tendon pathology variables in runners. After the systematic analysis described above, thirty-four studies were included. In order to assess the methodological quality of these studies, the modified Downs and Black scale (30) was used in which all the studies reported, at least, 8 points out of a total of 14. In order to obtain a better thematic and visual understanding, the results were shown in relation to 3 aspects: sample characteristics, running biomechanics variables and tendon pathology.

Sample characteristics

In relation with sample characteristics, the main variable that correlates with the morphology of the connective tissue is the running condition. In this way, runners showed higher tendons in terms of CSA and thickness than untrained people or other sports people. Other variables that positively correlate with the morphologic characteristics of the connective tissue are the ethnic, weight, height, sex and running performance.

For the AT characteristics, a large majority of the studies showed that runners had greater AT-CSA and AT-thickness compared to untrained subjects (16, 17, 31-36). Although some studies did not found differences (2, 3), in both studies, Kubo *et al.* studied sprinters (2, 3), while the rest of the studies assessed long distance-runners (16, 17, 31-36). It seems that running speciality might determine whether there exist differences, in terms of AT morphologic characteristics, between runners and untrained subjects. Thereby, long-distance runners apparently show higher CSA and thickness in such tendon. The greater weekly mileage run by these runners, and therefore a repeated load for a longer time, might justify why these differences occur in long-distance runners and not in sprinters.

In relation with the PT, Kubo *et al.* (17) and Wiesinger *et al.* (36) showed higher thickness and CSA, respectively, in long-distance runners compared to untrained subjects showing no differences for sprinters (2). Therefore, as already mentioned, running speciality seems to be determinant when analysing differences of the tendon morphologic characteristics between runners and untrained subjects.

It is worth mentioning that both thickness and CSA values were normalized to the body mass in some studies (16, 17, 36). In fact, in two of these studies (16, 17), the differences between runners and controls only happened for values normalized to body mass and not for absolute values. This finding highlights the importance of normalizing these values (*i.e.*, thickness and CSA) in relation to the body mass when these characteristics are correlated with other variables, especially when the sample characteristics are not very homogenous.

From all the studies considered, only one assessed the PF morphologic characteristics between runners and untrained people (42). Greater thickness was found among runners, but the results were no significant. The lack of consistency of the results and the reduced number of studies suggest that further research is needed to deepen the understanding in relation to the aforementioned structure.

The current literature shows that the thickness and CSA of the AT and PT were higher in long distance runners than untrained people suggesting that the chronic exposure of these tendons to repetitive impacts has resulted in significant tissue adaptation. When comparing with other sports, it seems that this chronic exposure to repetitive loading may explain why kayakers (37) and water-polo players (36), with less lower-limb tendon load, showed smaller AT-CSA and PT-CSA than long distance runners. These differences do not occur in sports with a higher AT and PT load such as volleyball (37) or ski-jump (36).

As mentioned above, there are other variables that are less studied (*i.e.*, ethnics, weight, height, sex and running experience), which seem to show a positive correlation with the morphology of the connective tissue in runners. Kenyan runners had a higher AT-CSA than their Japanese counterparts (41). AT-CSA showed a positive correlation with height (35, 40), weight (40) and sex (*i.e.*, males larger values) (40), while AT-Thickness correlates positively with height (35). Again, the repetitive loading over tendon appears as an influencing factor, since both running experience and kilometres run per week positively correlate with AT-CSA (40) and AT-Thickness (35, 40).

Considering the results, the exposure of the tendons to repetitive loading appears to be the main influencing factor on tendon characteristics. But not exclusively the load, but also the time that load has been repeated seems to be influential. The tendons of the sprinters bear high loads, but such loads are not as repeated in time as in the case of long-distance runners. Thus, the chronicity exposure to repetitive loading seems to be key when analysing differences of the tendon morphologic characteristics between runners and untrained people. To the best of the authors' knowledge, these differences remain uncertain and not well understood by professional and amateur runners. It would be worth

analysing whether tendon morphological characteristics is dependent upon training level within the same sport.
Other possible influencing factors that were studied but that did not show a significant relationship with the morphological characteristics of the connective tissue were running surface (38), running performance (39) and dominant side (40). The studies that have evaluated these aspects are few, so the scientific evidence is limited.

Running biomechanics variables

The two main running biomechanics variables that were analysed were the FSP (45, 46) and the footwear (44, 49). However, the results of these studies are controversial. FSP and footwear are closely related as it is known that runners wearing minimalist shoes typically show a forefoot strike pattern while runners using traditional footwear usually collide with the ground with the heel first (59). However, there is no consensus about the relation of these variables and the AT morphologic characteristics. While some studies supported that minimalist runners showed larger AT-CSA (44) and AT-Thickness (49), other studies did not find any differences between AT characteristics and foot strike pattern (45, 46). The fact that minimalist runners demand more the AT (60) could explain why these tendons are larger. Again, the loading repetition would result in tendon adaptations. One study that did not found any differences between foot strike pattern and AT characteristics was the study of Kubo *et al.* (46), where forefoot, midfoot and rearfoot strike patterns where compared. This fact could be explained because all the subjects of this study (46) were high level runners (*i.e.*, best official 5000 m record faster than 15'). Probably, the AT of all these highly trained subjects have been exposed to high repetitive loadings, regardless of the foot strike pattern, which could explain that no differences were found in relation to the foot strike pattern. The other study that did not found AT differences in relation with the foot strike pattern was Kernozek *et al.* (45). In this study, the sample was formed only by females. This fact could be key to explain this lack of differences, since in other study, Joseph *et al.* (61), found that greater AT adaptations, after a 6-month adaptation from traditional shod to minimalist shod, appeared only in males.
It seems that the chronic adaptations to the repetitive loading mentioned above also occurs acutely. It has been found that professional cross-country runners showed larger AT-CSA during cross country season, compared to pre-season and post-season (3-5 week after) (48). Therefore, these tendon adaptations could occur in a chronic way between subjects demanded to different loads (*i.e.*, runners *vs* untrained people), but acute intra-subject differences would also appear in response to moments of the season with greater loads. However, when the immediate response to the load is evaluated, the results corresponding to the tendon morphology are again contradictory. While, Neves *et al.* (47) found a decrease in the AT-CSA after 10´run, no changes were found by Farris *et al.* (43) after 30' run. More research seems necessary in this regard. It would also be interesting, when assessing the immediate adaptations of the tendon to the load, to take into account a factor that seems to influence such as the type of footwear.
Other running biomechanics variable, that showed a relation with tendon morphology is the lower limb stiffness, although this variable has been less studied. It seems that vertical stiffness (Kvert) has a positive correlation with AT-CSA (19) although further research is needed in this line. Finally only two studies (49, 50) analysed the relation between the PF morphologic characteristics and running biomechanics variables. The main finding in relation with this structure was that PF was thinner among runners using minimalist running shoes (49).
An outstanding finding in relation with running biomechanics variables is that none of the studies considered here analysed the influence of the PT characteristics. The lack of studies on PT is striking as it is a basic structure in running biomechanics. It is known that during running the lower limb behaves such as a spring that continually compresses and decompresses (9). During such mechanism, a proper function of the knee is needed and consequently the PT characteristics would be decisive.

Tendon pathology

Although eight studies assessed the connective tissue morphologic characteristics in relation with tendon pathology variables, only three of these studies (53, 54, 57) analysed the direct relation between these variables and the connective tissue characteristics. It was found that symptomatic AT were thicker compared to healthy AT (53, 57). Contrary to this finding, Lieberthal *et al.* (54) showed that the AT with abnormal ultrasound findings were thinner than normal AT. Probably, the variable used in each of these studies to consider the tendon as a "pathological tendon" can explain the reason for these contradictory results. In relation with the pathology model of the tendinopathy, Cook *et al.* (20) claimed that is a continuum. Thus, following this continuum, the tendinopathy process would comprise three states or phases: reactive tendinopathy, tendon disrepair and degenerative tendinopathy (20). In the first two phases, the presence of a thickening of the tendon structure is observed, while in the last of them the clinic of this tendon may show a thickening but also not occur (20). It would be worth knowing in which of these three phases were the subjects of the mentioned studies (53, 54, 57) as this could better explain

why this thickening occurs or not. Additionally, it should be noted that one of the inclusion criteria of Lieberthal *et al.* study was that the subjects must have run a marathon or half marathon in the last two years (54). The volume of kilometres necessary to complete the training of such efforts is usually quite important. In fact, the number of weekly kilometres run by the participants in the study of Lieberthal *et al.* (54) is almost double than that of the studies of Hirschmuller *et al.* (53) as Tillander *et al.* (57). It may happen that the participants of the study by Lieberthal *et al.* (54), having run a greater number of kilometres and therefore a greater load on the tendon, will be in a more advanced stage of tendinopathy. This could correspond to that phase of degenerative tendinopathy in which the tendon does not necessarily appear thickened. As is explained by Cook *et al.* (20), this last tendon pathology phase is characteristic of younger subjects or elite athletes with a chronically overloaded tendon, which is also a characteristic of the study sample of Lieberthal *et al.* (54). Furthermore, as Cook *et al.* explain in their recent update to their continuum model of tendon (59), not always all the pathologic and painful tendons show an altered ultrasound image.

Finally, only two studies (52, 58) analysed the PT and the PF in relation with pathology variables, without any significant result. Again, as in the previous sections, the main research focus was the AT. It would be interesting for future researchers to delve deeper into the influences and relationships of these structures, PT and PF.

From a practical point of view, it seems clear that runners present larger tendons as adaptations to the repetitive loading stimulus. These adaptations might lead to better running performance, so coaches should consider how the training load affects the tendon in order to optimize that adaptation. Especially, this attention to tendon adaptation should be a fundamental aspect in the training of amateur runners, whose tendons do not show such adaptations. However, when the running repetitive load is excessive, tendons might not be able to adapt properly increasing, thus, the likelihood of running related injuries. The ultrasonography is reliable method to control these morphologic tendon changes, and consequently assess these adaptations. Nevertheless, the clinicians should not consider this diagnostic imaging technique as an essential criteria, but rather as a complementary test, in the diagnosis of tendon pathology in runners. Given that tendon pathology may or may not present with morphological changes, detectable with ultrasound, the diagnosis of tendinopathy should be based on the tendon symptoms as well as its functional alteration.

CONCLUSIONS

The present systematic review about the morphologic characteristic of lower limb connective tissue and its influence in running biomechanics and tendon pathology variables of runners reveals that: 1) runners have higher AT and PT than control subjects (*i.e.*, untrained subjects) whose tendons are demanded at lower loads. When the characteristics of the sample are not homogeneous, it seems important to analyse the differences with the normalized connective tissue values in relation to body mass. 2) The footwear and Kvert seems to be the main running biomechanics variables that relate with the morphologic characteristics of the connective tissue. 3) In relation to the tendons of runners that present tendinopathy, there is no consensus regarding whether these tendons are thicker or thinner. Scientific evidence seems to indicate that this fact will depend on the phase of tendinopathy in which it is found.

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DATA AVAILABILITY

N/A.

CONTRIBTIONS

LERS, ARP: study design. LERS, ARP, FGP: study analysis. LERS, DJC, ACL, ARP: data collection. ARP, FGP: results interpretation. All authors contributed to the manuscript writing and have read and approved the final version.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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Fat Mass as an Independent Variable to Assess the Possibility of Predicting the Stability in Postmenopausal Women with and Without Osteoporosis

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SUMMARY

Purpose. We investigated the effect of fat mass value (based on skinfold thickness) and body mass index (BMI) on the center of pressure sway to assess the possibility of predicting the stability of postmenopausal women with and without osteoporosis during the standing position.

Methods. A total of 78 postmenopausal women participated voluntarily in the study. Postmenopausal (aged 55–75 years) women were divided into, osteoporotic, (lumbar T-score ≤ - 2.5, n = 51), and nonosteoporotic (lumbar T-score > - 1, n = 27) groups. Total body fat mass was assessed based on skinfold measurement. The isometric strength of the lower limb muscle groups was recorded using a digital hand-held dynamometer. Postural sway was determined by measuring the center of pressure (CoP) sway in the anterior-posterior (AP) and mediolateral (ML) direction during a comfortable double stance position, with eyes open.

Results. The fat mass value and BMI were significantly lower in the osteoporotic group than in the nonosteoporotic group. The isometric strength of all lower extremity muscle groups was considerably lower in osteoporotic subjects than in the nonosteoporotic group, except for dorsi flexors and knee extensors. In the mediolateral direction, the CoP sway displacement and velocity were significantly higher in the osteoporotic group than in the nonosteoporotic group. Fat mass was a significant independent variable for predicting CoP velocity in the ML direction (VIF = 2.51, p = 0.050).

Conclusions. The low-fat mass value is associated with mediolateral postural instability in postmenopausal women. Losing BMI-fat mass and muscle strength may affect postural instability in postmenopausal women. The cautiously strength-balance exercises may be effective for osteoporotic women to improve stability.

KEY WORDS

Osteoporosis; postmenopausal women; fat mass; center of pressure; instability.

INTRODUCTION

Estrogen deficiency related to menopause status could induce a net negative bone balance (postmenopausal osteoporosis) (1). Furthermore, if left untreated, half of the postmenopausal women may experience osteoporotic fractures in their remaining lifetime (2).

According to a systematic review in 2018, the overall prevalence of osteoporosis is estimated at 32% (confidence interval 95%) in postmenopausal Iranian women aged 50 to 55 years old (3). Also, it was reported that the economic burden of osteoporosis-related fracture is significant, costing approximately $ 17.9 billion and £ 4 billion per annum

in the USA and UK, respectively (4), which indicates the profound impact, in terms of mortality and morbidity on individuals, healthcare systems and communities as a whole. Hence, it highlights the importance of monitoring and screening the risk of falling and fracture in this population.
The fracture risk assessment algorithm is currently used to estimate fracture risk through Clinical Risk Factors (CRFs) and Bone Mineral Density (BMD). However, it has been reported that the addition of fall risks, as an independent risk factor, to other CRFs and BMD considerably increases the predictive worth of such algorithms (5). Moreover, postural instability has been proposed as the strongest predictor for falls and fractures (6, 7).
The (Center of Pressure) CoP-related parameters extracted from static posturography have been shown as strong predictors of falling risk among healthy community-dwelling elderly (8–10). The CoP parameters may provide valuable information for diagnosing people at risk of falling due to instability.
Aging is associated with increased CoP sway in the anterior-posterior/medial-lateral direction. Laughton *et al.* reported that older people who experienced falling had more CoP displacement in the anteroposterior direction (8). Silva *et al.* showed a higher anteroposterior CoP sway in osteoporotic women than in healthy women (9). Sinaki *et al.* revealed that osteoporotic-kyphotic individuals have higher CoP sway velocity and displacement in the medial-lateral direction than healthy age-matched individuals (10). Instability in osteoporotic women, associated with increased CoP sway, can be due to decreased muscle strength, increased fear of falling, weight loss, and muscle/bone mass ratio change (11).
Some previous studies demonstrated a relationship between the body's anthropometry and balance control (12, 13). The effect of obesity and BMI (Body Mass Index) on balance control has been regarded (14–16). According to a recent review finding, an increase in BMI and fat mass is proposed to predict postural instability, fall risk, and fracture (17). Neri *et al.* showed a positive association between waist circumference and risk of falls in older women; the obese women exhibited reduced postural balance control and increased fear of falling (18). However, there are conflicting results on the relationship between BMI-fat mass and balance control parameters. Some reports reveal no significant relationship between BMI-fat mass and balance function. Others linked obesity to a fall risk factor related to impaired postural control in older adults (19–22). A low BMI-fat mass has been proposed to be a relevant hip fracture risk factor (23).
Furthermore, it was reported that the relationship between BMI-fat mass and risk of fractures is site-specific with both detrimental and protective effects on fracture risk, with the protective effects explained by the endocrinal and load-related mechanical impact of adipose tissue on BMD (24). There is a paradoxical effect in which the increase in BMI-fat mass leads to a detrimental effect on postural control and a protective effect on BMD. In addition to the impact of fat mass on BMI, it has a gender-dependent effect on balance control (20).
Due to the high risk of falling in osteoporotic women and the changes that occur in weight and fat mass in these people, we designed this study to investigate the effect of the fat mass (based on the skinfold thickness) and BMI on the fall efficacy scale (FES) and static stability parameters in postmenopausal women with and without osteoporosis. Measuring the CoP displacement and velocity requires expensive and precise laboratory equipment such as an accurate force plate. Determining the relationship between fat mass (obtained through cheap and accessible tools such as skinfold thickness by caliper) and CoP sway may make it possible to regularly monitor and predict the equilibrium status of women with osteoporosis who are at high risk of falls. In this way, physiotherapists can be more effective in planning balance-strengthening exercises and reducing the risk of falling. So, we hypothesized that fat mass (based on the skinfold thickness) as an independent variable might predict the center of pressure sway in postmenopausal women with and without osteoporosis during the standing position.

METHODS

Study design and participants

This cross-sectional study was conducted (May 20, 2019 - Sep 22, 2019) at the Motion Analysis Laboratory of the Physical Therapy Department in Tarbiat Modares University, Tehran, Iran. The Medical Ethics Committee of Hamedan University of Medical Sciences approved the study (IR. UMSHA. 1398.069). The sample size was estimated based on the mean ± SD of CoP displacement in the anterior-posterior direction in a double standing position in Torkaman *et al.* (2015) with a 95% confidence interval and 80% power (25). Individuals were classified into osteoporosis (n = 51, lumbar T-score ≤ - 2.5) and non-osteoporosis (n = 27, Lumbar T-score > - 1) groups based on dual-energy X-ray absorption assay, which was performed 3-6 months before the study. A trained physiotherapist performed all assessments from 9.00 a.m. to 1.00 p.m., two sessions apart, within 24 hours.
Among postmenopausal women who became acquainted with the study by distributing promotional cards and counseling at the bone density centers, 84 women were willing to participate in the study voluntarily. The inclusion criteria included females 55–75 years of age, menopausal status for at least one year before the study, with no record of regular physical exercise for at least one year. In addition, the subjects who had secondary osteoporosis, a history of

osteoporotic fracture, osteopenia (- 2.5 > T-score ≤ - 1), the presence of neurogenic or myopathic disorders, diabetes, thyroid disease, rheumatoid diseases, any malignant neoplasia, and the use of drugs known to affect muscle strength were excluded from the study. Thus, a total of 78 volunteers were finally enrolled in the study. The methods and aims of the assessments were entirely explained to the subjects, and they signed consent forms before the evaluations.

Measurements

All subjects were assessed in two sessions. During the first session, anthropometric parameters, including age (year), height (m), and body mass index (BMI, weight/height^2, kg/m^2), were recorded. The skin-fold thickness was measured using a caliper to calculate fat and fat-free mass. The isometric strength was measured for the lower extremity muscular groups. The FES was administered through an interview using the Persian version of the FES questionnaire (26, 27), and finally, the self-reported duration of menopause was determined. After 24 hours, the static postural stability was assessed in the second session.

Fat mass measure

We measured the skinfold *thickness* in the triceps, supra iliac, and thigh regions using a caliper (Nederland b.v-Pomdernal-Huidplooidikte). Briefly, the skin fold was picked between the thumb and forefinger and pulled away slightly from the underlying tissue in each area. When the caliper jaws were applied to the skin fold, the thumb and forefinger were removed, then reading was taken after 2 or 3 s. All measurements were performed three times on the right side of the body in a standing position. The internal consistency *was excellent* for the triceps, supra iliac, and thigh regions (*Cronbach's alpha* 0.978, 0.979, and 0.939, respectively). First, the average value of fat mass was determined as a percentage of body weight by the nomogram proposed by Pollock *et al.* (26); then, the fat-free mass was calculated by the Siris equation (27).

Muscular strength measurement

The isometric strength of the hip abduction, adduction, extension and flexion, knee extension and flexion, and dorsi and plantar flexion of the ankle were measured bilaterally. The measurement was done by a digital hand-held dynamometer (Hand-held Dynamometer; Lafayette Instrument Co., Lafayette, IN, USA). An expert physiotherapist assessed each measure three times bilaterally, with at least one min of rest between repeated tests of the same muscle group; the mean value was used for analysis. The duration of each isometric test was 5 s, and the strength value was recorded in kilograms (kg).

Static postural stability measurement

The static postural stability assessment was performed on a force plate (9286B; Kistler Co., Winterthur, Switzerland). Data acquisition was performed by the sampling frequency of 100 Hz. All participants were assessed in a comfortable double stance position; Data were taken with eyes open, and registration time was 20 s. Data were processed by MATLAB software. The CoP displacement (Cm) and mean velocity of the CoP sway (cm/s) were determined in the anterior-posterior (AP) and mediolateral (ML) directions.

Data analysis

SPSS version of 16 (IBM, Armonk, NY, USA) was used for statistical analysis. The Shapiro-Wilk test showed the normal distribution of data ($p > 0.05$), so the independent-sample t-test was used to compare the variables between groups. The linear regression model was also performed in relation to the fat mass, fat-free mass, T-score value, FES score, and BMI with the CoP displacement and CoP sway velocity. Pearson's coefficient correlation assessed the correlation of the variables. Statistical significance was set at $p \leq 0.05$.

RESULTS

The flow diagram of participants is illustrated in **figure 1**. According to the include and exclude criteria, seventy-eight volunteers enrolled in the study and were assigned to the osteoporotic and nonosteoporotic (normal BMD) groups.

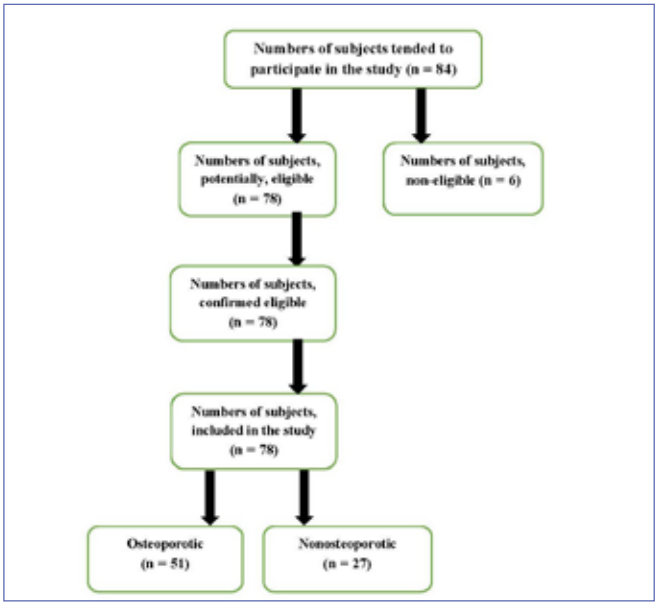

Figure 1. Diagram of the number of subjects at each stage of the study.

Anthropometric and basic variables are shown in **table I**. There were no significant differences in age, the duration of menopause, and fat-free mass ($p > 0.05$) between the two groups. The BMI and fat mass values were significantly higher in the nonosteoporotic (normal BMD) group than in the osteoporotic group ($p = 0.032$ and $p = 0.040$, respectively). The FES score in the osteoporotic group was higher than that of the nonosteoporotic group ($p = 0.012$).

According to **table II**, the isometric strength of all evaluated muscle groups was significantly lower in the osteoporotic group compared to the nonosteoporotic group ($p \leq 0.05$), except for dorsi flexors and knee extensor muscle groups, which values indicated no significant difference between two groups ($p > 0.05$).

In the osteoporotic group (**table III**), the ML CoP velocity and displacement were significantly higher than in the nonosteoporotic group ($p = 0.048$ and $p = 0.014$, respectively). The CoP sway velocity and displacement in the AP direction showed no significant difference between groups ($p > 0.05$).

Table IV shows a significant negative correlation between BMI, fat mass, fat-free mass, and lumbar T-score with CoP mean velocity in the ML direction ($p = 0.016$). Conversely, the FES correlated positively and significantly with CoP velocity in the ML direction ($p \leq 0.05$). In addition, FES correlated negatively with fat mass value ($p = 0.038$). Conversely, BMI, fat-free mass, and lumbar T-score showed a significant positive correlation with fat mass value ($p \leq 0.05$).

The correlated parameters with CoP velocity in the ML direction (**table IV**) were entered as the independent predictors in the linear regression analysis model to compare their value to predict the CoP velocity in the ML direction. Fat mass was observed as a significant independent variable for predicting CoP velocity in the ML direction ($p \leq 0.05$) (**table V**).

The collinearity test indicates no significant collinearity for the selected independent variables (VIFs ≤ 5).

Table I. Anthropometric and basic variables between the groups, Mean ± SD.

Variables	Osteoporotic (n = 51)	Nonosteoporotic (n = 27)	P-value
Age (years)	58.45 ± 5.03	56.22 ± 5.97	0.085
BMI (kg/m²)	27.24 ± 3.34	28.91 ± 2.70	0.032*
Menopause duration (years)	11.5 ± 6.88	8.61 ± 6.78	0.238
Fat mass (kg)	17.02 ± 4.74	19.36 ± 4.21	0.040*
Fat-free mass (kg)	49.05 ± 5.02	51.04 ± 4.39	0.094
Lumbar T-Score	-2.85 ± 0.70	0.05 ± 0.99	< 0.001*
FES score	17.78 ± 9.42	12.45 ± 3.43	0.012*

*Significant difference between two groups ($p \leq 0.05$). BMI: Body Mass Index; FES: Fall efficacy scale.

Table II. Isometric Strength of lower extremity muscle groups, Mean ± SD.

Muscular groups (Isometric strength) (Kg)	Osteoporotic (n = 51)	Nonosteoporotic (n = 27)	P-value
Dorsi flexors	11.98 ± 4.86	14.06 ± 6.37	0.116
Plantar flexors	11.08 ± 4.63	13.61 ± 4.18	0.026*
Hip abductors	15.23 ± 6.95	19.81 ± 12.01	0.040*
Hip adductors	10.85 ± 3.51	13.81 ± 5.76	0.007*
Hip flexors	11.30 ± 5.86	14.44 ± 7.87	0.050*
Hip extensors	11.58 ± 6.35	16.33 ± 9.10	0.010*
Knee flexors	9.27 ± 4.33	13.34 ± 9.83	0.016*
Knee extensors	18.02 ± 5.49	19.91 ± 9.33	0.277

*Significant difference between two groups ($p \leq 0.05$).

Table III. Static stability parameters, Mean ± SD.

Variables	Osteoporotic (n = 51)	Nonosteoporotic (n = 27)	P-value
CoP displacement in AP direction (cm)	0.162 ± 0.0047	0.022 ± 0.304	0.183
CoP displacement in ML direction (cm)	0.119 ± 0.0360	0.102 ± 0.030	0.048*
CoP velocity in AP direction (cm/s)	0.0082 ± 0.0019	0.0075 ± 0.0017	0.144
CoP velocity in ML direction (cm/s)	0.590 ± 0.1808	0.486 ± 0.1503	0.014*

*Significant difference between groups ($p \leq 0.05$); CoP: Center of Pressure; AP: Anterior-Posterior; ML: Mediolateral.

Table IV. Correlation between anthropometric parameters, FES, and CoP velocity in ML direction.

	CoP velocity in ML direction (cm/s)		Fat mass (kg)	
Variables	PCC	P-value	PCC	P-value
Age (years)	0.198	0.085	0.066	0.583
BMI (kg/m²)	- 0.453	0.000*	0.761	0.000*
Fat mass (kg)	- 0.309	0.008*	1	----
Fat-free mass (kg)	- 0.245	0.034*	0.432	0.000*
Lumbar T-score	- 0.239	0.037*	0.312	0.008*
FES	0.284	0.016*	-0.254	0.038*

*Significant Correlation ($p \leq 0.05$). PCC: Pearson Correlation Coefficient; BMI: Body Mass Index; FES: Fall Efficacy Scale.

Table V. Regression analysis with Entering method for prediction of CoP sways velocity in ML direction.

Variables	Standardized Beta Coefficient	t	P-value	Collinearity Statistics	
				Tolerance	VIF
BMI (kg/m²)	- 0.113	-0.613	0.542	0.337	2.97
Fat mass (kg)	- 0.338	-1.99	0.050*	0.397	2.51
Fat-free mass (kg)	- 0.023	-0.176	0.861	0.661	1.51
Lumbar T-score	- 0.145	-1.26	0.212	0.860	1.16
FES	0.203	1.68	0.098	0.781	1.28

*Significant Correlation ($p \leq 0.05$). VIF: Variance Inflation Factor; BMI: Body Mass Index; FES: Fall Efficacy Scale; t value: t Statistic is a measure of the precision of regression Beta Coefficient.

DISCUSSION

The present study was designed to investigate the effect of fat mass value (based on skinfold thickness) and BMI on the CoP sway to assess the possibility of predicting the stability of postmenopausal women with and without osteoporosis during the standing position. Although there are more accurate methods for calculating fat mass, such as Dexa, using simple and accessible techniques such as skinfold thickness allows for periodic follow-up of high-risk osteoporotic women. Suppose the relationship between fat mass and the CoP parameters is also determined. In that case, it can effectively predict the balance status, the risk of falling, and planning their therapeutic exercise.

The results revealed that osteoporotic women have lower BMI and fat mass values than nonosteoporotic women. Martinez-Ramirez *et al.* (2017) indicated that higher BMI

and total fat mass are associated with decreased risk of osteoporosis (28). In contrast, it is shown that excess fat mass may not protect against reductions in bone mass (23, 29). The adipose-derived hormones (estrogen, leptin, adiponectin) might affect the relationship between fat and bone mass through bone metabolism involvement. In other words, the adipocyte cells become the alternative estrogen source in estrogen-deficient subjects; thus, the protective role of fat tissue on bone mass might be highlighted in deficient hormonal subjects (29). Hence, postmenopausal women (with estrogen deficiency) with lower fat mass might be at risk of osteoporosis and fractures due to the lack of adequate alternative estrogen sources. The contrasting role of fat mass hormones on the bone and skeletal muscle mass complicates this relationship. In addition to the direct osteogenic effects on osteoblasts, leptin also has osteolytic effects by crossing the blood-brain barrier in the hypothalamus and suppressing serotonin production, which leads to a sympathetic nervous system (SNS) signaling via norepinephrine. This signaling pathway activates osteoclast differentiation, which leads to increased bone resorption (30).

Therefore, the lack of considering a specific optimal BMI-fat mass range for the subjects with hormone deficiency and ignoring the visceral fat mass value might cause inconsistency observed in the fat mass caliper data and bone mass relationship. In addition, high fat mass-BMI may positively affect bone metabolism by increasing bone loading during weight-bearing and daily functional activities. Applying mechanical loading to bones according to mechanostat theory can cause the bone formation and prevent bone resorption (31).

The static stability assessment showed a higher ML velocity sway and ML displacement in the osteoporotic group. This finding supports a recent study, which indicates the relationship between postural instability (increased CoP diversity) and osteoporosis in postmenopausal women (32).

BMI, fat mass, fat-free mass, lumbar T-score, and FES score were significantly associated with ML postural stability parameters. Linear regression analysis showed that fat mass is the only significant predictor of CoP sway velocity in the ML direction. At least, in the BMI range of our study, an increase in fat mass may significantly decrease CoP sway velocity in the ML direction. Our finding is inconsistent with Meng *et al.* (2020). They indicated that the rise in BMI and adiposity in older adults is associated with declines in postural control in the frontal plane (increase in CoP sway parameters) (33). Of course, they measured central adiposity in obese participants with BMI ≥ 30 kg/m^2 in both genders, which is different from our study. Rezaei pour *et al.* (2018) showed that the CoP sway velocity is lower in obese elderly females than the normal-weight females in the mediolateral direction (34). The role of obesity in increasing frontal stability is attributed to the anatomical changes in obesity, such as valgus knees, which keep feet apart, resulting in the frontal base of support expansion and increasing frontal postural stability (12). However, Blaszczyk *et al.* (2009) revealed that the increased fat mass-BMI reduces the risk of falls and fractures in the elderly, unlike young adults (35). Increased body inertia due to the excessive fat mass-BMI and high stance width related to the fat mass accumulation in the lower limbs increases frontal postural stability (35). The gender-dependent distribution of adipose tissue (gynoid type in females *versus* android type in males (36)), the hormonal status and age-dependent effects of fat mass on balance control (as mentioned by Blaszczyk *et al.* (35)), different adiposity measuring, and other associated parameters in the subjects (such as anxiety behaviors and fear of falling) maybe the reasons for the inconsistency observed in the studies about the fat mass and frontal CoP sway parameters relationship.

Interestingly, our results indicated that postmenopausal women with lower fat mass values experienced greater fear of falling (negative, significant correlation). There was a more incredible CoP sway velocity in the frontal plane due to the positive and significant correlation with FES. Subjects with higher self-judged fear values have more CoP sway variability than those with lower fear values (37). It was proposed that the effect of the fear of falling on postural control and fall risk is mediated by the changes in the allocation of attention or impaired attentional processing and related alteration in motor control (38). It was also indicated that the effects of anxiety condition or fear of falling on the balance control related to a complex interaction between neurophysiological changes (increase in muscle spindle sensitivity, the vestibular gain of balance, head and eye reflexes) and alteration in attentional processes (39). The fat mass has been well investigated as an alternative source of estrogen and other fat mass hormones. Some of these hormones, such as leptin, are anxiolytic and antidepressants (40). So, decreasing FES probably, mediated by the more plasma level of anxiolytic leptin in subjects with higher fat mass value. Therefore, the higher fat mass value due to the broader mediolateral base of support and also decreasing of FES may affect the frontal postural stability in postmenopausal women, mechanically and cognitively, which needs to be investigated in future studies.

In addition to the evaluated anthropometric parameters, which correlated to the postural instability, a contaminant decrease in muscle strength has been proposed as an important risk factor for balance impairment in osteoporotic subjects (41). Our results also showed a reduction in the isometric strength of lower limb muscle groups in osteoporotic women. The relation between muscular strength of lower extremity and postural instability in osteoporotic subjects related to the muscular alterations in adaptation to

the bone structure's deterioration, which leads to a change in center of gravity and, consequently, inefficient balance control falling, and fractures (42). For this reason, lower extremity muscle strengthening should be considered in rehabilitation programs to improve postural stability, mobility, and falling risk in older women (43, 44).

Postural instability is a significant risk factor for falls and fractures and may be associated with lower muscle strength, osteoporosis, and low-fat mass in postmenopausal women. Therefore, the BMI-fat mass values in women should maintain an optimal range in the decades following menopause. Furthermore, muscle strengthening may effectively prevent the development of osteosarcopenia in these women. On the other hand, weight loss without muscle strengthening and balance training may increase the risk of falling due to the undeniable role of muscle strength, especially in the lower limb, on the proper posture and balance.

It should be noted that the BMI-fat mass value in the present study was not at the level of obesity, and the positive effects of BMI-fat mass on BMD and postural stability are related to overweight status.

The small sample size for this cross-sectional study, the lack of consideration for visceral fat (waist circumference, as a simple proxy for visceral fat measurement), and the lack of measuring the plasma level of fat mass hormones were important limitations to the present study. Dexa body composition is a precise measurement of segmental body fat distribution in the arms, legs, waist (android), hips (gynoid), and trunk (torso) that we suggest investigating the relation with the stability parameters. Furthermore, assessing the status of sarcopenia compared to measuring muscle strength can provide more accurate information, so it's recommended for future studies. Although the age difference between the two groups was not significant, the wide range of participants' age (55-75 years) and relatively asymmetric age distribution was one of the study's inevitable limitations because women with osteoporosis are generally in the upper range. According to the criteria, we enrolled all eligible volunteers women with osteoporosis and normal BMD. It suggests that future studies limit the age range so that the distribution of individuals is more uniform in terms of age. Another limitation of the study is the exclusion of postmenopausal women with osteopenia. It suggests that the effect of fat mass (based on skinfold thickness) and BMI on CoP sway parameters to be investigated to predict stability in static and dynamic positions in postmenopausal women with normal BMD osteopenia and osteoporosis.

CONCLUSIONS

The results showed low-fat mass value is associated with mediolateral postural instability in postmenopausal women. Periodic assessment of muscle strength and fat /fat-free mass based on the skinfold thickness may provide important information about postural instability in osteoporotic women. Regarding the loss of BMI-fat mass and decreased muscle strength, it is recommended that strength-balance exercises be considered cautiously for osteoporotic women to improve stability.

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DATA AVAILABILITY

The data are available under reasonable request to the corresponding author.

CONTRIBUTIONS

GT, MM, SM: conceptualization, design. MD, SM, MM, ZB: acquisition of data. GT, SM, MD, MM, ZB, HN: data analysis and interpretation. GT, SM, MM, MD, ZB, HN: drafting of the manuscript. GT, SM, MM: critical revision. All authors read and approved the final version of the manuscript.

CONFLICT OF INTERSTS

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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Autograft Soaking in Vancomycin in Anterior Cruciate Ligament Reconstruction Alters Tendons Structure: an Histopathological Interpretation

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SUMMARY

Background. Anterior cruciate ligament rupture is a frequent injury in sports and infection after reconstruction is rare (0.14-1.8%). Intravenous antibiotic prophylaxis and soaking of autografts with vancomycin (tendons are soaked into a compress that contains 100ml of NaCl mixed with 500 mg of vancomycin for 10-15 min) have been applied in order to avoid infection after ACL reconstruction. The aim of this study was to determine if hamstrings soaking with vancomycin leads to histopathological changes than can produce functional impairment in patient's follow-up.

Methods. Assessment to the graft was made based on redundant tendon tissue from ACL reconstruction procedures of patients operated by the same surgeon. Tendons collected during surgery were used and surplus segments were submitted to histopathological study and elastin impregnation. Two samples from each tendon of eighteen patients were analyzed, with and without vancomycin impregnation.

Results. This study included 18 patients. The mean age at surgery was 29 years old, 83.3% were male and 16.7% female. There were no cases of documented septic arthritis. Our results showed a considerable reinforcement of elastin wavy configuration after soaking with vancomycin and apparent retraction of surrounding tissues.

Conclusions. Hamstrings autografts' structure is altered after vancomycin soaking. Histopathological analysis described in this study might produce a decrease in tendon resistance to tensile forces that was not correlated with an increased risk of rupture. Future studies are needed to clarify vancomycin effects on graft integrity.

KEY WORDS

Anterior cruciate ligament; vancomycin; antibiotic prophylaxis; knee infection; fibroblasts; elastin; collagen; hamstrings autografts.

INTRODUCTION

Anterior cruciate ligament (ACL) is one the most important stabilizing structures of the knee. Ruptures of ACL are common in sports, with an incidence > 200,000 per year at USA, most of them required surgical treatment (1). Septic arthritis after ACL reconstruction is a rare but potentially devastating complication, with an incidence rate between 0.14% and 1.8% (2, 3). The majority of these cases occurred between 2 and 6 weeks after surgery (4). Several risk factors are associated with septic arthritis such as diabetes, smoking, hamstrings autografts, drains, increased operative time, open procedures, previous surgery at the same knee or concomitant procedures, use of tourniquets, among others (2, 3, 5, 6). The two overwhelmingly prevalent organisms isolated are coagulase-negative staphylococcus (62.5%) and *Staphylococcus aureus* (21.9%). Other commonly reported pathogens include *Propionibacterium acnes*, Enterobacteriae and Pseudomonas (4). It has been verified a higher incidente of infection with hamstrings tendon autografts compared with bone-patellar tendon-bone autografts and allografts, this

may be attributable to a more complex harvesting technique (more time needed *vs* bone-patellar tendon-bone harvesting time) and the use of intra-articular suture material (2, 5, 7, 8). A few authors correlated the high number of infections linked to staphylococcus to graft contamination with skin comensal bacteria, potentially during the harvesting phase. It has been shown a 14-23% contamination rate during ACL autograft harvesting and manipulation phases (9). Septic artritis can lead to a poor outcome, chronic pain, arthrofibrosis, cartilage loss, degenerative arthritis and graft failure. Although graft removal can be avoided in most cases, several surgeries are needed as well as prolonged antibiotherapy, leading to significant socioeconomic costs. The apparent failure of antibiotic intravenous prophylaxis is thought to be due to the poor graft vascularization with levels of intravenous antibiotic below the minimum inhibitory concentration (MIC) capable to eliminate the most frequent pathogens (6). Vertullo *et al.* described in 2012 a novel surgical technique that besides the use of intravenous antibiotherapy associate presoaking of hamstrigns grafts with vancomycin. In this technique the harvested hamstrings graft is wrapped in a surgical sponge that had been previously soaked in a solution that contains 500 mg of vancomycin powder in 100ml of sterile saline solution. Autogratfs are then kept in this solution for 10-15 min before surgical application. Local vancomycin has routinely been used among anothers non-orthopaedic fields, such as vascular grafts and toracic and spine surgery (4, 10). The choice of vancomycin is due to the fact that is a bactericid agent against skin comensal gram-positive bacteries; its pharmacokinetic characteristics make it appealing to local use: low allergenicity, heat stability, safety for local use, water soluble, large volume of distribution and low resistance rate (2, 4, 11). Some *in vitro* studies have shown some evidence of its low osteoclast and chondrocite toxicity (12, 13). Recently, a study also reported that the use of vancomycin is cost-effective (6). Grayson *et al.* (12) have shown, *in vitro,* that the collagen fibres type I of the tendons, after soaking with vancomycin, can act as reservoirs for vancomycin, with the amount released and elution profile dependent on rinsing and tendon volume. Vancomycin elution was above the minimum inhibitory concentration for the most of bacteria. The elution of vancomycin is not sustained for long periods of time, in fact, the authors found that release occurs mainly between 10 and 60 minutes after impregnation.

All the studies carried out until today (although most are level IV evidence) have shown that vancomycin is effective in reducing the rate of infection after ACL reconstruction (2, 3, 17, 18, 5, 8, 10–12, 14–16), reducing infection rates to 0.1% (1).

The purpose of this study was to determine if hamstrings soaking with vancomycin leads to histopathological changes than might produce functional impairment in patient's *follow-up*.

MATERIALS AND METHODS

Material

We performed a prospective, case-control study. This study was approved by the Ethics Committee of our Department (favorable opinion on 22/04/2019, CE-003/2019). All patients included in this study signed an informed consent and are aware they will be part of a scientific publication. The study was conducted in accordance with the ethical standards established in the Declaration of Helsinki of 1946 (World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA 2013 Nov 27;310(20):2191-4. Doi: 10.1001/jama.2013.281053).

The datasets generated during the current study are available from the corresponding author on reasonable request.

The inclusion criteria in this study were: patients with acute-ACL ruptures submitted to ACL reconstruction with hamstring autografts by 2 senior surgeons from our Hospital, who applied the same surgical protocol. For graft fixation, the method used was the same in all patients – a suture button–based femoral cortical suspension (EndoButton fixation technique) and an interferential screw for tibial fixation. Patients with multi-ligamentous knee injury and chronic injuries were excluded.

All patients who met these criteria from May 2019 to May 2020 were included, corresponding to 18 patients (**table I**). The mean age at surgery was 29 years old, 15 patients were male (83.3%), and 3 patients were female gender (16.7%). All patients received antibiotic prophylaxis with 2 g of intravenous cefazolin, 30 min before starting of surgery (before tourniquet inflation) or 600 mg of clindamycin if there was a knowledge history of allergy to penicillin.

Table I. Study's population.

Case	Gender	Age at time of surgery
1	M	42
2	M	28
3	M	16
4	M	43
5	M	34
6	M	36
7	M	46
8	M	43
9	F	38
10	M	24
11	M	29
12	F	20

Case	Gender	Age at time of surgery
13	M	19
14	M	24
15	M	48
16	F	38
17	M	28
18	M	27

Methods

The first step of all surgeries was semitendinosus (ST) harvesting. Autologous grafts from semitendinosus tendons were collected. Simultaneously a solution with 500 mg of vancomycin powder in 100 ml of NaCl had been prepared. A surgical sponge was soaked in this solution. After harvesting and preparation of the hamstrings, they were wrapped in a surgical sponge soaked with a solution of 500 mg of vancomycin diluted in 100 ml of a saline solution and left there for 15 min, before being surgical applied.

Two samples were taken from each tendon:

- sample 1: obtained after graft harvesting (without vancomycin);
- sample 2: obtained after impregnation with vancomycin.
- Both samples were collected from redundant tendon tissue that was not use for graft preparation.

Afterwards, the collected samples of step 2, were formalin-fixed and formalin-embedded. Beyond Hematoxylin-Eosin, elastin fibres argentic impregnation was also realized. Histopathological study was developed at Institute of Anatomical and Molecular Pathology, Faculty of Medicine, University of Coimbra.

Optical interpretation was developed by comparing the two samples of each tendon.

A bibliographic review on the topic was carried out with consultation of the Medline database and taking into account the degree of clinical evidence.

RESULTS

Patient follow-up

There were 18 patients who met the inclusion criteria. All patients followed the same postoperative rehabilitation protocol. They were examined at 2 weeks, 6 weeks, 3 months, 6 months and 1 year of post-op. All patients completed at least 6-month follow-up.

There were no cases (0%) of documented septic arthritis or graft failure. Diagnosis of septic arthritis was based on clinical examination (fever, knee effusion, knee inflammatory signs), laboratory parameters (CRP and ESR) and bacterial cultures of synovial fluid of the knee joint.

There were no reported allergies or secondary effects to surgical procedure due to the preparation with vancomycin soaking. No patient had a recorded allergy or an adverse event.

Histopathological interpretation

Firstly, the structural changes in the autologous hamstring tendons of our group were evaluated by Hematoxylin and Eosin staining of tissue sections. Microscopy images showed an organized collagen network with tenocytes arranged in rows parallel to collagen fibres, with delicate way configuration, in hamstrings without vancomycin soaking (**figures 1**, **2**, **3**). All tendons with vancomycin soaking showed an increased wavy configuration. In addition, the alignment, or orientation, of the collagen fibres was altered and disrupted in the vancomycin soaking group (**figures 4**, **5**, **6**).

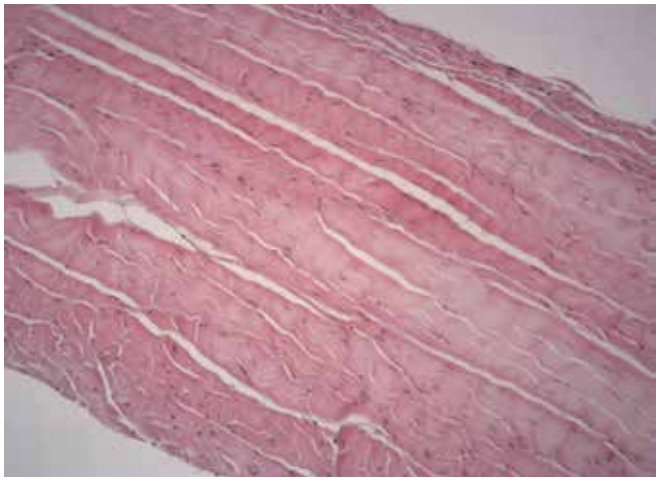

Figure 1. Section of hamstrings tendons stained by hematoxylin and eosin without vancomycin soaking - case 16.

Figure 2. Section of hamstrings tendons stained by hematoxylin and eosin without vancomycin soaking - case 12.

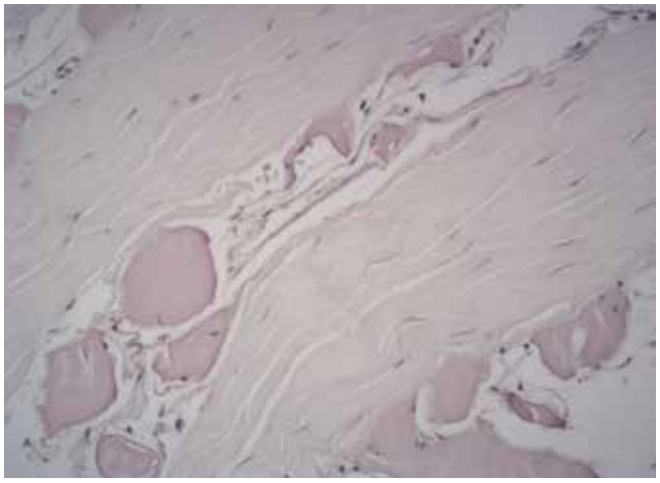

Figure 3. Section of hamstrings tendons stained by hematoxylin and eosin without vancomycin soaking - case 11.

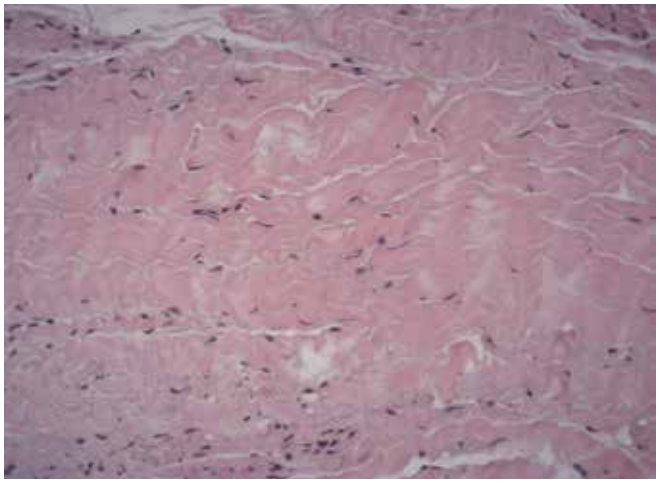

Figure 4. Section of hamstrings tendons stained by hematoxylin and eosin with vancomycin soaking - case 14.

Figure 5. Section of hamstrings tendons stained by hematoxylin and eosin with vancomycin soaking - case 12.

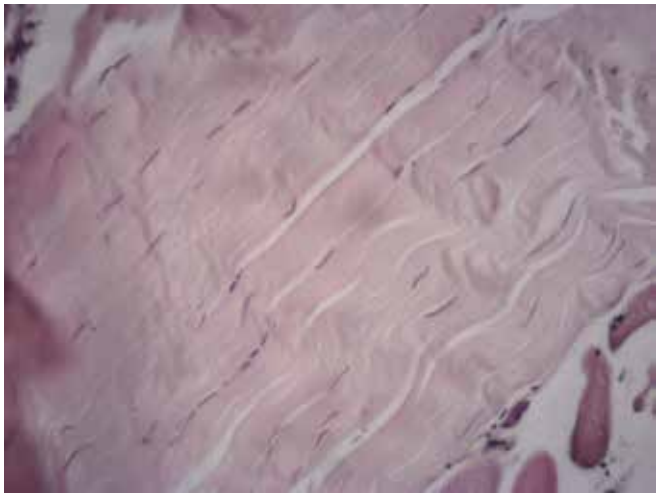

Figure 6. Section of hamstrings tendons stained by hematoxylin and eosin with vancomycin soaking - case 11.

Elastin impregnation

Elastic fibres are not clearly evident in traditional Hematoxylin and Eosin (H & E) stains of tissue sections. The applied technical procedures indicated that elastic fibres were densely distributed around tenocytes and between fascicles. Elastic fibres were longitudinally oriented along the tendon. **Figures 7**, **8** and **9** show the elastic fibres orientation without vancomycin impregnation. The observation showed considerable reinforcement of elastin wavy configuration after vancomycin soaking and apparent retraction of surrounding tissues (**figures 10**, **11**, **12**).

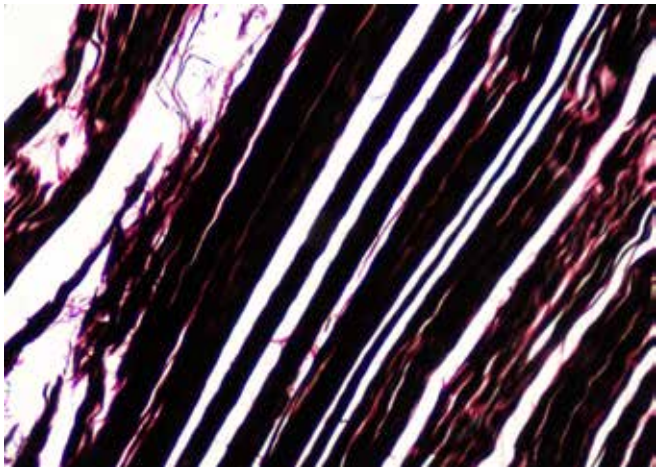

Figure 7. Elastin impregnation. Section of hamstrings tendons without vancomycin soaking - case 6.

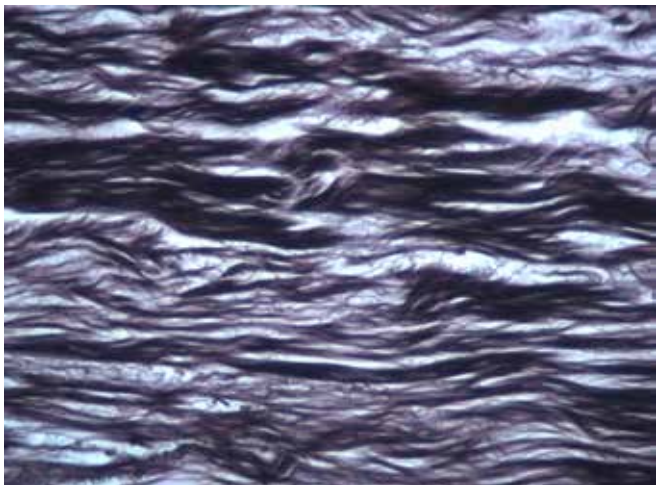

Figure 8. Elastin impregnation. Section of hamstrings tendons without vancomycin soaking - case 3.

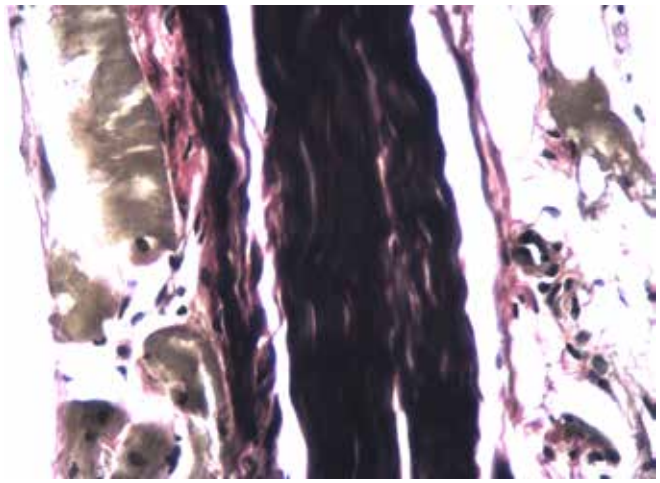

Figure 9. Elastin impregnation. Section of hamstrings tendons without vancomycin soaking - case 9.

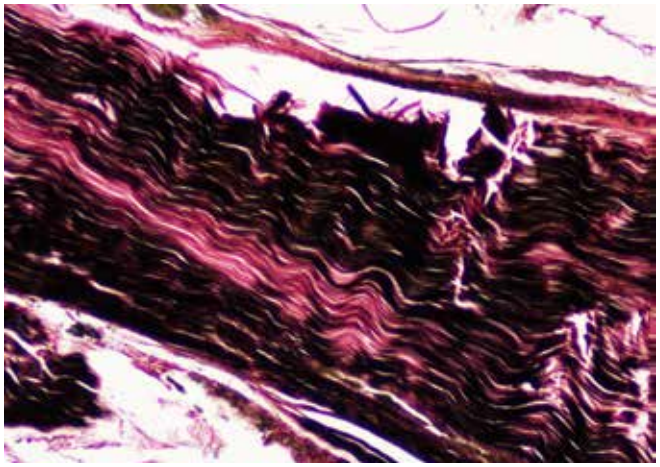

Figure 10. Elastin impregnation. Section of hamstrings tendons with vancomycin soaking - case 1.

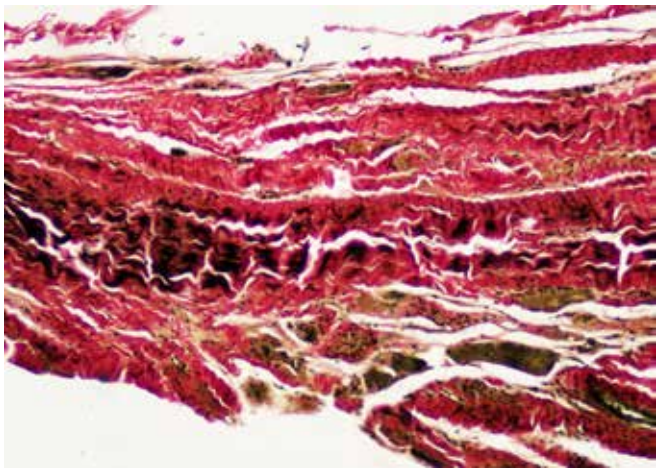

Figure 11. Elastin impregnation. Section of hamstrings tendons with vancomycin soaking - case 14.

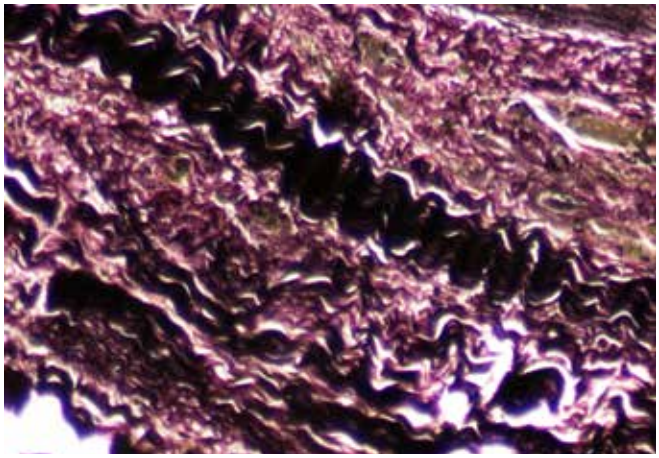

Figure 12. Elastin impregnation. Section of hamstrings tendons with vancomycin soaking - case 3.

DISCUSSION

To our knowledge, this is the first study to present the histopathological features of hamstring autografts soaking with vancomycin solution. The hamstring autografts used for ACL reconstruction showed marked histopathological changes.

Infection after ACL reconstruction is a rare but catastrophic complication. Major concerns following ACL-reconstruction infection are graft survival and long-term graft function. Although graft removal is rarely needed, long-term antibiotics and long hospital stays are usually necessary, with high socioeconomic costs associated. Several techniques have been studied to decrease infection rate. Recently, many studies showed that graft pre-soaking with vancomycin decrease infection rate. Vancomycin's choice lies in its pharmacokinetic proprieties such as low allergenicity, heat stability, safety for local use and large volume of distribution

(15). The use of vancomycin-soaked grafts was associated with a 10-flod reduction in the rate of postoperative infection after ACLR (16). For more than 10 years orthopedic surgeons have been used this technique but the literature surrounding this technique is very limited. Adverse effects, local and disseminated toxicity to cells and biomechanical proprieties are almost unknown. Every single study showed with no doubt that the use of vancomycin reduces the risk of infection after ACL-reconstruction from low to minimal (2, 5, 10, 14, 15), but there are some concerns about vancomycin safety, allergic reactions and potential antibiotic resistance development.

Recently, Braun *et al.* reported that even small concentrations of vancomycin have a negative effect on proliferation and functionalization of various cell types (19). They studied the cytotoxicity of vancomycin on osteoblasts, endothelial cells, fibroblasts and skeletal muscle cells. The most sensitive cells were the human skeletal muscle cells.

There are no guidelines for the local administration of antibiotics. Especially in local therapies the concentration of antibiotics is crucial to surrounding tissues viability. It´s known that tendons function as reservoirs of vancomycin (12). The amount of vancomycin released, and elution profile depend of tendon volume, soak solution concentration and on rising. It's unknown if the amount of vancomycin that stayed in autografts could damage tendon's structure. Liu *et al.* (20) concluded that continuous vancomycin exposure (48 h) has a significant cytotoxic effect on proliferating osteoblast and myoblasts at concentrations greater than or equal to 1 mg/cm², and for fibroblasts at concentrations greater than or equal to 3 mg/cm².

With this study we pretended to know histological effects of vancomycin in surrounding tissues, such as collagen and elastic fibres. The most important finding of this study was the change of tendon structure after vancomycin soaking. After vancomycin soaking tendons showed an increased wavy configuration, that fact was confirmed by histopathological interpretation in all cases. Several studies showed that in the resting state, the collagen fibres and fibrils of a tendon show a wavy configuration which appears already under the light microscope. This configuration disappears if the tendon is stretched slightly corresponding to a straightening of the collagen fibres. When the tensile force is released, the tendon resumes its normal wavy appearance (21). In this studied both of the samples were in resting state, and after vancomycin soaking an increased waving configuration was seen.

Recently investigators have been interested in a hypothetical mechanical role for elastic fibres in tendons. The mechanical stability of the tendinous collagen is the most important factor for the mechanical strength of a tendon. The function of elastic fibres is not entirely clear, but they may contribute to the recovery of the wavy configuration of the collagen fibres after tendinous stretch (21). Elastic fibres are present in low quantities in tendon (1-2%), where they are located both within fascicles near tenocytes and more broadly in the interfascicular matrix. Elastin is an important load-bearing tissue in the bodies of vertebrates, used specifically in tissues requiring high fatigue resistance or energy storage, as a result of its highly elastic mechanical behavior and resilience. Recent studies suggested that elastin had an important role in tendon healing process, elastin and fibrillin-1 are upregulated in torn tendons (22).

A recent study reported a slightly higher rate of postoperative arthrofibrosis in the vancomycin group (6), this study of 2018 was the first study to provide data regarding possible side effects of vancomycin such as graft failure, arthrofibrosis and subjective outcome measurements. Bohu *et al.* (17) affirmed that vancomycin presoaking does not increase the risk of ACL recurrent tears and that return to sport is not altered. Other studies had the same conclusions (18, 23). The biomechanical impact of vancomycin presoaking has not been well investigated until now and the majority of the studies are in animals (24). Ekdahl *et al.* (25) reported that only 8% of Swedish surgeons included in their study used vancomycin presoaking. The lack of guidelines on this subject should make us alert; studies in this area are urgently needed. It's questionable if the available data recommend the universal application of this technique for all patients undergoing ACL-reconstruction or if it only should be done in special conditions such as revisions surgeries, type of graft used (increased risk if HT autograft or allograft are used), patient risk factors (such as diabetes, smoking) and procedure-associated factors (such as increased operative time, concomitant open surgical procedures, long tourniquet inflation time, use of a drain).

New techniques describe the use of biodegradable polymeric materials, such as fast-resorbable hydrogel coating (Defensive Antibacterial Coating [DAC], Novagenit Srl, Mezzolombardo, Italy) that releases antibiotics in the knee joint after orthopedics procedures. A recent study (26) reported the use of DAC and vancomycin in ACL deficient patients and the authors said that is safe and effective. This method can be an option when tendons cannot be used – for example, in total knee joint arthroplasty. In ACL reconstruction the tendons function as reservoirs of vancomycin and there is no need to use these carriers.

Different limitations can be attributed to this study. First of all, the limited number of patients, our samples were taken from patients who underwent arthroscopic ACL reconstruction. The ideal sample should be the entire tendon, which could be done with allografts or animal samples. Another possible limitation of this study is the use of only semitendinosus autografts. We used only two staining methods (Haematoxylin and Eosin; Elastic impregnation). We

used these techniques because they were cost-effective, widely available and easy interpreted. Further techniques as electron microscopy weren't used, so we possible underestimate tendon abnormalities. It's difficult to have randomized controlled trials in this area because the number of required patients would be extremely high due to rare incidence of infections after ACL-reconstruction.

It's a fact that the structure of the tendons has been changed, but there's still an outstanding question remaining; if histopathological observed changes are result of duration of immersion in vancomycin or due to the concentration used. It's also unknown whether these structural changes are correlated with tendons biomechanics modifications and consequently an increased rupture rate after ACL reconstruction. Further studies are required to clarify vancomycin effects on graft integrity.

CONCLUSIONS

Vancomycin as bactericidal agent against skin commensal bacteria has shown evidence of low toxicity on chondrocytes and osteoclasts with releasing rate into tissues over time.

Although infection after ACLR is a rare complication, it can have catastrophic effects, leading to graft failure and joint disfunction.

Semitendinosus tendon structure is altered after vancomycin soaking. This study showed an increased wavy configuration in all patients of vancomycin group. In addition, the alignment, or orientation, of the collagen fibres was altered and disrupted in the vancomycin soaking group. Future studies are needed to clarify vancomycin effects on graft integrity beyond infection concern. These findings raise concern about ACL preserved function after surgery and the actual vancomycin application.

FUNDINGS

None.

DATA AVAILABILITY

Data are available under reasonable request to the corresponding author.

CONTRIBUTIONS

CQ, JPO, FF: study conceptualization and design. CQ, JPO, PM, AI, FF: data collection. CQ, JPO: analysis and interpretation of results. CQ: draft manuscript preparation.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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Inter-rater Reliability of Sports Medicine Physicians to Assess Healthy Individuals' Patellar Tendon with Conventional Ultrasound and Shear-wave Elastography

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LEVEL OF EVIDENCE: 3

SUMMARY

To evaluate the reproducibility of shear-wave elastography, thickness, power doppler, and hypoechoic region assessments taken from the different parts of the patellar tendon among sports medicine clinicians.

Twenty-one healthy, physically active individuals with dominant legs were included in the study. Shear-wave elastography, thickness, power doppler, and hypoechoic area assessment were evaluated from the proximal, middle, and distal part of the tendon both at 30-degree knee flexion and full extension. To evaluate the reproducibility within and among clinicians, the same measurements were performed at 4-7 days intervals. Evaluations of intra- and inter-rater reliability for examiners I and II were conducted via two-way random effects and absolute agreement type intraclass correlation coefficient (ICC). Gwet's AC1 (GC) agreement coefficient was used to evaluate categorical data agreement.

The intra-rater reliability of both sports physicians on shear-wave elastography was in good to excellent reproducibility in all three parts at the 0–30-degree measurements (intra-observer ICC: 0.60-0.88, inter-observer ICC: 0.67-0.87). Similar results were obtained for the thickness (intra-observer ICC: 0.83-0.94, inter-observer ICC: 0.65-0.83). As a result of the power doppler taken at two different angles were excellently (intra-observer GC: 0.86-1.00, inter-observer GC: 0.86-1.00) consistent both within and among the clinicians but not for the hypoechoic region evaluation (intra-observer GC: 0.58-1.00, inter-observer GC: 0.40-1.00).

This study shows that the reproducibility of local measurements made in different parts of the patellar tendon at two different angles within and among sports medicine physicians is at a good to an excellent level.

KEY WORDS

Patellar tendon; shear-wave elastography; power doppler; ultrasound; sports medicine.

INTRODUCTION

Patellar tendon transfers the force produced by the quadriceps muscle to the tibia as an essential component of the knee extensor mechanism (1). It also acts as a supporter in distributing the kinetic energy generated during the stroke after the jump to the other joints of the lower extremity (2). The tendon actively stores and releases energy with the demand on the knee extension in activities such as sitting, getting up, going up and downstairs in daily life, jumping, landing, sudden sprinting, and changing direction during

a sportive performance (1). Therefore, repetitive jumping, landing, sprinting, and changing direction, and not enough recovery may predispose to patellar tendinopathy, a common cause of anterior knee pain in athletes, especially in volleyball and basketball (1). It has been shown that the prevalence of patellar tendinopathy in elite-level athletes in volleyball and basketball can reach up to 45-32%, respectively (3).

As a common imaging modality, ultrasound (US) is used in tendon examinations to assist in diagnosing tendinopathy, monitor the effectiveness of treatments, and assess risk for symptom development (4). Especially compared to magnetic resonance imaging, ultrasound continues to gain more popularity among clinicians working with the musculoskeletal system due to its ease of application, less invasiveness, and rapid availability, and it has been a frequent subject of tendon-related research in the last two decades (4, 5). In this context, the changes that occur as a result of the degeneration of the tendon with the high load over time, that the local increase in the thickness (6) with hypoechoic regions (7), as can typically be detected with grayscale US, and neovascularization (8) be determined by power doppler US. The majority of these changes are observed according to Golman *et al.* (9) (> 70%) that it occurs in the proximal part of the tendon. Besides the advantages of ultrasound, there are also unclear sides. Minor damage to the tendon recently may have caused minimal changes that may be overlooked in gray-scale ultrasound imaging (4). In addition, structural changes observed mainly in athletes that cannot be clearly understood, whether they occur due to the physiological nature of the sport or as a part of the degenerative process because of loading, and which do not overlap with clinical outcomes such as pain and loss of function, even if they are revealed with gray-scale, or power doppler ultrasound may not even be sufficient to explain the individual's complaints (4). Clinical discrepancies with ultrasound raise questions for physicians dealing with the musculoskeletal system. In this context, the inconsistencies between the triad of pain, loss of function and pathology, and the effort to detect damage to the tendon at an early stage have caused alternative imaging methods to be the subject of more research in the field of the tendon (10, 11). Along with technological advances, it has enabled the development of a new ultrasound-based application called shear-wave elastography, which examines the elastic properties of healthy or damaged tissues quantitatively (12).

Although shear-wave elastography is a current method to assess tendon stiffness in tendon examination, it has shown promising results from a few studies (11, 13). While more consistent results were obtained, especially in studies on the Achilles tendon, current evidence has demonstrated that tendon stiffness decreases with Achilles tendinopathy (14). In the current literature, fewer studies have been conducted on the patellar tendon than the Achilles tendon, and the studies have no homogeneous results (14). While tendon stiffness increased in some studies (13, 15) some others observed the opposite (16, 17). This situation raises questions about the reproducibility of the methods and practitioners applied for the patellar tendon.

This study aimed to evaluate the reproducibility of shear-wave elastography, thickness, power doppler and hypoechoic region assessments taken from the different parts of the patellar tendon at two different angles among sports medicine clinicians.

MATERIALS AND METHODS

Twenty-one healthy, physically active individuals were included in our study after obtaining informed consent. All measurements were performed on the individuals' dominant legs (all right – 21 extremities). The Istanbul Faculty of Medicine Ethics Committee approved this study conducted according to the Declaration of Helsinki by Istanbul University, Faculty of Medicine (12.08.2020-134599 - Date of approval: 03/07/2020). As exclusion criteria: history of partial or full-thickness tear of the patellar tendon at any time, history of ligament or meniscus injury or operation in the dominant knee region in the last six months, ACL operation history with patellar tendon graft used at any time, history of chronic disease (diabetes, or rheumatic disease, *etc.*), history of active oral contraceptive use, presence of active infection in the knee or surrounding tissues were accepted. Male and female healthy individuals aged between 20-30 years who volunteered to participate in the study were included. Eligible individuals were taken to ultrasound measurements without vigorous or moderate-intensity physical activity or exercise for at least 24 hours. Each participant was evaluated blindly by two different sports medicine physicians independently with three years of experience in the field of ultrasound.

Procedure

All ultrasound measurements were performed with the Toshiba Aplio 500 (Toshiba Medical Systems Corporation, Otawara, Japan) and a 10 MHz (5-14 MHz) linear probe. Aquasonic 100 ultrasound gel was used (Parker Laboratories Inc, Fairfield, New Jersey) at room temperature (23 °C). In the neutral and supine position, patellar tendon examinations of all participants were performed on the ultrasound examination table, first with the knee at 0 degrees (full extension) and then at approximately 30 degrees of knee flexion. A goniometer was used to determine the knee angle. A cylindrical cushion with a length of 30 cm and a

diameter of 15 cm was placed under the knee to keep the knee at 30 degrees of flexion. Before starting the ultrasound measurements, the athletes rested on the ultrasound examination table in a neutral position for 10 minutes. The probe was held softly on the skin during the measurement so that the pressure-related values could not be altered, and a large amount of ultrasound gel was used. All measurements were carried out at least twice. A 5-minute rest was given for between all angle changes (from 0 degrees to 30 or from 30 degrees to 0). After starting all measurements with elastography, thickness, echogenicity, and power doppler evaluations were performed, respectively (**figure 1**).

Shear wave elastography (Toshiba Medical Systems Corporation) was used to evaluate the mechanical properties of the patellar tendon. At the three different points (proximal, middle, and distal) of the tendon, rectangular region of interest (ROI) boxes with a length of 1.5 cm and a width of 1.0 cm were placed longitudinally. Then, a circular ROI with a diameter of 0.3 cm was placed in these boxes, and after waiting for 5 seconds, the measurement in kPa was performed (**figures 1, 2**). If there was a difference of more than 20% between the previous measurement, a third and, if necessary, a fourth measurement was taken. At least 12 images (36 round ROIs) were recorded at two different angles of each individual's patellar tendon. (11).

The anteroposterior thicknesses of the patellar tendon were measured on the transverse plane with gray-scale ultrasound. The images were taken from 3 different points: from the point where the patella ends in the proximal, 0.5 cm proximal to the most prominent point of the tibial tuberosity in the distal, and the other measurement from the middle of the two proximal and distal points. Two different measurements were made from each point (**figure 1**). The values at the points where the tendon was thickest were recorded. A third measurement was taken if there was a difference of more than 0.5 mm between the two measurements (18). Hypoechoic areas in the patellar tendon were evaluated first in the longitudinal and then in the transverse plane on gray-scale imaging. The tendon was divided into three equal parts, and hypoechoic areas greater than 1 mm were recorded in the 1/3 proximal, middle, and distal (**figure 1**) (19).

0-3 grade classification was used for power doppler imaging. If there is no activation indicates Grade 0. If there is an increase in activation at 1-2 points outside of the tendon indicates Grade 1; 1-2 points within the tendon indicates Grade 2, and 3 or more points within the tendon indicate Grade 3 (**figure 1**) (20). Similar to other measurements, the tendon was divided into proximal, middle, and distal, and the power doppler activation were recorded in these regions. Both sports physicians performed shear-wave elastography, thickness, echogenicity, and power doppler imaging from the proximal, middle, and distal tendons, respectively, at two different angles. At least two measurements were made from all evaluation methods. One clinician performed at least 12 imaging (36 ROIs) for shear-wave elastography, 24 gray-scale ultrasound images (12 thicknesses, 12 hypoechoic fields), and 12 power doppler imaging at two different angles. Two clinicians performed all measurements of 1 participant in a single day, and the exact measurements were performed 4 to 7 days later in a randomized clinician order.

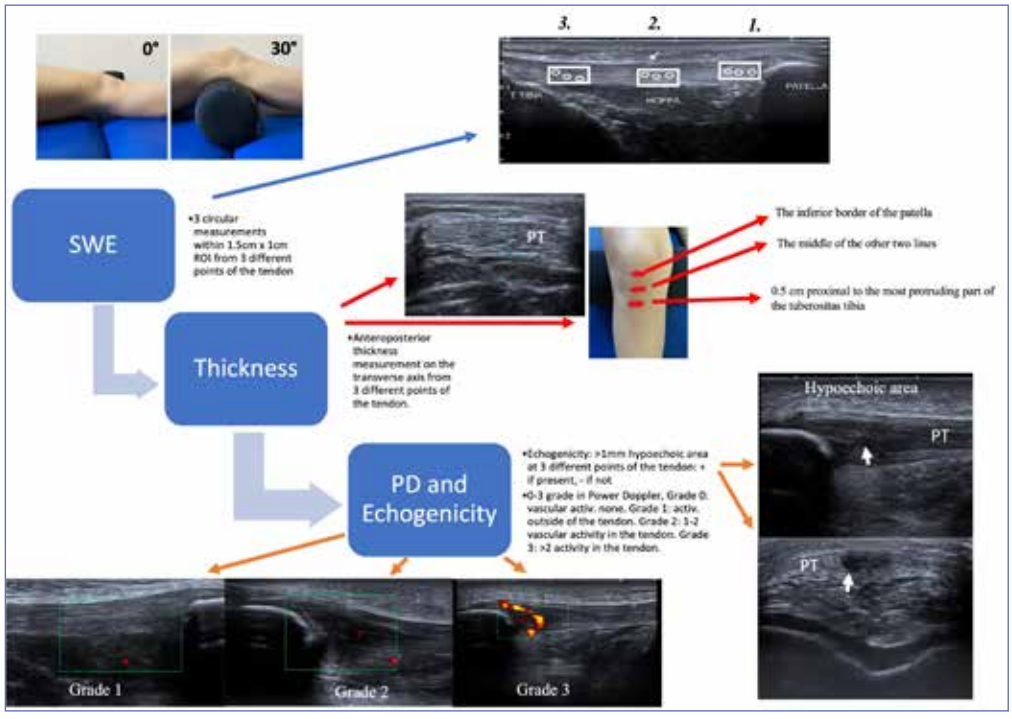

Figure 1. Schematic representation of all measurements made with ultrasound.

SWE: Shear-wave elastography; PD: power doppler; ROI: region of interest; PT: Patellar Tendon.

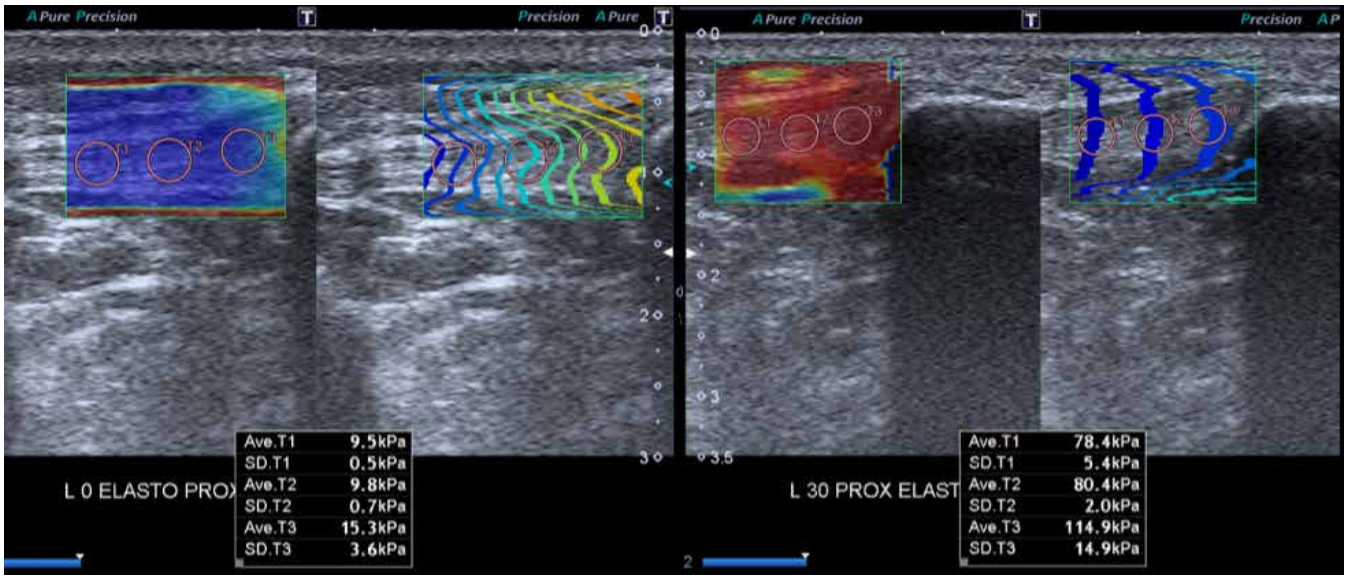

Figure 2. Shear-wave elastography images from the proximal part of the patellar tendon.
(A) 0 degrees, **(B)** 30 degrees of knee flexion.

Statistical analysis

Statistical analysis of the findings obtained in the study was performed with the SPSS (Statistical Package for Social Sciences) for Mac 21.0 program. Descriptive statistical methods (mean, standard deviation, number, percentage) were used while evaluating the study data. Evaluation of inter-rater reliability for examiner I and II was conducted via two-way random effects, single measure model (2,1), absolute agreement type intraclass correlation coefficient (ICC). Interrater reliability using the mean of first and second measurements was analyzed via two-way random effects, average measure model (2, k), absolute agreement type ICC. For evaluation of categorical data agreement, Gwet's AC1 agreement coefficient was used. A minimum sample size of 15 was calculated to provide sufficient statistical power (80%) with alpha = 0.05 error. All ICC results were evaluated according to Shrout *et al.* (21).

RESULTS

The demographic information of the individuals is summarized in **table I**. The intra-rater reliability of both sports physicians on shear-wave elastography were in excellent (intra-observer ICC: 0.76-0.88) reproducibility in all three parts at the 0-degree measurements (**table II**). However, at 30-degree measurements, they showed only good reproducibility in the proximal part (intra-observer ICC: 0.60-0.74), good to excellent reproducibility in the middle part (intra-observer ICC: 0.69-0.82), and excellent level of reproducibility in the distal part (intra-observer ICC: 0.75-0.81) (**table II**). Considering inter-rater levels, at 0 degrees, the proximal and distal parts were excellent (inter observer ICC: 0.84-0.87), the middle part was good (inter observer ICC: 0.68-0.74). At 30 degrees, they were excellent in the distal (inter observer ICC: 0.82) and good-excellent in the proximal and middle part (inter observer ICC: 0.67-0.79) (**table II**). When we look at the thickness, we observed that they were perfectly consistent (intra-rater) in all measurements taken at 0 and 30 degrees (intra-observer ICC: 0.83-0.95) (**table II**). But the measurements among clinicians were only good at all 3 parts at 0 degrees (inter observer ICC: 0.65-0.75): good to excellent in the proximal and middle parts (inter observer ICC: 0.66-0.83), excellent in the distal part at 30 degrees (inter observer ICC: 0.81-0.82) (**table II**).
As a result of the power doppler evaluation, measurements taken from all 3 parts of the tendon at two different angles were excellently consistent both within and among the clinicians (intra-observer ICC: 0.86-1.00; inter observer ICC: 0.86-1.00) (**table III**). In the evaluation of the hypoechoic regions, it was revealed that physicians were consistent at a fair to excellent level in the distal part at 0 degrees (intra-observer ICC: 0.58-0.93; inter observer ICC: 0.40-1.00), while it showed excellent reproducibility in all remaining measurements of the tendon considering the hypoechoic areas (intra-observer ICC: 0.77-1.00; inter observer ICC: 0.75-1.00) (**table III**).

Table I. Demographic features of the participans.

Demographic information		n (%)	Demographic information	Mean ± Std
Gender	Female	6 (50%)	**Age**	25.5 ± 2.6
	Male	15 (50%)	**Height**	177.0 ± 9.8
	Total	21 (100%)	**Weight**	72.5 ± 14.0

Table II. Intra Observer and Inter Observer ICC (Intraclass Correlation Coefficient) of the measurements were taken by two different observers at 0 and 30 degrees. A) Shear-wave Elastography; B) Thickness.

A)		Proximal		Middle		Distal	
		Observer 1	**Observer 2**	**Observer 1**	**Observer 2**	**Observer 1**	**Observer 2**
0°	Intra ICC	0.88	0.76	0.80	0.85	0.84	0.84
	95% CI	0.70-0.95	0.42-0.90	0.51-0.92	0.64-0.94	0.59-0.94	0.61-0.94
	P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
30°	Intra ICC	0.60	0.74	0.69	0.82	0.81	0.75
	95% CI	0.01-0.84	0.34-0.89	0.26-0.87	0.50-0.93	0.53-0.92	0.37-0.90
	P-value	0.026	0.003	0.005	< 0.001	< 0.001	0.002
		Measure 1	**Measure 2**	**Measure 1**	**Measure 2**	**Measure 1**	**Measure 2**
0°	Inter ICC	0.85	0.84	0.68	0.74	0.87	0.84
	95% CI	0.66-0.94	0.64-0.93	0.36-0.86	0.32-0.90	0.70-0.94	0.64-0.93
	P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
30°	Inter ICC	0.79	0.76	0.75	0.67	0.82	0.82
	95%CI	0.55-0.91	0.49-0.89	0.43-0.89	0.35-0.85	0.55-0.93	0.61-0.92
	P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

B)		Proximal		Middle		Distal	
		Observer 1	**Observer 2**	**Observer 1**	**Observer 2**	**Observer 1**	**Observer 2**
0°	Intra ICC	0.87	0.90	0.89	0.94	0.85	0.90
	95% CI	0.67-0.95	0.72-0.96	0.73-0.96	0.86-0.98	0.64-0.94	0.75-0.96
	P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
30°	Intra ICC	0.85	0.92	0.91	0.83	0.94	0.95
	95% CI	0.64-0.94	0.81-0.97	0.78-0.96	0.58-0.93	0.85-0.98	0.87-0.98
	P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
		Measure 1	**Measure 2**	**Measure 1**	**Measure 2**	**Measure 1**	**Measure 2**
0°	Inter ICC	0.68	0.75	0.69	0.72	0.65	0.73
	95% CI	0.33-0.86	0.42-0.89	0.30-0.87	0.38-0.88	0.01-0.87	0.32-0.89
	P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
30°	Inter ICC	0.76	0.66	0.73	0.83	0.81	0.82
	95% CI	0.31-0.91	- 0.10-0.90	0.45-0.88	0.62-0.93	0.31-0.93	0.49-0.93
	P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

ICC: less than 0.40 is low; 0.4-0.60 is fair; 0.60-0.75 is good; and greater than 0.75 indicates excellent reliability.

Table III. Intra Observer and Inter Observer GC (Gwet Coefficient) of the measurements were taken by two different observers at 0 and 30 degrees. SEM: Standard error of measurement A) Power Doppler (Grade 0-3); B) Presence Hypoechoic Region.

A)		**Proximal**		**Middle**		**Distal**	
		Observer 1	**Observer 2**	**Observer 1**	**Observer 2**	**Observer 1**	**Observer 2**
0°	Intra ICC	0.86	1.00	1.00	0.93	0.93	1.00
	SEM	0.11	-	-	0.07	0.07	-
	P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
30°	Intra ICC	0.93	1.00	1.00	0.93	0.93	1.00
	SEM	0.07	-	-	0.07	0.07	-
	P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
		Measure 1	**Measure 2**	**Measure 1**	**Measure 2**	**Measure 1**	**Measure 2**
0°	Inter ICC	1.00	0.86	0.93	1.00	1.00	0.93
	SEM	-	0.11	0.07	-	-	0.07
	P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
30°	Inter ICC	1.00	0.93	1.00	1.00	0.93	1.00
	SEM	-	0.07	-	-	0.07	-
	P-value	< 0.001	< 0.001	< 0.001	<0.001	< 0.001	< 0.001

B)		**Proximal**		**Middle**		**Distal**	
		Observer 1	Observer 2	Observer 1	Observer 2	Observer 1	Observer 2
0°	Intra GC	0.84	0.77	1.00	1.00	0.58	0.80
	SEM	0.12	0.14	-	-	0.20	0.14
	P-value	< 0.001	< 0.001	< 0.001	< 0.001	0.001	< 0.001
30°	Intra GC	1.00	0.86	1.00	1.00	0.93	0.75
	SEM	-	0.11	-	-	0.07	0.16
	P-value	< 0.001	< 0.001	<0.001	< 0.001	< 0.001	< 0.001
		Measure 1	**Measure 2**	**Measure 1**	**Measure 2**	**Measure 1**	**Measure 2**
0°	Inter GC	0.86	0.75	1.00	1.00	0.40	0.75
	SEM	0.11	0.15	-	-	0.25	0.15
	P-value	< 0.001	< 0.001	< 0.001	< 0.001	0.01	< 0.001
30°	Inter GC	1.00	1.00	1.00	1.00	0.93	1.00
	SEM	-	-	-	-	0.07	-
	P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

ICC: less than 0.40 is low; 0.4-0.60 is fair; 0.60-0.75 is good, and greater than 0.75 indicates excellent reliability.

DISCUSSION

We show that the evaluation of the SWE, thickness, power doppler, and the hypoechoic region are taken at two different angles from three different points of the patellar tendon can be measured both within the clinicians' measurements and between two different clinicians at a good to an excellent level consistently. The effect of angle differences on the reproducibility of the measurements was not significant. In the measurements taken at 30 degrees, the reproducibility of the SWE values in the proximal and middle parts was found to be less than the others. In this case, all measurements show good to excellent reproducibility.

In line with the results obtained in recent studies on SWE, it has been shown that stiffness changes that occur with-

out structural changes in the tendon may be a precursor to Achilles and patellar tendinopathy (11, 22). Galletti *et al.* (22) showed that sonoelastographic investigations could reveal tendon abnormalities of clinical relevance in an increased percentage of cases in individuals with normal ultrasound findings. The increasing interest in this field in the last decade raises the question of what effect the methodological differences in the studies will have on the results. Obst *et al.* (14), in their meta-analysis study, showed that the SWE (kPa) in patellar tendinopathy cases increased at 30 degrees both globally and locally in the proximal compared to the normal tendon, while at 0 degrees, the values were found to be lower than usual. Also, on the other hand, patellar tendinopathy study numbers were lower than Achilles tendinopathy studies (23). In this context, only three studies evaluated reproducibility among clinicians on the patellar tendon, and the ICC value varied from 0.71 to 0.97 (24-26). While in 2 of these studies (24, 25) the knee was flexed at 30 degrees, in 1 study (26), measurements were made at 90 degrees of knee flexion. No study was found that evaluated the reproducibility of measurements at 0 degrees. Hsiao *et al.* (26) showed that the tendon could differentiate locally due to tension on the tendon because of measurements taken at different angles. Basso *et al.* (27) showed that the amount of strain occurring in the tendon at different knee angles varies, and this change even differs locally in the anterior and posterior parts of the tendon. Eventually, Obst. *et al.* (14) showed differences in tendon structure and loading patterns (tension, compression, or both) wherein areas within the same tendon could display different mechanical or material properties. Moreover, variations during imaging (local, global measurements, and location and orientation of the 2D image plane and tendon load) may play a role. However, this does not affect the reproducibility of the local measurements within or between clinicians in our study.

Studies have shown in athletes that the tendon's thickness increases over time in the relationship of load adaptation (18, 28), and pathological tendons with patellar tendinopathy (6). Tendon thickness in patellar tendinopathy cases has been shown that especially the proximal part of the tendon is affected by this condition (9). This situation brings thickness measurements to the fore to follow-up tendon-related pathologies or load tendon response. In many studies about thickness: respectively, Dudley *et al.* (29) inter-rater ICC 0.69, intra-rater ICC 0.58-0.85, Toprak *et al.* (30) found intra-rater ICC as 0.87-0.99, Ekizos *et al.* (31) inter-rater ICC 0.69-0.79, intra-rater ICC 0.59, Skou *et al.* (32) found inter-rater ICC as 0.70-0.78, intra-rater ICC as 0.70-0.95, del Bano-Aledo *et al.* (33) found inter-rater ICC as 0.85, intra-rater ICC as 0.82-0.91, and Castro *et al.* (34) found the inter-rater ICC as 0.84-0.96 and the intra-rater ICC as 0.97-0.98. It is seen that the results in all studies had good to excellent reproducibility. Our study's results also have good-excellent reproducibility, which supports the literature. Intra-rater reproducibility rates were higher than inter-rater and did not show significant differences between regions.

Structural changes in the tendon that can be detected by ultrasound may not be clinically compatible. Patellar tendon abnormalities are common, especially in young asymptomatic athletes, and these structural changes may remain asymptomatic for a lifetime. In their meta-analysis study, McAuliffe *et al.* (4) found the rate of becoming symptomatic in the future as 21% of the tendons of asymptomatic athletes with increased thickness, vascular activation, and hypoechoic areas. Similarly, such structural tendon changes can be seen in the healthy sedentary population, although less frequently than in athletes (35). In our study, except for one measurement, the inter-rater and intra-rater reproducibility of power doppler and hypoechoic region presence in all the remaining parts' ICC was found to be excellent level. However, in the hypoechoic region measurements made from the distal of the tendon at 0 degrees, the intra-rater ICC of the 1st Observer was 0.58, while the second observer was 0.80. Inter-rater ICC was found to be between 0.40 and 0.75 at a fair to a good level. We thought that this situation might be due to the difficulty in interpreting the echogenic properties of the tendon due to the folding of the tendon on itself at the distal part during the measurement at 0 degrees. Two studies investigate clinicians' reproducibility about doppler activation on the patellar tendon in the literature. Watson *et al.* (36) found the inter-rater ICC as 0.8 and the intra-rater ICC as 0.95 in the power doppler measurements taken from the patellar and Achilles tendon. Macia-Villa *et al.* (37) obtained an excellent (ICC: 0.9) level of reproducibility in power doppler measurements taken from triceps, quadriceps, patellar and Achilles tendons. In another study examining the Achilles tendon, Sengkerji *et al.* (35) found the inter-observer ICC 0.85. When we look at the studies on echogenicity, few studies are in the literature. Castro *et al.* (34) found the inter-rater ICC as 0.83-0.90 and the intra-rater ICC as 0.97-0.98 from the patellar tendon. Cheng *et al.* (38) in the study examining the plantar fascia, showed that the inter-rater ICC was 0.76-0.79, and the intra-rater ICC was 0.86-0.92 with excellent reproducibility.

CONCLUSIONS

Today, ultrasound is widely used in the evaluation of tendon pathologies. With the increase in technological developments, the emergence of new examination methods raises questions about the reproducibility of these methods. This study shows that the reproducibility of local measurements made in different parts of the patellar tendon at two differ-

ent angles within and among sports medicine physicians is at a good to an excellent level.

FUNDINGS

None.

DATA AVAILABILITY

Data are available under reasonable request to the corresponding author.

CONTRIBUTIONS

SD: conceptualization, methodology, writing - original draft preparation. SD: data curation, writing - reviewing and editing. OE: visualization, investigation. OP: formal analysis, editing. BB: supervision.

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 Osteochondritis Dissecans of the Knee in Children with MRI. Arthroscopic Fixation with Bioabsorbable Chondral Darts. A Retrospective Study of 32 Knees

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LEVEL OF EVIDENCE: 3B

SUMMARY
Osteochondritis Dissecans (OCD) is an idiopathic, well-localized, pathological condition affecting the articular subchondral bone, and its overlying cartilage. In some cases it can progress to the detachment of the affected osteochondral region. Evaluating the stability of the lesion is a key part of providing the correct treatment. Stable lesions, particularly in juvenile patients, have a greater tendency to heal with non-surgical treatment, whereas unstable lesions usually require surgical management. The evaluation of instability of the affected osteochondral region can be assessed by magnetic resonance imaging and arthroscopy.
A total of 32 skeletally immature knees sustained OCD were included in this study. Each knee was evaluated for potential OCD instability by MRI and arthroscopy. The bioabsorbable chondral darts were used for the fixation of the thirty-two osteochondral fragments, to secure their stability and mainly to stimulate their healing. For evaluation of the knee function preoperatively and postoperatively we used the online International Knee Documentation Committee (IKDC) system.
The mean patient age at the time of surgery was 12.64 ± 0.98 years (range 10-14 years). The average follow-up was 2.58 ± 0.71 years (range, 2-4) years. The mean preoperative IKDC score was 31.67 ± 8.36 (range, 23-45) points. The mean postoperative IKDC score was 83.5 ± 1.52 (range, 81-86) points. The 2-tailed P-value < 0.001 was statistically significant.
MRI and arthroscopy are valuable tools for assessing the instability of the OCD lesion and its healing potential. The bioabsorbable chondral dart implant provides secure fixation and compression of the osteochondral fragment, increasing its healing potential.

KEY WORDS
Osteochondritis dissecans; MRI; arthroscopy; chondral darts; knee.

INTRODUCTION

Osteochondritis Dissecans (OCD) is an idiopathic, well-localized, pathological condition affecting the articular subchondral bone, and secondarily its overlying cartilage. This focal pathological condition can progress in some cases to the detachment of the affected osteochondral region. The cause of OCD remains controversial (1-4). Although several causes have been hypothesized, including inflammation, genetics, ischemia, ossification, and recurrent trauma, insufficient evidence remains to definitively support any of these causes for the time being. In addition, typical, "idiopathic" OCD must be differentiated from similar-appearing osteochondral lesions resulting from avascular necrosis associated with chemotherapy, hemoglobinopathy, and steroid

use. The Micro-trauma hypothesis is by far supported by the best level of evidence for typical OCD. There have been many reports in the literature regarding the potential role of trauma (either acute microtrauma or repetitive microtrauma) in the development of OCD, particularly because of the increasing prevalence among athletes (5). The incidence of this condition has been influenced in recent times by growing participation in competitive sports by children at younger ages across both genders (1). As a result, the mean age of the OCD onset seems to be decreasing, along with an increased prevalence among girls (1). The most affected joint is the knee joint, with the posterior lateral aspect of the medial femoral condyle being the most affected region in 70% of cases. Follows at a frequency the lateral femoral condyle in 20% of cases and the patella in 10% of cases. OCD is bilateral in 20-30% of cases. Symptoms are variable and range from the pain that is vague, poorly localized, and related to activity, to significant pain and locking (suggesting loose body formation). Unstable lesions are distinguishable by the presence of mechanical symptoms, and knee effusion. On physical examination, an antalgic, external rotation gait may be observed. On palpation, maximal tenderness can often be elicited over the anteromedial aspect of the knee with varying amounts of knee flexion. This corresponds to the most common site of the OCD lesions on the lateral aspect of the distal medial femoral condyle. Pain may be provoked with internal rotation of the tibia (Wilson sign). Atrophy of the quadriceps muscles provides a good indication of how long the lesion has been present. Evaluating the stability of the lesion is a key part of providing the correct treatment. Stable lesions, particularly in juvenile patients, have a greater tendency to heal with non-surgical treatment, whereas unstable lesions usually require surgical management. The evaluation of instability of the affected osteochondral region can be assessed by magnetic resonance imaging and arthroscopy. The assessment of the instability of the affected osteochondral region can be done with MRI by using the International Cartilage Repair Society System (ICRS classification system for OCD lesions) (**table I**). The ICRS was founded in 1997 (6, 7). MRI is useful for the assessment of the fragment's articular cartilage continuity and its potential instability (T2 weighted image: Type III , and IV), and for assessing the size and potential healing of its subchondral bone only by conservative means**.** In the T2 weighted image, the presence of a high signal at the interface between the osteochondral fragment and the underline bone is considered a bad prognostic sign of healing and indicates either granulation tissue or synovial fluid (MRI type, III, and IV). Arthroscopic exploration is the next necessary step for the confirmation of an unstable OCD lesion. The OCD lesions are classified based on articular cartilage integrity (open or closed) and the stability of the underlying subchondral bone and its bed (stable or unstable). The ICRS developed a four-grade classification based on findings upon inspection and palpation: Grade I: stable lesions with a continuous but softened area covered by intact cartilage. Grade II: lesions with partial discontinuity that are stable when probed. Grade III: lesions with a complete discontinuity that are not yet dislocated ("dead in situ"), and Grade IV: empty defects as well as defects with a dislocated fragment or a loose fragment within the bed (8) (**table II**). Drilling of the osteochondral lesion creates excellent outcomes if the lesion is stable. Unstable lesions require fixation. For loose but intact fragments with macroscopically normal cartilage surface and a layer of subchondral bone, fixation is also indicated.

Table I. ICRS TYPE, MRI evaluation for OCD lesions.

ICRS OCD I: small change on signal without clear margins of fragment
ICRS OCD II: osteochondral fragment with clear margins but without fluid between fragment and underline bone
ICRS OCD III: fluid is visible partially between fragment and underline bone
ICRS OCD IV: fluid is completely surrounding the fragment, but the fragment is still *in situ*
ICRS OCD V: fragment is completely detached and displaced (loose body)

Table II. ICRS STAGE, arthroscopic evaluation for OCD lesions.

ICRS OCD I: stable lesions with a continuous but softened area covered by intact cartilage
ICRS OCD II: lesions with partial discontinuity at the lesion and bone interface that are stable when probed
ICRS OCD III: lesions with a complete discontinuity that are not yet dislocated ("dead *in situ*")
ICRS OCD IV: empty defects with a dislocated fragment or a loose fragment within the bed.

MATERIALS AND METHODS

Between May 1990 and November 2017, a total of 64 consecutive immature patients (range 10-14 years old) (68 knees) had been referred to our institution with symptomatic OCD lesions for evaluation and treatment. Symptoms were variable and range from a pain that was vague, poorly localized, and related to activity, to significant pain and locking (suggesting loose body formation). The patients' knees were assessed first by clinical examination. The Knee OCD was then evaluated and classified according to its anatomical location and size with X-Rays (A/P, tunnel, and lateral view images) (**figure 1**). Next, the evaluation of the instability of the affected osteochondral region was made by magnetic resonance imaging (**figure 2**) using the International Cartilage Repair Society (ICRS Classification System for OCD Lesions) (**table I**). Twenty-eight patients (32 knees) who sustained symptomatic stable OCD, detected by MRI (ICRS Type I), responded very well to the conservative treatment they underwent (restraint of sports activities and immobilization of the knee for 6 weeks in a knee brace) and were excluded from our study. In four patients (4 knees), a free loose osteochondral fragment was detected by MRI (ICRS Type V) and confirmed by arthroscopy (ICRS stage IV). These four patients were excluded also from this study.

For the final evaluation of the OCD lesion, and its decisive treatment, an arthroscopic investigation was considered necessary in 36 knees. The arthroscopic findings were evaluated and classified using the International Cartilage Repair Society (ICRS) system (**table II**, **figure 3**). Surgical treatment to promote healing was performed on stable (immobile) lesions that did not respond to six months conservative treatment and on unstable (mobile) lesions. The patients' knee functionality was assessed by a subjective questionnaire (for pain and activity) and scored by using the online calculator of the International Knee Documentation Committee (IKDC) (87/87) system (4). The assessment was undertaken preoperatively and postoperatively, at 3, 6, and 12 months, and then at 6-month intervals. Postoperative MRI assessment was undertaken at 6, 12, 18, and 24 months postoperatively. The statistical analysis of the radiological, MRI, arthroscopic exploration, and patients' knee functionality results was performed by the author (M.P), with the contribution of the coauthor (M.O), using the SPSS v 23.0 software (SPSS Inc., Chicago, IL, USA). A paired t-test was used to analyze data, and a P-value of < 0.01 was considered statistically significant.

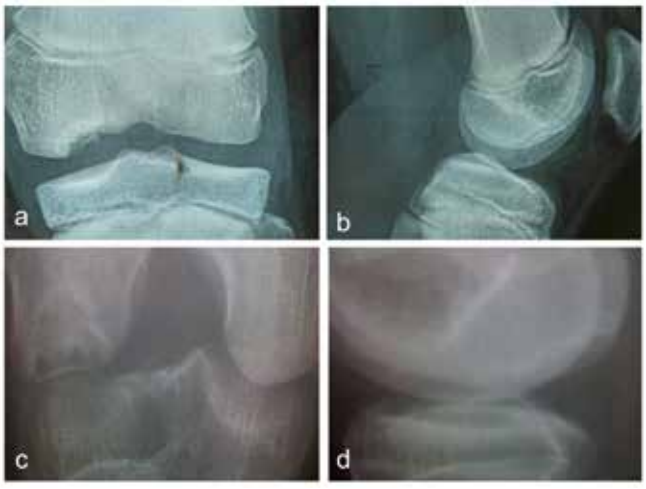

Figure 1. Evaluation of OCD lesion with X-Rays.

(a) OCD lesion of the medial femoral condyle, left knee, A/P view, **(b)** OCD lesion of the medial femoral condyle, left knee, Lateral view, **(c)** OCD lesion of the lateral femoral condyle, right knee, tunnel view, **(d)** OCD lesion of the lateral femoral condyle, right knee, lateral view.

Figure 2. Evaluation of OCD lesion with MRI.

(a) OCD lesion of the medial femoral condyle, left knee, T2 weighted MRI image, coronal view. Solid arrow: high signal at the interface between the fragment and the parent bone indicates the presence of either granulation tissue or synovial fluid, and it is a bad prognostic sign of healing of the osteochondral lesion by conservative means. Dotted arrow: interruption of the continuity of the fragment's cartilage is a strong indication of the potential instability of the osteochondral fragment MRI ICRS type III. **(b)** OCD lesion of the medial femoral condyle, left knee, T2 weighted MRI image, sagittal view. Dotted arrow: interruption of the continuity of the fragment's cartilage. **(c)** OCD lesion of the lateral femoral condyle, right knee, T1 weighted MRI image, coronal view. **(d)** OCD lesion of the lateral femoral condyle, right knee, T2 weighted MRI image, sagittal view. Solid arrow: high signal at the interface between the fragment and the parent bone, indicates the presence of either granulation tissue or synovial fluid, and it is a bad prognostic sign of healing of the osteochondral lesion by conservative means. Dotted arrow: interruption of the continuity of the fragment's cartilage, a strong indication of the potential instability of the osteochondral fragment MRI ICRS type III.

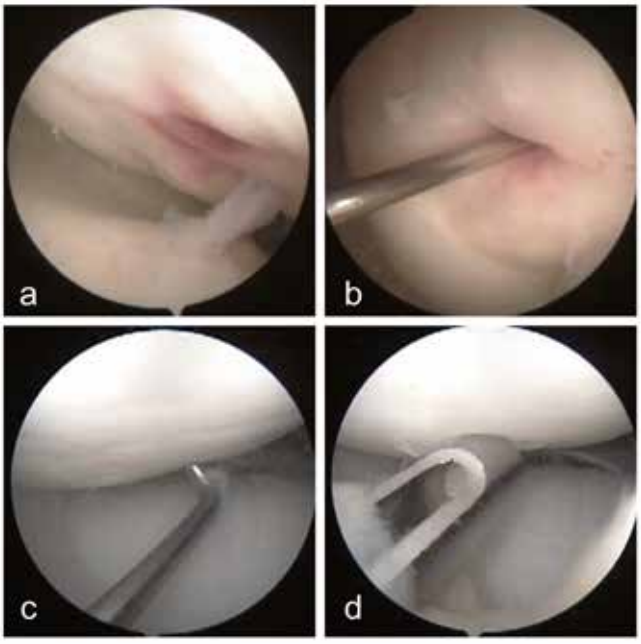

Figure 3. Arthroscopic evaluation of the OCD lesions.

(a) OCD lesion of the medial femoral condyle, left knee, **(b)** probing the OCD lesion of the medial femoral condyle, ICRS Stage III, **(c)** OCD lesion of the lateral femoral condyle, right knee, probing the OCD lesion ICRS Stage II, **(d)** Single-Shot Sheath in place, for chondral dart insertion.

Surgical technique

The procedures were performed arthroscopically with the patient under general anesthesia and using a thigh tourniquet. The anterolateral portal was used for the inspection of the M.F.C lesions, using a 4mm arthroscope, 30º oblique views (**figure 4 a**). For the instrumentation, necessary for the insertion of the chondral darts, the anteromedial portal was used to fix the M.F.C lesions (**figure 4 a**). By contrast, for the inspection of the L.F.C lesions, the anteromedial portal was used. Correspondingly, for the fixation of the L.F.C lesions, the anterolateral portal was used. A pilot hole within the osteochondral fragment to a depth of 20 mm was drilled with a drill pin through the Single Shot sheath (**figure 4 a**). A Chondral Dart 18 mm long was loaded into the end of the sheath (**figures 4 b, 5**). Once inserted into the sheath, a Single Shot Dart Inserter was used to deliver the dart into the pilot hole. With light taps on the inserter, confirmation was made that the Dart was seated, when the inserter contacts the back of the sheath, recessing the Dart 2 mm below the hyaline cartilage surface (**figure 4 c-f**)**.** To provide solid fixation of the osteochondral fragments, 3-5 chondral darts, 18 mm long, 1.3 mm diameter were inserted.

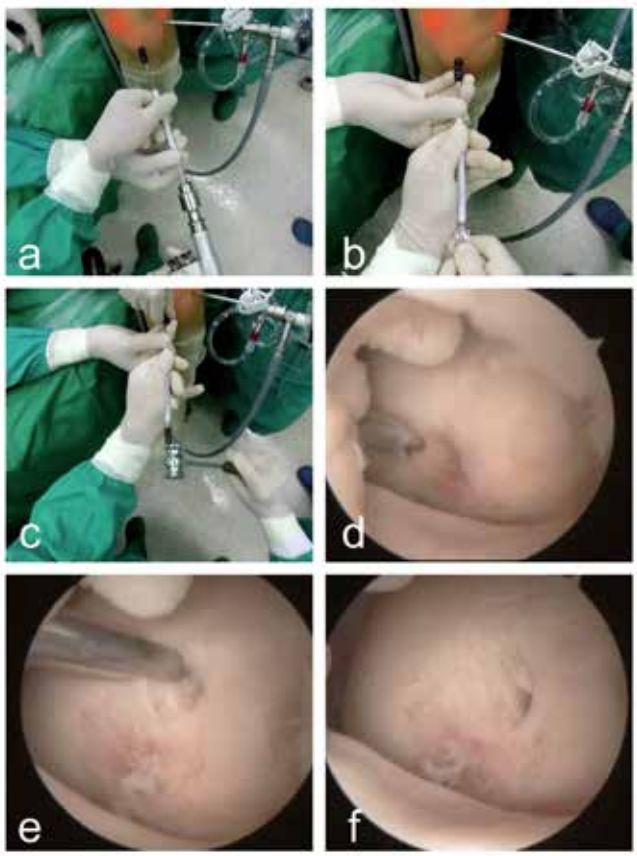

Figure 4. Arthroscopic fixation of the OCD lesion of the medial femoral condyle with chondral darts Surgical technique.

Anterolateral portal for the insertion of 4 mm arthroscope, 30º oblique views. Anteromedial portal for the instrumentation, to fix the M.F.C lesion with chondral darts. **(a)** A pilot hole within the osteochondral fragment to a depth of 20 mm is drilled with a drill pin through the Single Shot sheath. **(b)** A Chondral Dart 18 mm long is loaded into the end of the sheath. **(c)** Once inserted into the sheath, a Single Shot Dart Inserter is used to deliver the dart into the pilot hole, recessing the Dart 2 mm below the hyaline cartilage surface. With light taps on the inserter, confirmation that the Dart is seated is made when the inserter contacts the back of the sheath. **(d)** Arthroscopic view. Chondral dart in place, within the osteochondral fragment, recessing 2 mm below the hyaline cartilage surface. **(e)** Arthroscopic view. Third chondral dart in place. **(f)** Arthroscopic view. Final image. Fixation of the osteochondral fragment with four chondral darts.

Figure 5. Chondral Dart, 1.3 mm ×18 mm.

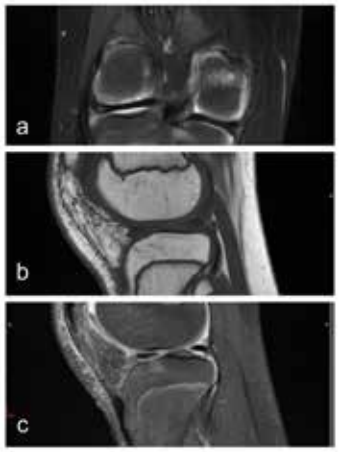

Figure 6. Postoperative MRI of the right knee, 18 months after the fixation of the L.F.C, OCD lesion with Chondral Darts. Complete healing of the OCD lesion.

(a) T2 weighted MRI image, coronal view, **(b)** T1 weighted MRI image, sagittal view, **(c)** T2 weighted MRI image, sagittal view.

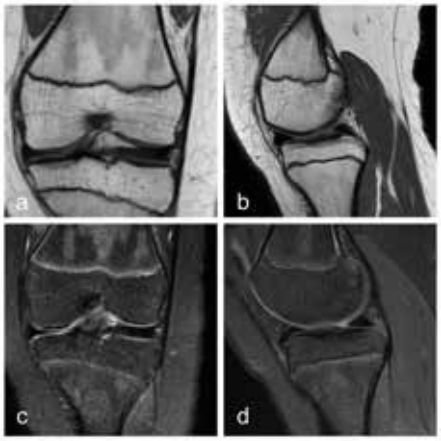

Figure 7. Postoperative MRI of the left knee, 24 months after the fixation of the M.F.C, OCD lesion with Chondral Darts. Complete healing of the OCD lesion.

(a) T1 weighted MRI image, coronal view, **(b)** T1 weighted MRI image, sagittal view, **(c)** T2 weighted MRI image, coronal view, **(d)** view image, proximal slice. Patella is in a good position.

RESULTS

A total of thirty-one patients (n = 31) (32 knees, 7 right knees and 15 left) were included in the analysis. Of these patients, 19 (61.3%) were males and 12 (38.7%) were females (**table III).** From the 32 knees, the M.F.C was affected in 21 knees (65.6%), and the L.F.C in 11 knees (34.4%) (**table IV**). Nine knees (9 patients), sustained symptomatic stable OCD, detected by MRI (ICRS type II) (28.1%) and confirmed by arthroscopy (ICRS stage I) (28.1%). These nine knees stable OCDs, remained symptomatic despite the conservative treatment they had for 6 months (restraint of sports activities and immobilization of the knee for 6 weeks in a knee brace). From the remaining twenty-three OCDs knees (22 patients), 17 were MRI: ICRS type III (53.1%), and six were MRI: ICRS type IV (18.8%) (**table IV**). The Arthroscopic exploration revealed five knees: ICRS stage II (15.6%), and 18 knees ICRS stage III (56.3%) (**table IV**). The bioabsorbable chondral darts were used for the fixation of the thirty-two osteochondral fragments, to secure their stability and mainly to stimulate their healing**.** The mean patient age at the time of surgery was 12.64 ± 0.98 years (range 10-14 years) (**table III**). The average follow-up was 2.58 ± 0.71 years (range, 2-4) years (**table III**). No complications occurred. MRI obtained approximately after a mean of 20 months postoperatively showed complete healing of the osteochondral lesion (**figures 6, 7**). The mean preoperative IKDC score was 31.67 ± 8.36 (range, 23-45) points (**table V**). The mean postoperative IKDC score was 83.5 ± 1.52 (range, 81-86) points (**table V**). The 2-tailed p value < 0.001 was statistically significant with t = 39.33 and degrees of freedom 30 (**table V**).

Ethics

All procedures performed in this study involving human participants were in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors. Informed consent was obtained from all individual participants included in the study.

Table III. Gender*, Knee Side*, Age¥, Follow-up¥.

Gender		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Male	19	61.3	61.3	61.3
	Female	12	38.7	38.7	38.7
	Total	31	100.0	100.0	100.0

Age	N	Minimum	Maximum	Mean	Std. Deviation
Age (years)	31	10.00	14.00	12.6452	0.98483
Valid N	31				

Knee side		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Right	17	53.1	53.1	53.1
	Left	15	46.9	46.9	46.9
	Total	32	100.0	100.0	100.0

Follow-up	N	Minimum	Maximum	Mean	Std. Deviation
Follow	31	2.00	4.00	2.5806	0.71992
Valid N	31				

*Frequency, valid percent; ¥Minimum-Maximum, Mean.

Table IV. *Femoral Condyle (Medial lateral), *MRI (ICRS Classification System - OCD Evaluation), *arthroscopy (ICRS Classification System, OCD Evaluation).

Femoral condyle: medial lateral		Frequency	Percent	Valid percent	Cumulative percent
Valid	M.F.C	21	65.6	65.6	65.6
	L.F.C	11	34.4	34.4	34.4
	Total	32	100.0	100.0	100.0
MRI: OCD evaluation		**Frequency**	**Percent**	**Valid percent**	**Cumulative percent**
Valid	ICRS II	9	28.1	28.1	28.1
	ICRS III	17	53.1	53.1	53.1
	ICRS IV	6	18.8	18.8	18.8
	Total	32	100.0	100.0	100.0
ARTHROSCOPY: OCD EVALUATION		**Frequency**	**Percent**	**Valid percent**	**Cumulative percent**
Valid	ICRS I	9	28.1	28.1	28.1
	ICRS II	5	15.6	15.6	15.6
	ICRS III	18	56.3	56.3	56.3
	Total	32	100.0	100.0	100.0

*Frequency, valid percent.

Table V. IKDC Preoperative and Postoperative Score.

Paired Samples Statistics		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	IKDC POSTOP.	83.5806	31	1.52259	0.27347
	IKDC PREOP.	31.6774	31	8.36017	1.50153

Paired Samples Test		Paired Differences					t	df	Sig.(2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	IKDC POST - IKDC_PR	51.90323	7.23581	1.29959	49.24911	54.55734	39.938	30	0.000

2-tailed P-value.

DISCUSSION

The early identification of the OCD allows the initiation of appropriate treatment that will prevent the aggravation of this pathological process and can lead to the healing of the localized, osteochondral lesion. MRI is useful for the assessment of the fragment's articular cartilage continuity, and the evaluation of the size and viability of its subchondral bone. The presence, in the T2 Weighted MRI image, of a high signal at the interface between the fragment and the parent bone, indicates the presence of either granulation tissue or synovial fluid, and it is additional evidence of possible instability of the OCD lesion. In addition, the presence of either granulation tissue or synovial fluid displays the weakness of healing of the osteochondral lesion by conservative means. Arthroscopic exploration is the next necessary step for the confirmation of an unstable OCD. The OCDs knees with bad prognostic evolution (MRI: ICRS type III and IV), (Arthroscopy: ICRS stage II and III) need arthroscopic fixation. Arthroscopic exploration and treatment are also necessary in the cases of symptomatic, stable OCDs knees in MRI evaluation (MRI: ICRS type I, and II), after the failure of six months of conservative treatment, consisting of restraint of sports activities and initial immobilization of the knee for 6 weeks in a knee brace. Several surgical procedures have previously been described for the fixation of symptomatic unstable OCD lesions. None of these surgical procedures have been globally successful. For many OCD lesions, a second operative intervention is often required. In 1957, Smillie (9) reported a technique that used metallic nails. Since then, many fixation implants have been evaluated (staples, screws with or without a head, and more recently, bio-absorbable nails (10) and screws) (11). Bioabsorbable implants are an alternative to metal and provide stable fixation (12-20). Bio-absorbable materials have gained preference in North America (21). The bioabsorbable Chondral Dart implant has a double-reversed-barbed design to facilitate superior fixation and compression of the osteochondral fragment. The 18 mm long, 1.3 mm diameter Chondral Dart implant provides secure fixation of the osteochondral fragment. Their important advantage is that no further procedure is required to remove the implant. Until now, no complications have been reported and observed from their use. For large OCD lesions, the Bio-Compression Screw may be used to fixate OCD lesions securely while eliminating the challenges of metal screw removal.

With regard to limitations on the use of bioabsorbable materials, in the International Literature, a case of systemic allergic reaction has been reported after the use of a multi-L-lactic acid biodegradable interference screw for anterior cruciate ligament reconstruction with a bone-patellar tendon-bone graft (22). There is also a case report of local fibroxanthoma occurrence after ACL reconstruction using a biodegradable interference screw (23). Finally, it should be emphasized that their use for the stabilization of purely focal chondral lesions is useless because articular cartilage does not contain vascular, nervous and lymphatic tissue and chondrocytes hardly participate in the healing or repair process of chondral tissue (24). Osteochondral autograft transplantation is a one stage procedure and repairs the lesion with hyaline cartilage. But its limitation is the lack of donor site availability.

In this study, the data analysis of the fixation results of the unstable OCD lesions with absorbable chondral darts, using the IKDC evaluation system, was similar to that of other researchers in the literature who used also absorbable chondral darts as fixation materials, and the IKDC evaluation system (10, 11, 25-27).

CONCLUSIONS

Surgical treatment to promote healing is recommended for stable (immobile) lesions that did not respond to six months of conservative treatment and for unstable (mobile) lesions.

FUNDINGS

None.

DATA AVAILABILITY

Data are available under reasonable request to the corresponding author.

CONTRIBUTIONS

PM: surgical technique, study design, performed measurements, manuscript preparation, photo editing, video editing, statistical analysis. OM: study design, manuscript preparation, photo editing, video editing, statistical analysis.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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Intervention Treating Kinetic Chain Factors *versus* Heavy-Slow Resistance Training in Athletes with Patellar Tendinopathy: Protocol for a Randomized Blind Clinical Trial

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LEVEL OF EVIDENCE: N/A

SUMMARY

Introduction. Heavy Slow Resistance Training (HSR) is one of the most recommended interventions for the treatment of patellar tendinopathy (PT). However, the HSR protocol does not address known risk factors for PT. Athletes with PT have been shown to have stiff jump-landings, decreased ankle dorsiflexion range of motion and hip extensors strength compared to asymptomatic athletes. This study aims to verify the effects of an intervention addressing kinetic chain factors in comparison to the HSR protocol on pain, symptoms severity and function, strength and flexibility of the lower limb and landing mechanics in athletes with PT.

Methods. Blind randomized, clinical trial consisting of 28 male recreational athletes, divided into two groups: Heavy slow resistance training group (HSG; n = 14) and kinetic chain group (KCG; n = 14). Both interventions will be delivered 3 times per week, for 12 weeks. Pain will be measured with a visual analog scale and disability will be measured with the VISA-P questionnaire, at baseline, after 6 weeks of intervention, immediately after the intervention, as well as at 6 months after the interventions. Lower limb strength and flexibility and landing mechanics will also be assessed before and after the 12-week interventions. The HSG intervention will involve the squat, Leg Press and Hack Squat, with progressive loads. The KCG intervention will involve the squat exercise, hip extensors and ankle plantar flexors strengthening, interventions to improve ankle dorsiflexion and a jump-landing training. General linear models will be used to compare the intervention effects.

Results. The results of this study will expand the evidence base on different exercise programs for patellar tendinopathy, and may aid clinicians in choosing the most appropriate program for the treatment of athletes with this condition.

Conclusions. This randomized controlled trial will compare the effectiveness of an intervention addressing kinetic chain factors to a standard patellar tendon progressive loading program for the rehabilitation of athletes with patellar tendinopathy.

Study registration. EnsaiosClinicos.gov.br (Identifier: RBR-74nhx9).

KEY WORDS

Knee joint; pain; jumper's knee; muscle strength; tendinitis.

INTRODUCTION

Patellar tendinopathy is a condition characterized by focal pain and dysfunction in the patellar tendon (1). It more frequently occurs in sports involving jumps such as basketball and volleyball, affecting 11.8% and 14.4% of recreational athletes of these modalities, respectively (2). Male athletes are at greater risk of developing patellar tendinopathy than female athletes (2). Patellar tendinopathy may be a rather limiting dysfunction for athletes. Often, athletes with patellar tendinopathy need to move away from training and competition for a long time and 53% of athletes terminate their careers because of this condition (3). The high prevalence of patellar tendinopathy in the athlete population and its impact in both sports career and daily life highlights the importance of identifying effective treatment options for this condition.

The progressive exercise program known as heavy slow resistance training (HSR) (4) has been highlighted by recent systematic reviews (5, 6) as one of the most recommended interventions for the rehabilitation of athletes with patellar tendinopathy. This intervention involves a progressive loading protocol for the quadriceps and, consequently, for the patellar tendon, resulting in improvement in the pain and function of athletes with patellar tendinopathy (4). Specifically, the HSR protocol involves three exercises: squat, leg press and hack squat; performed so that each repetition of each exercise involves 3 seconds for the eccentric phase and 3 seconds for the concentric phase. This protocol resulted in greater patellar tendon collagen turnover and greater athlete satisfaction when compared to an intervention composed solely of eccentric exercises (4), which is often considered the gold-standard intervention for patellar tendinopathy (5).

Rehabilitation programs that exclusively involve quadriceps exercises with progressive loads, such as the eccentric protocol or the HSR, may not be the best option for long-term symptoms resolution in athletes with patellar tendinopathy. Since these interventions focus only in providing the patellar tendon with progressive loads to improve its capacity, they do not address important risk factors for patellar tendinopathy (7). Interventions with a restricted look to the knee and the quadriceps muscle overlook variables that may be important to increase the patellar tendon forces in sports activities, potentially favoring recurrences. Kinetic chain factors, including hip and ankle joint strength and flexibility deficits and jump-landing alterations, have already been observed in athletes with patellar tendinopathy (7-9). In a prospective study with basketball athletes, Backman & Danielson (9) observed that restriction of ankle dorsiflexion range of motion is a risk factor for patellar tendinopathy. Restricted dorsiflexion movements may limit the eccentric action of the plantar flexor muscles in deceleration forces, with the ankle potentially becoming less efficient at force dissipation near end of range (7). This may alter the mechanics of the lower limb at landing, which may lead to an increase in the load on the patellar tendon and the risk of injury (7).

Scattone Silva *et al.* (7) observed that athletes with patellar tendinopathy present lower hip extensor torque when compared to asymptomatic athletes. Weakness in the extensor hip muscle probably increases the demand on knee extensors to dissipate ground reaction forces during jump landings, which could contribute to patellar tendinopathy (7). Despite these findings, the most frequently recommended interventions for the treatment of patellar tendinopathy (6) do not take into account deficits in strength and/or range of motion in the ankle and hip joints for rehabilitation of athletes.

Regarding jump landing, a recent systematic review concluded that athletes with patellar tendinopathy have a stiffer jump landing pattern when compared to asymptomatic controls (8). A stiffer landing requires energy to be dissipated more quickly, which leads to increased ground reaction forces and increased loads at the knee joint (10). Stiff landings, with an extended trunk, also decrease the contribution of the hip muscles for dissipation of the landing forces and increase the peak patellar tendon force (10). Thus, an abnormal landing may contribute to the development or perpetuation of patellar tendinopathy.

In this context, kinetic chain biomechanical alterations may contribute to increase patellar tendon stress, potentially contributing to patellar tendon overload in athletes (7). It is possible that a more comprehensive intervention, taking into account kinetic chain factors, may produce superior results than interventions directed at local factors (such as the HSR protocol) to treat athletes with patellar tendinopathy. However, the effect of an intervention directed to kinetic chain factors compared to a progressive loading protocol for the treatment of patellar tendinopathy in athletes has not been verified.

The primary objectives of the present study are to verify the short-term (after 6 weeks of intervention and immediately after 12 weeks of intervention) and long-term (6-month follow-up) effects of an intervention directed to kinetic chain factors in comparison to the HSR protocol in athletes with patellar tendinopathy in terms of knee pain, symptoms severity and function. The secondary objectives are to verify the short-term effects of these interventions in terms of lower limb isometric strength; of ankle dorsiflexion range of motion and jump landing mechanics.

MATERIALS AND METHODS

Design

This is a blind randomized clinical trial protocol, developed and reported in accordance with the Standard Protocol Items Recommendations for Intervention Trials (SPIRIT) (11). Participants will be randomized and allocated to the heavy-slow resistance training group (HSG) or the kinetic chain group (KCG).

The methods of this clinical trial will follow the recommendations and determinations of the Consolidated Standards of Reporting Trials (CONSORT) (12). This trial was registered in the Brazilian Registry of Clinical Trials (REBEC).

Recruitment

Participants will be recruited from the waiting list of patients of the Physiotherapy School Clinics of Federal University of Rio Grande do Norte. Announcements will also be made on local radios and social media and advertisements will be placed at training centers and schools in the cities of João Pessoa, Natal and Santa Cruz (Brazil).

Ethics

The project was approved by the Research Ethics Committee of the Federal University of Rio Grande do Norte (Number 3.577.145) and participants will sign an informed consent form. In the case of underage participants, the legal guardian will also sign a consent form. Results will be disseminated via publications in scientific journals, social media and presentations in scientific events.

Eligibility criteria

The eligibility criteria will be checked by means of a telephone screening by one of the authors. Suitable participants will be asked to attend the research site where they will be assessed for further eligibility screening.

Inclusion criteria

Male athletes with age between 15 and 40 years.

Practice physical activity involving activities with high demand for the knee extensor mechanism (volleyball, basketball, handball, *etc.*) at least 2 times a week.

Present with localized pain in the patellar tendon, confirmed by palpation, during activities that impose load to the patellar tendon (jumping, squatting, *etc.*) with a duration of 3 months or longer (7).

Have patellar tendon pain during the single-leg decline squat test (13, 14).

Present with a positive Royal London Hospital Test (15).

Present with a score lower than 88 points in the Victorian Institute of Sport Assessment-Patella (VISA-P) questionnaire (16).

Exclusion criteria

Use corticosteroid-based medication in the last 6 months.

Symptoms related to trauma or previous knee surgery.

Knee symptoms associated with other dysfunctions, such as intra-articular lesions, patellofemoral pain, patellar instability and Osgood-Schlatter or Sinding-Larsen-Johansson disease (7).

Inability or unwillingness to commit with conducting treatment during the intervention period.

Sample calculation

The sample size was calculated based on the results of a previous study comparing different interventions with exercises for the rehabilitation of athletes with patellar tendinopathy (4). The primary outcome variable used for the calculations was the Victorian Institute of Sport Assessment-Patella (VISA-P) questionnaire score, The sample size calculation considered $\alpha = 0.05$ and a power of 80%, with a difference between groups in the VISA-P of 22 points. This difference in the VISA-P score is greater than the minimal clinically important difference of the questionnaire (17). Based on these calculations, a sample size of 12 individuals per group was obtained. Considering a sample loss of 15%, which is expected in clinical studies of this nature (18), the sample size was determined to be of 28 subjects. The study flow chart is presented in **figure 1**.

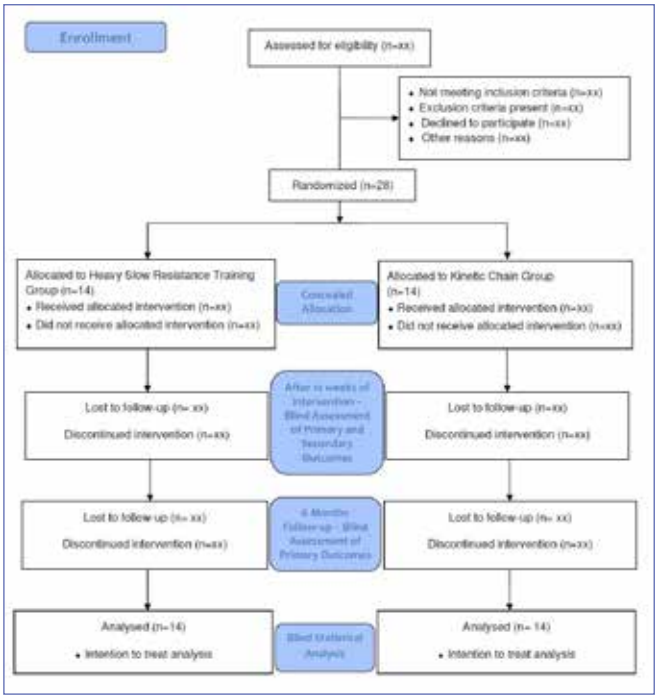

Figure 1. Participants flow chart.

Randomization and strategies for bias minimization

For the participant's randomization, a sample randomization system (blocks of four) will be used by generating a list of random numbers on a computer (http://www.randomization.org). Consecutively numbered opaque envelopes will be filled in and sealed with the numbers generated prior to the start of the study. An individual with no knowledge of the participants will perform this procedure and their allocation in the HSG and KCG groups. Randomization will be performed prior to the initial evaluation and participants will remain blinded to the group to which they were allocated. In order to ensure this, the participants of different groups will perform the treatment sessions separately, not knowing about the exercises performed by the other group (19).

The interventions will be conducted at a training center with fitness gym equipment localized in the cities of Natal, Santa Cruz and João Pessoa (Brazil). Given the nature of the intervention in question, it will not be possible to blind the therapist who will carry out the interventions. All evaluations, before and after the intervention, will be performed by a researcher who will not be involved with the administration of the interventions (blind assessor). In addition, data analysis will be performed by a researcher who will remain blind to the treatment groups.

Evaluations

Initially, participants will complete an identification form, containing personal information, including body mass, height, age and information regarding the sports modality they practiced. The Brazilian Portuguese short version of the International Physical Activity Questionnaire (IPAQ) will be used for the evaluation of the participants' level of habitual physical activity practice (20). The short version of the IPAQ consists of eight open questions and its information allows an estimation of the time spent per week in different dimensions of physical activity and physical inactivity (20).

Primary outcome measures

Pain rating

A 10-cm visual analogue scale (VAS) will be used to evaluate the participants' pain, with 0 indicating no pain and 10 indicating the worst imaginable pain (21). Pain measurement will be performed in two ways: the worst pain in the previous week 18 and pain during the single-leg decline squat test (13). The latter is a validated provocative test, where the participant, on standing on a platform with a 25° inclination and keeping the trunk upright, performs a single-leg squat up to 90° of knee flexion (13). The VAS is a reliable for the evaluation of individuals with knee pain, with a minimal important difference of 2 cm (22).

Function and severity of symptoms

The VISA-P is an 8-item questionnaire for assessing the severity of symptoms and disability of individuals with patellar tendinopathy (23). The total score in VISA-P ranges from 0 to 100 points, with a maximum score indicating absence of symptoms and disability (23). Changes greater than 13 points after interventions in the VISA-P questionnaire are considered clinically relevant (17). The Brazilian Portuguese version of the questionnaire will be used (24).

Global perception of change

A 15-point Likert scale that measures the impression of change in health status after a treatment intervention will be used. The scale ranges from - 7 ("much worse") to + 7 ("much better"), so a score of 0 indicates "no change" (25). Changes equal to or greater than four points on this scale have been considered clinically important in the treatment of patellar tendinopathy (26).

The primary outcomes will be assessed before the intervention, after 6 weeks of intervention, immediately after the end of the interventions (at 12 weeks) and six months after the end of the interventions, by a researcher who will remain blind to group allocation.

Secondary outcome measures

Isometric strength test

A portable handheld dynamometer (Lafayette Instrument Company, Lafayette, IN) will be used to assess lower limb strength. Four repetitions of each strength test will be performed, one to familiarize the participant with the procedure, followed by 3 valid repetitions, which will be used for analysis. At each repetition, the participant will be required to exert maximum force for 5 seconds, with a 15 second interval between repetitions (7). The assessments described below have been shown to be reliable, with intraclass correlation coefficients ($ICC_{3,3}$) ranging from 0.78 to 0.93 (7).

In order to measure hip extensors strength, the participant will be positioned in the prone lying with the knee of the limb to be tested at 90° of flexion and the dynamometer positioned immediately above the popliteal fossa (7). An inelastic belt will be positioned around the participant's hip and the examination table in order to stabilize the trunk, and another belt will be positioned in the distal thigh region to stabilize the dynamometer and resist the movement (7).

To assess the knee extensors strength, the participant will be positioned in supine, with 30° of knee flexion and approximately 20° of hip flexion (7). A foam roll will be placed

in the posterior region of the knee to maintain the desired angle. The dynamometer will be positioned in the midpoint between the malleoli, with an inelastic belt positioned to stabilize the equipment and resist the movement (7).
The strength of the ankle plantar flexors will be measured with the participant in prone lying, with the foot of the evaluated lower limb positioned outside of the examination table (7). The dynamometer will be positioned in the plantar aspect of the metatarsal heads with an inelastic belt stabilizing the equipment and resisting the movement (7).
The peak force results will be converted to torque values [force (N) × action length of the segment (m)]. The torque data will be normalized by the body mass and height of each participant (7).

Dorsiflexion range of motion

The lunge test (7, 9) will be performed using an inclinometer (Baseline Bubble, NY, USA), positioned 15 cm distal to the tibial tuberosity. For this measurement, the participant will be instructed to flex the knee to touch a wall with the patella without removing the heel from the ground. At the maximum distance that the participant can touch the patella on the wall without removing the heel from the ground, the examiner will verify the angle relative to the vertical will be recorded for analysis. Good reliability was observed for this test in a previous study ($ICC_{3,3}$ = 0.90) (7).

Landing mechanics

The Landing Error Scoring System (LESS) is a clinical tool described for evaluation of jump landing biomechanics (27). For this evaluation, two cameras (Panasonic NV-GS180, Matsushita Group, JP) will be positioned 3 m away from a 30 cm box, one in the frontal plane and the other in the sagittal plane. In front of the box, a landing zone will be demarcated, at a distance of 50% of the height of the participant. The test consists of two jumps, one from the box to the landing zone and another, immediately after, upwards with maximum effort. The landing mechanics will be measured through a 17 items tool, which considers the position of the lower limbs and trunk at the time of initial contact with the ground as well as the peak movements during landing, both in the sagittal and frontal planes. Higher values indicate worst landing quality. This evaluation presents good reliability ($ICC_{2,1}$ = 0.91) and high agreement with three-dimensional movement evaluations (27).
The secondary outcomes will be assessed before the intervention and immediately after the end of the interventions (at 12 weeks), by a researcher who will remain blind to group allocation. The symptomatic lower limb will be evaluated and, in cases of bilateral symptoms, the most symptomatic limb will be submitted to the evaluations. The time points of all evaluation of this trial are summarized in **figure 2**.

		STUDY PERIOD					
	Enrolment	Allocation	Post-Allocation				Close-out
TIMEPOINT	$-t_1$	0	t_1	t_2	t_3	t_4	t_5
ENROLMENT:							
Eligibility Screening	X						
Informed Consent	X						
Allocation		X					
INTERVENTIONS:							
Heavy Slow Resistance Training (Squat, Hack Squat, Leg Press)			←		→		
Kinetic Chain Group (Hip and knee extensors and ankle plantar flexors strengthening, improving dorsiflexion range of motion and landing training)			←		→		
ASSESSMENTS:							
Primary Outcomes (Pain, severity of symptoms and disability, perception of change)		X		X		X	X
Secondary Outcomes (Strength, lower limb flexibility and jump-landing mechanics)		X				X	

$-t_1$=Before allocation; 0=Allocation and baseline evaluations; t_1=1st Month; t_2=2nd Month; t_3=3rd Month; t_4=Evaluations immediately after the interventions; t_5=6 Months after the interventions

Figure 2. Description of the Standard Protocol Items Recommendations for Intervention Trials (SPIRIT).

Interventions

Participants of both the KCG and HSR will be treated during 12 weeks of rehabilitation, with the interventions of both groups lasting approximately 50 minutes in each session. Participants will be encouraged to come to the treatment setting 3 times/week for in-person supervised rehabilitation. If that is not possible for any reason, the treating therapist will be available to supervise the participant's session via telehealth. If that is also not possible, the participant will be instructed to conduct that specific session unsupervised, following the previously provided instructions and filling a training diary which will include information about the number of repetitions, load and pain during each exercise.
During the strengthening exercises in both groups, each participant will be using a headset and will have the auditory stimulus of a metronome adjusted to a rate of 60 beats per minute, to control the concentric and eccentric phases of each exercise. Recently, it has been suggested that auditory stimuli are important in the treatment of tendinopathies to improve motor control, increasing muscle excitability and decreasing muscle inhibition (28, 29). In the sessions when the participant is unavailable for in-person supervised treatment or for telehealth supervised treatment, he

will be instructed to perform the exercises using his phone or computer with the metronome provided by the Google platform (available at https://g.co/kgs/paexw4). In the first supervised session, the participants will be given instructions on how to access this and how to set the frequency to 60 beats/minute.

Heavy Slow Resistance Training Group intervention

The HSG participants will be submitted to the Heavy-Slow Resistance Training (HSR) protocol proposed by Kongsgaard *et al.* (4). The HSR consists of three exercises: squat, hack squat and leg press (**figure 3**). The parameters of each exercise are described in **table I**. This intervention has already been shown to be effective to reduce pain and improve function in athletes with patellar tendinopathy, both in short and in long term (4).

During the HSR protocol, knee pain will be acceptable as long as it is of a maximum intensity of 3/10 in the VAS (4). If pain intensity during the intervention is greater than 3, the exercise will be modified with a reduction in range of motion and/or load.

Table I. Heavy Slow Resistance Training Detailed Protocol.

Exercises
Squat, Leg Press and Hack Squat
Progressive load for strengthening exercises
1st week: 4 × 15 – Load 15 repetition maximum (RM)
2nd / 3rd weeks: 4 × 12 – Load 12RM
4th / 5th week: 4 ×10 – Load 10RM
6th-8th week: 4 × 8 – Load 8RM
9th-12th weeks: 4 × 6 – Load 6RM
Muscle activation time
Concentric phase - 3s
Eccentric phase - 3s
Rest time between sets: 2 minutes
Range of motion: 0º (full extension) to 90º of knee flexion

Figure 3. Heavy Slow Resistance Training Protocol.
Squat **(A)**, Leg Press **(B)** and Hack Squat **(C)**.

Kinetic Chain Group intervention

Participants assigned to the KCG will undergo a protocol involving not only knee extensors strengthening, but also strengthening of the hip extensors and ankle plantar flexors, interventions to improve ankle dorsiflexion range of motion (stretching/mobilization) and a jump-landing training (**figure 4**). The parameters of all the strengthening exercises of the KCG intervention will be identical to the parameters used in the HSG exercises and are described in **table II**.

Figure 4. Kinetic Chain Group Intervention.
Joint mobilization to improve dorsiflexion with an inelastic belt **(A)** and soleus muscle stretching **(B)**. Hip extensor strengthening exercise – Single-limb deadlift initial position **(C)** and final position **(D)**. Ankle plantar flexors strengthening exercise – Seated heel-rise initial position **(E)** and final position **(F)**. Squat exercise initial position **(G)** and final position **(H)**. Jump landing training emphasizing soft landing – Initial position (I) and final position, landing with trunk flexion and backwards hip projection **(J)**.

Table II. Kinetic Chain Group Detailed Protocol.

Joint mobilization for dorsiflexion: 4 ×10 s (20 s rest)
Soleus muscle stretching: 3 × 30 s (10 s rest)
Exercises: Single-Limb Deadlift, Squat and Seated Heel Raise
Progressive load for the strengthening exercises:
1st week: 4 × 15 – Load 15 repetition maximum (RM)
2nd / 3rd weeks: 4 × 12 – Load 12RM
4th / 5th week: 4 ×10 – Load 10RM
6th-8th week: 4 × 8 – Load 8RM
9th-12th weeks: 4 × 6 – Load 6RM
Muscle activation time
Concentric phase - 3s
Eccentric phase - 3s
Rest time between sets: Squat **-** 2 minutes; Remaining exercises **-** 1 minute
Range of motion: Squat - 0º (full extension) to 90º of knee flexion; Seated heel raise - Full range; Single-limb deadlift - 0º (trunk upright) to 90º of hip flexion.
Jumping landing strategy modification training (after the 4th week): 2 × 10

Ankle plantar flexors strengthening
The seated heel raise will be the exercise used to strengthen the ankle plantar flexors of the KCG. For this exercise, the participant will be positioned in sitting with 90° of hip and knee flexion, with the tips of the feet resting on a 15 cm step. The exercise will consist of raising the heel as much as possible, keeping the toes in contact with the step, followed by a slow lowering of the heel to the maximum possible dorsiflexion range of motion (**figure 4**) (30). Additional load for this exercise will be imposed with a bar with weights which will be positioned on the thighs of the participant, the bar surrounded by padding to avoid discomfort.

Hip Extensors strengthening
The single-limb deadlift will be the exercise performed to strengthen the hip extensors of the KCG athletes. For this exercise, the participant will stand only in one leg and performs an anterior lean of the trunk keeping the spine erect until the trunk is parallel to the ground, later returning to the initial position (**figure 4**) (31). Loads will be added with dumbbells in the hands of the participant. This exercise will be carried out bilaterally to avoid asymmetric loads during the landing of a jump (26).

Knee Extensors strengthening
The squat exercise (**figure 4**) will also be performed by the KCG athletes, in order to strengthen the knee extensor muscles, which have been shown to be weak in this population (32), and to provide progressive load to the patellar tendon (4). The exercise will be performed with the exact same parameters used by the HSG (**table I**).

Jump Landing Strategy Modification training
From the fourth week of intervention, in addition to strengthening exercises, participants will perform an exercise to change their landing strategy. The participant will receive verbal instructions to land softly, minimizing the impact of the landing. Landing with a forward trunk lean and greater hip flexion will be encouraged, with the aim of increasing the contribution of the hip muscles to dissipate ground reaction force, in order to decrease the forces in the patellar tendons (26). The instructions given to the participant will be as follows: "Keep your knees slightly bent before landing and try to land as softly as possible. Lean your trunk forward and project your hips back as you bend your knees, in order to minimize the impact of the landing. Pay attention to the sound of your landing and use this information to help you to land more softly". After each jump landing, feedback will be given to the participant if he does not incorporate one or more of these recommendations into his strategy. At each session of the intervention, the participant will perform drop vertical jump landings with a bipodal support of a 30 cm box in 2 sets of 10 repetitions, with 5 seconds rest between landings and 1 minute between sets (**figure 4**) (26).

Interventions to improve dorsiflexion
A cut-off angle of 36° in the lunge test has been previously determined as a risk factor for patellar tendinopathy in athletes (9). For participants of the KCG with dorsiflexion movement restriction (lunge results < 36°), interventions to improve range of motion will be pragmatically implemented. It has been suggested that interventions directed at tissue that is limiting the movement are more effective to improve ankle dorsiflexion range of motion (33). In this sense, both joint mobilizations and muscle stretching would be important to increase the range of motion and, therefore, will be performed in these participants of the KCG.
Joint mobilizations with movement will be performed with an inelastic belt around the tibia of the participant's affected limb and the waist of the therapist. For the mobilization technique, the therapist will apply a sustained posteroanterior glide to the tibia through the belt by leaning backwards, which causes a posterior glide of the talus in the ankle mortise (34). Then, the participant will be instructed to perform a slow dorsiflexion movement until the first onset of pain or end of range (**figure 4**). Once this point is reached, the glide will be maintained for 10 seconds. The participant will then return to the initial standing position and the mobilization force will be released. Each set of mobilization will consist of 4 glides, followed by a

20-second rest period. Four sets of mobilizations with movement will be applied in each treatment session (34).

The soleus muscle stretch will be done with the participant standing with his hands on a wall, the lower limb to be stretched positioned behind, keeping the entire foot in contact with the ground and the toes pointing forward. From this position the participant will flex both knees, until reaching the limit of movement without lifting the heel of the ground (**figure 4**) (35). The stretch position will be held for 30 seconds and will be repeated 3 times, with a period of 10 seconds between the stretches.

Load monitoring during daily activities and adverse effects

Participants will be allowed to continue practicing the sporting modality they usually practice, only being asked to avoid activities that cause pain greater than or equal to 3/10 in the VAS (4, 26). Participants will be instructed not to change their daily routines and to record the number and duration of training sections. Participants will be asked to refrain from seeking other treatments during the study period, but analgesia and anti-inflammatory drugs will be permitted (18). All medication use and co-interventions will be recorded. If any pain medication is used during the intervention period, the participants will be asked to record the amount and dosage of each drug, reporting this information in the post-intervention evaluation (18, 36). If the participants experience any adverse event at any time during the study, they will be asked to report it to the investigators. All adverse events will be included in the final manuscript.

Statistical analysis

Statistical analyses will be performed using SPSS software (version 17.0; SPSS Inc, Chicago, IL). Data normality and homoscedasticity will be verified with the Shapiro-Wilk and Levene tests, respectively. General linear models will be used to compare the results obtained at baseline, after 6 weeks of interventions, immediately after the interventions and six months after the interventions of both groups, with a significance level of 5% in all analyses. An intention-to-treat analysis will be used for the analysis and the statistician will remain blind to groups.

DISCUSSION

Considering that strength and range on motion impairments in different segments of the kinetic chain may increase patellar tendon overload, rehabilitation programs that focus only on progressively loading the patellar tendon with quadriceps-focused exercises may not be ideal, especially for the long-term. To our knowledge, this is the first clinical trial that focuses on verifying the effects of an intervention addressing kinetic chain factors known to be associated with patellar tendinopathy, in comparison to a progressive patellar tendon loading program. A recent study from our research group found that an in-season prevention program addressing kinetic chain factors, such as hip extensors strength and ankle dorsiflexion range of motion, significantly decreased the incidence of patellar tendinopathy in jumping athletes (37). In a previous study we also observed promising long-term results of pain and function improvements in a volleyball athlete with patellar tendinopathy that was treated solely with hip extensors strengthening exercises and a jump-landing strategy modification (26). It is possible that these promising results are occurring due to a better load distribution between the different joints of the lower limb during their sport participation, which potentially decreased the overload in the knee joint. However, randomized controlled trials are necessary to verify the effects of interventions addressing kinetic factors in athletes with patellar tendinopathy.

The main strengths of this study come from the scientific rigor in terms of randomization, concealed allocation, blinding and intention-to-treat analyses. These are important aspects to be considered because if they are not accounted for, they are strong sources of bias in clinical trials (38-40). In addition to performing adequate randomization and allocation procedures, we will blind the participants, the researcher responsible for the evaluation of the outcomes and the researcher responsible for the statistical analyses. An intention-to-treat approach will also be used for data analysis in order to ensure an unbiased estimate of treatment effect (41).

Some limitations are also expected in the study. Blinding of the therapist delivering the interventions will not be possible due to the study design. As a way to minimize this limitation, the therapist will deliver the treatments using standardized procedures. Another limitation is the fact that, although participants will be encouraged to undergo three supervised treatments per week, they will be allowed to take part in only one supervised treatment session per week, with the possibility of telehealth or home unsupervised exercises for the remaining two weekly sessions. This limitation will be minimized by the use of a training diary where the participant will report the details of his unsupervised session (repetitions, load, *etc.*). Although this is a limitation, we believe this approach more closely resembles the reality of treatment of these patients. Finally, we will not control the participants' medication intake throughout the study. However, medication intake will be monitored during the study by means of a diary that will be delivered to participants on their baseline assessment. If significant differences in medication intake are observed during the study, this variable will be accounted for in data analyses.

CONCLUSIONS

In conclusion, this randomized controlled trial will compare the effectiveness of an intervention addressing kinetic chain factors to a standard patellar tendon progressive loading program for the rehabilitation of athletes with patellar tendinopathy. The results of this study will expand the evidence base on different exercise programs for patellar tendinopathy, and may aid clinicians in choosing the most appropriate program for the treatment of athletes with this condition.

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DATA AVAILABILITY

Data are available under reasonable request to the corresponding author.

CONTRIBUTIONS

EHDA, NR, RSS: the initial draft manuscript writing. All authors contributed to and revised subsequent drafts and approved the final version.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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Patients' Perception of Technology: an Update of Patients' Understanding of Robotics and Navigation in Total Joint Arthroplasty

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SUMMARY

Background. Given the increasing use of RN in orthopaedics, our aim is to evaluate TJA patients' understanding of RN technology.

Methods. A survey based cross sectional study was conducted in one joint replacement clinic from January- March 2021. Questions pertaining to demographic information, robotics and navigation prevalence, autonomy, benefits, drawbacks, costs, and patient experiences and attitudes were asked.

Results. Ninety-seven completed questionnaires were included in our results. 70.1% of patients knew that some orthopaedic surgeons use robotics and navigation systems in surgery. 71.1% had not undergone or did not know a first degree relative who had undergone an orthopaedic surgery performed with RN assistance. 86.6% of patients thought that >10% of operations were performed using a RN system. 58.7%, 49.4% and 49.4% of the patients believed that RN makes surgeries more accurate, easier, and quicker, respectively. 73.2% believed that robotic surgery would increase the price of their surgery. 46.3% answered that RN costs > $ 100,000. 55.7% of the patients responded that they would be willing to pay extra for a RN assisted surgery. 39.2% of patients were unsure of whether they would like to have RN surgery.

Conclusions. Patients' understanding of robotic and navigation in arthroplasty seems to be limited and inconsistent. With increased direct-to-consumer marketing of these technologies, it is critical for physicians and healthcare systems to promote balanced and complete information.

KEY WORDS

Arthroplasty; joint replacement; robotics; navigation; technology.

INTRODUCTION

Total Joint Arthroplasty (TJA) is amongst the most frequent and most successful orthopaedic surgeries performed (1) With increasing average age, prevalence of obesity and improved patient reported outcomes (PRO), the number of TJA is steadily increasing (1). Many studies have assessed patient satisfaction and understanding of total joint replacement (2-7). The literature demonstrates that patient expectations are a strong contributor to their overall satisfaction (8). Since the advent of robotic and navigation (RN) assisted surgery in orthopaedics, RN has demonstrated promising joint replacement outcomes (9, 10). RN are used to help with presurgical planning, aid in precision of cuts and mechanical axis alignment (9). The technology's use in arthroplasty is rising steadily and is becoming a target for healthcare marketing (11). With its increasing use and publicity, it is important to evaluate patients' understanding of RN and how its use impacts their expectations of surgical outcomes. Though

research is revealing the utility of the technology in arthroplasty, little is known about how much orthopaedic patients themselves currently understand about it (12). Given the increasing use of RN in our institutions and around the world, it is necessary to evaluate patients' understanding of these evolving technologies. We hypothesize that patients have a poor understanding of the cost and role of these systems, and that this may be impacted by patient age, education level, and race.

METHODS

Study protocol

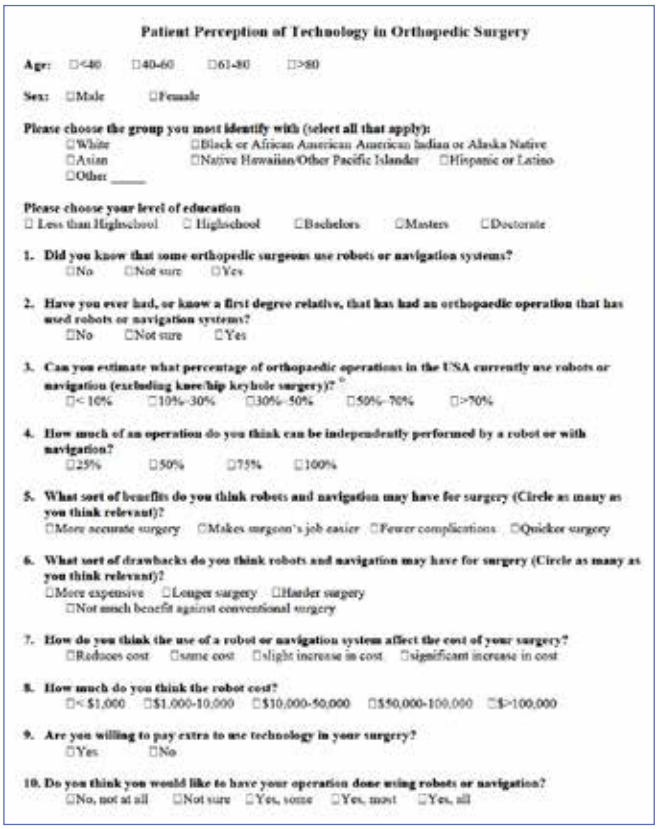
The current study was conducted in accordance with the Declaration of Helsinki and institutional ethical standards. Institutional Review Board Policy and Procedure Committee (IRB) approval was obtained from the University of Miami Hospital (20210388 - Date of approval: September 2020). The study is an observational cross-sectional study and was completed in accordance with STROBE guidelines. Data was prospectively collected through administration of a survey-based questionnaire. All collected information was deidentified. Patient consent for participation in the current study was obtained verbally prior to the administration of the questionnaire as required by our institutions IRB.

Setting and eligibility criteria

Survey based questionnaires were administered in one adult reconstruction clinic at the University of Miami Hospital between January and March 2021. Patients were included if they were over the age of 18, had the capacity to consent, and were interested in or had undergone an elective joint replacement surgery. Patients were excluded if they did not meet inclusion criteria. All incomplete surveys were excluded from the final results.

Questionnaire

97 paper-based questionnaires were distributed to the patients of one adult reconstruction clinic. The survey was reviewed and approved by our institution's IRB. Prior to an appointment with their joint replacement surgeon, research fellow SH approached patients to determine eligibility and willingness to partake in the current study. Verbal consent was acquired if inclusion criteria was met, and patients were given time to complete the survey independently. The survey administrator was available to answer questions and address concerns. Surveys were available in both English and Spanish. No identifying information was recorded. Questions regarding demographic information, robotics and navigation prevalence, autonomy, benefits, drawbacks, costs, and patient experiences and attitudes were included (**figure 1**). Participants had the option to include more than one race in their demographic responses.



Patient Perception of Technology in Orthopedic Surgery

Age: ☐<40 ☐40-60 ☐61-80 ☐>80

Sex: ☐Male ☐Female

Please choose the group you most identify with (select all that apply):
☐White ☐Black or African American ☐American Indian or Alaska Native
☐Asian ☐Native Hawaiian/Other Pacific Islander ☐Hispanic or Latino
☐Other _____

Please choose your level of education
☐ Less than Highschool ☐ Highschool ☐Bachelors ☐Masters ☐Doctorate

1. **Did you know that some orthopedic surgeons use robots or navigation systems?**
☐No ☐Not sure ☐Yes
2. **Have you ever had, or know a first degree relative, that has had an orthopaedic operation that has used robots or navigation systems?**
☐No ☐Not sure ☐Yes
3. **Can you estimate what percentage of orthopaedic operations in the USA currently use robots or navigation (excluding knee/hip keyhole surgery)?**
☐< 10% ☐10%-30% ☐30%-50% ☐50%-70% ☐>70%
4. **How much of an operation do you think can be independently performed by a robot or with navigation?**
☐25% ☐50% ☐75% ☐100%
5. **What sort of benefits do you think robots and navigation may have for surgery (Circle as many as you think relevant)?**
☐More accurate surgery ☐Makes surgeon's job easier ☐Fewer complications ☐Quicker surgery
6. **What sort of drawbacks do you think robots and navigation may have for surgery (Circle as many as you think relevant)?**
☐More expensive ☐Longer surgery ☐Harder surgery
☐Not much benefit against conventional surgery
7. **How do you think the use of a robot or navigation system affect the cost of your surgery?**
☐Reduces cost ☐same cost ☐slight increase in cost ☐significant increase in cost
8. **How much do you think the robot cost?**
☐< $1,000 ☐$1,000-10,000 ☐$10,000-50,000 ☐$50,000-100,000 ☐$>100,000
9. **Are you willing to pay extra to use technology in your surgery?**
☐Yes ☐No
10. **Do you think you would like to have your operation done using robots or navigation?**
☐No, not at all ☐Not sure ☐Yes, some ☐Yes, most ☐Yes, all

Figure 1. Questionnaire on Robotics and Navigation in Orthopedic Surgery.

Outcomes of interest

Our aim was to obtain roughly 100 survey responses from adult reconstruction patients with the goal of assessing patients' understanding of robotics and navigation technology. Secondarily, we sought to analyze how patient demographics, including age, sex, race, and education level impacted their answer choices.

Statistical analysis

Surveys were assessed for completeness. All completed responses were tabulated in Microsoft Excel 2021. Statistical analysis was conducted using SPSS (Version 27.0.1.0, 2020) by author SH at the University of Miami Hospital, Florida, USA. A Pearson's chi-squared analysis and Fisher's exact test were used to assess association between demographic sex, gender, race, and education and answer choices.

All data was assessed as categorical data. Multiple response questions 5 and 6 were broken down into yes/no responses for each answer choices and assessed categorically. Race was assessed categorically as yes/no for choices: White, Asian, Black, Hispanic, and Other. A P-value of 0.05 was used as a cut of for statistical significance.

RESULTS

Cohort

100 surveys were administered from January-March 2021. Three surveys were excluded due to incomplete responses, leaving 97 surveys included in our final analysis.

Patient demographics

The median age of respondents was between 61-80 years old (range < 40 - > 80). Fifty-four patients were female (55.7%). Patients' racial identifications included 51 White, 2 Asian, 23 Black, 0 Native Islander, 0 Native American, 29 Hispanic, and 2 others. Eight of the patients responded as bi-racial or multi-racial. Patients' highest education level included 1 less than high school, 41 completed high school, 30 completed a bachelors, 15 completed a masters, and 10 completed a PhD (**table I**).

Responses

Most patients (70.1%) knew that some orthopaedic surgeons use robotics and navigation systems in surgery (**figure 2**). Most (71.1%) had not undergone or did not know a first degree relative who had undergone an orthopaedic surgery performed with RN assistance (**figure 3**). 86.6% of patients thought that > 10% of operations were performed using a RN system; 4.1% indicating > 70% and 13.4% indicating < 10% (**figure 4**). 29.9%, 33.0%, 29.9%, 7.2% of patients believed that 25%, 50%, 75%, 100% of an operation could be independently performed by using RN, respectively. 58.7%, 49.4% and 49.4% of the patients believed that RN makes surgeries more accurate, easier, and quicker, respectively. A majority, 67.0%, believed RN makes arthroplasty more costly as a whole (**figure 5**) and 73.2% believed that robotic surgery would increase the price of their surgery; conversely, 26.8% believed that robotics would not change or reduce the price of their surgery (**figure 6**). 46.3% answered that RN costs > $ 100,000 (**figure 7**). 55.7% of the patients responded that they would be willing to pay extra for a RN assisted surgery. 39.2% of patients were unsure of whether they would like to have RN surgery, 13.4% did not want involvement of RN in their surgery, 9.3% wanted all of

Table I. Socio-demographic characteristics of the participants.

Characteristic	Frequency (n)	Percentage (%)
Total	97	100%
Gender		
Male	43	44.3%
Female	54	55.7%
Race		
White	51	52.5%
Asian	2	2.1%
Black	23	23.7%
Native Islander	0	0%
Native American	0	0%
Hispanic	29	29.9%
Other	2	2.1%
Bi- or Multi- Racial	8	8.2%
Education		
< High School	1	1.0%
High School	41	42.3%
Bachelors	30	30.9%
Masters	15	15.5%
PhD	10	9.7%

the surgery performed by RN, and 27.8% wanted some or most of the surgery to be performed with RN (**figure 8**).

Figure 2. Pie Chart of Question 1 Responses.

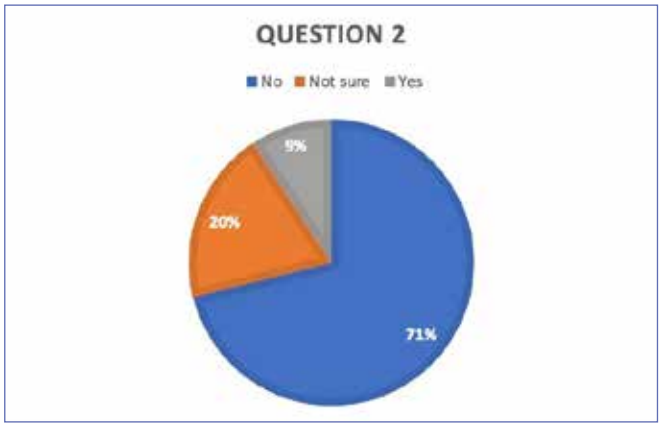


Figure 3. Pie Chart of Question 2 Responses.

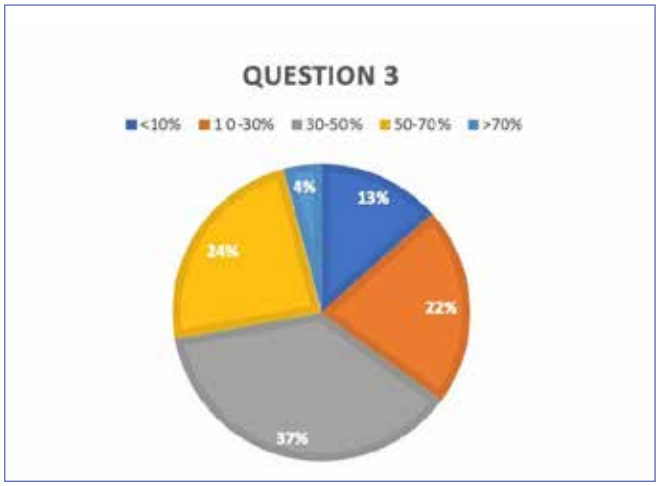


Figure 4. Pie Chart of Question 3 Responses.

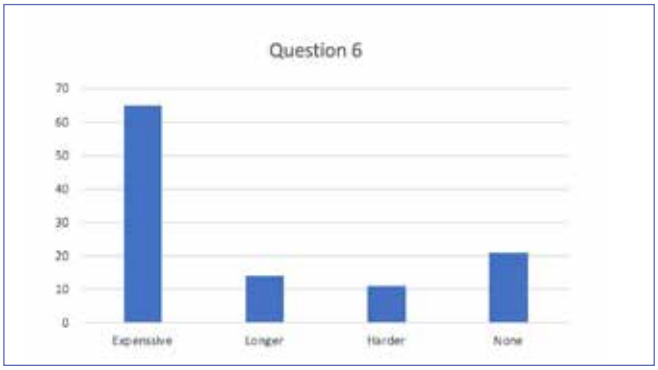


Figure 5. Bar Chart of Question 6 Responses.

Figure 6. Bar Chart of Question 7 Responses.

Figure 7. Pie Chart of Question 8 Responses.

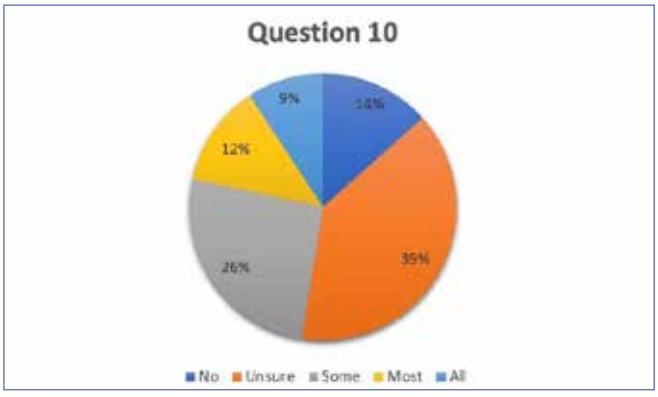


Figure 8. Pie Chart of Question 10 Responses.

Demographic impact on response choice

Age and sex were independently associated with all answer choices. Education was associated with answer choice for question 2 and 5a. Education was therefore associated with respondents undergoing RN surgery or knowledge of a first degree relative who has had RN assisted orthopaedic surgery ($p = 0.029$). Education was also associated with whether respondents believed RN assisted surgery made surgery more accurate or not ($p = 0.048$). Several racial demographics had associations with answer choices. White race was associated with questions 1, 9, 10. Therefore, Whites had an association with whether they knew some orthopaedic surgeons use RN assisted surgery or not ($p = 0.004$); they had an association with whether they would pay extra for robotic surgery or not ($p = 0.008$) and there was also an association with choice of level of RN involvement in their surgery ($p = 0.014$). Black race was associated with answer choices for questions 5a and 10. Therefore Blacks choice of whether RN was more accurate or not was not independent of race ($p = 0.007$); there was also an association with their choice of level of RN involvement in surgery ($p = 0.026$). Hispanic race was associated with questions 1 and 2. Therefore Hispanics choice of whether they knew that some orthopaedic surgeons use RN was not independent of their race ($p = 0.033$). Hispanics also had an association with whether they had or knew a first degree relative that has had an RN assisted orthopaedic surgery or not ($p = 0.032$).

DISCUSSION

Given the rise of RN in orthopaedics, our study adds to the literature by serving as an evaluation of patients understanding of the new technology. Our results demonstrate that most patients know of RN assisted surgery in orthopaedics, though there seems to be no consistent understanding of details on the subject. A majority either did not know, or were unsure if they knew, someone who had an orthopaedic robotic surgery in the past, though most believed RN surgery comprised > 10 % (median of 30-50%) of surgeries. Perceived level of autonomy and impact of the technology on surgical costs was likewise diverse amongst patients. Over 50% incorrectly believed RN systems cost < $ 100,000. Likewise, greater than 50% of patients were willing to pay extra for RN assisted surgery though 39.1% were unsure whether they would like a robotic surgery at all. When stratifying our results by age, gender, race, and education we found associations between education and race and several answer choices (**table II** and **table III**).

A similar study was conducted by Jassim Benjamin-Laing *et al.*'s 2014 study from the United Kingdom (12). Our total cohorts were comparable in size and results. Comparably, 9% of our cohort and 12% of theirs had or knew someone who had undergone a robotic surgery; 37% of our cohort believed that most or all (75% or 100%) of an operation could be performed by a RN system, similar to their cohort (33%). 87% of our cohort compared to 75% of their cohort believed > 10% of orthopaedic surgeries were performed using RN. Similarly, roughly 50% of both cohorts believed that RN made surgery more accurate, easier, or quicker compared to manual TJA. Roughly 70% of both cohorts also correctly believed RN to be more expensive than conventional surgery, and roughly 20% of both believed RN had no benefit compared to conventional surgery. Around 50% of both cohorts would have at least some involvements of RN in their surgery. 13% of ours compared to 20% of their cohort would like no involvement of RN in their TJA.

Abundant marketing campaigns for RN surgeries exist with advertising typically suggesting personalized, faster, less painful, and easier surgeries (13). Our cohorts' responses on benefits and drawbacks of RN surgery largely agreed with these claims. In studies on RN in TKA, THA and UKA, robotics have in fact largely demonstrated superior precision in metrics such as implant positioning, offset, leg length and mechanical access alignment (14). Most of the evidence for improved clinical outcomes, however, is supportive of UKA to a greater degree than THA or TKA. Studies assessing UKA specifically, demonstrate improved implant survivorship, reduced pain, function scores and time to discharge compared to conventional surgery (15, 16). Conversely, RN in THA and TKA has demonstrated some benefit in pain and functional outcomes, though systematic reviews and meta-analysis have yet to demonstrate superiority compared to conventional surgery (14, 17). Established drawback of RN include, increased surgical time, learning curve and increased costs (14). Time has been shown to increase up to 25 minutes per surgery adding up to $ 1625 (14). At baseline value RN systems cost $ 800,000 though many are closer to $ 1,000,000 and can add several thousand dollars to patient cost (14). The most agreed upon drawback in our cohort was expense, to which 54 patients responded that they would be willing to pay more for robotic surgery. It is unlikely that RN companies include the drawbacks and lack of documented superiority to patients, possibly contributing to this majority. A 2012 study investigating direct-to-consumer internet advertising in robotic prostatectomy found unbalanced and misleading information (18). They therefore suggested government and medical societies increase their efforts in promotion of balanced education to patients (18). A similar study from 2007 demonstrated that over 52% of orthopaedic patients experienced some sort of direct-to-consumer advertising, which was correlated with higher requests for specific surgery or implant type (19). This becoming increas-

Table II. Questions associated with education stratified.

Question 2 (p = .029)		**No**	**Unsure**	**Yes**
	< HS	100% (1/1)		
	HS	68.3% (28/41)	31.7% (13/41)	
	BA	66.7% (20/30)	10% (3/30)	23.3% (7/30)
	MS	86.7% (13/15)	20% (2/10)	10% (1/10)
	PhD	70% (7/10)	20% (2/10)	10% (1/10)
Question 5a (p = .048)		**Accurate**		
	< HS	0% (0/1)		
	HS	51.2% (21/41)		
	BA	60.0% (18/30)		
	MS	53.3% (8/15)		
	PhD	100% (10/10)		

Table III. Questions associated with race stratified.

White Question 1 (p = 0.004)	**No**	**Unsure**	**Yes**		
	17.6% (9/51)	0% (0/51)	72.5% (37/51)		
White Question 9 (p = 0.007)	**Yes**	**No**			
	68.6% (35/51)	31.4% (16/51)			
White Question 10 (p = 0.014)	**No**	**Unsure**	**Some**	**Most**	**All**
	5.9% (3/51)	31.4% (16/51)	35.3% (18/51)	13.7% (7/51)	13.7% (7/51)
Black Question 5a (p = .007)	**Accurate**				
	34.8% (8/23)				
Black Question 10 (p = .026)	**No**	**Unsure**	**Some**	**Most**	**All**
	26.1% (6/23)	52.2% (12/23)	4.3% (1/23)	13.0% (3/23)	4.3% (1/23)
Hispanic Question 1 (p = 0.033)	**No**	**Unsure**	**Yes**		
	37.9% (11/29)	10.3% (3/29)	51.7% (15/29)		
Hispanic Question 2 (p = 0.032)	**No**	**Unsure**	**Yes**		
	89.7% (26/29)	6.9% (2/29)	3.4% (1/29)		

ingly relevant as the spread of fragmented and misinformation only continues to grow with the ubiquity of handheld smartphones and increased access to social media application.

Patients are left with decisions on how they would like their arthroplasty completed, though it appears many have fragmented knowledge on the subject. Though many options exist, RN is not as ubiquitous as patients might perceive. In our cohort, for example, most patients knew RN was performed by some orthopaedic surgeons, though a greater majority did not know or were unsure of someone who was operated with RN assistance. Two recently published studies on trends of robotic surgery indicated that surgeries performed using this relatively new technology number less than 10%. Using the National Inpatient Sample Database, Antonios and Korber *et al*. demonstrated that RA surgeries in TKA grew from 0.1% to 0.8% of all from 2009-2013 and that computer navigation use grew from 1.2% to 6.3% (11). Similarly, Boylan and Suchman *et al.* used the Statewide Planning and Research Cooperative System and demonstrated that technology assistance was used in 5.1% of all total hip, knee or UKA surgeries between 2008-2015 with a steady increase each year (20). RN was also more likely to be used in knee *versus* hip arthroplasty, high volume *versus* low volume hospitals, and on private insurance patients *versus* Medicaid and Medicare patients (20). This data represented metrics of 5 or more years ago and based on our search through online journals like PubMed and readily accessible search engines like Google, no recent data from reputable sources exists. Perhaps the lack of accessible information contributes to some degree of our patients markedly incorrect assessment of RN prevalence (most believing RN is involved in > 30% of surgeries).

Robot autonomy categories include passive, semi-autonomous (haptic), and autonomous. Passive indicates the machine completes a portion of the surgery under directs control of the surgeon; semi-autonomous require surgeon feedback to complete a task; autonomous can complete tasks independently of surgeon feedback. Earlier autonomous systems such as CASPER and ROBODOC showed potential in improving implant alignment and patient satisfaction though they also were found to have higher complication rate, surgical time and aborted surgeries (21). Passive systems like the Acrobat have demonstrated some utility in positioning TKA prosthesis, but under full control of the surgeon (10). More commonly used haptic systems, such as the Mako, couple preoperative planning with intraoperative adjustments. Mako has growing evidence of effectiveness in reducing intraoperative complications, improving patient activity and satisfaction scores, and placing implants with greater precision than manual total joint arthroplasty (22). The variety of RN systems available may have led to our cohort's variable estimates of independence.

Our study is not without limitation. Firstly, we administered our survey to one clinic's patients. Therefore, our results are not likely generalizable to the entire country but may reflect patients of the central Miami and surrounding areas. Our cohort did however include a group of patients diverse in age, education level, and race. Additionally, our sample size was relatively small, consisting of 97 patients. Our study was also not extensively piloted prior to administration, though a similar study has been conducted on 98 patients in the UK (12). The survey was also only conducted once per patient. Therefore, we were unable to assess if exposure to educational material would improve participants' understanding of the technology. Lastly, robotics and navigation were included as one entity in all questions, so to not confuse patients.

Further studies should investigate patients' understanding of RN in orthopaedics using a larger cohort, across multiple institutions. Researchers should also aim to assess the effect of unbiased educational material on participants' understanding of robotics and navigation. Furthermore, there is a need for more updated studies on the trends in utilization and prevalence of robotics and navigation in joint replacement.

CONCLUSIONS

Patients understanding of robotic and navigation in arthroplasty seems to be limited and inconsistent. Our cohort responded similarly to Jassim and Benjamin-Laing *et al*'s cohort from 2011, despite a decade time gap (12). Racial and education level's influence on answer choices were present. With increased direct-to-consumer marketing of these technologies, it is critical for patient satisfaction met their expectations, physicians and healthcare systems needs to promote balanced and complete information as part of patient's education.

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DATA AVAILABILITY

Data are available under reasonable request to the corresponding author.

CONTRIBUTIONS

All authors contributed equally to this work.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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A Discussion on The Utility of Discharge Location Prediction Models for Total Joint Arthroplasty Surgery

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SUMMARY

Objective. Total Joint Arthroplasty (TJA) remains one of the highest frequency elective surgical procedures. Medicare is expected to spend close to $ 50 Billion on TJAs by 2030. Predicting discharge location could allow for cost mitigation and the ability to set appropriate expectations for patients preoperatively. Our aim is to determine the validity of one predictive model. We hypothesize that this tool will demonstrate comparable predictive value as in the pilot study.

Methods. We conducted a cross-sectional study of unilateral, primary, total joint replacements from January 2020 through February 2021. Nine variables were input into a predictive model at https://dukeriskcalculators.shinyapps.io/Dispo/ and percent likelihood of discharge to SNF/rehabilitation facility was recorded and analyzed. Receiver operating characteristics curve (ROC) analysis was utilized to evaluate the model's predictive capability.

Results. Our cohort consisted of 264 patients. 9.1% of patients were discharged to an SNF/rehabilitation facility. ROC analysis demonstrated an area under the curve (AUC) of 0.72 indicating good predictive value. The mean percent likelihood of discharge to SNF/rehab was 31% ± 9% (mean ± 95% confidence interval) for patients whose final discharge location was an acute rehabilitation facility; The mean percent likelihood of discharge to SNF/rehab was 15% ± 2% for patients who were discharged home.

Conclusions. The predictive model analyzed uses nine easily accessible variables and demonstrates good predictive capability. Discharge to acute rehabilitation is a costly and often unnecessary intervention. Predictive models can reduce discharge to these facilities, reduce healthcare costs, and improve patient outcomes.

KEY WORDS

Joint replacement; arthroplasty; acute rehabilitation; skilled nursing facility; discharge location.

INTRODUCTION

Total Joint Arthroplasty (TJA) remains one of the highest frequency elective procedures in United States with estimates of over one-million joint replacement surgeries to be performed annually by 2030 (1). In 2014, total annual costs related to TJAs approached $7 billion (2), and Medicare is expected to spend closer to $ 50 Billion on TJAs by 2030 (3). To mitigate costs and improve quality, alternative payment models such as the Bundled Payments for Care Improvement Initiative (BPCI) and the Comprehensive Care for Joint Replacement (CJR) were established by the Centers for Medicare and Medicaid (CMS), the major contributor

to TJA payments (4). Under these models, providers are either rewarded or penalized if costs of the index procedure through postoperative care deviate from a predetermined value (2). Promising results have led Medicare to adopt this relatively new payment method in place of the fee-for-service model (5). Evidence demonstrates cost, lengths of stay, and readmission rate improvements for TJA patients (5, 6). Moreover, patient outcomes are not negatively impacted by resource consumption reductions (7).

Our group has previously demonstrated that post-discharge costs are significantly higher for patients discharged to an acute rehabilitation center compared to those discharged home following TJA; yet, both cohorts have comparable short-term outcomes (8). Others show that skilled nursing and rehabilitation facilities (SNF/rehab) discharge is associated with significant complications and morbidity (9). Bozic and Ward *et al*. estimate that post discharge care can cost over one third of total costs in TJA (10). Likewise, Slover and Mullaly *et al*. demonstrate that extended acute hospital care is financially preferable to acute care discharge with no negative ramifications (11). Such evidence has led to successful reduction of SNF/rehab discharge, some demonstrating this to be the greatest single contributor to recent TJA cost reduction (12, 13). In attempt to further reduce discharge to acute rehabilitation facilities, several publications have proposed discharge location predication models (14, 15). Validation of these models are necessary because they allow providers to determine at-risk patients, direct their discharge location, and adjust their preoperative expectations (14). Ideally, modifiable risk factors can be addressed to improve their outcomes and lower total cost.

Goltz and Ryan *et al*. recently developed a predictive model which they describe as both convenient and highly accurate (16). Our aim is to determine the validity of this tool. We intend to determine if the tool is as predictive as in the initial study. We hypothesize that this tool will demonstrate comparable predictive value.

METHODS

Study protocol

The current study was conducted in accordance with the Declaration of Helsinki and institutional ethical standards. Institutional Review Board Policy and Procedure Committee (IRB) approval was obtained from the University of Miami Hospital (20210388 - Date of approval: May 2021). The study is an observational cross-sectional study and was completed in accordance with STROBE guidelines. Data was collected through a retrospective chart review. All collected information was deidentified. Patient consent was not required by our institutions IRB.

Setting and eligibility criteria

University of Miami Hospital electronic medical records were reviewed for information on TJA patients between January 2020 and February 2021. Patients were included if they were over the age of 18 and had undergone a primary, unilateral total joint replacement. Patients were excluded if their index procedure involved revision, bilateral, emergent, oncological, and partial joint replacement surgery. The final cohort of 264 patients included all patients meeting inclusion criteria with available data in the specified timeframe.

Data collection

Nine preoperative data points were collected through retrospective chart review. These included age, marital status, American Society of Anesthesiologists (ASA) score, body mass index (BMI), gender, neurologic disease status, electrolyte disorder status, paralysis status, and pulmonary circulation disease status. All quantitative variables were defined using the Elixhauser Comorbidity Index.

Each patient's data was entered into a discharge location prediction model at the following website https://dukeriskcalculators.shinyapps.io/Dispo/. Using these nine variables, the model determined a percent likelihood of discharge to an SNF/rehab facility with a 95% confidence interval. Patient's medical records were then reviewed for discharge location following TJA (home or acute rehabilitation). Variables of interest included predicted likelihood of discharge to acute rehabilitation and true discharge location. These data points were recorded and compared as described in the statistical analysis section.

Statistical analysis

Data was summarized using descriptive statistics for continuous and categorical variables. Inpatients were defined as a patient admitted to our orthopaedic unit from the Post-Anesthesia Care Unit (PACU).

Statistical analysis was completed in SPSS (27.0.1.0, 2020). A P-value of < 0.05 was used as a cut off for statistical significance.

The predictive accuracy of the model was assessed using a receiver operating characteristic curve (ROC) analysis. A ROC curve was created using predicted likelihood of discharge to acute rehabilitation and true discharge location. The final outcome measure of predictive accuracy was the area under the curve. An area under the curve of 0.7 or greater definied good predictive value (16). Data was also demonstrated through the use of a scatter plot.

RESULTS

Patient demographics

264 patients met inclusion criteria and were included in the final cohort. Patient demographics are presented in **table I**. Inpatients demographics are described in **table II**.

Results syntheses

ROC curve analysis demonstrated an AUC of 0.72 ($p < 0.05$), thus indicating good predictive value of the model (**figure 1**). Using Youden's method, the optimal cutoff off for sensitivity and specificity occurred at 10.9% (0.79 and 0.60, respectively). Stratified analysis for inpatients demonstrated an AUC of 0.75 ($p < 0.05$), indicating good predictive value.

The mean percent likelihood of discharge to SNF/rehab was 31% ± 9% (mean ± 95% confidence interval) for patients whose true discharge location was an acute rehabilitation facility; The mean percent likelihood of discharge to SNF/rehab was 15% ± 2% for patients who were discharged home (**figure 2**).

Table I. Descriptive summary of entire cohort.

Variable of Interest	N	Prevalence or mean
Cohort	264	
Discharged to SNF/rehab	24	9.1%
Inpatient	90	34.10%
Hips	135	51.10%
Gender (Female)	156	59.1%
Age		63.80
BMI		30.10
ASA (% 1/2/3/4)	(1/133/128/2)	2.49
Marital Status (single/divorced/widowed)	132	50.0%
Elixhauser Comorbidity Index		
other neurological disorders	11	4.2%
electrolyte disorder	1	0.4%
paralysis	2	0.8%
pulmonary circulation disease	0	0.0%

Table II. Descriptive summary of inpatients.

Variable of Interest	N	Prevalence or mean
Inpatients	90	
Discharged to SNF/rehab	16	17.8%
Hips	58	64.4%
Gender (Female)	53	58.9%
Age		68.00
BMI		29.70
ASA (% 1/2/3/4)	(0/40/49/1)	2.47
Marital Status (single/divorced/widowed)	43	47.8%
Elixhauser Comorbidity Index		
other neurological disorders	9	10.0%
electrolyte disorder	0	0.0%
paralysis	2	2.2%
pulmonary circulation disease	0	0.0%

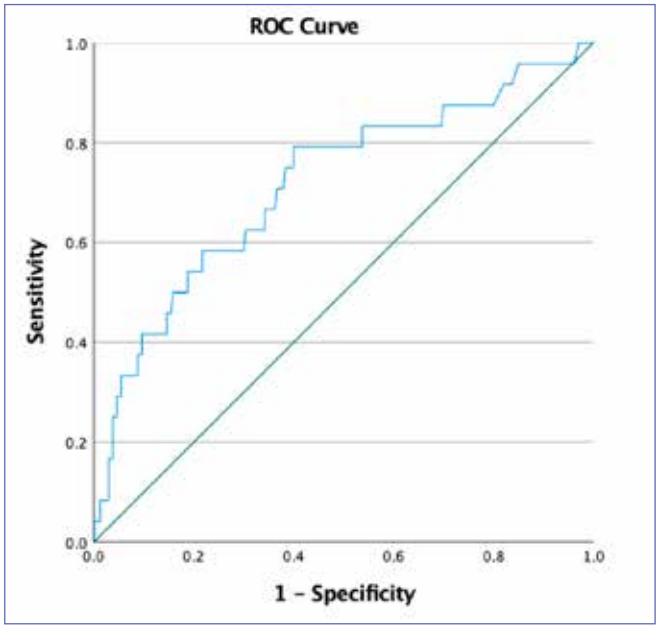

Figure 1. Entire cohort receiver operating characteristic curve analysis of Goltz and Ryan *et al.*'s calculator.

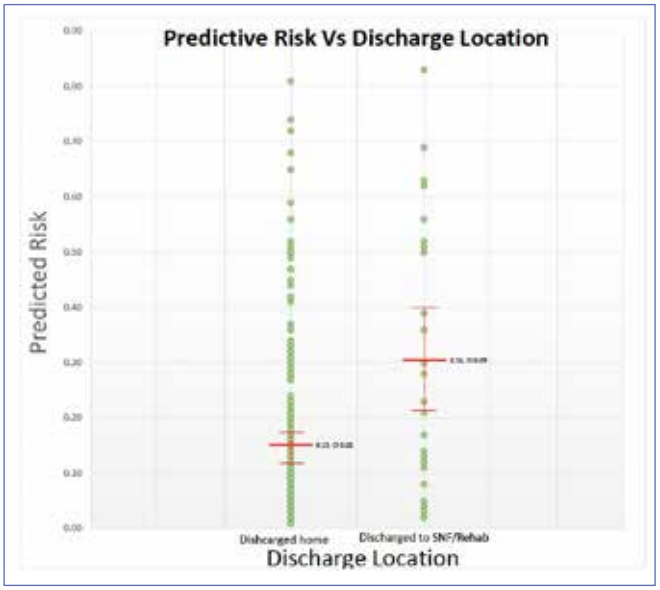

Figure 2. Scatter Plot of predicted risk *vs* discharge location.

DISCUSSION

Arthritis is one of the most common debilitating conditions in the United States (17). Joint replacement surgery is a cost-effective intervention for the disease, which restores patient function and quality of life (18). Unfortunately, patients often elect for surgical intervention late in the course of their disease, therefore mitigating the treatment's full potential (19). Delay is associated with poor baseline pain and function (19). These patients require extensive postoperative physical therapy and rehabilitation (19). Though most achieve adequate function, some will never achieve the maximal benefits of surgery. As a result, orthopaedic surgeons aim to optimize modifiable factors to ensure adequate outcomes.

Advances in surgical muscle sparing techniques and rapid recovery protocols have increased the effectiveness of arthroplasty surgeries (20, 21). Nowadays, patients can be discharged the same day as their surgery (22). Despite this progress, many patients have comorbidities, social issues, and medical conditions which require long inpatient stays and discharge to acute rehabilitation facilities (23). For this population, there has been an increased emphasis on advanced preoperative planning pathways and risk stratification (24, 25).

Predictive models are useful to practitioners in facilitating optimal patient recovery and in reducing care costs (26). For example, the well-studied RAPT score has demonstrated an ability to reduce hospital lengths of stay (27). Literature shows that patients' discharge preference has the strongest impact on discharge location and that lack of education influences these preferences (28). Preoperative risk assessment may allow surgeons to better counsel their patients regarding recovery expectation.

A recent model published in 2021 by Goltz and Ryan *et al.* sought to predict post-operative discharge location following TJA. Their model uses nine easily accessible variables and has demonstrated high predictive utility (16). Unlike other models, this model emphasizes objective preoperative variables to ensure its applicability in preoperative planning (16). Conversely, the 6-Clicks score requires a postoperative physical therapy assessment and the RAPT score entails collection of challenging variables (27, 29). Other models have proven less predictive under similar statistical analyses (14, 30). Though Goltz and Ryan *et al.*'s calculator seems promising, it has yet to be validated by an outside source. Our article adds to the literature by evaluating this recently published model for predictive accuracy and discussing its application into current practice.

Our data demonstrates that the predictive model developed by Goltz and Ryan *et al.* has "good" predictive value (AUC 0.72) (**figure 1**) (16). This finding indicates the model has enough predictive capability to be used in practice. Though encouraging, our findings are less impressive than in the pilot paper (16). The originally published paper found an AUC of 0.82 following the same methodology, thus indicating excellent predictive capability (16). However, given the simplicity of the calculator and easy accessibility of the data points, "good" may be sufficient from an effort *vs* benefit perspective. Furthermore, we have demonstrated promis-

ing results in an alternative manner. We found that patients who were discharged to an acute rehabilitation facility had a mean predicted percent of discharge to SNF/rehab of 31%, more than double when compared to patients who were discharged home (15%) (**figure 2**); the large dichotomy between the two groups supports this model's use in clinical practice. Upon subgroup ROC analysis, the model demonstrated better predictive capability for inpatients than for the complete cohort (AUC 0.75 *vs* AUC 0.72, respectively). Thus, practitioners may benefit from the model's use both preoperatively, as intended, and postoperatively when counselling patients on their discharge course. The model may also be beneficial in reducing global healthcare costs.

Given the growing financial burden associated with TJA, it is imperative to minimize costs without compromising patient care (1–3). In recent years, medical care has begun shifting from a fee-for-service model to alternative payment services (31). The initiative has demonstrated effective cost control and improved surgical outcomes (5, 6, 32–34). Under new payment plans, expensive discharge to post-acute care has been minimized without negatively affecting patient outcomes (10, 11). Factors, such as age, ASA score, and the presence of a Medicare Major Complications/Comorbid Conditions (MCC) modifier are valuable predictors of cost. Some have suggested that bundled payment compensation should be adjusted upwards for high-risk individuals, though these implementations have yet to be incorporated (35). Predictive models which include such factors can help healthcare providers to optimize patients pre- and post-operatively to ultimately improve their chances of a successful and cost-effective recovery (36). Goltz and Ryan *et al.*'s model may provide the greatest benefit for the effort required to implement it (14, 15, 29, 36). Overall, we found this discharge calculator to have good predictive value in determining discharge location. While the calculator did not produce "excellent" predictive utility as in the pilot paper, this may be due to limitation within our study.

Notable limitations include our cohort's relatively small size of 264 patients. Using a larger population, such as that in Goltz and Ryan *et al.* (10, 15), could have produced stronger results (16). Additionally, we analyzed patients from January 2020-February 2021 during the COVID-19 pandemic, which may have contributed to a selection bias. During the early stages of the pandemic, many elective procedures were canceled, and orthopaedic surgeons transitioned to outpatient TJAs. Per International Census Group and Research Committee of the American Association of Hip and Knee Surgeons guidelines, it was recommended that elective surgeries be deferred for patients with significant comorbidities (37). Predictive models have been determined to be less useful for intermediate risk patients, which may have contributed to our lower AUC compared to the initial study. Our institution also had a relatively small number of patients discharged to SNF/rehab (9.1%) compared to Goltz and Ryan *et al.*'s 17.6%. This may indicate a positive trend of our institutions reduced discharge to acute rehabilitation brought on by the pandemic. Our study was completed retrospectively and in one facility, which decreases the generalizability of our findings. Lastly, with a retrospective chart review study there is an inability to determine the temporal relationship of data points. For example, a patient with a documented neurological disease may have been diagnosed after their TJA therefore inflating the predicted percent of discharge to an acute rehabilitation facility. However, this is unlikely as the data collection was completed within a few months of all index procedures. Future validation studies using a prospective cohort may result in more accurate findings.

Goltz and Ryan *et al.*'s model may provide an accurate way for orthopaedic surgeons to determine discharge location preoperatively. The models use in current practice could allow for cost mitigations associated with reduced discharge to acute rehabilitation as has been demonstrated in the literature. Additionally, patients would benefit from improved post-surgical outcomes. To fully validate this model, we recommend a prospective cohort be used; this would determine if the model is truly useful in real time.

CONCLUSIONS

In summary, our data showed Goltz and Ryan *et al.*'s predictive model has good predictive value (AUC 0.72). Our findings indicate better utility for inpatients (AUC 0.75). Given the model's easy use, predictive value, and potential for reducing costs and managing patient expectations, we advocate for its judicious implementation into current practice.

FUNDINGS

None.

DATA AVAILABILITY

Data are available under reasonable request to the corresponding author.

CONTRIBUTIONS

All authors contributed equally to this work.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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Even Patients with Very Chronic Symptoms of Greater Trochanteric Pain Syndrome (GTPS) may Report Improvements Following Radial Extracorporeal Shockwave Therapy (rESWT), but no Single Baseline Factor Predicts Response

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SUMMARY

Objective. Do any measures at baseline predict response from radial Extracorporeal Shockwave Therapy (rESWT) for patients with GTPS?

Methods. Setting: single UK NHS Sports Medicine Clinic. Patients: 260 patients following rESWT for GTPS. Mean age 60.0 ± 11.9 years, 81% female, mean duration of symptoms 44.5 ± 44.7 months (range: 3 months-20years). Interventions: participants received three sessions of rESWT plus structured home exercise programme (flexibility, strength, and balance). Main outcome measures: follow-up was 3-months, and 6-months. Outcome measures of self-reported "average pain", "worst pain", and VISA-G score. Baseline PROMS (Non-Arthritic Hip score, and Oxford Hip Score), pain (painDETECT, S-LANSS, CSI), "ability" (ODI, MSK-HQ), mood (HADS).

Results. Improvement in "average pain" of 30% at 3-months, and 37% at 6-months. VISA-G improved by more than 10% points at 3-months and 6-months. Several weak or very-weak correlations were identified, but no single baseline variable corelated strongly to the improvements seen at follow-up time-points.

Conclusions. There were clinical and statistically-significant improvements seen following rESWT for patients with GTPS, even in those with very long duration of symptoms, irrespective of age or symptom duration. There was a statistically significant difference for gender (greater benefit in female patients) which did not reach clinical significance. Greatest improvements in self-reported pain were seen in those with the worst baseline symptoms, particularly with variables more sensitive to non-arthritic hip pain (NAHS, VISA-G) than "arthritic pain" measures (OHS). Baseline measures such as Oswestry Disability Inventory or the MSK-HQ have weak correlations to improvements in some factors seen, with fewer correlations seen for markers of chronic, neuropathic, or centralised pain, or mood.

KEY WORDS

Extracorporeal shockwave therapy; tendons; outcome assessment; Greater Trochanteric Pain Syndrome

INTRODUCTION

Greater Trochanteric Pain Syndrome is a common cause of lateral hip pain, has an incidence of 1.8/1000 patients per year in primary care, affecting women more than men, most typically in their 50's and 60's (1-4). "Greater trochanteric pain syndrome" has held various names over the last few decades, indicating the ongoing confusion as to the pathological processes involved; for the purposes of this article

the phrase "greater trochanteric pain syndrome" (abbreviated to "GTPS") will be used, although the limitations of this terminology are recognised (3, 5, 6).

GTPS describes an area of reproducible lateral hip pain over the area of the greater trochanter, typically spreading to the buttock, upper lateral thigh, or occasionally further, which can overlap with symptoms from other conditions including hip joint, spinal, or radicular pathologies (3, 7). GTPS is more common in patients with pre-existing osteoarthritis of the knee or low back pain, with conflicting evidence as to whether it may be more common in overweight or obese patients (3, 8). Examination typically reveals maximal tenderness in the posterolateral area of the greater trochanter, but the majority of clinical tests have limited sensitivity and poorly differentiate GTPS from other causes of lateral hip pain (9, 10). Investigations can be useful in aiding diagnoisis, and 88% of patients with GTPS symptoms have evidence of gluteus tendinopathy on MRI compared to 50% of those with hip pain but without specific greater trochanteric symptoms (5, 11). However as gluteal tendinopathy can be often reported on MRI in those without specific GTPS-symptoms, rather than a diagnostic tool, investigations may be more useful in ruling out other conditions such as osteoarthritis of the hip, or tears of the gluteal tendons (11).

Radial extracorporeal shockwave therapy (rESWT), involves the use of inaudible high-energy sound waves which are generated externally to the body and spread aspherically into soft-tissue as a pressure wave, losing power as they pass deeper into tissue, but potentially covering a broad area (12). rESWT is most typically performed over three sessions, at weekly intervals, in order to promote a healing response alongside a structured rehabilitation programme (13). However the treatment numbers vary between studies with ongoing uncertainty as to "optimal treatment" (14), with evidence from a case-control study suggesting benefits of a single treatment session of rESWT in patients with GTPS (15), and another case series that used between one and eleven sessions (mean 5.6 sessions) of rESWT (16). Recent research however has questioned the protocol typically recommended, with equal benefit demonstrated in patients following 3 sessions of "maximum tolerated" *versus* "minimal dose" rESWT in a double-blinded RCT (17).

Similar to other treatments and conditions, there appears to be great heterogeneity in response to rESWT for patients with GTPS. Benefits are typically seen in most, but not all, patients but this can take 3-months to 6-months for clinically appreciated benefits to occur (17, 18). To date there is no research looking to identify any specific factors at baseline which may be of value in predicting response to treatment and which may guide individualised pathways of care. This research seeks to identify if any self-reported measures at baseline may have value in predicting response to rESWT in patients with chronic GTPS.

MATERIALS AND METHODS

Procedure logs were examined from a single UK hospital outpatient clinic, which has a regional reputation for the management of patients with chronic tendinopathy. A total of 260 patients who had previously been treated with rESWT for symptoms of Greater Trochanteric Pain Syndrome (GTPS) were identified from procedure logs.

Baseline and outcome measurements

Baseline assessments were conducted prior to the rESWT procedure. There is no single "gold standard" for assessing patients with GTPS, therefore a 0-10 numerical rating scale of self-reported "average pain" and self-reported "worst pain" were used initially, alongside validated PROMS: the Oxford Hip Score (OHS) (19, 20), and the Non-Arthritic Hip Score (NAHS) (21, 22). Additional hip-specific measure of function was undertaken with participants partway through the longitudinal case series with the Victorian Institute of Sport Assessment - Gluteal Tendon questionnaire (VISA-G) (23) and the Musculoskeletal Health Questionnaire (MKS-HQ) (24) for global musculoskeletal functioning. These measures were recorded contemporaneously at baseline before rESWT treatment, and also at 3-months and 6-months after rESWT treatment.

Additional measurements were taken across a broad range of aspects of function to assess baseline function, with subsequent analysis undertaken to assess if measurements at baseline predicted subsequent outcome. These included validated measures of neuropathic pain or central sensitisation: painDETECT (25, 26), and Leeds Assessment of Neuropathic Symptoms and Signs (S-LANSS) (27, 28), or Central Sensitisation Inventory (CSI) (29, 30) respectively. Assessments of anxiety and depression symptoms were assessed through the Hospital Anxiety and Depression Scale (HADS) (31, 32) plus PHQ-4, GAD-7 & PHQ-9 (33-35). Markers of chronic pain were assessed via the Pain Catastrophising Scale (PCS) (36) and the Psychological Inflexibility in Pain Scale (PIPS) (37). Additional measures of global function and ability were assessed via EuroQol 5Dimensions (EQ-5D) (38, 39) and the Oswestry Disability Index (ODI) (40). These combined questionnaires took an average of 30 minutes to complete by the patient prior to treatment.

Interventions

Patients received three rESWT treatment sessions performed at weekly intervals of rESWT from a "Intelect" RPW ESWT machine (DJO Global Chattanooga), with

energy level controlled by the operator to the "maximum comfortably tolerated dose" for the individual patient in line with manufacturer instructions. The treatment protocol was for rESWT delivered at a frequency of 20 Hz with 2000 shocks per treatment session. The mean value of the pressure waves generated was 2.3 ± 0.2, 2.9 ± 0.3, and 3.4 ± 0.4 bar for the 1st, 2nd, and 3rd treatment sessions respectively.
In addition to the rESWT, all patients were given a structure home exercise programme and taught how to progress this programme alongside the rESWT treatment. This programme included components of mobilisation, stretching, balance and strengthening exercises and is documented in previous work (17). Patients were encouraged to start the HEP prior to rESWT and to continue this throughout the period of follow-up, progressing this as their symptoms allowed. They were all given written material to support their compliance with the HEP.

Follow-up

Patients were reviewed in routine clinics at three-months and at six-months following the final rESWT treatment procedure. Follow-up data for outcome measurements was recorded at the follow-up time-points, the patients' general progress was assessed, their compliance and ability with the home exercise programme was reviewed and further instruction given if appropriate.

Ethics statements

This specific project does not fulfil the criteria of research as stipulated by HRA, therefore formal NHS ethics approvals were not required for this project. This specific project, which examines data at baseline and compares these to outcomes seen following treatment is a part of a wider ongoing body of work examining different aspects of chronic tendinopathy which is fully registered with the hospital Trust and relevant authorities.

Statistical analysis

Anonymised data from the procedural logs were inputted into a bespoke Excel spreadsheet (MS Excel for Mac – current version 16.50) by the author. All data was anonymised prior to analysis and held/used in accordance with hospital procedures. From this, group values (including means, standard deviations, and ranges) were calculated for the patient group as a whole, and for different sub-groups. Most data collected were scale data, with some data recorded as either ordinal or nominal categories. Data were analysed with SPSS (v27) and the Shapiro-Wilk test was performed to assess normality. Most data were not normally distributed, therefore non-parametric testing was used throughout, typically Wilcoxon Signed-Rank or Chi-Square testing. Spearman's correlation was used to assess relationships between variables. Statistical significance was set at $p < 0.05$. Missing Value Analysis (MVA) was not undertaken.

Ethical approvals

This project utilised anonymised data from questionnaires that patients who attended this outpatient department completed as a part of their routine clinical care. Patients were advised that these questions were designed to better understand their pain and the impact that their symptoms had on their quality of life, and they were free to choose not to complete the questionnaires if they wished, and gave permission for use of anonymised data. This specific project, which examines data at baseline and compares these to outcomes seen following treatment is a part of a wider ongoing body of work examining different aspects of chronic tendinopathy which is fully registered with the hospital Trust and relevant authorities. This specific project does not fulfil the criteria of research as stipulated by HRA, therefore formal NHS ethics approvals were not required for this project.

RESULTS

260 patients who had been treated with rESWT for GTPS were identified from procedural records who had follow-up recorded at either 3-months (n = 251/260) or 6-months (n = 229/260), or both (n = 219/260). There was a mean age of 60.0 ± 11.9 years, 81% (n = 211/260) were female, and there was a mean duration of symptoms of 44.5 ± 44.7 months (range: 3-240). These data are displayed in **table I**.
Baseline data were available for self-recorded values of "average pain" for all patients, and 256/260 patients for values of "worst pain". In addition, baseline values were available for the following PROMS: VISA-G (n = 213/260), NAHS (n = 256/260), OHS (n = 256/260) and MSK-HQ (n = 93/260).
Follow-up data for patients at 3-months showed a reduction in self-reported measures of "average pain" and "worst pain" of 30% and 25% respectively, and at 6-months these figures were 37% and 30%. There were statistically significant improvements in all outcome measures comparing baseline to 3-months, and baseline to 6-months (all $p < 0.001$), and these data are displayed in **table II**.

Table I. Demographics (n = 260 total).

Age	60.0 ± 11.9 years
Gender (%female/%male)	81% female/19% male (211 F/49 M)
Duration of symptoms	44.5 months ± 44.7 months (Range: 3-240 months)

Data are mean ± SD.

Table II. PROMs at baseline and follow-up.

	Baseline	3-month follow-up	6-month follow-up
Self-reported "average pain" (0-10)	6.3 ± 1.8 (n = 260)	4.4 ± 2.6* (n = 251)	4.0 ± 2.7* (n = 228)
Self-reported "worst pain" (0-10)	8.0 ± 1.5 (n = 256)	6.0 ± 2.7* (n = 241)	5.6 ± 2.9* (n = 226)
VISA-G	48.4% ± 16.2% (n = 213)	58.4% ± 19.1%* (n = 195)	61.3% ± 20.6%* (n = 197)
NAHS (total score)	43.0 ± 13.1 (n = 256)	51.4 ± 14.9* (n = 239)	53.0 ± 15.6* (n = 225)
Oxford Hip Score	25.7 ± 8.4 (n = 256)	32.1 ± 9.2* (n = 239)	33.0 ± 9.3* (n = 225)
MSK-HQ	31.0 ± 8.4 (n = 93)	36.2 ± 10.1* (n = 88)	37.8 ± 10.7* (n = 84)

Data are mean ± SD. Into brackets: number of patients with data. Asterisks (*) represent significant change from baseline value (all $p < 0.001$). VISA-G: Victoria Institute of Sports Gluteal Tendon score; NAHS: Non-Arthritic Hip Score (total score displayed); OHS: Oxford Hip Score; MSK-HQ: Musculoskeletal Health Questionnaire.

The presence of neuropathic pain as a component of pain has been suggested both as a concept, and has been found in previous cohort study across multiple different tendon conditions (26). Patients were categorised according to their baseline painDETECT score (n = 254/260) as to the likelihood of a neuropathic pain component. Overall, the painDETECT score was high enough to categorise as neuropathic pain was "likely" (painDETECT 19-38) in 22% of the patients (n = 55/254). Of the remainder, 23% (n = 58/254) scored in the "equivocal" category (painDETECT 13-18), and 56% (n =141/254) were identified as neuropathic pain was "unlikely" (painDETECT 0-12). Mean scores for self-reported average and worst pain calculated for those in each of these categories. These demonstrated that there were significant between-groups differences at baseline, with those who scored more highly on the painDETECT questionnaire at baseline (hence being more likely to have neuropathic pain) scoring higher on both 0-10 scales of "average pain" and "worst pain". However, these differences were not consistently seen at follow-up periods. These data are displayed in **table III**.

A further measure of neuropathic pain was used with the self-reported version of Leeds Assessment of Neuropathic Symptoms and Signs (S-LANSS, n = 93). Using this questionnaire, 27% (n = 31/93) scored highly enough to suspect the presence of neuropathic pain (S-LANSS score 12+). Additional baseline measures were taken for symptoms of central sensitisation using the Central Sensitisation Inventory (CSI, n = 201). Using this questionnaire 27% (n = 54/201) were judged to be more likely to have central sensitisation (CSI score > 40) at baseline.

Additional measures of global ability/function were recorded with the Oswestry Disability Inventory (ODI, n = 206) and the EQ-5D %health measure (n = 254). Quantification of patient mood state for anxiety and depressive symptoms was made with Hospital Anxiety and Depression Scale (HADS, n = 252), PHQ-4 (n = 204), GAD-7 (n = 203), and PHQ-9 (n = 204). Measurement was also undertaken with the Pain Catastrophising Scale (PCS, n = 93) and Psychological Inflexibility in Pain Scale (PIPS, n = 93) as additional markers of interest. The baseline values for these different PROMs are displayed in **table IV**.

To attempt to identify possible predictors of outcome following rESWT, independent correlation analysis was undertaken between the different baseline values for the different PROMS and improvements in the self-reported values of "average pain", "worst pain", and the changes in the VISA-G score at 3-months and 6-months.

At 3-months weak or very weak correlations were found between a change in self-reported "worst pain" and baseline "average pain" (but not "worst pain"), MSK-HQ and ODI questionnaires. Improvements in the VISA-G score correlated with other hip function PROMS at baseline.

At 6-months follow-up, very weak correlations for improvements in self-reported "average pain" and 'worst pain" were found for baseline values of the ODI and painDETECT questionnaires. There were also found to be weak correlations between improvements in the VISA-G score and several of the other hip questionnaires (r_s all - 0.2 to - 0.4), but also a very weak negative correlation between improvements in the VISA-G score

Table III. Average/worst self-reported pain (0-10) for different categories of baseline painDETECT score.

	Self-reported "average pain"		
	Baseline	**3-month follow-up**	**6-month follow-up**
"Neuropathic pain unlikely" painDETECT (0-12) (n = 141)	6.0 ± 1.9	4.2 ± 2.6	4.1 ± 2.6
"Equivocal" painDETECT (13-18) (n = 58)	6.1 ± 1.7	4.2 ± 2.4	3.8 ± 2.6
"Neuropathic pain more likely" painDETECT (19-38) (n = 55)	7.2 ± 1.6	4.9 ± 2.7	4.4 ± 2.9
P-value	0.007 *	0.255	0.518
	Self-reported "worst pain"		
	Baseline	**3-month follow-up**	**6-month follow-up**
"Neuropathic pain unlikely" painDETECT (0-12) (n = 141)	7.7 ± 1.6	5.9 ± 2.7	5.7 ± 2.9
"Equivocal" painDETECT (13-18) (n = 58)	7.9 ± 1.4	5.8 ± 2.7	4.9 ± 2.9
"Neuropathic pain more likely" painDETECT (19-38) (n = 55)	8.7 ± 1.2	6.4 ± 2.7	6.1 ± 2.9
P-value	0.027*	0.452	0.013*

Data are mean ± SD; P-values demonstrate significance of difference within time-period group.

Table IV. Baseline PROMs.

Measure	**PROM**	**Baseline value**	**n**
Neuropathic pain	painDETECT	12.1 ± 7.2	254
	S-LANSS	9.1 ± 5.6	93
Central Sensitisation	CSI	32% ± 15%	201
Chronic pain	PCS	16.3 ± 11.3	93
	PIPS	60.1 ± 18.6	93
Global ability/function	ODI	34% ± 15%	206
	EQ-5D % health scale	68% ± 19%	254
Mental health (anxiety/depression)	HADS (anxiety scale)	5.8 ± 3.7	251
	HADS (depression scale)	4.9 ± 3.3	252
	PHQ-4	1.6 ± 2.2	204
	GAD-7	3.6 ± 3.9	203
	PHQ-9	5.2 ± 4.7	204

Data are mean ± SD.

at 6-months and the symptom duration (r_s = 0.160, p = 0.026*), n = 193, indicating those with shorter symptom duration may have had slightly greater improvements seen at 6-months.

These are described under the different sub-headings below, with key findings explored. All relevant data is shown in **table V** for the significance of any correlations at 3-months, and **table VI** for correlations at 6-months.

Table V. Correlations at 3-months.

	% change in "average pain"	% change in "worst pain"	Change in VISA-G
Age	N	N	N
Gender	p = 0.028*	N	N
Symptom duration	N	N	N
Number of previous injections	N	(r_s = - 0.140, p = 0.034), n = 231	N
Rehab frequency (@3M)	N	N	N
Rehab regularity (@3M)	N	N	N
Baseline			
"Average pain" (0-10)	N	(r_s = - 0.135, p = 0.038*) n = 237	N
"Worst pain" (0-10)	N	N	N
VISA-G	N	N	(r_s = - 0.325, p < 0.001*), n = 195
NAHS	N	N	(r_s = - 0.227, p = 0.001*), n = 195
OHS	N	N	(r_s = - 0.220, p = 0.002*), n = 195
MSK-HQ	N	(r_s = 0.220, p = 0.046*), n = 83	N
ODI	N	(r_s = - 0.143, p = 0.049*), n = 190	N
EQ-5D %health	N	N	N
painDETECT (score)	N	N	N
painDETECT (category)	N	N	N
S-LANSS (score)	N	N	N
S-LANSS (category)	N	N	N
CSI (score)	N	N	N
CSI (category)	N	N	N
PCS	N	N	N
PIPS	N	N	N
HADS (Anxiety)	N	N	N
HADS (Depression)	N	N	N
PHQ-4	N	N	N
GAD-7	N	N	N
PHQ-9	N	N	N

N: non-significant statistical relationship; rs < 0.2 "very weak" correlation, > 0.2 "weak" correlation", > 0.4 "moderate" correlation" … (not seen in data set).

Table VI. Correlations at 6-months.

	% change in "average pain"	% change in "worst pain"	Change in VISA-G
Age	N	N	N
Gender	N	N	N
Symptom duration	N	N	(r_s = 0.160, p = 0.026 *), n = 193
Number of previous injections	N	N	N
Rehab frequency (at 6M)	N	N	N
Rehab regularity (at 6M)	N	N	N
Baseline			
"Average pain" (0-10)	N	N	N
"Worst pain" (0-10)	N	N	N
VISA-G	N	N	(r_s = - 0.308, p < 0.001*), n = 197
NAHS	N	N	(r_s = - 0.176, p = 0.013*), n = 197
OHS	N	N	N
MSK-HQ	N	N	N
ODI	(r_s = - 0.151, p = 0.037 *) n = 191	(r_s = - 0.175, p = 0.015*), n = 190	N
EQ-5D %health	N	N	N
painDETECT (score)	N	(r_s = 0.139, p = 0.039*) n = 221	N
painDETECT (category)	(p = 0.039*) n = 222	N	N
S-LANSS (score)	N	N	N
S-LANSS (category)	N	N	N
CSI (score)	N	N	N
CSI (category)	N	N	N
PCS	N	N	N
PIPS	N	N	N
HADS (Anxiety)	N	N	N
HADS (Depression)	N	N	N
PHQ-4	N	N	N
GAD-7	N	N	N
PHQ-9	N	N	(r_s = - 0.153, p = 0.035*), n = 189

N: non-significant statistical relationship. r_s < 0.2 "very weak" correlation, > 0.2 "weak" correlation", > 0.4 "moderate" correlation" … (not seen in data set).

Patient demographics

The patient age did not have any significant correlations with any of the changes in outcome measures seen at either 3-months or 6-months.

Patient gender was found to have a statistically significant difference in % change in self-reported "average pain" at 3-months. However, this is unlikely to have been clinically significant with female patients improving by 30% ± 38%

at 3-months, compared to male patients improving by 20% ± 55%, p = 0.028*. There were no statistically significant relationships between gender and changes in "worst pain" at 3-months (p = 0.127), nor changes in either variable at 6-months.

Symptom duration was found to have a statistically significant, but very weak correlation with improvements in the VISA-G score at 6-months (r_s = 0.160, p = 0.026*), n = 193, but not at 3-months (r_s = 0.075, p = 0.303), n = 190. There were no significant correlations with any changes in self-reported "average" or "worst" pain scales at either 3-months or 6-months.

Rehabilitation programme compliance

Although previous research has suggested that rESWT needs to be performed alongside a graded home exercise programme (13), there were no significant correlations seen identified either the self-reported frequency (how many times per day were the exercise undertaken), or how compliant patients were with the rehabilitation programme and any of the tracked improvements in pain or function at 3-months (**table V**) or 6-months (**table VI**). This may be limited by the accuracy of the self-reporting of this question.

Baseline pain and local hip function

Baseline assessments were undertaken with self-reported measures of "average" pain, "worst pain" and validated measures including VISA-G, NAHS, and OHS. None of these baseline measures correlated significantly with improvements in "average pain" measured at 3-months (**table V**) or 6-months (**table VI**). There were very weak negative correlations seen between baseline average pain, and improvements in self-related pain at 3-months, *i.e.*, those with highest levels of average pain at baseline reported the greatest % improvement in worst pain at 3-months, (r_s = - 0.135, p = 0.038*), n = 237. Similar correlation was not seen at 6-months which raises questions about any clinical significance of this.

Several weak or very weak correlations were seen for different baseline parameters and improvements in VISA-G scores seen at either 3-months (**table V**) and 6-months (**table VI**). This included baseline VISA-G score (3- and 6-months), NAHS (3-months), and OHS (3-months only). The data is displayed in **table V** (3-months) and **table VI** (6-months).

Global function

Baseline aspects of more global function was undertaken with the MSK-HQ, ODI, and EQ-5D-5L questionnaires. The baseline % health scale of EQ-5D did not have any statistically significant correlations with the three outcome measures studied at follow-up.

The baseline MSK-HQ score was shown to have a weak correlation with improvements in "worst pain" at 3-months (r_s = 0.220, p = 0.046*), n = 83, but not with improvements in average pain or VISA-G at 3-months, or any of these markers at 6-months.

The baseline ODI questionnaire had a weak correlation with improvements in "worst pain" at 3-months (r_s = - 0.143, p = 0.049*), n = 190, but not with improvements in "average pain" or VISA-G at 3-months. At 6-months weak correlations were found with self-reported "average pain" (r_s = - 0.151, p = 0.037*) n = 191 and "worst pain" (r_s = - 0.175, p = 0.015*), n = 190 but not with changes in VISA-G.

Markers of chronic, neuropathic, and centralised pain

Markers of chronic, neuropathic, and centralised pain were undertaken at baseline with painDETECT, S-LANSS, CSI, plus PCS and PIPS. Of these baseline assessments, the only statistically significant correlation with outcome was at 6-months with weak correlations between the baseline painDETECT score and improvements in "worst pain" (r_s = 0.139, p = 0.039*) n = 221 and between baseline painDETECT category and improvements in "average pain" (p = 0.039*) n = 222.

Mental health/anxiety and depression scales

Baseline assessment of anxiety and depression symptoms was undertaken with HAD (anxiety and depression sub-scales), PHQ-4, GAD-7, PHQ-9. The only baseline variable that had a statistically significant correlation with outcomes measured was a weak negative correlation with improvement in VISA-G score at 6-months (r_s = - 0.153, p = 0.035*), n = 189.

DISCUSSION

This large case series of 260 patients with chronic GTPS has demonstrated statistically and clinically significant improvements in average pain, worst pain, and a number of validated hip-region PROMS at 3-months and 6-months. However, the nature of this case series data means that benefits seen cannot be ascribed solely to the rESWT intervention and other factors may have contributed, such as the nature of time itself. However, the long average duration of symptoms prior to rESWT-treatment, and the lack of correlation between duration of symptoms and benefits seen, may suggest that time-alone is not the leading cause of the symptom improvements that were seen in this case series. The

benefits seen in this study replicate benefits seen in other studies (2, 15, 18). However, outside this study group, previously published work has only investigated "hip-related" outcome measures, rather than assessing the wider aspects of function used here.

There were statistically significant correlations between multiple baseline factors in aspects of local pain, global function, neuropathic pain, and mental health function and improvements in measures of self-reported "average pain", "worst pain", or VISA-G score at either 3-months or 6-months. However, the majority of these were very weak strength correlations, limiting the clinical significance of these. It may be that multi-level regression may produce a model that could better predict response to treatment, but it is not clear how clinically useful such a complex model may be. Instead, these results demonstrate that no single baseline variable measured in this study had a meaningful value in predicting response to radial shockwave therapy in patients with chronic GTPS.

One of the important limitations of the findings from this study which may influence its generalisability was the duration of symptoms of the patients involved with this project. There was a mean ± SD of symptoms of 44.5months ± 44.7months with a range of 3-months to 20-years. This cohort represents a group of patients who have often had a very long duration of symptoms, and whilst were representative of the patients seen in this tertiary clinic, the outcomes may not be representative of patients with a shorter duration of symptoms, and previous studies have reported success in patients with symptoms of about 12-14months average (15). However, in this study the symptom duration had only a very-weak correlation with changes in VISA-G score at 6-months, and not at 3-months, and no statistically significant correlations with changes in markers of "average pain" or "worst pain" at either time-period studied. Further research could be undertaken with a cohort of patients with shorter duration of symptoms to see if the findings are replicated, or whether the findings from this research are as a result of the chronicity of symptoms.

A strength of this study is the large size of the cohort that were involved (n = 260 total) which gives weight to the findings that were presented here, and exceeds most published studies for this treatment in this condition. This is data from a pragmatic real-world clinic and different numbers of data were available for different measures studied, but the large overall group size has allowed the study of multiple baseline parameters and assessment as to value, if any, in predicting response to treatment. The patients' ages, gender, or duration of symptoms have not been consistently shown to affect outcomes seen. The influence of factors of chronic or centralised pain have not been shown to influence outcomes either positively or negatively, therefore these should not necessarily be barriers to considering rESWT. Local measures of hip pain did weakly correlate to outcome, with stronger correlations between VISA-G (23) and NAHS (21, 22) which may be representative of non-arthritic hip problems, than the OHS (19, 20) which may be more representative of symptoms from hip osteoarthritis. This could be the focus of further work.

CONCLUSIONS

In summary, the data presented here suggests that there is an average improvement in measure of "average" pain of about 30% at 3-months, and 37% at 6-months following rESWT. The improvement in measure of "worst pain" improved by 25% at 3-months, and 30% at 6-months. These reached statistical significance and exceeded the minimally important clinical difference (MCID) in a patient cohort with very long average duration of symptoms. Heterogenous outcomes were identified following rESWT, and no single factor was found to have a moderate or strong correlations with the outcome measures that were studied.

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None.

DATA AVAILABILITY

Data are available under reasonable request to the corresponding author.

CONTRIBUTIONS

The author collated and analysed the data, and wrote the manuscript.

CONFLICT OF INTERESTS

The author declares that he has no conflict of interests.

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ORIGINAL ARTICLE

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Is Posterolateral Incision Better than Direct Posterior Incision in Chronic Tear of Achilles Tendon Reconstruction? A comparative Study of Series of Cases

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SUMMARY

Background. Wound gaping and dehiscence following open repair or reconstruction for chronic (more than 4 weeks old) Achilles tendon ruptures is a great concern and the subject of debate. The location of the incision is crucial. There is no consensus as to the safest incision location. This study aims to observe the effects of posterior midline and posterolateral surgical incision (modified skin incision) on wound dehiscence.

Methods. It was hypothesized that using Posterolateral incision improves wound healing and minimizes chance of wound dehiscence. In our study, open reconstruction was performed for 172 active young soldiers with chronic Achilles tendon ruptures. We consider tear as chronic when it was older than 4 weeks duration and was untreated. Patients were divided into Group A (posterolateral) and group B (direct posterior approach) and TA tendon reconstruction was performed using the Bosworth technique. Patients were followed for two years for wound complication. We used the American Orthopedic Foot, Ankle Society (AOFAS) score, ankle-hind foot score, the Achilles tendon Total Rupture Score (ATRS), VAS for pain and functional evaluation using Heel rise height index (HRHI).

Results. In our comparative study between 2 groups, in group B, 7 patients (11.6%) developed wound dehiscence whereas none in group A. We did not record any sural nerve injury, limitation with ankle motion, footwear related complication and skin adhesion in posterolateral incision group. The AOFAS score averaged 93.5 ± 4 in posterolateral incision (group A) and 78.8 ± 7 in direct posterior incision (group B). Three soldiers developed re-rupture following the fresh injury in group A.

Conclusions. Based on our results, posterolateral skin incision gives equally good results as direct posterior incision however, this approach minimizes the risk of wound-related complication especially wound dehiscence following TA tendon reconstruction.

KEY WORDS

Posterolateral skin incision; wound dehiscence; Achilles tendon rupture; open reconstruction.

INTRODUCTION

Achilles tendon rupture is one of the most common tendon injuries in orthopaedics practice (1, 2). A chronic or neglected tear always required surgical reconstruction and possess great challenges in wound healing. Chronic tear is defined as tear older that 4weeks (3). However, Maffulli N. defined as it is 6 weeks (4). In chronic tear primary repair is very difficult because of two reasons firstly due to retraction of torn tendon ends and secondly increased in gap (5). Regarding choice of tendon graft is concerned Maffulli N. reported that if gap between torn tendon ends is less than 6 cm peroneus brevis is effective whereas if gap is more than 6 cm semitendinosus tendon graft is indicated (6). Maffulli N., in his study on 62 patients of chronic TA Tendon rupture 21 patient who had more than 6 cm gap underwent less invasive technique of reconstruction using free ipsilateral semitendinosus graft, 20 patients who had less than 6 cm gap underwent reconstruction using peroneus brevis, and another set of 21 patients who also had less than 6 cm gap underwent reconstruction using flexor hallucis longus, and he did not note any significant advantage of one technique over the others (7). Wound related complication following surgical reconstruction of chronic tear is noted by many authors ranging from minor wound infection, wound dehiscence, keloid formation, skin necrosis (8-10).

In the literature the wound complication rate following reconstruction of chronic Achilles tendon rupture varies from 8% to 9.7% (9-11). Yepes *et al.* (11) noted that the location of the surgical incision may be a risk factor for wound complication, and he further noted that since Achilles tendon is covered with a peritenon, fascia, and a thin layer of skin. The ideal skin incision must provide adequate exposure and good tissue healing with minimal scar formation. Hammit *et al.* (12) however, suggested posterior direct approach rather than posterolateral or posteromedial approach. He noted that this approach provides excellent exposure with minimal wound related complications. The explanation for less wound complication was that the approach provides dissection between angiosomes, which preserved the blood supply to the skin flaps. In the present literature, a debate regarding the safest incision is continuing. Whether direct midline incision over Tendon which lies between the two angiosomes or slightly off the midline medial or lateral incision to avoid the vascular watershed area is still subject of study (13, 14). Taylor noted that the posteromedial aspect is supplied by the posterior tibial vessels of the ankle and the posterolateral aspect by Peroneal vessels (14). Yepes *et al.* (11) in a cadaveric study using angiography of the skin and subcutaneous tissues covering the Achilles tendon noted that the posterior cutaneous midline was less vascular as compared to that of the medial and lateral areas adjacent to the tendon which has better vascularity. He further suggested that the skin incisions be placed one cm medial to the Achilles tendon and to incise the peritenon without dissecting it from the subcutaneous fat. However, Attinger *et al.* (13) have recommended that the safest incision between two angiosomes and thus incisions be made along the central raphe over the Achilles tendon in between the peroneal and posterior tibial angiosomes.

The sural nerve injury is a great concern for posterolateral incision as it is located about 1-2 cm lateral to the lateral border of the Achilles tendon. It crosses the Achilles tendon 8 to 10 cm proximal to the superior aspect of the calcaneal tuberosity (15). Traumatic or iatrogenic sural never injury leads to loss of sensation in the lateral mid and hindfoot. Since its lateral location in the lower leg, the posteromedial incision is safer as compared to the posterolateral incision.

The present study aimed to compare the rates of wound dehiscence between direct posterior midline incision and posterolateral incision.

MATERIALS AND METHODS

This is a prospective randomized study of 172 soldiers who underwent TA tendon reconstruction of chronic tear (tear older than 4 weeks duration) between Mar 2010 and Feb 2019 with 2 years follow up. The study was conducted in accordance with institutional and international (Declaration of Helsinki) standards. Ethical approval was obtained from the Ethics Committee of Base Hospital Guwahati, India (File no: 151/BH/EC). Patients with traumatic confirmed full-thickness TA rupture were included. Acute tear, previous history of surgery on Achilles tendon, open injuries, or a percutaneous repair was excluded. All patients were soldiers without any medical co- morbidities. A pre-anesthesia checkup was performed before taking up for surgery. If any medical co-morbidity was detected during pre-anaesthesia checkup, he was excluded from the study. History of alcoholism and smoking was taken and were recorded. In group A, 7 patients had history of alcoholism (60 ml twice week), 10 patients had history smoking (5-8 cigarettes per days) and 28 had both, however, in group A 5 patients had history of alcoholism (60 ml twice week), 8 patients had history smoking (5-8 cigarettes per days) and 17 had both. All patients mentioned supra were underwent assessment of the ankle-brachial index (ABI) and Duplex ultrasonography to rule out any subclinical Peripheral vascular disease. In the present study if patients had history of alcoholism or smoking for more than 5 years were excluded in the study.

112 patients of age group 21 to 50 years (mean 35.5 years) were assigned as group A (modified posterolateral incision group) and 60 patients as control group B (direct posterior incision group). Randomization was done with every third

patient and postoperative evaluation was done by independent surgeon not participating in the present study. We followed sequentially numbered, opaque, sealed envelopes for concealment of patient allocation to minimize bias.

Study design

Total 220 soldiers with Tendo Achilles tendon tear reported to emergency room of Orthopaedic department. Only 172 who met with inclusion criteria were included in the study, 30 soldiers were excluded from study as they did not meet inclusion criteria, 14 soldiers declined to participated in study and 4 soldiers were excluded from study due to other reasons (**figure 1**). All included patient were available to follow-up till 2 years post-surgery.

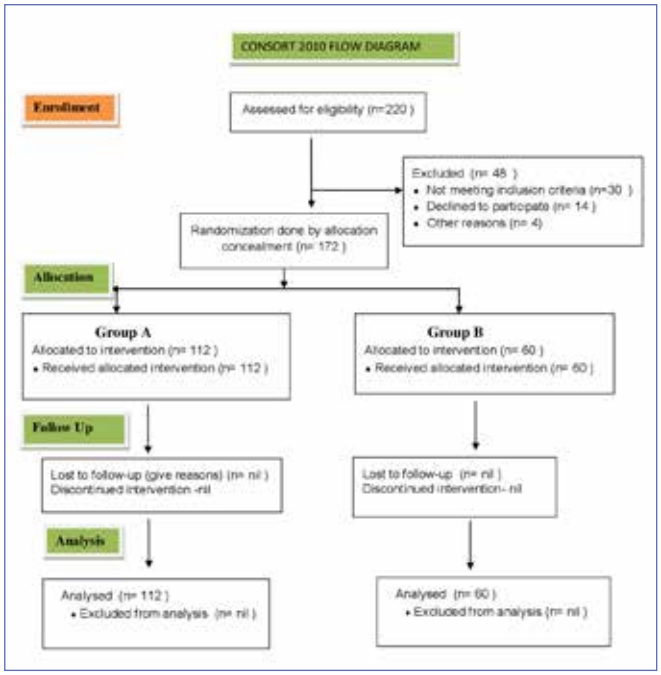

Figure 1. Study design.

Statistical analysis

The calf circumference, ankle range of motion, footwear restriction, duration of wound healing, VAS score and AOFAS score were compared between two groups using the paired sample t-test; $p < 0.05$ was considered significant. Data collected were analyzed with a multi-purpose computer analysis program, Statistical Package for the Social Sciences version 19.0 (IBM; SPSS, Chicago, IL, USA) was used.

Operative technique

All patients underwent surgical reconstruction using the Bosworth procedure in prone position under spinal anesthesia and under tourniquet control. A straight posterior midline incision starting proximally 10 cm below the knee joint was given (**figure 2 a**). The incision was continued distally and then gently curved laterally about 6 cm above the insertion of the tendon. After the skin incision, the sural nerve and short saphenous vein were isolated. Graft 1.5–2-cm-wide strip was marked and harvested from the central portion of the raphe (**figure 2 b**). The graft was left attached just two cm proximal to the rupture site. The entire graft passed through the proximal ruptured tendon and passed through the distal tendon stump by making a split in it. (**figure 2 c**). If the distal stump was small, a drill hole was made in calcaneum 1 cm below and a graft was passed through it. The graft was passed in a lateral to medial direction ensuring free graft movement in tunnel then it was sutured with proximal part of main tendon (**figure 2 d**). The graft was then sutured in proper tension in plantar flexion of the ankle (**figure 2 e**). The wound was then closed, firstly closure of paratenon using 2.0 Vicryl, secondly the subcutaneous tissue using 2.0 Vicryl and finally skin closure using staplers and silk (stapler was used in proximal part and 2.0 Silk suture in distal part of incision).

After surgery removable plaster cast in 20° plantar flexion and the knee in 30° flexion was applied for 4 weeks. Intravenous antibiotics were given for three days postoperative. Wound dressing was changed after 48 hrs and then at 7th postoperative day and suture removed on 15th postoperative days.

Plaster was removed after 4 weeks, below knee cast was applied for another 4 weeks in 20° plantar flexion. A walking short leg cast was applied with the ankle plantigrade for

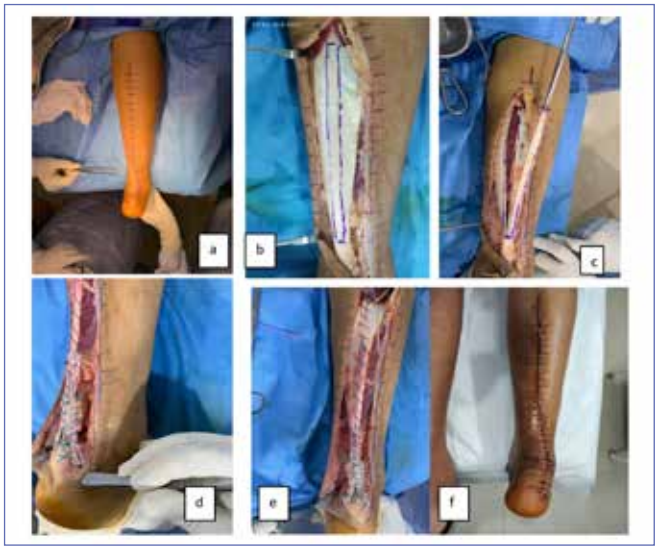

Figure 2. Posterolateral incision group A.
From top clockwise, **(a)** modified skin incision posterolateral, **(b)** marking of graft, **(c)** harvesting of graft, **(d)** passing of graft through Calcanium tunnel, **(e)** final suturing of graft, and, **(f)** final wound closure.

4 more weeks. The plaster was finally removed at 3 months and gradual calf strengthening and stretching exercises were started along with weight-bearing. Post operative rehabilitation was same in both groups.

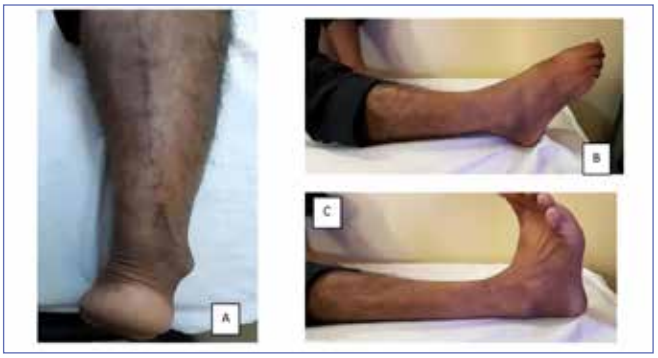

Figure 3. Healed surgical posterolateral scar, range of ankle motion and endurance at 6 month of follow-up group A.
(A) Healed surgical scar, **(B)** Planter flexion, and **(C)** Dorsiflexion.

Figure 4. Healed surgical posterolateral scar and full weight bearing at 6 month of follow-up group A.

Evaluation at follow-up

Each patient was evaluated in follow-up at 3 months, 6-month 12 months, 24 months and finally at two years using the scoring system described by Leppilahti *et al.* (16) Status of wound and range of ankle motion, and full weight bearing at 6 months of follow-up has been shown in **figures 3** and **4**. Heel rise height index (HRHI) was performed on a single leg and on both leg while the patients was standing, with his both knees extended position. When the heel was raised, the distance of the heel bottom from the floor was measured with a tape. Measurement was taken on both side the operated and unaffected sides was calculated **figure 5**.

Wound complication in direct posterior incision group has shown in **figure 6**.
The scoring included subjective factors (pain, stiffness, muscle weakness, and footwear restrictions) as well as objective factors (range of ankle motion and isokinetic calf muscle strength). Out of 100 points, ≥ 90 points were considered as excellent, 75–89 points as good, 60–74 points as fair and < 60 points considered as poor. A subjective symptoms questionnaire was given to all patients and were asked to fill the response.

Figure 5. Heel rise height index (HRHI) inspection and evaluation.
Patient was standing on his toes and measurement was taken from heel to floor on both sides to compare HRHI. Black line indicates level of heel and level of ground. Yellow line indicate distance between heel and floor.

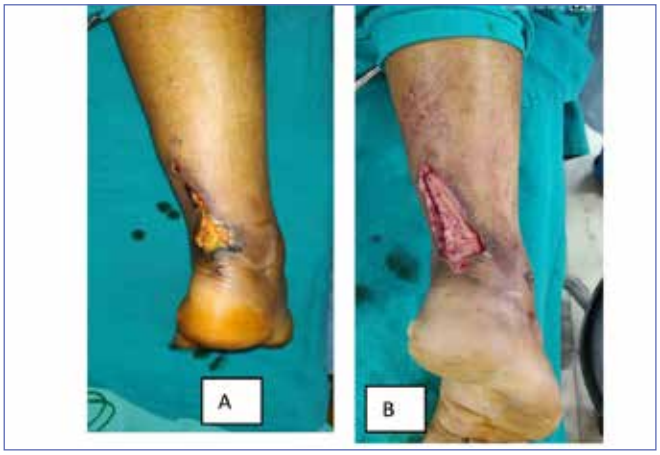

Figure 6. Wound complication in Direct Posterior incision.
(A) Wound dehiscence after 3 weeks postoperative. **(B)** Wound dehiscence that needs flap cover.

RESULTS

The demographical characteristics of the included patients were shown in **table I**. BMI does not possess any significant risk factor in rupture however, sports injury was a significant risk factor (p = 0.032) for tendon rupture. History of alcoholism and smoking was also not a risk factor too.

We used the Leppilahti scoring system for evaluation (**table II**). At the end of two year follow up in group A, 98 patients had excellent results, 9 had good results, 3 had fair results and 2 patients had poor results. Whereas in-group B, 45 patients had excellent results, 8 had good results, 4 had fair results and 2 had poor results.

Preoperative and post operative scores (at 2 years follow up) in both groups have been summarized in **table III**.

In group A, mean AOFAS scores increased from 60.27 ± 10.71points preoperatively to 93.5 ± 4.2 points at the latest follow-up (p = 0.0334), however it was 63.24 ± 9.23 and 78.8 ± 7.4 respectively in group B. Mean ATRS score also showed significant improvements from 15.57 ± 6.27 points preoperatively to 88.73 ± 11.95 points at the last follow-up (p = 0.0251) in group A however it was 11.26 ± 4.21 and 69.03 ± 7.26 respectively in group B. Pain during weight bearing was assessed by the VAS which improved from a mean of 8.2 + 0.3 (range: 6 to 10) preoperatively to a mean of 1.2 + 0.1 (range: 0 to 2) at the last follow-up in group A, however it was 7.9 + 0.1 and 2.8 + 0.8 respectively in group B (**table III**). Thus, a significant difference was seen in VAS score in group A than in group B.

A significant clinical difference was noted between two groups at two years follow up in the AOFAS score (p = 0.0334). The mean AOFAS score was 93.5 ± 4.2 in group A and 78.8 ± 7.4 in group B. The difference between VAS scores was also significant (p = 0.0211). Mean VAS score was 1.2 + 0.1 in group A and 2.8 + 0.8 in group B (**table IV**). All patients were able to perform a single-limb heel rise test and had returned to their preinjury level. Returned to their preinjury level was assessed by Heel rise height index (HRHI) adopted by Imaya (17). HRHI was noted preoperatively in group A and B, 45.18 ± 14.6 and 55.24 ± 23.56 respectively (p = 0.713). However, the same was noted postoperatively in group A and B, 89.76 ± 21 91.44 ± 11.11 respectively (p = 0.683) which was statistically non-significant. Hence, as far as reconstruction of TA tendon is concerned, we did not note any significant difference in HRHI scores. Three months after surgery, MRI scan was done which showed some signs of inflammation, which disappeared at the end of 6 months and two years postoperatively. At the latest follow-up, MRI scan showed full continuity of the reconstructed Achilles tendon.

In group B, deep infection was noted in 4 (6.6%) patients however it was as less as 1.7% in group A (p = 0.0217), delayed wound healing was noted in 9 (15%) patients in group B (p = 0.0344) and wound dehiscence was seen in 7 (11.6%) in group B (p = 0.0154). None of the group A patient showed wound dehiscence. When infection involved tendon, we considered as deep infection and was managed by vacuum assisted dressing and antibiotics according to culture report. The infection has healed in 3-week time.

Table I. Demographical characteristics of included patients in both groups.

Characteristics	No of patients		P-value
	Group A	Group B	
Age (mean) 35.5 years	112	60	
Mode of injury			
Sports	91	39	0.032
Training	12	8	
Direct hit	9	13	
Duration of rupture			
30 -90 days	101	51	0.042
> 90 days	11	9	
BMI			
< 18.5 kg/m²	32	10	0.632
18.5 - 25 kg/m²	63	21	0.685
> 25 kg/m2	17	29	0.730
Alcoholic (60 ml twice a week)	7	5	0.221
Smoking (5-8 per days)	10	8	0.732
Both	28	17	0.341
Mean length of gap between TA tendon ends (in mm)	58 (27-105)	55 (24-104)	0.776

Table II. Leppilahti scoring system of both groups at final follow up at 2 years.

Factor	Point	No of patients at 6 months		No of patients at 1 year		No of patients at 2 years	
		Group A	Group B	Group A	Group B	Group A	Group B
Pain							
Non	15	102	39	103	52	109	55
Mild, no limitations in recreational activities	10	5	9	9	2	3	1
Moderate, limitations in recreational, but not daily activities	5	3	5	-	2	-	2
Severe, limitations in recreational and daily activities	0	2	7	-	4	-	3
Stiffness							
Non	15	90	51	97	52	109	56
Mild, no limitations in recreational activities	10	6	5	5	2	3	2
Moderate, limitations in recreational, but not daily activities	5	4	1	7	4	-	1
Severe, limitations in recreational and daily activities	0	2	3	3	2	-	-
Calf muscle weakness (subjective)							
Non	15	99	53	103	56	112	58
Mild, no limitations in recreational activities	10	7	3	9	3	-	1
Moderate, limitations in recreational, but not daily activities	5	5	2	-	1	-	1
Severe, limitations in recreational and daily activities	0	1	2	-	-	-	-
Footwear restrictions							
None	15	87	7	106	18	112	34
Mild	5	21	22	6	13	-	9
Moderate, unable to tolerate fashionable shoes, required modified shoes	0	4	31	-	29	-	17
Active range of motion (ROM) difference between ankles							
Normal (< 6°)	15	102	48	106	42	112	53
Mild (6°–10°)	10	7	8	3	6	-	4
Moderate (11°–15°)	5	2	3	3	5	-	2
Severe (> 15°)	0	1	1	-	7	-	1
Isokinetic muscle strength (score)							
Excellent	15	83	48	97	53	112	56

Factor	Point	No of patients at 6 months		No of patients at 1 year		No of patients at 2 years	
		Group A	Group B	Group A	Group B	Group A	Group B
Good	10	19	9	13	3	-	2
Fair	5	4	3	1	4	-	2
Poor	0	6	-	1	-	-	-
Leppilahti score							
Excellent	90-100	77	38	89	41	98	45
Good	75-89	24	11	15	11	9	8
Fair	60-74	8	4	6	3	3	4
Poor	< 60	3	7	2	5	2	3
Satisfaction level							
Fully satisfied	15	97	36	108	38	110	55
Satisfied with minor issues	10	10	17	4	11	2	1
Satisfied with minor issues	5	5	4	-	3	-	2
Dissatisfied	0	-	3	-	2	-	2

Table III. Preoperative and two years post operative scores in both groups.

Score	AOFAS (mean SD)		ATRS (mean SD)		VAS (mean SD)		HRHI (mean SD)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Group A	60.27 ± 10.71	93.5 ± 4.2	15.57 ± 6.27	88.7 3 ± 11.95	8.2 + 0.3	1.2 + 0.1	45.18 ± 14.6	89.76 ± 21
Group B	63.24 ± 9.23	78. 8± 7.4	11.26 ± 4.21	69.03 ± 7.26	7.9 + 0.1	2.8 + 0.8	55.24 ± 23.56	91.44 ± 11.11
P-value*	0.742	0.0334	0.612	0.0251	0.651	0.0211	0.713	0.683

*Paired sample t-test; Pre-Preoperative; Post-Postoperative.

Table IV. Comparing the calf circumference, ankle range of motion and Leppilahti score between both groups.

Parameters at 4 years	Group A	Group B	P-value*
Calf circumference (cm)	35 ± 3.9 (31-43)	33 ± 2.8 (27-38)	0.0766
Ankle motion (°) Plantar flexion	32 ± 7 (19-44)	28 ± 6 (15-34)	0.0013
Ankle motion (°) Dorsi flexion	15 ± 5 (10-20)	13 ± 5 (9-18)	0.0643
Leppilahti score (at 2 year) Excellent	98 (112) 87.5%	45 (60) (75%)	0.0221
Footwear restrictions (at 2 year)			
None	112/112 (100%)	34/60 (56.6%)	0.0235
Mild	-	9/60 (15%)	0.0213
Need footwear modification	-	17/60 (28.3%)	0.0430
VAS (Mean SD)	1.2 + 0.1	2.8 + 0.8	0.0211
AOFAS score (Mean SD)	93.5 ± 4.2	78.8 ± 7.4	0.0334
ATRS Score (Mean SD)	88.73 ± 11.95	69.03 ± 7.26	0.0251

*Paired sample t-test.

DISCUSSION

There is a constant debate what will be the best management of TA tendon rupture in the present literature. Maffulli G. (18) in his study included 26 patients with TA tendon rupture and compared three different types of management in acute rupture, including non-surgical plaster immobilization, traditional open surgery and percutaneous repair. He noted that surgical repair, percutaneous and open repair gives better functional outcomes than conservative management.

As far as chronicity of tear and outcome after repair is concerned Maffulli N. included 21 patient who presented between 2 weeks to 30 days from day of Achilles tendon tear and underwent minimally invasive technique of repair, and noted that there was no difference in outcome as compared with those underwent repair within 14 days of rupture (19). Maffulli N. *et al.*, enrolled 21 patients of re-ruptured of TA tendon for minimally invasive reconstruction with the ipsilateral peroneus brevis (5 patients) or the semitendinosus tendon graft with or without interference screw fixation (10 and 6 patients, respectively). He observed that minimally invasive reconstruction is safe and effective surgical procedures for reconstruction of re-rupture of the Achilles tendon (20).

In the present literature there is debate on which incision in better, direct posterior incision, posteromedial or posterolateral incision. However, direct posterior incision has some wound healing complication. In present study we like to present our experience of using posterolateral skin incision which is equally good than other incision as for as exposure and TA tendon reconstruction is concerned but it has definite lesser wound healing complication than direct posterior approach.

In our study with modified posterolateral incision, none of the patients showed delayed wound healing or wound dehiscence, which was more common in direct posterior midline incision.

In young patient generally open surgical repair/ reconstruction is preferred over conservative treatment and especially where the patient is a soldier. Open surgery provides good outcome, rapid recovery, early rehabilitation and early return to duty and to sports. Many published data suggest that after open repair risk of re-rupture is less (11, 21, 22).

However, non-surgical treatment is preferred for elderly, diabetics, and patient with peripheral vascular disease (21, 22). The major concern following TA tendon reconstruction is delayed wound healing and wound dehiscence (23-25). It has been documented in the literature that the delayed wound healing may be due to thin skin and poor blood supply over tendon area (13). In the present literature the wound complications after open reconstruction vary from 11-21% (24) and 8-9.7% (26). Bruggeman *et al.* reported 17 wound (10.4%) complication in 164 patients treated by open TA tendon repairs (9). Cretnik *et al.* (27) reported that minimally invasive repair minimizes wound complication however some author did not note any significant difference between open *versus* minimally invasive repair (28-30).

As far as muscle strength is concerned, we did not note any weakness in gastrocnemius, this finding is supported by study done by Goren on 20 patients with chronic TA tendon rupture (more than 6 months old rupture) underwent repair (10 patients were treated by open surgery, and another 10 patients were treated percutaneously). He noted that there was no difference in functional outcome and the biomechanical strength (31).

However, few author noted Calf muscle endurance was affected after repair and noted various risk factors like, increased tendon compliance, tendon lengthening, inadequate rehabilitation, persistent pain, gender and level of activity (32-35).

Many meta-analyses have shown that Achilles tendon ruptures repaired by open methods have a significantly lower re-rupture rate compared to nonsurgical treatment, but open reconstruction/repair has other complications such as superficial and deep infections, sural nerve injury, delayed wound healing and wound dehiscence requiring secondary surgeries like flap cover (36-39).

In the search of a method to minimize wound complications after reconstruction, we noted that there are very few studies based on blood supply at ankle area. In this regard, Yepes *et al.* (11) have shown that the deep fascia and anterior paratenon, which usually remain intact in Tendon rupture that, have very good blood supply from the posterior tibial and peroneal arteries. He suggested that after reconstruction tendon should be covered with these well-perfused tissues which may compensate the inadequate blood supply in this region and may minimize wound complication. He also recommended that medial incision can be used to minimize wound complications. Skin adhesions (superficial skin tethering) and deep adhesions causing tendon pain, and difficulty in using footwear have been reported as complications of both surgical and non-surgical management with rates of 5–7.1% (40-42). Thus, superficial and deep adhesion interfere with use of footwear and ankle motion.

In present study, we noted that even covering the repaired tendon with peritenon, which has rich vasculature, the placement of skin incision plays an important role in wound healing. Placing skin incision directly over the tendon has higher wound complication rate as compared to posterolateral incision.

Limitation of study

The study was conduction on the military soldiers without any medical co-morbidities and thus result may not be applicable to whose who has medical co-morbidities, as latter may be a risk factor for wound healing and infection.

CONCLUSIONS

In this study direct posterior appears to be associated with high wound complication rate for delayed reconstruction of chronic TA tendon rupture. This may be due to skin over the TA tendon being less vascular as compared with skin on the posterolateral side. The direct posterior skin incision is also associated with postoperative skin adhesion to underlying tendon and the use of footwear is delayed because of its direct contact with footwear. Our study suggests that posterolateral incision has promising results in chronic TA rupture reconstruction as compared to direct posterior incision.

FUNDINGS

None.

DATA AVAILABILITY

Data are available under reasonable request to the corresponding author.

CONTRIBUTIONS

SKR: critical analysis and writing. SKR, TPG. MB: performed surgeries. TPG: conceptualize and design of the study. GKG, ABK: English writing. SKR. GKG, OS, MB, ABK: data collection. OS: statistical analysis.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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Skeletal Muscle Fibre Characteristics of the Lumbar Multifidus Muscle in Patients Undergoing Microdiscectomy for Unilateral Lumbar Disc Herniation

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SUMMARY

Background. Lumbar disc herniation (LDH) is the most common diagnosed degenerative pathology in the lumbar spine. Because of its role in spinal stability there is an increased interest in the role of the *Lumbar Multifidus* muscle in low back pain research. Despite surgical treatment long-term, disability and pain remain a persistent problem. The aim of the study is to compare side-to-side *Lumbar Multifidus* muscle fibre characteristics in unilateral LDH patients, and compare both sides to a healthy control group.

Methods. Thirty patients (n = 17 men and n = 13 women) scheduled for microdiscectomy for unilateral disc herniation and ten healthy controls (n = 5 men and n = 5 women) were included in this study. Biopsies of the *Lumbar Multifidus* muscle were analysed by means of immunohistochemistry combined with immunofluorescence microscopy to determine type I and type II muscle fibre type distribution, cross-sectional area, myonuclear- and satellite cell content, inflammation and various indices of muscle fibre capillarisation.

Results. The proportion of muscle fibres with centrally located myonuclei, various indicis of muscle fibre capillarisation and pro-and anti-inflammatory cell content were higher in the patients compared with the healthy controls. No differences were observed in type I and type II muscle fibre characteristics between the injured and uninjured side within the LDH patients.

Conclusions. This study shows clear differences in *Lumbar Multifidus* muscle fibre characteristics between LDH patients, irrespective of injured or uninjured side, and healthy controls. Additional studies are warranted to establish the clinical significance of these differences in muscle fibre morphology in LDH compared with healthy controls.

Study registration. This trial was registered on ClinicalTrials.gov under the identification number NCT03753711.

KEY WORDS

Biopsy; lumbar disc herniation; multifidus; satellite cells; skeletal muscle fibres.

INTRODUCTION

Low back pain is one of the most frequent and debilitating musculoskeletal conditions globally causing high socioeconomic burden (1). The most common diagnosed degenerative pathology in the lumbar spine is lumbar disc herniation (LDH) (2). It is characterised by localised or focal displacement of disc material beyond the limits of the intervertebral disc space, most often in the posterolateral region (3). Nowadays it is not clear which loading conditions are responsible for lumbar intervertebral disc failure. Due to similar mechanical behaviour of the ovine and human intervertebral disc, the ovine model can be used to investigate the mechanical loading on the intervertebral disc itself (4).

In a selected group of patients experiencing progressive neurological deficits, red flags, or failure of conservative management, surgery may be required (5). Although surgery provides short time relief of leg pain when compared to conservative management, no significant long-term differences in clinical outcomes have been observed (6, 7). Despite surgical treatment up to 36% of patients keep experiencing persistent low back pain symptoms (8). This could indicate that surrounding tissues outside the epidural space contribute to pain persistence and long-term disability. The *Lumbar Multifidus* muscle (LMM) is an increasing subject of interest in low back pain research, especially because of its functional role in spinal stability and control of spinal motion (9, 10). Recent human research has shown that changes in muscle mass and muscle quality of the paraspinal muscles (*e.g.*, fat infiltration, fibrosis) are often associated with unilateral LDH, especially at the side of radiculopathy (11). Moreover, degenerative changes of the LMM such as atrophy, fat infiltration and fibrosis have been observed in different animal intervertebral disc injury models (12-16). Inflammatory dysregulation has been proposed as a potential mechanism of LMM degeneration, stepping away from simple denervation atrophy or reflex inhibition paradigm (17). This mechanism is supported by *in vivo* studies showing increased numbers of inflammatory, fibro-adipogenic, and satellite cells in skeletal muscle of spinal pathologies (18-21). These observations indicate that a more complex process than just simple denervation or disuse atrophy is involved. It should be noticed that these studies (18-21) are all lacking an age-matched healthy control group, which makes it difficult to draw firm conclusions.

Currently, the mechanism of LMM degeneration and atrophy *in vivo* in humans remains largely unknown. The aim of the present study is to compare side-to-side LMM fibre characteristics and muscle cell type content in unilateral LDH patients, and compare both sides to a healthy control group. This study provides insight into the LMM characteristics of hernia patients and the potential importance of specific cell types in the development of LMM degeneration and atrophy. Subsequently this may help to improve existing and/or develop new intervention strategies to more effectively prevent or reverse the deconditioning of paraspinal muscles after LDH.

MATERIALS AND METHODS

Subjects

Thirty adults with symptoms of unilateral LDH, scheduled for minimally invasive discectomy, were recruited from July 2018 until December 2019 at the Jessa Hospital, Hasselt, Belgium. Participants were informed about the opportunity to participate in the study by their neurosurgeon during their preoperative consultation if they met the following criteria: unilateral LDH diagnosed using medical imaging, age between 18 and 55 years old, and fluent in Dutch (both spoken and written). Participants were excluded when they had undergone surgery within the last year, had degenerative or other spinal pathologies, or had other known pathologies that could interfere with muscle biology. Healthy participants were recruited by convenience sampling using local advertisements. Participants, between 25 and 60 years of age and able to understand the Dutch language, were included if they either had no acute or chronic low back pain (> 3 months). Healthy subjects were excluded if they had been in rehabilitation or exercise therapy for an acute condition within the last 3 months. All subjects were informed about all the aspects of the study and were included in the study after providing their informed consent. Ethical approval was given by the Medical Ethics Committee from the Jessa Hospital and Hasselt University of Belgium (B243201836859 - Date of approval June 27, 2018).

Muscle samples and clinical data

LMM samples were taken during minimally invasive microdiscectomy for unilateral LDH. After patients were anaesthetised and placed in the genu-pectoral position a midline incision was made over the spinous process of the involved segment. A fine needle biopsy technique was used to obtain a muscle sample from the contralateral (non-herniated side) LMM, using the 12G semi-automated Bard® Mission® Core Biopsy Instrument. The ipsilateral biopsy sample (herniated side) was taken directly from the LMM when surgically preparing the access to the posterior lamina at the level of surgery. LMM samples in the healthy control group were taken at the right side of the body at the spinal level of the spinous process of vertebra L4, as described previously (22). All samples were placed on cork and embedded in optimum cutting compound. Samples were immediately

frozen using isopentane cooled in liquid nitrogen. Frozen samples were stored at - 80 °C in the clinical biobank until further analyses.
Demographic data (*e.g.*, age, gender, body mass index (kg/m²)) were obtained from all participants. Leg and back pain duration and intensity were assessed using the Visual Analogue Scale (VAS) using a 10-point Likert scale. The presence and duration of motor deficits was assessed by manual muscle testing (23). Disability was assessed using the Oswestry Disability Index (24).

Immunohistochemistry

Frozen muscle biopsies were cut into 5 µm thick cryosections using a cryostat at - 20 °C and thaw mounted on uncoated pre-cleaned glass slides. Samples from the ipsilateral and contralateral sides of the LMM were mounted on the same slide. Samples were stained with antibodies against CD31 (1:50, endothelial cell mouse IgGg1 Dako M0823), CD68 (1:100, monoclonal mouse Anti-Human IgG1, Dako M0718), CD206 (1:200, Rabbit polyclonal to Mannose Receptor IgG, Abcam 64693), PAX-7 (1:1, cell supernatant from cells obtained from the DSHB, USA), myosin heavy chain I (1:25 mouse IgM A4.840 (slow isoform), DSHB, USA), laminin (1:50, sigma-aldrich, |L9393 USA). For immunofluorescent detection, secondary antibodies used were as follows: Pax7 (biotin-vector BA-2000, 1:200, streptavidin 488 1:200, Invitrogen, Molecular Probes, Carlsbad, CA, USA); myosin heavy chain type I (clone A4.480) (goat anti-mouse IgM Alexa Fluor 488, 1:500, Invitrogen); laminin (goat anti-rabbit IgG Alexa Fluor 647, 1:400, Invitrogen); CD68 (1:200, goat anti-mouse IgG 488, Invitrogen); CD206 (1:200, goat anti-rabbit 555, Invitrogen); CD31 (biotin-vector BA-2000, 1:200, Avidin Texas red 555 1:400, Invitrogen, Molecular Probes, Carlsbad, CA, USA). Nuclei were labelled with DAPI (4',6-diamidino-2-phenylindole) (1:20000, Sigma-Aldrich, Oakville, ON, Canada). Prior to cover slipping slides with fluorescent mounting media (DAKO, Burlington, ON, Canada). Slides were viewed with the Nikon Eclipse Ti Microscope (Nikon Instruments Inc., USA), equipped with a high-resolution Photometrics CoolSNAP HQ2 fluorescent camera (Nikon Instruments, Melville, NY, USA). Images were captured and analysed using the Nikon NIS Elements AR 3.2 software (Nikon Instruments Inc., USA).
For muscle fibre size an average of 218 ± 204 (range 52-1324) fibres were analysed per fibre type in each biopsy sample to determine muscle fibre type distribution, muscle fibre size, percentage of fibres with central nuclei, and myonuclear content and domain size. To quantify capillaries an average of 45 ± 20 (range 18-119) fibres were analysed per fibre type/cross-section based on the work of Hepple *et al.* (25). Quantification was made of (I) capillary contacts (CC), (II) the capillary-to-fibre ratio (C/Fi), (III) capillary-to-fibre perimeter exchange (CFPE) index and (IV) capillary density, (CD). To quantify satellite cell content an average of 218±130 (range 67-685) fibres were analysed per fibre type/cross-section, and for macrophages an average of 210 ± 83 (range 78-438) fibres/cross-section were analysed.

Statistical analysis

Anthropometric and clinical data are displayed as mean ± SD. Main outcome variables (fibre distribution and size, myonuclear content, satellite cell and macrophage content) were analysed using JMP Pro 14.1.0 software (SAS Institute Inc, Cary, NC, USA, 1989 –2007). A mixed model was performed with fibre type and group (injured *vs* uninjured *vs* healthy) as within subject factors. For secondary measurements, the patients in the LDH group were subdivided into two groups based on the duration of radicular pain (acute < 12weeks, chronic > 12weeks). For secondary subgroup analysis a mixed model was performed with fibre type and side (injured *vs* uninjured) and fibre type and duration (acute *vs* chronic) as within subject factors. Normality of the data was checked using the normal quartile plots calculated from the conditional residuals. When a normal distribution was not assumed, the data set was transformed using a square root transformation. Significance was set at the 5% point with a confidence interval of 95%. When a significant interaction or main group effect (whenever 3 groups were used) was found, a *post-hoc* Tukey-HSD was used to locate the differences between groups. For the secondary outcome measures, a *post-hoc* t-test was performed when there was a significant interaction effect. Results are reported as mean ± SE.

RESULTS

Participants' characteristics

All LDH patients

Twenty-nine patients diagnosed with a unilateral LDH (age: 40 ± 9 y) and ten healthy controls (age: 42 ± 8 y) were included in the final analyses. From one patient the biopsy sample was deemed not usable because of insufficient quality. Participants' characteristics are displayed in **table I**. No significant differences were observed in age, weight, height, gender or BMI between groups. Fifteen patients underwent surgery at the spinal level L4-L5, and 14 patients at the level L5-S1. Based on VAS-scores low back pain was present in 87% of all unilateral LDH patients, with an average pain severity score of 4.7 ± 2.9 on a 10-point Likert scale. All

patients experienced leg pain with an average VAS score of 5.8 ± 2.1 on a 10-point Likert scale. Average duration of low back and leg pain was 23 ± 34 and 6 ± 11 months, respectively. Motor deficits (*e.g.*, reduced strength or paralysis) were present in 60% of the unilateral LDH patients with an average duration of 7 ± 11 months. Patients experienced severe disability with an average score of 41 ± 17% on the Oswestry Disability Index.

Acute vs chronic LDH patients

Subgroup analyses were performed to assess lateralisation between the injured and uninjured side between patients experiencing acute radicular pain (< 12weeks, n = 14) or patients experiencing more chronic radicular pain (> 12 weeks, n = 15). No between group differences except for the duration of radicular pain ($p < 0.05$) could be observed, indicating no different patient characteristics between groups (**table I**).

Muscle fibre type composition and size

All LDH patient's vs controls

In both the LDH patients and control subjects type I muscle fibres were significantly greater compared with type II muscle fibres (main effect of fibre type $p < 0.0001$, **figure 1**). When comparing side-to-side differences within the LDH group, no significant differences were observed in type I and type II muscle fibre size or fibre type composition (**figure 1**). When comparing the heathy control group to the LDH group (injured and uninjured side) no significant differences were found for muscle fibre size and type composition (**figure 1**). Detailed values are displayed in **table II**.

Table I. Participants' characteristics of healthy controls and all included lumbar disc hernia patients, and subdivided in an acute (< 12weeks) and chronic (> 12 weeks) group.

	Control subjects	LDH patients		
	All (n = 10)	**All (n = 29)**	**Acute (n = 14)**	**Chronic (n = 15)**
Age (yr)	42 ± 8	40 ± 9	40 ± 9	40 ± 9
Length (m)	1.79 ± 0.07	1.76 ± 0.10	1.77 ± 0.11	1.75 ± 0.10
Weight (kg)	83.9 ± 17.4	81.8 ± 19.5	81.9 ± 19.3	81.7 ± 20.4
BMI (kg/m²)	26.0 ± 4.0	26.5 ± 4.8	26.8 ± 4.7	26.2 ± 5.0
Gender (male:female)	5:5	17:12	9:5	8:7
LBP (months)	-	17.3 ± 27.1	9.0 ± 13.9	24.7 ± 34.1
Leg pain (months)	-	6.01 ± 9.13	1.20 ± 0.75	10.4 ± 11.07*
Motor (months)	-	4.30 ± 9.51	0.68 ± 0.69	7.53 ± 12.52
Back (VAS)	-	4.74 ± 2.97	4.64 ± 3.34	4.90 ± 2.73
Leg (VAS)	-	5.88 ± 2.17	5.08 ± 2.43	6.75 ± 1.56
Level (L4-L5:L5-S1)	-	15:14	8:6	7:8
ODI (%)	-	39.8 ± 17.2	37.4 ± 18.6	43.1 ± 16.2

BMI: body mass index; LBP: low back pain; VAS: visual analogue scale; ODI: Oswestry disability index; LDH: lumbar disc hernia; *Indicating a significant difference compared to the acute subgroup, $p < 0.05$. Data represent mean ± SD.

Table II. Muscle fibre characteristics in healthy controls and lumbar disc herniation patients, both at the injured and uninjured side.

		Controls (n = 10)	LHD injured (n = 29)	LDH uninjured (n = 29)
Muscle fibre type composition and size				
Fibre size (µm²)	I	5824 ± 419	5819 ± 299	5965 ± 312
	II	3932 ± 459*	3341 ± 305*	3625 ± 239*
Fibre type composition (%)	I	63 ± 4	56 ± 3	61 ± 3

		Controls (n = 10)	LHD injured (n = 29)	LDH uninjured (n = 29)
	II	36 ± 4*	44 ± 3*	39 ± 3*
Muscle fibre myonuclear and satellite cell content				
Myonuclear content (number / fibre)	I	4.01 ± 0.28	4.78 ± 0.19†	4.95 ± 0.2†
	II	2.61 ± 0.31*	3.21 ± 0.19*†	3.43 ± 0.21*†
Myonuclear domain (µm²)	I	1453 ± 71	1208 ± 47†	1196 ± 50†
	II	1483 ± 79*	1011 ± 48*†	1060 ± 53*†
Central nuclei (%)	I	1.93 ± 1.95	7.58 ± 1.2	8.57 ± 1.25
	II	2.8 ± 2.33	4.74 ± 1.25	3.11 ± 1.4
Satellite cell content (number / 100 fibres)	I	8.79 ± 1.45	12.61 ± 0.8	10.46 ± 0.8
	II	4.01 ± 1.45*	3.71 ± 0.8*	4.24 ± 0.85*
Muscle fibre capillarization				
CC	I	2.83 ± 0.24	3.76 ± 0.16†	3.72 ± 0.17†
	II	2.66 ± 0.27*	3.03 ± 0.16*	2.96 ± 0.17*
C/Fi	I	0.77 ± 0.12	1.82 ± 0.08†	1.76 ± 0.08†
	II	0.27 ± 0.13*	1.03 ± 0.08*†	0.98 ± 0.08*†
CFPE (capillaries / 1000 µm)	I	4.64 ± 0.26	5.84 ± 0.18†	5.74 ± 0.19†
	II	3.5 ± 0.29*	4.28 ± 0.18*†	4.29 ± 0.19*†
CD (capillaries / mm²)	I	286 ± 30	361 ± 19†	356 ± 20†
	II	291 ± 34	370 ± 19†	360 ± 20†
Muscle fibre inflammatory cells				
M1 (number / mm²)	MIX	0.97 ± 0.47	2.01 ± 0.27	2.76 ± 0.28†
M2 (number / mm²)	MIX	8.87 ± 2.23	15.09 ± 1.26†	13.59 ± 1.31

Data represent mean ± SE. CC: capillary contacts; C/Fi: capillary-to-fibre ratio; CFPE index: capillary-to-fibre perimeter exchange; CD: capillary density; LDH: lumbar disc hernia; Type I: type I muscle fibres; Type II: type II muscle fibres; MIX: mixed muscle fibre types; M1: cells positive for CD68 and DAPI; M2: cells positive for CD68, CD206 and DAPI. *Significant different compared with type I muscle fibres, $p < 0.05$; †*Post-hoc* significantly different compared to healthy control group, $p < 0.05$.

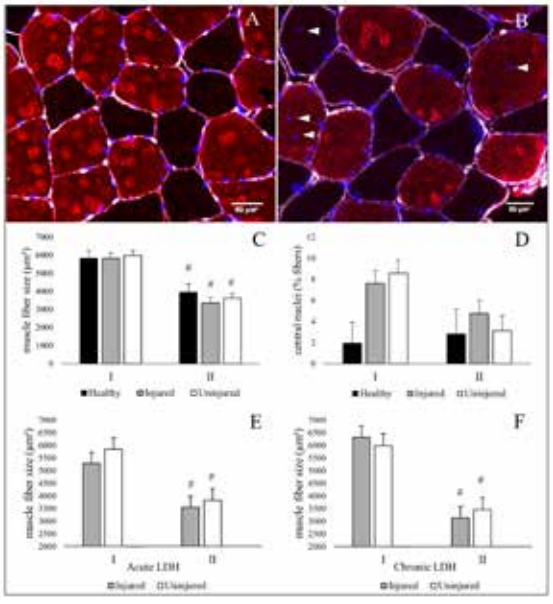

Figure 1. A, B: Representative image of immunohistochemical analyses of the lumbar multifidus muscle cross-section in healthy control **(A)** and lumbar disc herniation (LDH) patients **(B)**: myosin heavy chain I (red), laminin (white), dapi (blue), with white arrows indicating central nuclei. **C-D**: Type I and type II muscle fibre size **(C)** and central myonuclei **(D)** in healthy controls and LDH patient (injured and uninjured side). **E, F**: Type I and type II fibre size in the acute **(E)** (n = 14) and chronic **(F)** (n = 15) subgroups of the LDH patients for both injured and uninjured side.

Data are expressed as mean ± SE. #Indicating a significance with type I muscle fibres $p > 0.05$.

Acute vs chronic LDH patients

Although no significant differences were observed for muscle fibre size for the whole LDH group, the acute patient subgroup showed smaller type I (- 11%) and type II (- 8%) muscle fibre sizes when comparing the injured to the uninjured side (main effect of side p = 0.0735, **figure 1**). Fibre type distribution was not different between the acute and chronic subgroup, or between the injured and uninjured side within the groups. Detailed values are displayed in supplementary **appendix 1**.

Muscle fibre myonuclear and satellite cell content

All LDH patient's vs controls

No significant side-to-side differences were found for myonuclear content and satellite cell content within the LDH group (**table II**). Type I and II muscle fibre myonuclear content of the uninjured and injured side LDH patient were significantly greater when compared with healthy control subjects (main effect of group $p < 0.05$, **table II**). Type I and type II myonuclear domain size was significantly smaller in the LDH compared with the healthy control group (main effect of group, $p < 0.0001$, **table II**). In the LDH patients, the percentage of muscle fibres containing one or more central myonuclei was greater in type I compared to type II fibres (main effect of fibre type p= 0.0597, **figure 1**). In addition, the percentage of muscle fibres containing one or more central myonuclei was greater in LDH patients (both sides) compared with healthy controls (main effect of group p = 0.0951, **figure 1**). In both the LDH patient (injured and uninjured side) and control subjects type I muscle fibre satellite cell content was significantly greater compared with type II muscle fibres (main effect of fibre type $p < 0.0001$, **figure 2**). No significant differences were observed in type I and type II muscle fibre satellite cell content between LDH patients and healthy controls (**figure 2**). Detailed values are displayed in **table II**.

Acute vs chronic LDH patients

The injured side showed a significant smaller myonuclear domain size compared to the uninjured side within the acute subgroup (main effect of side $p < 0.05$, supplementary **appendix 1**). Type I myonuclear domain was significant larger in the chronic group compared to the acute group at the injured side (main effect of duration $p < 0.05$, supplementary **appendix 1**). No differences in muscle fibre myonuclear or satellite cell content were observed between the acute and chronic group, or between the injured and uninjured side (supplementary **appendix 1**).

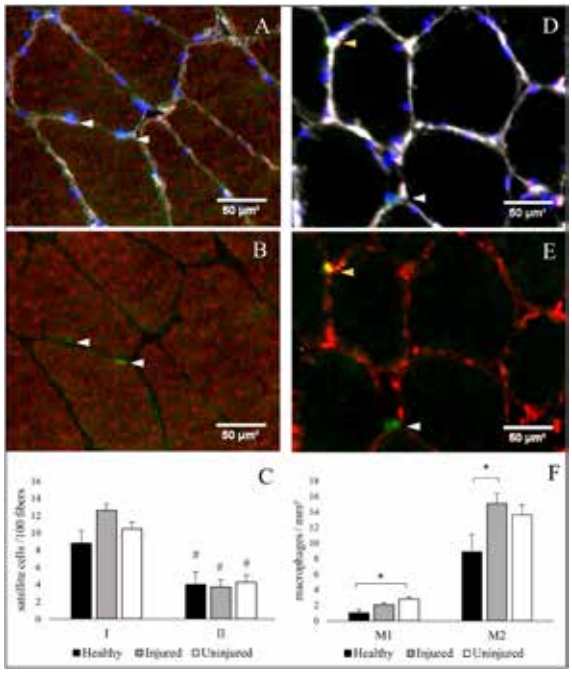

Figure 2. A, B: Representative image of immunohistochemical analyses of the lumbar multifidus muscle cross-section stained for satellite cells. **(A)**: myosin heavy chain I (red), laminin (white), dapi (blue), white arrows indicating satellite cells. **(B)**: myosin heavy chain I (red), pax-7$^+$ cells (green), white arrows indicating satellite cells. **(C)**: type I and type II muscle fibre satellite cells/100 muscle fibres in healthy controls and LDH patient (injured and uninjured side). **D, E**: representative image of immunohistochemical analyses of the lumbar multifidus muscle cross-section stained for macrophages. **(D)**: laminin (white), dapi (blue), white arrow indicating CD68$^+$ M1 macrophage, yellow arrow indicating CD206$^+$ M2 macrophage costained with CD68$^+$. **(E)**: CD68$^+$ cells (green), CD206$^+$ cells (red), white arrow indicating CD68$^+$ M1 macrophage, yellow arrow indicating CD206$^+$ M2 macrophage costained with CD68$^+$. **(F)**: number of M1 and M2 macrophages/mm^2 in healthy controls and LDH patient (injured and uninjured side).

Data are expressed as mean ± SE. *Indicating a significant between group difference $p < 0.05$. #Iindicating a significance with type I muscle fibres $p > 0.05$.

Muscle fibre capillarisation

All LDH patient's vs controls

No significant side-to-side differences were found for any of the muscle fibre capillarisation indices in the LDH group. In all groups, type I muscle fibre capillarisation was significantly greater compared with type II muscle fibres (main effect of fibre type $p < 0.0001$, **figure 3**), except for CD (**table II**).

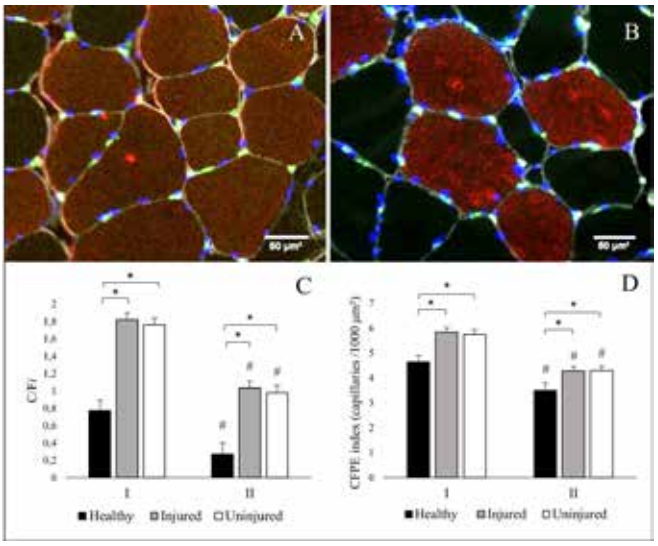

Figure 3. A, B: Representative image of immunohistochemical analyses of the lumbar multifidu muscle cross-section in healthy control **(A)** and lumbar disc hernia (LDH) patients **(B)**: myosin heavy chain I (red), laminin (white), dapi (blue), CD31 (green). **C, D**: Type I and type II muscle fibre capillary to fibre ratio (C/Fi) (C) and capillary to fibre perimeter exchange (CFPE) index (D) in healthy controls, and LDH patient (injured and uninjured side).

Data are expressed as mean ± SE. *Indicating a significant between group difference $p < 0.05$. #Indicating a significance with type I muscle fibres $p > 0.05$.

Type I and II muscle fibre CC, C/F*i*, CD as well as CFPE-index were greater in LDH patients compared with healthy controls (main effect of group $p < 0.05$, **figure 3**). Detailed values are displayed in **table II**.

Acute vs chronic LDH patients

Type I and type II muscle fibre capillarisation were not different between the acute and chronic group, or between the injured and uninjured side within the groups (supplemental **appendix 1**).

Muscle fibre inflammatory cells

All LDH patient's vs controls

No significant side-to-side differences were found for muscle fibre inflammatory cell density in LDH patients (**figure 2**). A significant greater number of pro-inflammatory M1 cells was found between the LDH group and healthy controls (main effect of group $p > 0.05$, **figure 2**), while for the anti-inflammatory M2 cells a borderline significant difference was found (main effect of group $p = 0.0595$, **figure 2**). Detailed values are displayed in **table II**.

Acute vs chronic patients

No differences in muscle fibre inflammatory cells were observed between the acute and chronic group, or between the injured and uninjured side (supplemental **appendix 1**).

DISCUSSION

The present study is the first to compare LMM fibre characteristics both at the injured and uninjured side between unilateral LDH patients and age-matched healthy controls. Although muscle fibre size did not differ between groups, the proportion of muscle fibres with centrally located myonuclei, as well as muscle fibre inflammatory cell content, was greater in the LMM of unilateral LDH patients when compared with healthy controls. Interestingly, type I and II muscle fibre capillarisation were considerably greater in unilateral LDH patients compared with healthy controls. Finally, no significant differences were observed in satellite or inflammatory cell content between the injured and uninjured sides or between groups.

Previous studies suggested type I and II muscle fibres to be larger in patients with LDH compared to healthy control subjects (26, 27). However, these studies used post-mortem biopsies to serve as controls, which may have influenced their findings as cellular breakdown, autolytic activity, and structural alterations of muscle tissue cannot be excluded (28). In the present study, *in vivo* LMM samples were obtained from age-matched healthy controls, providing a more accurate evaluation of the true differences in LMM morphology between healthy individuals and people suffering from LDH. In contrast to our expectations, no significant differences were observed in type I and II muscle fibre size between LDH patients and healthy controls. As lateralisation has been observed following lumbar disc herniation, we also aimed to assess differences in LMM fibre characteristics between the injured and uninjured side in patients with LDH. Previous studies have reported significantly smaller LMM muscle fibres (type I and II) at the injured compared to the uninjured side in LDH patient (29-31). In our study, we observed no differences in muscle fibre size (or fibre type distribution) between the injured and non-injured side of LDH patients. The lack of differences may, in part, be explained by the timing of the muscle biopsy sampling in relation to the onset of low back pain. Previous animal studies report profound muscle atrophy in the acute (3-6 days) phase, that was no longer present following a more prolonged period (3-6 months) (12, 14). Hence, we additionally (retrospectively) evaluated differences in muscle fibre characteristics between patients having an acute (< 12 weeks, n = 14) or chronic radiculopathy (> 12 weeks, n = 15) at the time of muscle biopsy sampling. Although not significant, patients

in the acute subgroup tended to show a greater difference in type I (~ - 11%) and type II (~ - 8%) muscle fibre size between the injured and uninjured side, compared with the chronic subgroup (type I ~ + 5% and type II ~ - 10%). The current study is the first to show potential evidence for acute, but not chronic, atrophy (type I) in human LMM samples in patients suffering from LDH. As the LMM is the primary stabiliser of the lumbar spine (32), this muscle relies especially on the type I muscle fibres for their stabilising function (33). In previous research, we have already reported a greater proportion of type I muscle fibres in patients with low back pain compared to healthy controls, without differences in type I muscle fibre size (34). We speculate that patients with acute LDH have decreased motor neuron activity (*e.g.*, inhibition) of the LMM, (35) resulting in a decrease in type I muscle fibre size. In chronic low back pain patients, LMM motor neuron activity is likely to be higher (36) leading the recovery of muscle fibre size compared to the more acute phase LDH. However, clearly more *in vivo* human research is warranted to confirm these speculations.

Skeletal muscle fibres are multinucleated cells, with each muscle fibre containing hundreds to thousands of myonuclei. These myonuclei provide the transcriptional activity required to regulate myocellular homeostasis, and support muscle reconditioning (37). In the present study, we observed a significantly greater myonuclear content in LDH patients when compared to controls. This may indicate that muscle fibres in LDH patients require increased transcriptional capacity to support muscle protein synthesis to recover from the acute phase of atrophy or are in need of increased transcriptional capacity due to higher protein turnover rates following increased muscular activity as seen in low back pain (38). On other hand, the greater myonuclear content could also represent a reduced efficiency of the existing myonuclei in these patients, a phenomenon that has been suggested to occur in age-related muscle fibre atrophy (39). Interestingly, the percentage of muscle fibres with centrally located myonuclei was three times higher in muscle tissue collected from LDH patients when compared with healthy controls. Centrally located myonuclei are one of the hallmarks of muscle fibre regeneration/repair following injury and, as such, have been suggested to be a proxy for muscle fibre damage (40). Hence, our findings suggest that muscle tissue of LDH patients is in a state of muscle fibre repair/regeneration when compared with healthy controls. As myonuclei are post-mitotic, provision of additional or replacing damaged myonuclei to support fibre homeostasis depends on a pool of myogenic precursor cells, also known as satellite cells (41). Muscle satellite cells are essential in skeletal muscle fibre regeneration and repair (42, 43). In addition, a decline in satellite cell content has been hypothesised to be an important contributing factor in the development of skeletal muscle fibre atrophy in aging as well as various other myopathies (44, 45). Although the muscle fibres of LDH patients clearly show more muscle fibre damage based on centrally located myonuclei, satellite cell content was not different compared with healthy controls. In addition, satellite cell content did not differ between the injured and uninjured side or between acute and chronic LDH patients. As satellite cell content does not seem to be reduced in muscle tissue of the LDH patients, these data suggest that muscle fibre repair/regeneration and reconditioning is not limited by satellite cell number in the LMM of these patients.

The delivery of oxygen and metabolic substrates, as well as removal of waste products, are of critical importance for muscle fibre homeostasis and reconditioning. Capillary rarefaction has been shown to be associated with muscle fibre atrophy and may limit muscle fibre size recovery during exercise training/rehabilitation (45, 46). The present study shows that various indices of muscle fibre capillarisation were substantially higher (~ 22 to 281%) in muscle tissue of LDH patients (both on the injured as well as uninjured side) when compared with the control group. These results appear to be in line with the study by Strobel *et al.* who reported increased oxygen tension with a concomitant increase in muscle tension in patients with low back pain, suggesting a high capillary content to be related to increased muscle tension (47). Previous studies have reported an average of 276 ± 69 capillaries/mm^2 in patients with degenerative spinal pathologies, (18) which is in line with our findings in healthy controls (268 ± 30 capillaries/mm^2). We observed a significantly higher capillary density within our patient sample, suggesting a difference in capillary content between patients in this study and elderly patients with advanced degenerative spinal disorders (18). Although the increase in muscle capillary contents may be in line with the increased muscle tension hypothesis leading to hypoxia, stimulating angiogenesis, there may also be alternative explanation for this observation. (48, 49). A different mechanism that could cause the increase in capillary content is inflammatory dysregulation of the LMM, which has been suggested as in important mechanism of LMM degeneration (35). Macrophages play an essential role in the regulation of vascularisation in skeletal muscle tissue (50). A relative high density of especially anti-inflammatory macrophages (M2) has been associated with increased neovascularisation (51). Although we observed a greater number of anti-inflammatory macrophages within the LMM muscle tissue collected in our LDH patients, no correlations were observed between the number of anti-inflammatory macrophages and various indices of muscle fibre capillarisation. This may suggest that the number of macrophages is not likely the primary reason for the higher muscle fibre capillary density observed in LDH

patients. Hence, further investigations are needed to unravel underlying mechanisms.

Clinical significance and limitations

The present study is the first extensive evaluation of *in vivo* muscle fibre characteristics in a large group of LDH patients (injured and uninjured side) and age-matched healthy controls. Although these data provide novel insights in the potential underlying mechanisms on the morphological changes in muscle fibre characteristic as a result of LDH, the cross-sectional nature does not allow us to establish causality. Hence, we cannot exclude the possibility that alterations in muscle fibre characteristics were already present on beforehand, and may even have contributed to the development of disc injury itself (*e.g.*, increased spinal loading). Insight in skeletal muscle fibre morphological changes following LDH is of clinical importance as it provide insight to optimise existing and develop new exercise rehabilitation intervention strategies in these patients. Thus far, exercise rehabilitation programs have been mainly based on morphological animal studies by Hodges and colleagues (12-14). These studies show muscle size reduction (by muscle inhibition) and early fatty infiltration, based upon which they correctly recommend that acute low back pain should be treated using motor control training to overcome initial muscle inhibition. In addition, they recommend to progressively add resistance and endurance exercise training when evolving to a chronic phase of low back pain to prevent atrophy, fibrosis, fatty infiltration and promote anti-inflammatory effects (35). Furthermore, Dohnert *et al*. showed that exercise therapy in patients with disc protrusion reduced pain and improved function (52). Based on the present study results (*i.e.*, type I fibre atrophy in acute, and type II muscle fibre atrophy in both acute and chronic LDH patients), it could be suggested to incorporate both motor control as well as progressive resistance exercise training during the initial phase of rehabilitation. Including resistance exercise training may be of particular importance as it is likely vital to overcome, or maybe even prevent, type II fibre atrophy following LDH. Clearly, more long-term human follow-up studies are warranted to determine if the differences observed in this study may have an impact on the rehabilitation strategy of the LMM.

CONCLUSIONS

Clear differences in LMM fibre characteristics are apparent between LDH patients, irrespectively of the injured or uninjured side, and healthy control subjects. With the exception of acute muscle atrophy in the initial stage after LDH, bilateral involvement of various muscle fibre associated structures/cells are evident. Although this study provides further insight into the potential underlying mechanisms of changes in muscle fibre characteristics in the LMM, future studies should investigate whether exercise interventions can change LMM muscle fibre characteristics and improve clinical outcomes.

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None.

DATA AVAILABILITY

Data are available under reasonable request to the corresponding author.

CONTRIBUTIONS

SS, FV, AA., LvL, TS, MP, AT: research conceptualization and design. SS, FV, AA, MP, SB, TA, MB: experiments performance. SS, FV, AA, TS, TA, MB, SB: data analysis. SS, FV, AA, LvL, TS, AT: results interpretation. SS, FV, AA: manuscript drafting. SS, AA, FV: figures preparation. SS, FV, AA, TS, MP, SB, TA, MB, AT, LvL: manuscript editing and revision. All authors approved the final version of the manuscript.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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SUPPLEMENTS

Appendix 1. Muscle fibre characteristics for acute and chronic lumbar disc herniation patients in both the injured *vs* uninjured side.

		Acute LDH (n = 14)		Chronic LDH (n = 15)	
		Injured	**Uninjured**	**Injured**	**Uninjured**
Muscle fibre size and type composition					
Fibre size (µm²)	I	5283 ± 430	5847 ± 441	6313 ± 450	5987 ± 467
	II	3543 ± 435*	3825 ± 466*	3127 ± 457*	3452 ± 477*
Fibre type composition	I	56.23 ± 4.25	61.60 ± 4.50	56.82 ± 3.79	62.77 ± 4.00
(%)	II	45.27 ± 4.25*	39.92 ± 4.64*	43.18 ± 3.79*	37.24 ± 4.00*
Muscle fibre myonuclear and satellite cell content					
Myonuclear content (number/fibre)	I	4.75 ± 0.24	5.02 ± 0.26	4.75 ± 0.30	4.77 ± 0.32
	II	340 ± 0.25*	3.39 ± 0.29*	2.96 ± 0.31*	3.35 ± 0.33*
Myonuclear domain size (µm²)	I	1108 ± 76	1172 ± 78†	1324 ± 56#	1254 ± 60
	II	1020 ± 77	1140 ± 82†	1020 ± 58*	1052 ± 62*
Central nuclei (%)	I	7.91 ± 2.03	8.49 ± 2.21	7.16 ± 1.62	8.17 ± 1.62
	II	5.27 ± 2.11	2.00 ± 2.80	4.06 ± 1.70*	3.37 ± 1.70*
Satellite cell content (number/100 fibres)	I	11.94 ± 1.03	10.40 ± 1.03	13.28 ± 1.28	10.55 ± 1.28
	II	4.93 ± 1.03*	4.43 ± 1.12*	2.61 ± 1.28*	4.06 ± 1.32*
Muscle fibre capillarization					
CC	I	3.63 ± 0.20	3.67 ± 0.22	3.82 ± 0.25	3.63 ± 0.26
	II	3.17 ± 0.21*	2.97 ± 0.22*	2.83 ± 0.25*	2.83 ± 0.26*
C/Fi	I	1.81 ± 0.11	1.76 ± 0.12	1.80 ± 0.12	1.71 ± 0.13
	II	1.05 ± 0.11*	1.01 ± 0.12*	0.98 ± 0.12*	0.91 ± 0.13*
CFPE (capillaries/1000 µm)	I	6.01 ± 0.25	5.74 ± 0.27	5.64 ± 0.29	5.63 ± 0.31
	II	4.41 ± 0.26*	4.19 ± 0.27*	4.13 ± 0.29*	4.27 ± 0.31*
CD (capillaries/mm²)	I	390 ± 29	359 ± 32	333 ± 28	351 ± 30
	II	374 ± 30	339 ± 32	366 ± 28	379 ± 30
Muscle fibre inflammatory cells					
M1 (number/mm²)	Mix	1.76 ± 0.43	2.71 ± 0.43	2.23 ± 0.38	2.80 ± 0.41
M2 (number/mm²)	Mix	13.46 ± 2.14	13.66 ± 2.14	16.53 ± 4.56	13.36 ± 1.69

Data represent mean ± SE. CC: capillary contacts; C/Fi: capillary-to-fibre ratio; CFPE: capillary-to-fibre perimeter exchange; CD: capillary density; LDH: lumbar disc herniation; Type I: type I muscle fibres; Type II: type II muscle fibres; Mix: mixed muscle fibre types; M1: cells positive for CD68 and DAPI; M2: cells positive for CD68, CD206 and DAPI. *Significant effect of type; †Significant effect of side. #Significant effect of duration (acute *vs* chronic).

ORIGINAL ARTICLE

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Gender Differences in Ultrasound Imaging of Lateral Abdominal Muscle Thickness and Trunk Mobility

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SUMMARY

Background. A number of gender differences are of great importance in the research interpretation, being a differentiating variable in many of them. The example is a varied structure of the musculoskeletal system. The objective of this study was to determine corelations between gender and thickness of the abdominal muscles as well as selected trunk mobility parameters.

Methods. The studied group consisted of 80 subjects: 42 women and 38 men aged 18-45. The inclusion criteria were: age 18-45, absence of pain of the spine and peripheral joints; Body Mass Index (BMI) ≤ 29.9; absence of neurological symptoms; absence of chronic diseases affecting the musculoskeletal system; absence of postoperative scars within the abdominal wall; not being pregnant. The research procedure consisted of an interview, morphological analysis with body composition assessment, measurement of abdominal muscle thickness by ultrasound imaging and mobility tests: Fingertip-to-Floor, Sit and Reach Test and lateral flexion of the spine. The ANOVA, the Pearson correlation coefficient (r) and Intraclass Correlation Coefficient (ICC3.1) were used.

Results. Statistically significant differences between the groups were demonstrated in the thickness of internal ($p = 0.0057$) and external oblique muscles ($p = 0.0079$) and in the total measurement TrA + IO + EO ($p = 0.0020$), indicating a greater mean thickness in the male group. The results from functional trunk mobility tests did not differ significantly between men and women ($p > 0.05$). There was a weak correlation between the selected morphological variables and the thickness of the abdominal muscles in both groups.

Conclusions. Gender differentiates the thickness of the muscles of the lateral abdominal wall. In the group of men there is a greater thickness of the internal and external oblique muscle, as well as the entire muscle complex. No relationship between sex and the thickness of the transverse abdominal muscle was demonstrated. There were no differences in the mobility of the trunk between women and men.

KEY WORDS

Gender; abdominal muscle thickness; mobility; ultrasonography; trunk movements.

INTRODUCTION

A number of gender differences are of great importance in the research interpretation, being a differentiating variable in many of them, especially in the area of sport, physical activity and public health. An example may be the different structure of the musculoskeletal system beetwen men and women expressed by dissimilarities in the overall somatic structure of the body *e.g.*, body weight, height, body composition and in the selected anthropometric measurements (1-5). Moreover, the endocrine system plays a significant role in this matter as well - especially sex hormones such as estrogens, testosterone, thyroid hormones, growth hormone and others. They determine the difference in muscle mass percentage, which affects greater strength and power generated by men later on (6-8). Both these parameters are elements of motor performance skills and strongly influence sport results achieved by women and men which causes a significant disproportion, especially visible and common in the early stages of children's and youth's sporting performance (9-11).

In addition to the difference in muscle mass, there is also a distinct proportion of bone and fat mass, which is respectively 15:36:30 (bone: muscle: fat) for women and 20:40:20 for men (12, 13). The bone and fat mass density is also regulated by sex hormones (including androgens in men and estrogens in women) (14, 15).

The mobility and stability of the trunk are part of the fundamental human motor skills, on the basis of which a proper motor function develops (16). That motor function is stimulated in the nervous system by the force of gravity (17). In the case of a child which develops new motor skills in the first year of life, differences resulting from gender are yet to be visible. Changes occurring at the level of basic motor skills begin to manifest later in life and are not yet fully known nor understood (18, 19).

The relation between mobility and stability is of high importance because the feasible range of motion increases as the stabilizing function of the trunk improves (20). Additionally, the stability of the trunk is the result of the interaction of multiple systems, ranging from neuromuscular control, control of the neutral zone of joints, their anatomical structure, flexibility and stiffness of the soft tissues (21-23).

The assessment of stability and mobility of a trunk as elements of the motor function is exceedingly challenging, because those two factors' outcomes must be expressed in a quantitative manner. Conversely, for clinicians and other practitioners concerning themselves with this matter, the qualitative assessment of the movement pattern is significantly more important, but also considerably more difficult to rank (16, 24, 25). Therefore, in this study, the thickness of abdominal muscles was measured by examining the variance in their mass, which has a direct effect on postural stability in case of deep muscle function and ability to generate strength and power in superficial muscles (20, 26, 27). Mobility was assessed as the range of motion in selected functional tests.

The aim of this study was to determine corelations beetwen gender and thickness of the abdominal muscles as well as selected trunk mobility parameters.

MATERIALS AND METHODS

Characteristics of the subjects

The studied group consisted of 80 subjects: 42 women and 38 men, who consituted respectively 52.5% and 47.5% of all respondents. The inclusion criteria were the following: age 18-45, absence of pain of the spine and peripheral joints; Body Mass Index (BMI) ≤ 29.9; absence of neurological symptoms; absence of chronic diseases affecting the musculoskeletal system; absence of postoperative scars within the abdominal wall; not being pregnant.

The exclusion criteria included presence of back pain and/or with accompanying neurological symptoms; abdominal wall surgery; diagnosis of a chronic disease; in the group of women - pregnancy; missing at least one training session or measurement procedure; current pharmacotherapy (including analgesics during the study); resignation at any stage of the study. The variables describing the study group are presented in **table I**. There were no statistically significant differences in the level of physical activity between the groups, assessed with the IPAQ questionnaire ($p = 0.6143$). Moreover, none of the women mentioned having children in the interview.

The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by the Bioethics Committee of the Medical University of Silesia, Katowice, Poland (Resolution No KNW/ 0022/KB46-7/18). Each of the subjects gave their written consent to participate in the research.

Methods

The research procedure consisted of the interview which was the initial qualification, morphological analysis including body composition assessment, assessment of abdominal muscle thickness and basic mobility tests. The obtained results were recorded in the research questionnaire. The study was preceded by the pilot examination for the sake of refinement and validation of the measurement procedures and elimination of potential errors.

Table I. Statistics of age and morphological parameters.

Variable	Sex	N	X	Min	Max	CI - 95%	CI 95%	SD	p*
Age (years)	Women	42	23.71	19.00	37.00	22.64	24.79	3.44	0.0837
	Men	38	27.03	19.00	45.00	24.99	29.07	6.21	
Height (cm)	Women	42	167.95	158.00	179.00	166.26	169.65	5.44	0.0000
	Men	38	180.42	165.00	195.00	178.19	182.65	6.79	
Body mass (kg)	Women	42	59.71	46.80	72.70	57.76	61.67	6.28	0.0000
	Men	38	79.46	59.80	102.80	76.42	82.49	9.24	
BMI (kg/m2)	Women	42	21.21	17.20	27.00	20.48	21.94	2.35	0.0000
	Men	38	24.37	20.40	28.20	23.66	25.08	2.17	
Fat mass (%)	Women	42	26.54	17.20	37.00	25.06	28.03	4.76	0.0000
	Men	38	18.56	8.80	25.80	17.36	19.76	3.64	
Fat free mass (kg)	Women	42	43.65	37.70	51.50	42.67	44.63	3.13	0.2390
	Men	38	63.37	16.20	80.00	60.08	66.66	10.02	

*Analysis of variance ANOVA.

Participatnts who met the inclusion criteria were informed about changing into sports clothes (t-shirt and shorts) and were directed to warm-up activities, including riding a spinning bike with a load of 70 watts (W) and a duration of 5 minutes (min) and an elliptical bike afterwards (power: 80W, cadence of 70 RPM, 5 min). The tests were performed without shoes.

Morphological analysis

Body composition analysis was performed using the Tanita scale (Tanita BC-418, Tanita Corp., Tokyo, Japan). A bioelectric resistance method was used to assess the following parameters: body weight (kg), BMI (kg/m^2), body fat mass (%), fat free mass (kg). Height (cm) was measured using Wall-Mounted Height Rod (Tanita HR-200, Tanita Corp., Tokyo, Japan). The measurement was performed in accordance with the manufacturer's instructions. Due to the dress code, all participants were set to -0.5 kg body weight before the measurement.

International Physical Activity Questionnaire

The IPAQ short questionnaire is a tool designed primarily for population surveillance of physical activity among adults. The specific types of activity are assessed as walking, moderate-intensity activities and vigorous-intensity activities (28). The respondent answered on his own and the questions concerned 7 days before the assesment.

Assessment of abdominal muscle thickness

The thickness of abdominal muscles was measured utilizing ultrasound system (Edan DUS 60, Edan Instruments, Shenzhen, China) (USG) with a linear probe of 10 MHz frequency, B-mode. The study was conducted and performed by a certified researcher with 3 years of experience in the field of musculoskeletal system ultrasound examinations and based on the methology elaborated by Niewiadomy *et al.* (29).

The interpretation included thickness of three abdominal muscles: transverse abdominal muscle (TrA), internal oblique muscle (IO) and external oblique muscle (EO). Moreover, the results were also analyzed with a particular consideration of the total thickness of all three muscles (TrA + IO + EO) from the lower edge of TrA to the upper edge of EO and not including the intermuscular septums. The measurement was noted with an accuracy of 0.01 mm and was repeated five times.

Fingertip-to-Floor Test

In the Fingertip-to-Floor Test, the subjects started in a free standing position on the platform specially designed for this test so that the spinal flexion range could be evaluated in the maximum trunk forward bend with legs remaining straight - bending the knees was not allowed (30, 31). The placement of hands on a centimeter scale in their final position provided a score ranging from - 30 cm to + 30

cm, which means that the higher the value, the greater the mobility range. Placing a 5 centimeters (cm) high original FMS platform (Functional Movement System, Inc., Chatham**,** USA) under the feet was a modification of the test. The changes were applied in two ways: by placing the platform under the subject's heel and under the forefoot to influence the tension of the posterior myofascial chain. The measurement, being repeated three times, was noted with an accuracy of 1 cm.

Sit and Reach Test

The Sit and Reach Test assessed the maximum forward flexion of the trunk in a sitting position with the knees extended, feet together and soles of the feet placed against the edge of the box (32). This test was also performed using the dedicated platform (the same as in the Fingertip to floor test) and continued until three results were obtained. The calculations were noted with an accuracy of 1 cm.

Lateral flexion of the spine

The lateral flexion of spine was tested and evaluated with the subject sitting on the edge of the chair with the feet hip-width apart. The plane of motion was marked by a pole placed on participant's shoulders. Measurements were made with a digital inclinometer (Saunders Group Inc., Chaska, USA) placed in the center of the pole. The subject alternately executed the movement of lateral flexion in both directions (31). The movement was repeated three times with an accuracy of 1°.

Statictical analysis

Statistical analysis was performed on the basis of the Statistica 13.3 (TIBCO Software Inc., CA., USA) program. The Kolmogorov-Smirnov test (K-S) with Lileforse's correction was used to assess the normality of the distribution. Gathered quantitative variables are presented in the form of position measures with arithmetical mean (x), and measures of variability with standard deviation (SD). Additionally, the minimum (min) and maximum (max) values and Confidence Intervals (CI) are given. The Analysis of Variance Test ANOVA was used to calculate the level of intergroup differences for quantitative variables with the estimation of the F-value, while to assess the relationship between the variables, the Pearson correlation coefficient (r) was computed. For the tests, an Intraclass Correlation Coefficient was calculated for one researcher (ICC3.1). Statistical significance level was determined as $p < 0.05$. Moreover, the test power was calculated for individualy analyzed variables in relation to the number of variables. In all cases, the power of the test was valid for the null hypothesis.

RESULTS

Both abdominal muscle thickness measurements and the outcomes of mobility tests are summarized in **tables II** and **III**. Statistically significant differences between the groups were demonstrated in the thickness of IO ($p = 0.0057$), EO ($p = 0.0079$) and in the total measurement of TrA + IO + EO ($p = 0.0020$). The mean values of muscle thickness in male group were greater than in women and equaled 10.19 mm ± 2.8 for IO; 9.1 mm ± 3.6 for EO and 22.47 mm ± 5.7 for TrA + IO + EO. However in female group, the mean thickness was 8.77 mm ± 1.6 for IO, 7.44 ± 1.6 for EO and 19.16 mm ± 3 for total measurement of TrA + IO + EO.

Table II. Abdominal muscle thickness measured by ultrasound, divided by gender.

Variable	Sex	N	X	Min	Max	CI - 95%	CI 95%	SD	p*
TrA (mm)	Women	42	2.96	1.48	4.44	2.73	3.18	0.72	0.2452 F-1.37
	Men	38	3.17	1.76	5.52	2.87	3.47	0.92	
IO (mm)	Women	42	8.77	5.19	11.64	8.28	9.25	1.56	0.0057 F-8.1
	Men	38	10.19	6.52	17.68	9.27	11.11	2.80	
EO (mm)	Women	42	7.44	3.72	12.12	6.93	7.94	1.61	0.0079 F-7.4
	Men	38	9.11	4.76	21.34	7.92	10.29	3.60	
TrA + IO + EO (without muscle septum) (mm)	Women	42	19.16	12.17	24.34	18.21	20.11	3.05	0.0020 F-10.8
	Men	38	22.47	14.04	35.42	20.59	24.34	5.69	

*Analysis of variance ANOVA.

Table III. Test results: Fingertip-to-Floor, Fingertip-to-Floor (heel), Fingertip-to-Floor (inverted heel), Sit and Reach, lateral flexion to the right and to the left, divided by gender.

Variable	Sex	N	x	Min	Max	CI - 95%	CI 95%	SD	p*
Fingertip-to-Floor Test (cm)	Women	42	1.89	- 22.33	19.00	- 1.11	4.89	9.63	0.3476
	Men	38	4.05	- 18.33	24.00	0.49	7.61	10.85	
Fingertip-to-Floor (heel), (cm)	Women	42	-0.33	- 24.00	15.33	- 3.07	2.40	8.78	0.5919
	Men	38	0.82	- 20.33	19.33	- 2.61	4.26	10.45	
Fingertip-to-Floor (inverted heel) (cm)	Women	42	-2.22	- 24.00	15.00	- 5.24	0.80	9.70	0.3503
	Men	38	0.02	- 21.00	23.33	- 3.80	3.83	11.61	
Sit and Reach Test (cm)	Women	42	5.06	- 16.00	20.33	2.19	7.94	9.22	0.4771
	Men	38	6.65	- 14.33	24.00	3.15	10.14	10.63	
Lateral flexion to the right (degrees)	Women	42	55.48	33.33	75.67	52.33	58.64	10.14	0.8000
	Men	38	56.11	31.00	90.00	52.15	60.07	12.05	
Lateral flexion to the left (degrees)	Women	42	53.93	31.33	74.00	50.63	57.23	10.59	0.6197
	Men	38	55.17	37.00	89.33	51.34	58.99	11.64	

*Analysis of variance ANOVA.

The mean values of TrA thickness and the Fingertip-to-Floor, Sit and Reach and lateral spinal flexion tests did not differ significantly between men and women.

By analyzing the influence of the remaining morphological variables on the thickness of abdominal muscles, statistically significant weak correlations were obtained in the group of women between: muscle mass and IO ($r = - 0.308$, $p = 0.047$); fat mass and TrA ($r = - 0.3305$, $p = 0.033$). However, in the group of men significant correlations were found between: body weight and IO ($r = - 0.3743$, $p = 0.021$) as well as TrA + IO + EO ($r = - 0.3899$, $p = 0.016$); between BMI and IO ($r = - 0.3495$, $p = 0.031$) as well as TrA + IO + EO ($r = - 0.3279$, $p = 0.045$); between fat mass and IO ($r = - 0.3316$, $p = 0.042$); as well as between fat free mass and EO ($r = - 0.6492$, $p = 0.000$) and TrA + IO + EO ($r = - 0.4688$, $p = 0.003$).

The correlation between muscle thickness and mobility tests was only shown in the group of women between the thickness of TrA and lateral spinal flexion: to the right ($r = 0.3492$, $p = 0.023$); to the left ($r = 0.3181$, $p = 0.040$). This correlation also proved weak.

All the tests used in the study have shown excellent reliability: ICC3.1 = 0.98 for abdominal muscles thickness, ICC3.1 = 0.99 for Fingertip-to-Floor Test, ICC3.1 = 0.98 for The Sit and Reach Test and ICC3.1 = 0.97 for measurement of Lateral flexion of the spine in the frontal plane.

DISCUSSION

The best known differences in body composition and proportions of men and women according to common knowledge were confirmed in this study such as height, body mass, BMI and fat free mass - higher in the group of men, and fat mass higher in woman. Furthermore, an ultrasound was used to measure the thickness of the abdominal muscles, which is a common method for assessing morphological changes of muscles. The high reliability of this tool at the ICC level of 0.98, also confirmed by other authors, should also be noted (29, 33-35). In the group of men, greater thickness of the internal (on average by 1.42 mm) and external oblique abdominal muscles (on average by 1.67 mm) was obtained, as well as in total measurement of the muscle complex of the lateral abdominal wall (on average by 3.31 mm) compared to the group of women. Assessing by their proportion, regardless of gender, the thickest muscles of the complex is the internal oblique muscle. In both groups, the internal oblique muscle constituted slightly more than 45% of the entire complex thickness. This relationship has been confirmed in other studies (34-36).

Interestingly, no statistically significant differences were found at the level of the transverse abdominal muscle, which is main active stabilizer of the lumbar spine. This can be explained by a deliberate selecion of the subjects, which accounted for healthy, young and professionally active people with no chronic low back pain. Hence, no atrophic changes in the abdominal muscles resulting from impaired motor function, weakened stabilization of the lumbo-pelvic-hip complex or other degenerative deviations in the spine or intervertebral disc were to be expected. In addition, the transverse abdominal muscle is a local muscle composed in the most part of tonic, slow-twitch muscle fibers (ST). In

the study by Linek *et al.* (37), thickness of that muscle was assessed by additionally optimizing the measurement with normalization of body weight according to described algorithm. As in our own study, no differences in its thickness relative to sex were found. On the contrary, the external oblique muscle consists predominantly of fast-twitch phasic fibers, and this type of muscle has different characteristics. The study by Jee *et al.* (38) estimated the influence of both sex and the aging process on the mechanical functions of the vastus lateralis muscle by examining its single fiber. It has been demonstrated that the mechanical and functional properties of the muscle, including its power, the fibers' shortening velocity and the maximum force of contraction is influenced by gender to a greater extent than the aging process. These discrepancies were also apparent in the muscle's cross section. Successively, Krivickas *et al.* (39) showed that the quality of single muscle fibers in older women and men is the same, so it cannot explain the differences in the strength, power and function of the entire muscle. It appeares that it is the tonic or phasic composition of muscle fibers and the muscle function they determine that are of greatest importance, and not the sex differences themselves. The transverse abdominal muscle as a trunk stabilizer shows no difference in thickness between men and women, but several differences are observed in the global muscles that are responsible for one's performance; including strength, speed or power.

There are studies that indicate differences in the thickness of the TrA muscle in men and women, but in our judgement, their limitation lies in the methodology - the use of convex probe instead of linear probe, which significantly affects the objectivization of measurements (40) as well as conducting research on a small number of subjects (41).

In our study, no statistically significant differences were found in the mobility of the trunk in the frontal and sagittal planes. The men obtained higher mean scores on each test. Many authors have used the Fingertip-to-Floor and Sit and Reach tests in various ways to evaluate mobility. For instance, Kuszewski *et al.* (42) in their study focused on the assessment of mobility in two groups - physically active and inactive people, of which the first group had better mobility results. A similar study was conducted by Knapik *et al.* (43), in which, using the Fingertip-to-Floor, Sit and Reach tests and lateral flexion of the spine tests, better results were also obtained in the group of physically active people. Gender was not taken into account as a differentiating variable in this research. In the study by Minarro *et al.* (44) the range of the spinal flexion was approached by measuring the thoracic and lumbar angles during performing numerous variants of the Sit and Reach and Fingertip-to-Floor tests. Interestingly, in all tests, the group of men acquired higher values of the thoracic angle than the group of women. The authors also point out the exceptional consistency of these trials, which have been used unchangingly for 50 years. Nevertheless, Jackson and Langford (45), while analyzing male and female Sit and Reach test results, discovered that women, in turn, obtained better results. As in this study, they used a centimeter scale. Finally Youdas *et al.* (46) in Sit and Reach test noticed a greater range of flexion of the hip joints also in the group of women compared to men.

An analysis of the research conducted so far shows that failure of the abdominal muscles, which act as local stabilizers, may result in compensation in the form of shortening of the hamstring muscle group, and thus a reduction in mobility (44-49). However, in this study it was not possible to establish a relationship between the thickness of abdominal muscles and the results of selected mobility tests, which is probably related to the lack of dysfunctional symptoms of the lumbar spine in the group of respondents taken into account in other publications.

In summary, the methodology used in this publication is the mobility tests and measurements of the abdominal muscle thickness utilizing an ultrasound is reliable and often used by reaserchers. Differences in the basic morphological parameters characterizing the studied group and in the thickness of abdominal muscles have been demonstrated.

In our opinion, the lack of differences between sex in the transverse abdominal muscle allows for the interpretation of its results without division into sex. It is different in the case of internal and external oblique muscles, where this division should be taken into account. It should be assessed whether other deep muscles, apart from the transverse abdominal muscle, are differentiated by gender. This requires further research into the mechanism of central stabilization.

Some limitations were highlighted in the study.The lack of divergence between men and women in the results of the mobility tests may be related to the selection criteria for the comprised group - which was a limitation to the results. The subjects were young, healthy people with a correct body weight and standard physical and professional activity. Their BMI value was a condition for obtaining high reliability of ultrasound measurements.

CONCLUSIONS

Gender differentiates the thickness of the muscles of the lateral abdominal wall. In the group of men there is a greater thickness of the internal and external oblique muscle, as well as the entire TrA + OI + OE muscle complex.

No relationship between sex and the thickness of the transverse abdominal muscle was demonstrated.

There were no differences in the mobility of the trunk between women and men.

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DATA AVAILABILITY
Data are available under reasonable request to the corresponding author.

CONTRIBUTIONS
All authors contributed equally to the manuscript and read and approved the final version of the manuscript.

CONFLICT OF INTERESTS
The authors declare that they have no conflict of interests.

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