

I.S.Mu.L.T. Recommendations for Intra and Periarticular Injections during COVID-19 Pandemic

F. Oliva¹, F. Vittadini², A. Frizziero³, C. Costantino³, A. Fusco⁴, A. Gaii Via⁵, C. Foti⁶, G. Nanni⁷, A. Forte⁸, C. Biz⁹, M.C. Vulpiani¹⁰, A. De Carli¹⁰, A.C. Berardi¹¹, M. Fini¹², J. Padulo¹³, N. Maffulli¹

¹Department of Musculoskeletal Disorders, Faculty of Medicine and Surgery, University of Salerno, Salerno, Italy

²Department of Physical and Rehabilitation Medicine, Casa di Cura Policlinico San Marco, Venice, Italy

³Department of Medicine and Surgery, University of Parma, Parma, Italy

⁴Clinical Laboratory of Experimental Neurorehabilitation, I.R.C.C.S. Santa Lucia Foundation, Rome, Italy

⁵Department of Emergency and Acceptance, Orthopaedics and Traumatology Unit, San Camillo-Forlanini Hospital, Rome, Italy

⁶Physical and Rehabilitation Medicine, University of Rome Tor Vergata, Rome, Italy

⁷Isokinetic Medical Group, FIFA Medical Centre of Excellence, Bologna, Italy

⁸Biomedical Research Centre, Gruppo Forte, Salerno, Italy

⁹Department of Surgery, Oncology and Gastroenterology DiSCOG, Orthopedic Clinic, University of Padova, Padova, Italy

¹⁰Physical Medicine and Rehabilitation Unit, Sant'Andrea Hospital, "Sapienza" University of Rome, Rome, Italy

¹¹UOC Immunohematology and Transfusion Medicine Laboratories, Laboratory of Stem Cells, Spirito Santo Hospital, Pescara, Italy

¹²Laboratory of Preclinical and Surgical Studies, Rizzoli Orthopedic Institute, Bologna, Italy

¹³Department of Biomedical Sciences for Health, Università degli Studi di Milano, Milan, Italy

DOI:

10.32098/mltj.03.2020.00

INTRODUCTION

SARS-CoV-2 is the coronavirus involved for the ongoing pandemic, responsible of the disease that has been identified as COVID-19 (COronaVIrus Disease-19) (1,2).

In most cases, transmission occurs via saliva droplets in closed or poorly ventilated environments (particularly public and workplaces), unclean hands and rarely via contaminated surfaces (1).

At present, it is unclear whether SARS-CoV-2, can be harbored in the synovial fluid, though musculoskeletal symptoms as myalgia, arthralgia and asthenia are frequent (3-5). Rapid tests based on the identification of specific IgM and IgG antibodies should not replace the molecular test based

on the identification of viral RNA from nasopharyngeal swabs (6,7).

Serological testing can be used for research and retrospective epidemiological purposes, while the possible role in diagnosing ongoing SARS-CoV-2 infection need further evidence considering high false negative and false positive risk (5).

PRE-TRIAGE AND OUTPATIENT ACCESS

The first outpatient access should be preceded by a telephone triage 48-72 hours before the consultation, in which the operator should be supported by a dedicated question-

naire (figure 1), aimed to unveil the possible presence of suspicious symptoms or close contacts. Triage should be repeated at each subsequent access, and patients should be instructed to alert the operators whether any change in health status occurred.

By telephone, the operator should also provide information on access rules:


1. The patient must wear a surgical mask.
2. The patient can be accompanied by only one person.

When suspicious symptoms are identified during telephone triage, the doctor should contact the patient directly,

and invite them to contact the general practitioner (GP). GPs should also be alerted when patients meet the criteria of suspected or close contact of a confirmed case. For the purpose of the European Centre for Disease Prevention and Control, a close contact of a confirmed case, has been clearly defined (8).

When a patient attends the clinic should be measured the temperature with a contactless device.

When temperature is inferior to 37.5°C, patients are allowed to access, providing them with all the necessary PPE. On the other hand, if the temperature exceeds 37.5°C, the patient cannot access and will be referred to the GP.



**ISMuLT TRIAGE QUESTIONNAIRE FOR
COVID-19 PREVENTION**

TELEPHONE TRIAGE AMBULATORY TRIAGE

SURNAME	NAME
DATE OF BIRTH	BIRTHPLACE
RESIDENCE	PROVINCE

Do you have or have you had one or more of these symptoms in the last 14 days?

<input type="radio"/> Temperature	<input type="radio"/> Alteration in taste
<input type="radio"/> Cough	<input type="radio"/> Alteration in smell
<input type="radio"/> Sore Throat	<input type="radio"/> Fatigue
<input type="radio"/> Headache	<input type="radio"/> Dyspnea
<input type="radio"/> Diarrhea	<input type="radio"/> Vomit

One of the following has occurred in the last 14 days:

I have had confirmed family members / cohabitants / close contacts with COVID-19

I have had family members / cohabitants / close contacts considered suspected / probable for COVID-19

I have executed COVID-19 swab on (day/month/year) _____ resulted:

<input type="radio"/> POSITIVE	<input type="radio"/> NEGATIVE
<input type="radio"/> WAITING FOR REPORTING	<input type="radio"/> DOUBT

I declare that I am cured of COVID-19 (attach certificate of healing)

I declare that I am in the mandatory quarantine status

The undersigned

Date _____

The healthcare worker

Date _____

Figure 1. ISMuLT questionnaire.

AMBULATORY SETTING AND PREVENTIVE MEASURES

Healthcare workers should always be equipped with PPE:

- since the injection procedure does not expose to droplets, the surgical mask should be sufficient (suspected or symptomatic cases cannot access to the procedure);
- the nurse equipped with a surgical mask may be present together with the doctor;
- the accompanying person should remain in the waiting room.

In ultrasound-guided and ultrasound-assisted procedures, the probe must be properly disinfected and a disposable probe cover should always be used (9).

It is mandatory to disinfect the contact surfaces (table, chairs) and the bench at each appointment.

Furthermore, appropriate ventilation of the environment must be ensured.

INJECTION PROCEDURE

Intra-articular and peri-articular injections can be practised in an ambulatory setting or in hospital outpatient department (10), according to a sequential procedure (**table I**).

Written or verbal informed consent should be obtained. In case of injection procedures, it is always necessary to discuss the risks, benefits and alternatives with the patient (12).

The presence of contraindications should be investigated:

- systemic infections;
- suspect or presence of infections at injection skin site or selected joint;
- fractures;
- prosthesis at injection site;
- coagulopathies;
- hypersensitivity to products.

Relative contraindications include:

- presence of infection in another site of the body;
- hypersensitivity to other injection products (not used);
- Diabetes Mellitus (10-12).

Injection therapy in patients on anticoagulant drugs or with known bleeding diathesis should be approached with great caution. All coagulation parameters should be evaluated in these patients, including prothrombin time (PT), activated partial thromboplastin time (aPTT), international normalized ratio (INR), and platelet count. Injections should be avoided with prolonged bleeding time, INR > 1.2, and platelet count < 100,000 / μ l(12).

Various side effects can occur during the injection procedure; however, most adverse events are minor, transient and localized at the injection site (13).

Table I. Step-by-step procedure of intra-articular and periarticular injection (10-12).

Clinical exam; evaluate injection indications; evaluate comorbidities
Explain and sign the informed consent; discuss possible risks and advantages
Preparation of the necessary instruments (disinfectant, sterile material, drugs, needles, syringes, dressings); select needle length and gauge
Prepare patient; identify anatomical landmarks
Skin disinfection; prepare sterile field
Proceed with arthrocentesis when needed
Proceed with injection
Remove the needle rapidly after injection and perform local compression with sterile gauze
Disinfection and patch application
Instruct the patient to notice if any symptom or infection sign is detected; schedule follow-up visit

The most common complications are sudden onset of local pain lasting no more than 72 hours (2-10% of cases), skin atrophy (1%), skin fat atrophy (1%), and appearance of redness of the face (1-12%) (13).

Less commonly reported side effects may include iatrogenic infection (risk of 1 in 1,000) and tendon rupture (less than 1%). The risk of tendon rupture is higher during soft tissue injections around the Achilles tendon and plantar fascia (14).

Vasovagal episodes can occur with any type of injection procedure due to the nociceptive stimulation effect of the needle. The treatment is mainly supportive; it is rarely necessary to administer fluids and / or oxygen (12).

Required equipment

The equipment required is:

- gloves / sterile gloves;
- sterile swabs and sterile draps;
- prepacked sterile needles and syringes;
- disinfectant (iodopovidone / chlorhexidine);
- synovial fluid collection bottles;
- sterile ultrasound kit (only for US-guided injection);
- emergency kit (10,15,16).

The choice of the right needle is mandatory, considering the characteristics of the target joint. A needle of 21 gauge should be preferred in large joints such as the shoulder or the knee, while 23-25 gauge needles are indicated in small joints. Deep joints such as the hip required spinal needles (length of 3,5 18 inches; 8-9 centimeters) (10,12).

HYALURONIC ACID PROPERTIES

Several studies support the role of viscosupplementation with hyaluronic acid (HA) in knee and hip osteoarthritis, in comparison to intra-articular corticosteroids (10,17-19). Emerging evidences suggest that the use of intra-articular corticosteroids could also determine a higher risk of viral infections, such as influenza, while no evidence about coronaviruses are currently found (20).

HA protects the articular environment through several mechanisms of action: anti-inflammatory effect, chondroprotection, analgesic effect, subchondral bone protection and increased production of endogenous HA (21).

REFERENCES

1. Contini C, Di Nuzzo M, Barp N, *et al.* The novel zoonotic COVID-19 pandemic: An expected global health concern. *J Infect Dev Ctries* 2020;14(3):254-264.
2. Ng OW, Tan YJ. Understanding bat SARS-like coronaviruses for the preparation of future coronavirus outbreaks - Implications for coronavirus vaccine development. *Hum Vaccin Immunother* 2017;13(1):186-189.
3. Cipollaro L, Giordano L, Padulo J, Oliva F, Maffulli N. Musculoskeletal symptoms in SARS-CoV-2 (COVID-19) patients. *J Orthop Surg Res* 2020;15(1):178. doi: 10.1186/s13018-020-01702-w.
4. López-González MdC, Peral-Garrido ML, Calabuig I, *et al.* Case series of acute arthritis during COVID-19 admission. *Ann Rheum Dis* 2020 <http://dx.doi.org/10.1136/annrheumdis-2020-217914>.
5. Alivernini S, Cingolani A, Gessi M, *et al.* Comparative analysis of synovial inflammation after SARS-CoV-2 infection. *Ann Rheum Dis* 2020;annrheumdis-2020-218315. doi: 10.1136/annrheumdis-2020-218315.
6. WHO. Laboratory testing for coronavirus disease (COVID-19) in suspected human cases. Interim Guidance March 2020.
7. WHO. Laboratory testing strategy recommendations for COVID-19. Interim Guidance March 2020.
8. European Centre for Disease Prevention and Control. www.ecdc.europa.eu/en/covid-19/surveillance/surveillance-definitions.
9. Nyhsen CM, Humphreys H, Nicolau C, Mostbeck G, Claudon M. Infection Prevention and Ultrasound Probe Decontamination Practices in Europe: A Survey of the European Society of Radiology. *Insights Imaging* 2016;7(6):841-847.
10. Frizziero A, Vittadini F, Oliva F, *et al.* I.S.Mu.L.T. Hyaluronic acid injections in musculoskeletal disorders guidelines MLTJ 2018;8(3)364-398. doi: 10.32098/mltj.03.2018.04.
11. Stephens MB, Beutler AI, O'Connor FG. Musculoskeletal injections: a review of the evidence. *Am Fam Physician* 2008;78(8):971-6.
12. Douglas L, PCRS Members. The Primary Care Rheumatology Society Joint and Soft Tissue Injection Guidelines. *The Primary Care Rheumatology Society* 2014;12 p.
13. Falco FJE, Obi Onyewu C, Lee Irwin F Jr, Kim DW, Zhu J. Peripheral Joint, Soft Tissue and Spinal Injection Techniques. In: Braddom RL (eds). *Physical Medicine and Rehabilitation*. 3rd edition. Elsevier Health Science, 2006;pp. 541-562.
14. Courtney P, Doherty M. Joint aspiration and injection. *Best Pract Res Clin Rheumatol* 2005;19(3):345-69.
15. Kaux JF, Samson A, Crielaard JM. Hyaluronic acid and tendon lesions. *Mus Lig Ten J* 2015;5(4):264-269. doi: 10.11138/mltj/2015.5.3.264.
16. Jackson DW, Evans NA, Thomas BM. Accuracy of needle placement into the intra-articular space of the knee. *J Bone Joint Surg Am* 2002;84-A(9):1522-7.
17. Piccirilli E, Oliva F, Aconstantinesci Murè M, *et al.* Viscosupplementation with intra-articular hyaluronic acid for hip disorders. A systematic review and meta-analysis. *Mus Lig Ten J* 2016;6(3):293-299. doi: 10.11138/mltj/2016.6.3.293.
18. Henrotin Y, Raman R, Richette P, *et al.* Consensus statement on viscosupplementation with hyaluronic acid for the management of osteoarthritis. *Semin Arthritis Rheum* 2015;45(2):140-9.
19. Ayhan E, Kesmezacar H, Akgun I. Intraarticular injections (corticosteroid, hyaluronic acid, platelet rich plasma) for the knee osteoarthritis. *World J Orthop* 2014;5(3):351-61.
20. Sytsma TT, Greenlund LK, Greenlund LS. Joint Corticosteroid Injection Associated With Increased Influenza Risk. *Mayo Clin Proc Innov Qual Outcomes* 2018;2(2):194-198.
21. Altman RD, Manjoo A, Fierlinger A, Niazi F, Nicholls M. The mechanism of action for hyaluronic acid treatment in the osteoarthritic knee: a systematic review. *BMC Musculoskel Dis* 2015;16:321.

Three-Dimensional Architecture of the Acetabular Transverse Ligament and its Connection with the Acetabular Labrum

N. Kaku¹, T. Shimada², R. Nogami³, H. Tagomori¹, H. Tsumura¹

¹ Department of Orthopaedic Surgery, Faculty of Medicine, Oita University, Yufu City, Oita, Japan

² Oita College of Judo Therapy and Acupuncture-Moxibustion, Oita City, Japan

³ Oita University Graduate School of Medicine, Graduate School of Orthopedic Surgery, Yufu City, Oita, Japan

CORRESPONDING AUTHOR:

Nobuhiro Kaku
Department of Orthopaedic Surgery
Faculty of Medicine, Oita University
Idaigaoka Hazama-machi
879-5593 Yufu City, Oita, Japan
E-mail: nobuhiro@oita-u.ac.jp

DOI:

10.32098/mltj.03.2020.01

LEVEL OF EVIDENCE: 5

SUMMARY

Background. We clarified the microstructure of the acetabular transverse ligament in terms of the construction and arrangement of the collagen fibers constituting the ligament and their connection with the acetabular labrum using light and scanning electron microscopy (SEM).

Methods. Deparaffinized blocks were treated with 2N NaOH to digest the cell matrix, allowing the collagen fibers constituting the ligament and cartilage to be observed under SEM.

Results. The acetabular transverse ligament had parallel collagen fibers with a width of 30–50 μm , which consisted of bundles with type I collagen fibrils. The collagen fibers of the transverse ligament were interwoven between the cartilage tissues of the acetabular labrum, forming a layered structure. These components seemed to be directly joined to each other histologically. However, in SEM images, the collagen fibers were not directly connected; rather, fine collagen fibers with varying diameters extending from the structures were interwoven.

Conclusions. The acetabular transverse ligament had a distinctly different microstructure than the acetabular labrum. At the connection of both tissues, we found that the microstructure was not a direct adhesion but rather a structural bond, at least with respect to collagen. The results suggest that ligaments potentially attach to tissues other than bone.

KEY WORDS

Acetabular labrum; acetabular transverse ligament; collagen fibrils; microstructure; scanning electron microscopy.

BACKGROUND

The acetabular labrum of the hip joint is not continuous, but rather horseshoe-shaped, with its base connected by the acetabular transverse ligament. This is unlike the shoulder joint with the labrum, which exists all around the glenoid fossa. The acetabular transverse ligament crosses the acetabular incision and visibly connects to the acetabular labrum at the anterior and posterior parts of the acetabular notch (1). The ligament adheres not only to the acetabular labrum but also to the bone, forming a sucker-like structure surrounding the lower part of the femoral head. It has elements that function in conjunction with the acetabular labrum which

helps prevent the microinstability of the hip joint (2,3). The shape of the acetabulum varies among ethnic groups(4), and the acetabular transverse ligament and labrum are easily affected, such as in cases of inversion or damage to them, when acetabular dysplasia is present (5,6).

The acetabular diameter is smaller than the femoral head diameter when unloaded (7). The anterior and posterior corners of the lunar surface open with loading to disperse the load, but the acetabular transverse ligament is positioned anteriorly and posteriorly and is thought to spread the force between the corners and participate in load distribution (1). However, a study reported that the amount of

load on the lunar surface does not change after acetabular transverse ligament excision (8). Thus, there are many uncertainties regarding the function of the acetabular transverse ligament. However, the acetabular transverse ligament is considered to be a factor that limits femoral head reduction in the developmental dislocation of the hip, and its excision and resection release the acetabular entrance, rendering the femoral head easier to reduce to the acetabulum (9,10). The acetabular transverse ligament is easy to visualize, even in the joints with arthritic changes; thus, it is clinically useful (e.g., as an indicator of acetabular component placement in total hip arthroplasty) (11-13).

Ligaments usually connect to bones; however, the transverse ligament also connects to the anterior and posterior angles of the acetabulum. It is rare for a ligament to appear clearly attached to a tissue other than bone, as noted in the case of the acetabular labrum. Thus, it would be interesting to observe how these two systems are connected. To achieve this goal, the microstructure of the main collagen fiber components of the acetabular transverse ligament must be clarified. Treatment of a deparaffinized block with 2N NaOH enables the digestion of the cellular matrix and observation of the collagen fibers that make up the ligament, cartilage, and bone (14).

By observing these samples three-dimensionally using scanning electron microscopy (SEM), it has become possible to observe the microstructure, namely, the collagen fibers, acetabular labrum, and ligamentum teres of the hip joint (15,16). In addition, type I collagen fibrils in the collagen tissue and tendon have a diameter of approximately 150 and 190 nm, respectively (17). The ligament is predominantly composed of type I collagen (17,18), whereas the diameter of type II collagen fibrils in the hyaline cartilage is 75 nm on average, and the predominant collagen type is type II (17,19). This knowledge is useful for collagen typing when performing SEM. However, the microstructure of the acetabular transverse ligament itself, namely of its collagen fibers, has not yet been clarified as it has in other ligaments. Furthermore, the mechanism underlying the connection between the histologically distinct collagen fibers of the acetabular transverse ligament and those of the acetabular labrum remains unknown. Therefore, the purpose of this study was to clarify the microstructure of the acetabular transverse ligament in terms of the construction and arrangement of the collagen fibers and their connection to the acetabular labrum using light microscopy (LM) and SEM.

MATERIALS AND METHODS

The acetabular transverse ligaments and labrums obtained from a 69-year-old woman and an 82-year-old woman during

total hip replacement with femoral head necrosis and femoral neck fracture, respectively, were used for the experiments. The acetabular transverse ligaments and labrums were fixed with 10% formalin immediately after sample collection. After fixation, the specimens were immersed in a 5% ethylenediaminetetraacetic acid (EDTA; Wako Pure Chemical Industries, Osaka, Japan) solution for 2 weeks for decalcification. The specimens were then cut into 5- to 10-mm squares, fixed in 10% formalin or 4% paraformaldehyde in phosphate buffer (pH 7.4), dehydrated, and embedded in paraffin.

LM examination

Paraffin sections (5–10 μ m) were stained with hematoxylin and eosin (HE; pH 2.5) or alcian blue (AB; pH 1.0 and 2.5) to assess the carbohydrate reactions histochemically and observed under LM (Nikon ECLIPSE Ci-L, Tokyo, Japan) at 2 \times or 4 \times magnification.

The slides were deparaffinated and rehydrated with decreasing ethanol passages. Masson's trichrome staining was performed as described below. After washing with running water for 3 minutes and rinsing with distilled water, the slides were treated for 10 minutes with a dedicated mordant (Muto, Tokyo, Japan) for Masson's trichrome staining. Next, the slides were washed with running water for 5 minutes and rinsed with distilled water. To differentiate the nuclei, the slides were then immersed in Carrazzi's hematoxylin for 15 minutes and rinsed in 0.5% hydrochloric acid water. In order to stain the cytoplasm and erythrocytes, the slides were submerged in Masson B for 15 minutes and then rinsed twice with 1% acetic acid water. Next, the slides were treated with phosphotungstic acid solution, a mordant, for another 6 minutes. After washing them twice with 1% acetic acid, the slides were immediately submerged in aniline blue solution for 3 minutes to stain the fibroblasts and collagen. After subsequent washing with 1% acetic acid twice, the slides were dehydrated quickly in 100% ethanol, cleared in xylene, and mounted onto coverslips using a mounting medium.

SEM examination

The remaining paraffin blocks were deparaffinized with xylene overnight and re-fixed with a 2.5% glutaraldehyde and 2.0% formaldehyde solution for SEM after observation under an optical microscope. Furthermore, after being immersed in the 2N NaOH solution at 37°C for 3 hours to digest the extracellular matrix, the samples were thoroughly washed with physiological saline and placed in 1% osmium tetroxide, 1% tannic acid, and 1% osmium tetroxide for 1 hour each, dehydrated with alcohol, and then freeze-dried with butyl alcohol. The dried specimens were coated with

a 4-nm thick gold layer (E102 Ion Sputter, Hitachi, Tokyo, Japan) and examined using SEM (S-4800, Hitachi, Tokyo, Japan) at 5 Kv (14).

Fifty collagen fibrils from multiple microphotographs were used to determine the mean diameter of the collagen fibrils using Java ImageJ software version 1.46d (National Institute of Health, Bethesda, MD). The mean chondrocyte size was determined using 25 chondrocytes from different microphotographs at the same magnification (1000×). To investigate the acetabular labrum's fibrous cartilage and transverse ligament, the collagen was classified as type I or II based on the diameter of the collagen fibrils (type I, approximately 150 nm; type II, 75 nm) (17).

The study procedures were conducted ethically in accordance with international standards, as required by the journal and as described by Padulo *et al.* (20). This study was approved by the Ethics Committee of Oita University (approval number, 1053; approval date, 22 July 2016). All experimental procedures were performed in accordance with the Declaration of Helsinki. Informed consent was obtained from all patients for study participation and publication of their clinical information.

RESULTS

Macroscopic findings

A schematic drawing of the constitution of the acetabular labrum and transverse ligament is shown in **figure 1a**. In a

normal joint, tissues of the acetabular transverse ligament and the labrum are continuous with one another (**figure 1b**).

LM findings

The acetabular transverse ligament is composed of bundles of collagen fibers and was, thus, eosinophilic in HE-stained specimens but negatively stained in AB-stained (pH 1.0 and 2.5) specimens. In contrast, the acetabular labrum is composed of fibrocartilage; thus, it stained bright in HE-stained specimens and strongly in AB-stained specimens (**figure 2**). The collagen fibers of the acetabular transverse ligament appeared to be penetrating and surrounding the cartilage tissue of the acetabular labrum in enlarged LM images of the connection of both tissues (**figure 3**). Masson trichrome staining revealed clear wave-depressing collagen fibers in the acetabular transverse ligament (**figure 4**).

SEM examination

In low-magnification SEM images of the acetabular transverse ligament, bundles of 30–50- μ m wide collagen fibers were arranged in parallel (**figure 5**). The collagen fibrils composing the acetabular transverse ligament had a mean diameter of 161.28 ± 16.79 (range: 133.3–189.7) nm, forming collagen fibrils classified as type I (**figure 6**).

In SEM images of the acetabular labrum, the cartilage tissue was composed of chondrocytes and extracellular matrix (**figure 7**). In high-magnification SEM images of the chon-

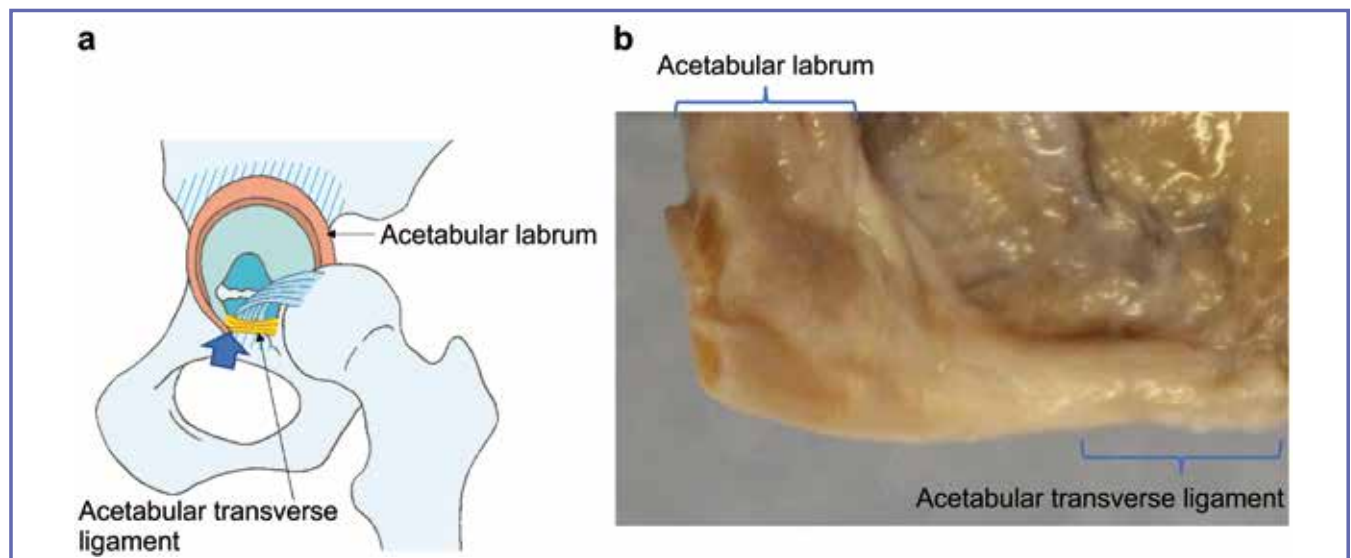


Figure 1. Macroscopic appearance of the transitional part of the acetabular transverse ligament (L) and acetabular labrum (AL). **a.** The large arrow in the schematic drawing indicates the junction between the AL and the L. **b.** Both tissues appear to be connected without clear boundaries.

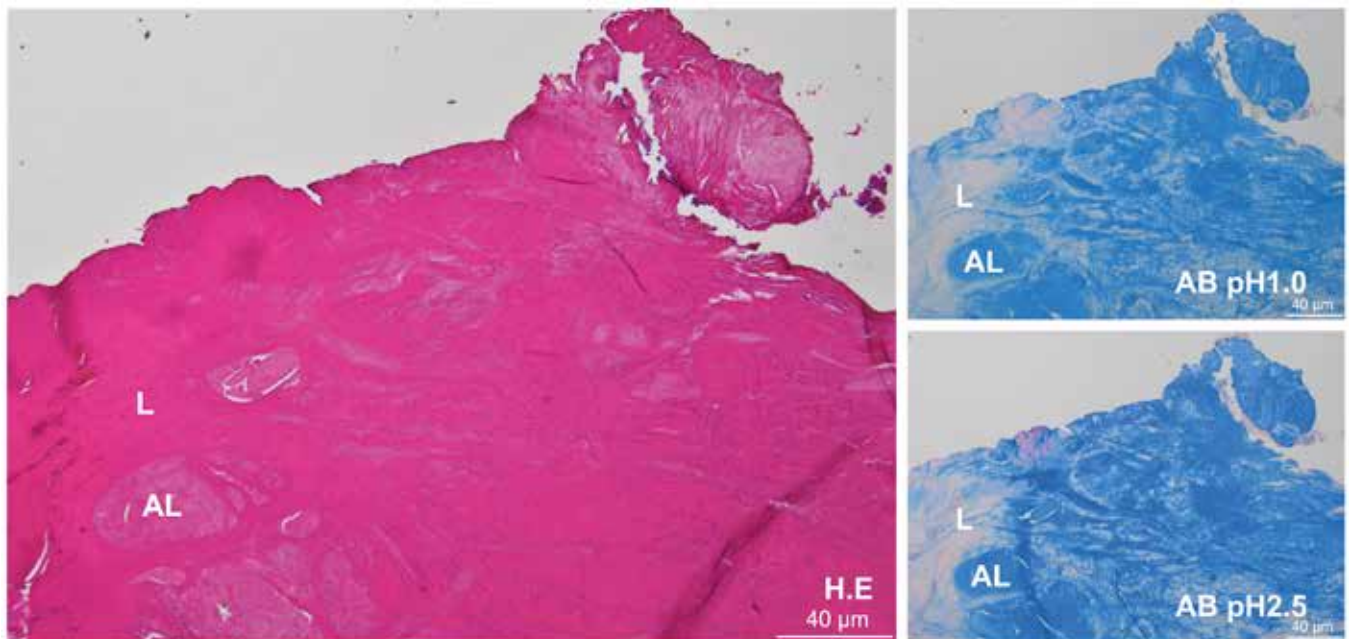


Figure 2. Optical microscopy images of the transition between the acetabular transverse ligament and labrum. The acetabular transverse ligament (L) consists of collagen fibers and stains negative on alcian blue (AB) staining (pH 1.0 and 2.5). The acetabular labrum (AL), on the other hand, is composed of fibrocartilage, stains slightly brighter on eosin staining, and is strongly positive for AB staining (pH 1.0 and 2.5).

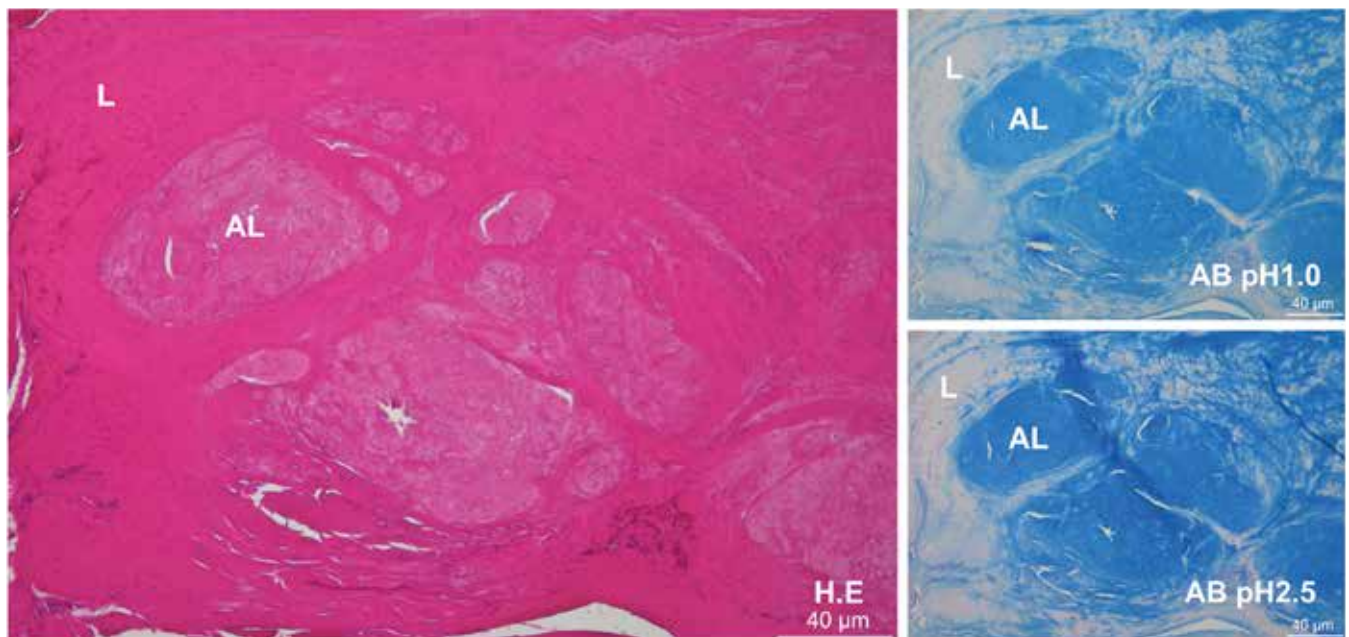


Figure 3. Magnified optical microscopy images of the transitional part of the acetabular transverse ligament (L) and acetabular labrum (AL). Collagen fibers composing the L appear to penetrate the cartilage tissue.

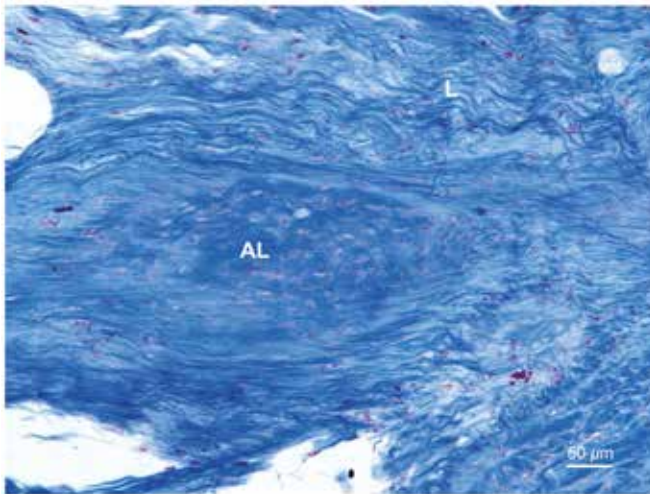


Figure 4. Optical microscopy image of the transition between the acetabular transverse ligament (L) and acetabular labrum (AL) with Masson's trichrome staining. The L shows clear wave-depressing fibers, while the AL shows rich cellular components around; no clear wave-depressing fibers appear to be mixed at the junction of the two tissue types.

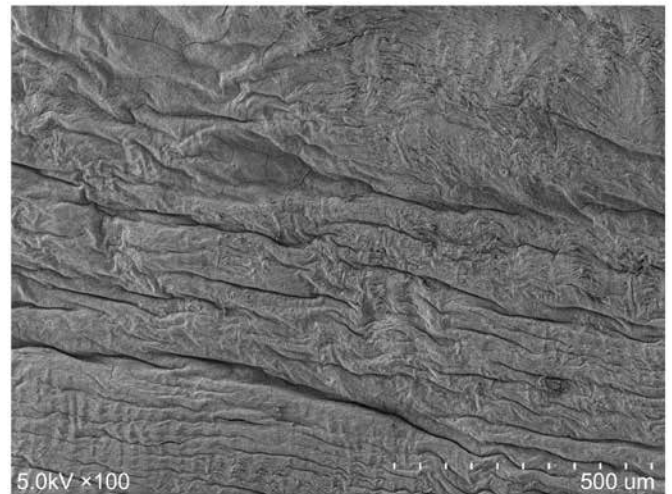


Figure 5. Low-magnification scanning electron (SEM) microscopy image of the acetabular transverse ligament (L). The L consists of bundles of collagen fibers with a width of 30–50 nm that are arranged in parallel.

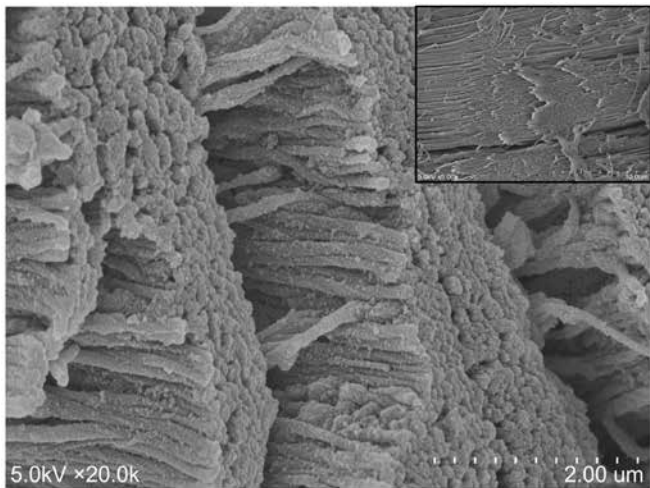


Figure 6. Horizontal ligament collagen fibers in high-magnification scanning electron microscopy (SEM) images. As treatment with 2N NaOH digests the extracellular matrix, the collagen fibrils that make up the collagen fibers are clearly observed in the inserted figure. The type I collagen fibrils are arranged in parallel and have a mean diameter of 161.28 ± 16.79 (range: 133.3–189.7) nm.

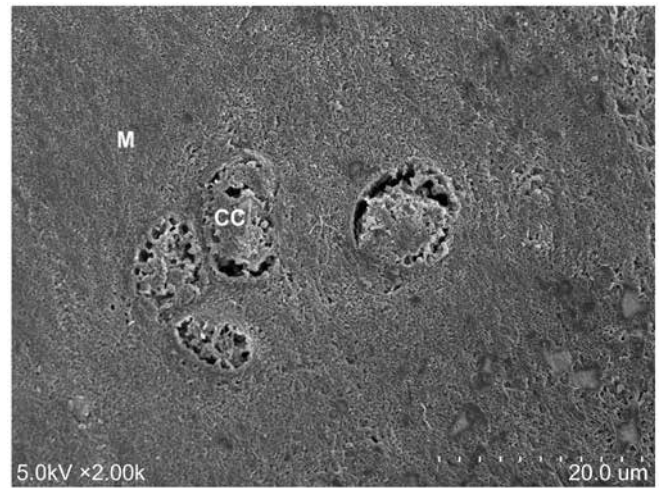


Figure 7. Scanning electron microscopy (SEM) image of the joint lip cartilage tissue. The cartilage tissue consists of chondrocytes (CC) and extracellular matrix (M).

drocytes and extracellular matrix, the extracellular matrix was densely packed with type II collagen, with a chondrocyte size of 12.52 ± 1.71 (range: 10–15.17) μm (**figure 8**).

Type I collagen was also present in the acetabular labrum and appeared to cause cartilage tissue disruption, showing a parallel arrangement as seen in the transverse ligament.

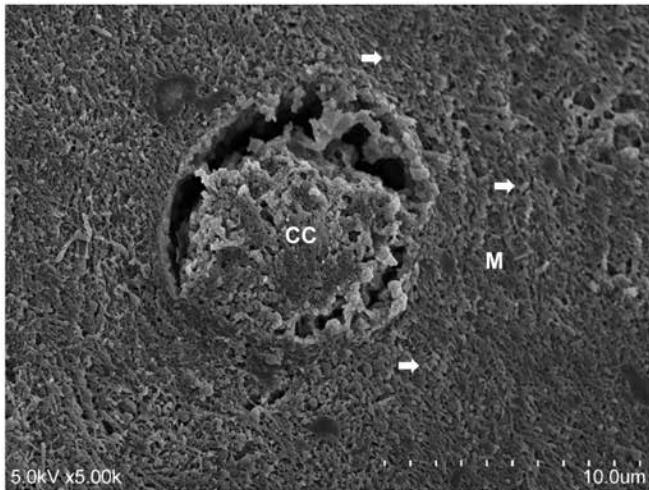


Figure 8. High-magnification scanning electron microscopy (SEM) image of the chondrocytes (CC) and extracellular matrix (M). In the M, the thinner collagen fibrils are dense and have a major axis of 12.52 ± 1.71 (range: 10–15.17) μm .

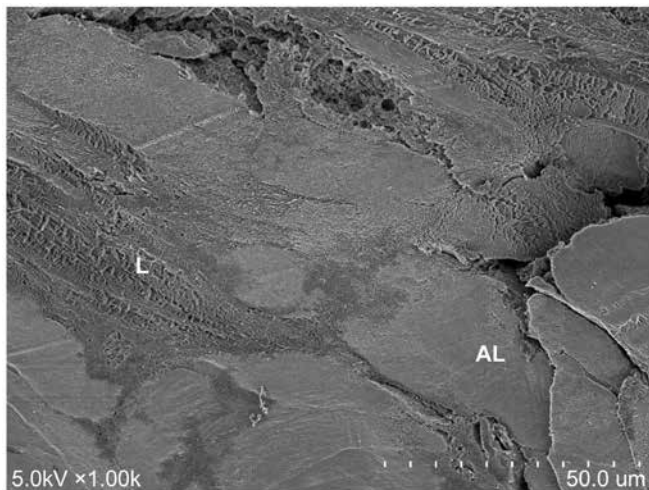


Figure 10. Scanning electron microscopy (SEM) image of the junction between the acetabular transverse ligament (L) and the acetabular labrum (AL). The L is penetrated by cartilage tissue that constitutes the AL.

Ultra-low magnification SEM images of the transitional zone between the acetabular transverse ligament and acetabular labrum revealed that the acetabular transverse ligament penetrated the cartilage tissue composing the acetabular labrum, as seen in LM images (**figure 9**). Furthermore, the collagen fibers of the acetabular trans-

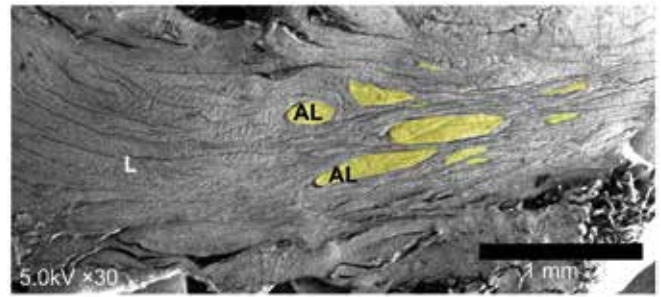


Figure 9. Ultra-low magnification scanning electron microscopy (SEM) image of the transition zone between the acetabular transverse ligament (L) and acetabular labrum (AL). The cartilage tissues are colored to differentiate between both tissues in the L and AL. The tissues of the AL seem to penetrate the tissues of the L.

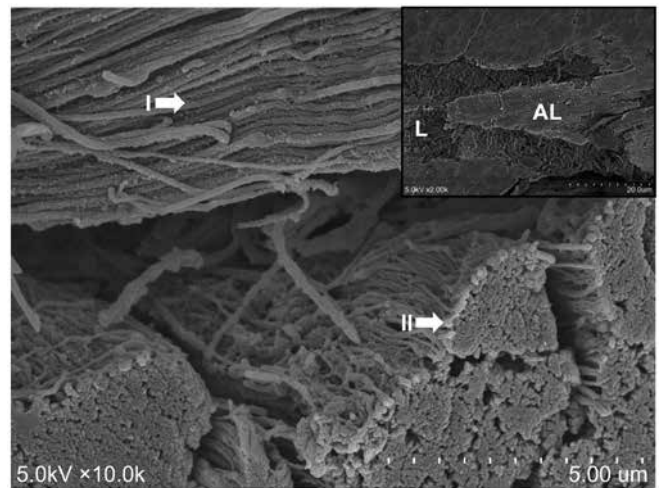


Figure 11. Scanning electron microscopy (SEM) images of the collagen fibrils in contact with the cartilage tissue. The collagen fibrils that constitute the acetabular transverse ligament (L) are dense and surround the cartilage tissue that constitutes the acetabular labrum (AL) in the inserted figure. Higher magnification SEM images show that type II collagen of the AL, with a diameter of 78.08 ± 13.04 (range: 61.8–100.5) nm, did not directly bind to type I collagen constituting the L.

verse ligament were interspersed in the acetabular labrum cartilage (**figure 10**); they also surrounded and were densely surrounded by cartilage (**figure 11**). At the junction of the acetabular transverse ligament and acetabular labrum, type I collagen of the transverse ligament and type II collagen of the acetabular labrum, which had a diameter of

73.65±8.56 (range: 61.8–89.56) nm, did not bind directly. Instead, the collagen fibers of the acetabular transverse ligament and labrum were interwoven.

DISCUSSION

We assessed the microstructure of the acetabular transverse ligament with respect to the construction and arrangement of the collagen fibers constituting the ligament and their connection with the acetabular labrum. We found that the collagen fibers were interwoven between the cartilage tissues of the acetabular labrum; these structures seemed to be directly joined histologically with one another. The SEM images, however, revealed that the collagen fibers were not directly connected; instead, the fine collagen fibers with varying diameters extending from the structures were interwoven.

The present study revealed the microstructure of the acetabular transverse ligament and its connection with the acetabular labrum by delineating collagen for the first time using SEM. A limitation of this study is that the effects of sex and age were not evaluated because only two samples were collected, and both subjects were older women. We also did not evaluate the attachment between the acetabular transverse ligament and bone, which may have indicated a different histological connection from that of the present result. The acetabular labrum has abundant sensory fibers, mechanoreceptors, and blood vessels in the fibrous connective tissue (16). Kapetanakis *et al.* also found that free nerve endings and nerve end organ presence was greater in the ventral side of the acetabular labrum (21). On the other hand, although Gerhardt *et al.* reported that sensory fibers and mechanoreceptors are present in the acetabular transverse ligament using LM after histological staining (22), Kılıçarslan *et al.* concluded that it showed good vascularity with abundant free nerve fibers within the fibrous connective tissue; however, the latter group of authors did not find mechanoreceptors using neurofilament protein and S-100 protein immunohistochemistry and microscopy (23). In addition, no chondrocytes have been reported to be present in the transverse ligament, unlike in the acetabular labrum (24). However, very little is known about the microstructure of the acetabular transverse ligament. The acetabular transverse ligament is unusual in that it is a ligament that connects to tissues other than bone.

In the present study, the type I collagen fibrils were found to form bundles and collagen fibers, had a width of 30–50 µm, and were arranged in parallel in the acetabular transverse ligament, as seen in other ligaments. The present study also revealed that the histological features of the acetabular transverse ligament were completely different from those of the cartilage tissue of the acetabular labrum, which is

mainly composed of type II collagen fibers. The diameter of the type I fibrils of the acetabular transverse ligament was, on average, 161.28±16.79 (range: 133.3–189.7) nm in this study, which is greater than that of general type I collagen and lower than that of tendon collagen (12). In contrast, although the diameter of the type II fibrils in the acetabular labrum, which have a fibrous cartilage structure, was 73.65±8.56 (range: 61.8–89.56) nm, it was similar to that of the collagen fibrils in the hyaline cartilage(17). Further, the collagen fibers of the acetabular transverse ligament were interwoven with the acetabular labrum cartilage, forming a layered structure with collagen fibers of different diameters. These components histologically seemed to be directly joined to each other. However, in high-magnification SEM images, the diameter of the collagen fibers was rather uniform, and their distribution patterns were well segregated from the bundles composed of smaller fibers. Nogami *et al.* (16) reported that multiple layers of thin type I collagen are interwoven with the cartilage tissue of the acetabular labrum. Conversely, in the adhesive portion of the acetabular transverse ligament, many relatively thick bundles consisting of type I collagen were present and formed a layered structure. Therefore, type II and type I collagen can coexist and seem to give rise to various structures with distinct histological features. Regarding the attachment of the ligament to tissues other than bone, it has been found that a part of the ligamentum teres composed of type I collagen is attached to the surface and inside of the articular cartilage of the femoral head (15).

CONCLUSIONS

The present study revealed the microstructure of the transverse ligament and its attachment to the acetabular labrum. In the macrostructure of the acetabular transverse ligament, collagen fibers with a width of 30–50 µm were arranged in parallel which consisted of bundle with type I collagen fibrils. The acetabular transverse ligament had a distinctly different microstructure than the acetabular labrum with cartilage tissues. In the connection between the acetabular transverse ligament and the labrum, it was found that the microstructure was not a direct adhesion, but rather a structural bond, at least with respect to collagen. Although ligaments usually attach to bone, the results suggest that ligaments can attach to tissues other than bone. As this study only reports findings based on morphological observation, quantitative analysis, such as determining the number of cells or conducting protein analysis, should be performed in detail in future studies. New findings from future studies will contribute to the elucidation of the mechanism underlying the connection of different tissues.

UNBLINDED ETHICS STATEMENT

The study procedures were conducted ethically in accordance with international standards, as required by the journal and as described in Padulo *et al.* 15. This study was approved by the Ethics Committee of Oita University (approval number, 1053; approval date, 22 July 2016). All experimental procedures were performed in accordance with the Declaration of Helsinki. Informed consent was obtained from all patients for study participation and publication of their clinical information.

ACKNOWLEDGEMENTS

We would like to thank Editage (www.editage.com) for English language editing.

CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interests.

REFERENCES

1. Löhle F, Eckstein F, Sauer T, Putz R. Structure, strain and function of the transverse acetabular ligament. *Acta Anat (Basel)* 1996;157:315-323.
2. Aprato A, Giachino M, Masse A. Arthroscopic approach and anatomy of the hip. *Muscles Ligaments Tendons J* 2016;6:309-316.
3. Bolia I, Chahla J, Locks R, Briggs K, Philippon MJ. Micro-instability of the hip: a previously unrecognized pathology. *Muscles Ligaments Tendons J* 2016;6:354-360.
4. Tran Trung D, Pham Trung H, Truong Cong M, Maffulli N, Nguyen Trung T. The anatomical characteristics of Vietnamese adult hip joint: a multiplanar reconstruction computer tomographic study. *Muscles Ligaments Tendons J* 2019;9:165-172.
5. Chmielewski J, Albiñana J. Failures of open reduction in developmental dislocation of the hip. *J Pediatr Orthop B* 2002;11:284-289.
6. Gala L, Clohisy JC, Beaulé PE. Hip Dysplasia in the Young Adult. *J Bone Joint Surg Am* 2016;98:63-73.
7. Bullough P, Goodfellow J, Greenwald AS, O'Connor J. Incongruent surfaces in the human hip joint. *Nature* 1968;217:1290.
8. Konrath GA, Hamel AJ, Olson SA, Bay B, Sharkey NA. The role of the acetabular labrum and the transverse acetabular ligament in load transmission in the hip. *J Bone Joint Surg Am* 1998;80:1781-1788.
9. McCluskey WP, Bassett GS, Mora-Garcia G, MacEwen GD. Treatment of failed open reduction for congenital dislocation of the hip. *J Pediatr Orthop* 1989;9:633-639.
10. Hsieh SM, Huang SC. Treatment of developmental dysplasia of the hip after failed open reduction. *J Formos Med Assoc* 1998;97:763-769.
11. Archbold HA, Mockford B, Molloy D, McConway J, Ogonda L, Beverland D. The transverse acetabular ligament: an aid to orientation of the acetabular component during primary total hip replacement: a preliminary study of 1000 cases investigating postoperative stability. *J Bone Joint Surg Br* 2006;88:883-886.
12. Fujita K, Kabata T, Maeda T, et al. The use of the transverse acetabular ligament in total hip replacement: An analysis of the orientation of the trial acetabular component using a navigation system. *Bone Joint J* 2014;96-B:306-311.
13. Griffin AR, Perriman DM, Bolton CJ, Smith PN. An in vivo comparison of the orientation of the transverse acetabular ligament and the acetabulum. *J Arthroplasty* 2014;29:574-579.
14. Shimada T, Sato F, Zhang L, Ina K, Kitamura H. Three-dimensional visualization of the aorta and elastic cartilage after removal of extracellular ground substance with a modified NaOH maceration method. *J Electron Microscop* 1993;42:328-333.
15. Kaku N, Shimada T, Tabata T, et al. Three-dimensional architecture of the ligamentum teres in the human hip joint. *Muscles Ligaments Tendons J* 2018;7:442-448.
16. Nogami R, Kaku N, Shimada T, Tabata T, Tagomori H, Tsumura H. Three-dimensional architecture of the acetabular labrum in the human hip joint. *Med Mol Morphol* 2019. doi:10.1007/s00795-019-00228-3.
17. Fawcett DW, Bloom W. A textbook of histology. New York/London; Chapman & Hall. 1994.
18. Kew SJ, Gwynne JH, Enea D, et al. Regeneration and repair of tendon and ligament tissue using collagen fibre biomaterials. *Acta Biomater* 2011;7:3237-3247.
19. Almarza AJ, Athanasiou KA. Design characteristics for the tissue engineering of cartilaginous tissues. *Ann Biomed Eng* 2004;32:2-17.
20. Padulo J, Oliva F, Frizziero A, Maffulli N. *Muscles, Ligaments and Tendons Journal - Basic principles and recommendations in clinical and field Science Research: 2016 Update.* *Muscles Ligaments Tendons J* 2016;19:1-5.
21. Kapetanakis S, Gkantsinikoudis N, Dermon A, Kommata V, Papatheanasiou J, Soukagos P, Dermon C. Normal microscopic architecture of acetabular labrum of hip joint: a qualitative original study with clinical aspects. *Muscles Ligaments Tendons J* 2017;7:279-285.
22. Gerhardt M, Johnson K, Atkinson R, et al. Characterisation and classification of the neural anatomy in the human hip joint. *Hip Int* 2012;22:75-81.
23. Kılıçarslan K, Kılıçarslan A, Demirkale İ, Aytekin MN, Akseki-li MA, Uğurlu M. Immunohistochemical analysis of mechanoreceptors in transverse acetabular ligament and labrum: a prospective analysis of 35 cases. *Acta Orthop Traumatol Turc* 2015;49:394-398.
24. Gray H, Lewis WH. *Anatomy of the Human Body.* Philadelphia; Lea & Febiger 2010.

Biomechanical Analysis of Krackow Suture Fixation Strength of Cadaver Achilles Tendon with Loop Distance Variance of 5 mm, 7.5 mm, and 10 mm

K. A. M. Luthfi, H. N. Rasyid, W. Arsa, Y. D. Ismiarto

Department of Orthopaedics and Traumatology, School of Medicine, Padjadjaran University/Dr. Hasan Sadikin General Hospital, Bandung City, Indonesia

CORRESPONDING AUTHOR:

Yoyos Dias Ismiarto
Head of Study Program
Department of Orthopaedics and
Traumatology, Faculty of Medicine Universitas
Padjadjaran/ Dr. Hasan Sadikin General
Hospital
Jalan Pasteur No. 38 Kota Bandung,
Indonesia
E-mail: yoyos@unpad.ac.id

DOI:

10.32098/mltj.03.2020.02

LEVEL OF EVIDENCE: 3

SUMMARY

Background. The incidence of Achilles tendon rupture continues to increase significantly from year to year. The Krackow technique for Achilles tendon repair has proven to be stronger than two clinical standards, Kessler modification technique, and Bunnel technique. However, the incidence of re-rupture and complications in the Krackow technique is still high if an earlier mobilization is carried out. From several studies, it appears that the distance between knots can affect the strength of suture fixation. The purpose of this study is to analyze: How does the difference in loop distance in Krackow suture affect the strength of suture fixation.

Methods. This is a comparative analytic study with an experimental design. Cadaveric Achilles tendon was made rupture by 4 cm incision above its insertion. Then, it was repaired using Krackow suture with a loop distance of 5 mm (group 1), 7.5 mm (group 2), and 10 mm (group 3). Suture fixation strength was assessed with a standard load to failure test. The results were analyzed statistically using a one-way analysis of variance and post hoc test.

Results and Discussion. The mean value of Krackow suture fixation strength in group 1 was 81.2 ± 18.9 N, group 2 was 106.8 ± 16.5 N, and group 3 was 132.3 ± 28.6 N. There was statistically significant mean difference between the strength of tendon fixation in the loop distance of 5 mm, 7.5 mm and 10 mm (p -value=0.002). The most effective group was group 3 (10 mm) because it had the highest mean difference (51.10000, $p = 0.000$). Possible mechanisms that can increase the strength of tendon fixation in greater loop distance (10 mm) are a greater suture-tendon interaction, a more secure grip power of the sutures on the tendon surface, expanding stress concentration to the tendon surface area, and increased stiffness to counteract tensile forces.

Conclusions. The 10 mm loop distance from the Krackow suture results in a significantly stronger suture fixation than the 7.5 mm and 5 mm loop distance for Achilles tendon rupture in cadaver.

KEY WORDS

Biomechanical testing; cadaveric Achilles tendon; Krackow suture; loop distance.

BACKGROUND

The incidence of Achilles tendon rupture continues to increase significantly from year to year, mostly due to sports injuries (1-4). In Finland, the incidence of rupture increased from 2.1/100,000 in 1979 to 21.5/100,000 in 2011(1). Meanwhile in Canada, the incidence of rupture was 18.0/100,000 in 2003, rising to 29.3/100,000 in 2013(3). Likewise in the United States, the incidence of rupture

also increased from 1.8/100,000 in 2012 to 2.5/100,000 in 2016(4). The increased incidence of rupture is due to the growth and rising interest in sports activities, especially in young men (1-4) Most of Achilles tendon rupture (75%) is related to recreational activities in particular in soccer, basketball, tennis, and squash, but 25 % of ruptures may occur in sedentary patients(2). Badminton is the leading cause of the rupture in Finland.1 Meanwhile, basketball is

most often involved in the United States(4). Patients aged 40-59 years had the most considerable rise (78%) in the incidence of ruptures(4). Possibly predisposing factor was passive mechanical properties change of the tendon in older patients (5).

Treatment of Achilles tendon rupture can be non-operative or operative. Non-operative treatment is generally through immobilization by casting and functional bracing (6,7). As for operative treatment, three main suture techniques have shown excellent results, namely the Bunnell, Kessler, and Krackow techniques(6,7). Operative treatment (open surgery or percutaneous repair) showed significantly improved clinical and functional outcomes than non-operative treatment (8). In 1940, Sterling Bunnell introduced a suture technique that permits cross-penetration between tendon fibers (9). Then, the grasping suture technique was described by Isidor Kessler and Fuad Nissim in 1969, which later became a popular method for repairing flexor tendons (10,11) In 1986, Kenneth A. Krackow introduced a unique tendon suture technique using locking loops (12,13). The Krackow technique for Achilles tendon repair has proven to be superior from two clinical standards, Kessler modification technique, and Bunnell technique (14,15). The average strength of tendon fixation from the Krackow technique (147 newtons) proved significantly stronger than the Kessler modification technique (85 newtons) and the Bunnell technique (93 newtons)(15). However, there was no significant difference in suture strength between the Krackow, Bunnell, and Kessler techniques that were performed with a double suture weave (16). The Krackow technique also stronger compared to the percutaneous method (17). Some research on the Krackow technique is also widely done, including those that modify the number of loops (2, 4, 6 loops) where the results were no statistical differences in peak load to failure (18). Research by McKeon et al. showed that the addition of second interlocking suture placed at 90° to the first proved to be stronger (18). Other research by Hapa et al. showed that the number of locking loops might have an influence on the Krackow suture strength using the larger diameter, high-strength sutures (14). But, it did not examine whether loop distance variance of the Krackow suture can influence the suture fixation strength. A study from Ortiz et al. showed the triple-strand Dresden technique significantly stronger than the Dresden technique, a modified oblique Dresden technique, and a Krackow technique (19).

Several meta-analysis studies showed that the incidence of re-rupture in operative treatment varies from 3.1% - 5.0% compared to 8.8% - 13% in conservative treatment (20-23). A meta-analysis from Soroceanu et al., and Van der Eng et al., showed that operative management of Achilles tendon rupture did not show better results than conservative treat-

ment. It was because of the same re-rupture rate and higher complications if earlier weight-bearing was carried out after four weeks (24,25). Meanwhile, operative treatment using the Krackow technique showed a low re-rupture rate of 2.5% (26). However, a prospective study from Twaddle et al. showed an increased re-rupture rate by 10% in operative treatment with the Krackow technique if an early motion was carried out after ten days, and 4.5% in conservative management (27). In fact, an early motion has proven beneficial for healing and tendon function, both in animal and human studies (26).

The occurrence of high re-rupture rates can be caused by the weakness of the suture due to differences in the loop distance of the Krackow technique. Also, suture materials can influence tendon repair on the biological level (28). Good suture techniques for Achilles tendon repair are needed because strong suture fixation can facilitate an early rehabilitation program to prevent joint stiffness. Strong suture fixation and good suture technique are associated with a lower re-rupture rate (7,18, 29-31). According to Y.F. Wu and J.B. Tang, a factor that can influence suture fixation strength, is the distance between core suture placement and the cut end of the tendon. Lengthening the distance can effectively increase the suture fixation strength, with the optimal length between 0.7 - 1.0 cm (32). Also, N. Kozono et al., examined the Kessler core suture with 6 Pennington locking loop at the edges made asymmetrically at distances of 1 mm, 2 mm, 3 mm, 4 mm, and 5 mm. It showed that the distance 3 mm or more had significantly stronger suture fixation than 1 mm (33).

From these studies, it appears that the loop distance of the suture can influence the suture fixation strength. However, in the Krackow technique, there was no standardization of optimal loop distance to provide high suture fixation strength. Moreover, no biomechanical studies were examining whether the loop distance variance of the Krackow suture can influence the suture fixation strength. The purpose of this study was to analyze: biomechanical of the Krackow suture fixation strength of cadaver Achilles tendon with loop distance variance of 5 mm, 7.5 mm, and 10 mm.

MATERIALS AND METHODS

This is a comparative analytic study with an experimental design. Eleven human cadavers (21 Achilles tendons, age range between 40 to 60 years old, six males and five females) were obtained from the Department of Forensic and Legal Medicine, Faculty of Medicine Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital Bandung, Indonesia. The cadaver was embalmed with a formalin-containing solution (34). This study was performed after receiving ethical clearance from the Health Research Ethics Committee of our

institution and followed the international ethical principles as well as the ethical standards of the Muscle, Ligaments and Tendons Journal (35).

Cadavers were positioned prone, and gastrocnemius muscle-Achilles tendon complex was dissected. Achilles tendons were made rupture with a scalpel 4 cm proximal to the calcaneal insertion (avascular zone 2-6 cm from its insertion)(22-24). Then, the tendon was repaired with Ethibond No. 5 (Braided, nonabsorbable, polyester fiber; Ethicon, USA) in one of three ways with seven specimens in each group. The Krackow technique was used starting 7 mm from the cut end of the tendon with a configuration of 3 locking loops and 2 strands (12,13,32,36). The locking loop distance was performed 5 mm in group 1, 7.5 mm in group 2, and 10 mm in group 3. For accurate range, measurements were made with a ruler and marked with markers on the tendon (see **figures 1** and **figures 2**).

The specimens were secured onto a tensile testing machine (CV. Abdi Tunggal Perkasa – Fanatron Indonesia, West Java, Indonesia) with a maximum tensile load of 100 pounds (444.8 N), and a maximum traction length of 12 inches

(30.48 cm). The tensile testing machine was calibrated by the National Accreditation Committee (Indonesia) following the Japanese Industrial Standards (JIS Z 2241: Tensile Testing). A Steinmann pin was drilled transversely through the calcaneus and affixed to the machine. The soft tissue of the gastrocnemius muscle was secured proximally onto a custom made nylon clamp (**figure 4**). The clamp was made asymmetrical teeth jaw shape to prevent tendon slippage (37,38). The Steinmann pin and the clamp were not found to be weak points in the machine construct. Because the calcaneus bone was not dissected free from the cadaveric body, the Achilles tendon is rotated 90 degrees on its insertion. However, the direction of pull was still parallel with the longitudinal axis of the Achilles tendon. After that, the specimens were loaded in tension until failure of the Achilles tendon (see **figure 3** and **figure 4**). The ultimate strength was considered the peak force recorded by the machine.

Before the statistical test is performed, the numerical data is assessed by the normality test using the Shapiro Wilks test to examine whether the data is normally distributed. Then, the results were analyzed statistically using a one-way analysis



Figure 1. A. Cadaveric Position, B. Marking of the incision and suture area of the Achilles tendon, C. The sharp incision of the Achilles tendon, D. Tendon repair with the Krackow Technique.

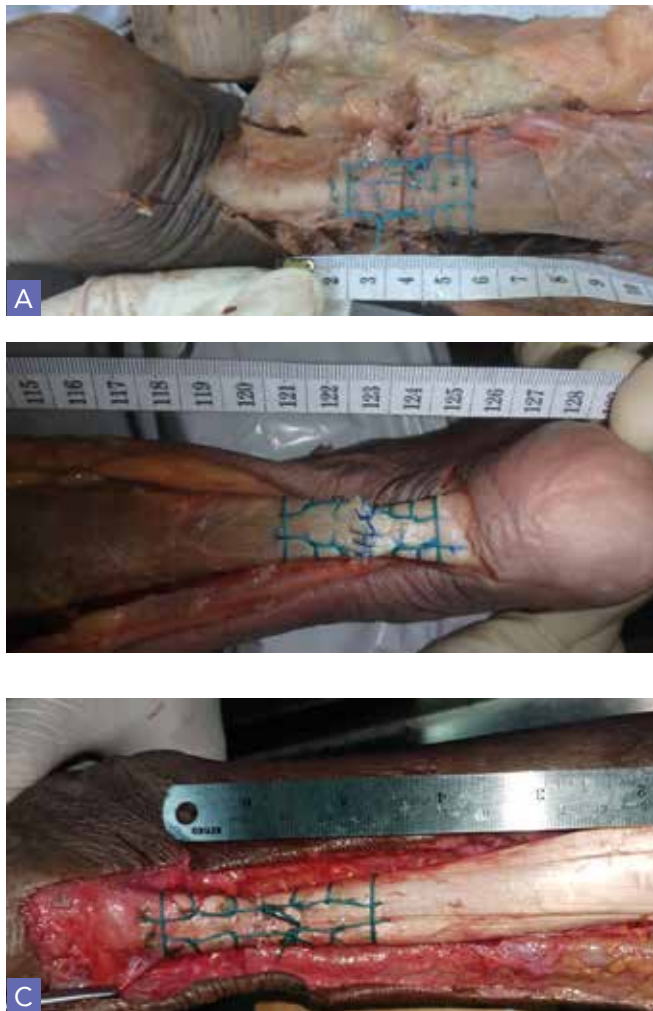


Figure 2. The Krackow Technique with locking loop distances of 5 mm (A), 7.5 mm (B) and 10 mm (C).



Figure 3. Tensile testing machine

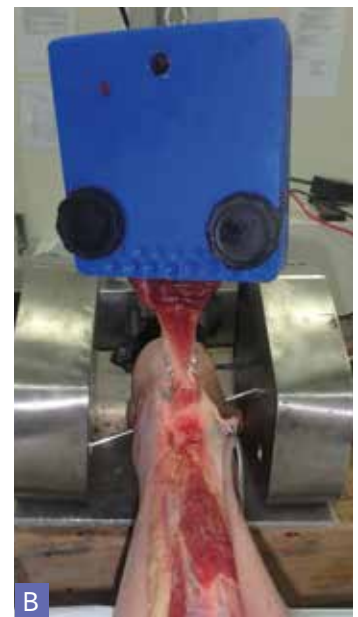


Figure 4. Achilles tendon secured onto a tensile testing machine (A, B). The monitor showed the ultimate strength (C).

of variance (ANOVA) comparing the three groups of specimens to assess if there was an effect of loop distance variance of 5 mm, 7.5 mm, and 10 mm to the Krackow suture fixation strength. If there was an effect, post hoc tests using Fisher's least significant difference (LSD) were performed to assess differences between the loop distance variance. An α level of 0.05 was assumed to be statistically significant.

RESULTS

The results are summarized in **table I**. From all groups, the Krackow suture fixation strength had a minimum value of 53.9 N, a maximum value of 170.9 N, and a mean value of 106.8 N with a standard deviation of 29.9 N.

In group 1 (loop distance 5 mm), a minimum value of suture fixation strength was 53.9 N, a maximum value was 109.7 N, and a mean value was 81.2 N with a standard deviation of 18.9 N. Whereas in group 2 (loop distance 7.5 mm), a minimum value of suture fixation strength was 95.3 N, a maximum value was 142.9 N, and a mean value was 106.8

N with a standard deviation of 16.5 N. Finally, in group 3 (loop distance 10 mm), a minimum value of suture fixation strength was 103.4 N, a maximum value of 170.9 N, and mean value of 132.3 N with a standard deviation of 28.6 N. Shapiro Wilks test showed that the data are normally distributed (p -value = 0.177, >0.05), so a parametric test was used. The mean value of the Krackow suture fixation strength in group 1 was 81.2 N, group 2 was 106.8 N, and group 3 was 132.3 N (**figure 5**). From the one-way analysis of variance test results, the p -value was 0.002 ($p < 0.05$), which means there was a statistically significant difference between Krackow suture fixation strength with a loop distance variance of 5 mm, 7.5 mm, and 10 mm. Fisher's least significant difference testing revealed that for all three groups, group 3 were stronger than both group 1 and group 2 because group 3 had the highest mean difference of 51.1 compared to group 1 with p -value = 0.000 ($p < 0.05$) and mean difference of 25.5 compared to group 2 with p -value = 0.043 ($p < 0.05$) (**table II**).

Table I. Characteristics of the Krackow suture fixation strength based on loop distance.

Variable	Loop distance			Σ N=21
	Group 1 5 mm N=7	Group 2 7.5 mm N=7	Group 3 10 mm N=7	
The Krackow Suture Fixation Strength (N)				
Mean \pm SD	81.2 \pm 18.9	106.8 \pm 16.5	132.3 \pm 28.6	106.8 \pm 29.9
Median	79.8	100.0	130.4	103.4
Range (minimum-maximum)	53.9-109.7	95.3-142.9	103.4-170.9	53.9-170.9

Table II. Post Hoc Test of Krackow suture fixation strength based on loop distance.

Loop Distance		Mean Difference Krackow suture fixation strength (I-J)	p
(I) Group	(J) Group		
5 mm	7.5 mm	-25.6*	0.043
	10 mm	-51.1*	0.000
7.5 mm	5 mm	25.6*	0.043
	10 mm	-25.5*	0.043
10 mm	5 mm	51.1*	0.000
	7.5 mm	25.5*	0.043

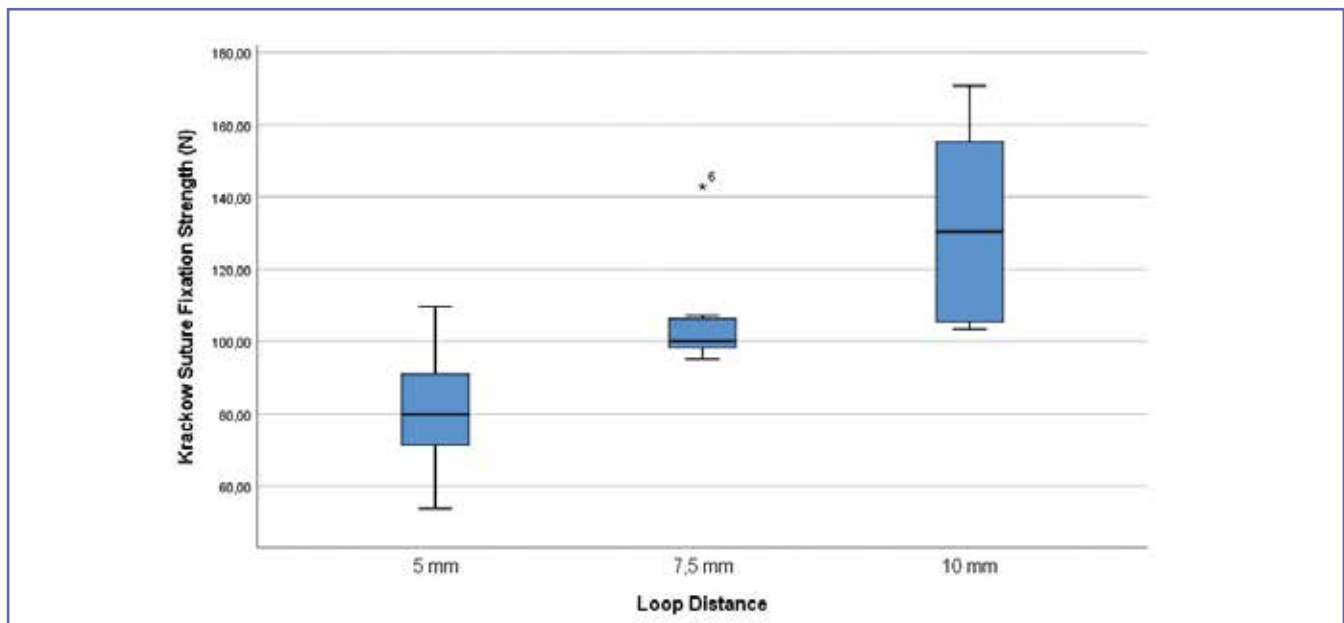


Figure 5. Krackow suture fixation strength based on loop distance.

DISCUSSION

The results showed that there was a statistically significant mean difference between the Krackow suture fixation strength in all groups with a loop distance variance of 5 mm, 7.5 mm, and 10 mm. From all groups, the most effective was group 3 (loop distance 10 mm) because it had the highest mean value of tendon fixation strength compared to group 1 (5 mm) and group 2 (7.5 mm). Kenneth A. Krackow first introduced this unique tendon suture technique using locking loops in 1986 (12,13). The average strength of tendon fixation from the Krackow technique (147 newtons) proved significantly stronger than the Kessler modification technique (85 newtons) and the Bunnell technique (93 newtons) (15,39). But, there was no significant difference in strength between the Krackow, Bunnell, and Kessler suture techniques, when each was performed with a double suture weave (16). The Krackow technique also stronger compared to the percutaneous method (17). Some research on the Krackow technique is also widely done, including research by McKeon et al. showed that the addition of second interlocking suture placed at 90° to the first proved to be stronger (18).

An early motion has proven beneficial for healing and tendon function, both in animal and human studies (26). However, an early motion could increase the re-rupture rate by 10% in operative treatment with the Krackow technique (27). The occurrence of high re-rupture rates can be

caused by the weakness of the suture due to differences in the loop distance of the Krackow technique. Strong suture fixation and good suture technique are associated with a lower re-rupture rate (7,18, 29-31). Several factors that can influence the suture fixation strength include the number of suture strands, suture materials, locking or grasping loop configuration, suture knots, knots or loop distance, and core suture tension (32). Several meta-analysis studies showed that the incidence of re-rupture in operative treatment varies from 3.1% - 5.0% compared to 8.8% - 13% in conservative treatment (20-23). A meta-analysis from Soroceanu et al., and Van der Eng et al., showed that operative management of Achilles tendon rupture did not show better results than conservative treatment. It was because of the same re-rupture rate and higher complications if earlier weight-bearing was carried out after four weeks (24,25) Meanwhile, operative treatment using the Krackow technique showed a low re-rupture rate of 2.5% (26).

According to Y.F. Wu and J.B. Tang, a factor that can influence suture fixation strength, is the distance between core suture placement and the cut end of the tendon. Lengthening the distance can effectively increase the suture fixation strength, with the optimal length between 0.7 - 1.0 cm (32). Also, N. Kozono et al., examined the Kessler core suture with 6 Pennington locking loop at the edges made asymmetrically at distances of 1 mm, 2 mm, 3 mm, 4 mm, and 5 mm. It showed that the distance 3 mm or more had significantly

stronger suture fixation than 1 mm (33). Also, N. Kozono et al., examined the Kessler core suture with 6 Pennington locking loop at the edges made asymmetrically at distances of 1 mm, 2 mm, 3 mm, 4 mm, and 5 mm. It showed that the distance 3 mm or more had significantly stronger suture fixation than 1 mm (33). From these studies, it appears that the loop distance of the suture can influence the suture fixation strength.

However, in the Krackow technique, there was no standardization of optimal loop distance to provide high suture fixation strength. In this study, the loop distance variance of the Krackow technique proved to influence the suture fixation strength significantly. From the biomechanical studies that have been conducted, the loop distance of 10 mm in the Krackow technique has the highest mean value of suture fixation strength (132.3 N) compared to the loop distance of 5 mm and 7.5 mm. The mechanism that can increase the suture fixation strength is the longer the suture distance, the higher the interaction between tendons and sutures (32). Also, greater loop distance can increase the strength of the suture grip on the tendon surface and further strengthen the suture fixation (32). A long loop distance can also divide the stress concentration to a greater surface area, thereby increasing the suture fixation strength (33,40) In addition, the greater loop distance can increase stiffness to neutralize the tensile forces so that the suture fixation strength is stronger (32,40,41) Tendon stiffness indicates the magnitude of the force required to produce elongation from a tendon segment. The suture tendon stiffness suggests the ability of the suture to resist tendon deformation to the stress force (32,41). Therefore, in this study, the suture fixation strength was highest at the 10 mm loop distance because it had high stiffness to resist tendon elongation from the tensile force.

The results of this study are supported by research from Yi Cao et al., who examined locking cruciate sutures on the flexor tendon with the core purchase distance 10 mm and 4 mm. Sutures purchase of 4 mm have suture fixation strength 20-45% lower than suture purchase of 10 mm (41). Other studies from Kim et al., and Lee et al., also supported this research. It showed that the tendons with a core suture purchase of 10 mm had a high suture fixation strength (42,43). The distance of the core suture to the cut end of the tendon determines the number of tendon segments that interact with the suture. Extending the length can increase the suture fixation strength due to the more significant interaction between the suture and tendons (32,41). Likewise, with the locking loop suture in the Krackow technique, the longer the loop distance, the more Achilles tendon segments interact with the Krackow suture, thereby increasing the suture fixation strength.

It was proven in this study, the loop distance of 10 mm showed a mean value of suture fixation strength that was significantly stronger than the loop distance of 7.5 mm and 5 mm.

Although the results showed that the mean value of suture fixation strength is significantly higher in the Krackow technique with a loop distance of 10 mm in this study, some weaknesses cannot be avoided. First, the researcher could have subjectivity in conducting this research. It can be minimized by performing according to standards and using a ruler and marker so that the measurements were correct. Second, differences in the size of the cadaveric tendon can also cause bias in this study. Therefore, this study used adult cadavers, age 40-60 years old, so that the size of the tendon is not too varied. Third, the elasticity of tendons can be different due to differences in gender and age, so that it can affect the ultimate failure of the tendon (44,45,46). Fourth, Achilles tendon was not placed along with its physiological boundary condition (aligned with the tibia). The calcaneus bone was not dissected free from the cadaveric body because of an ethical issue in our institution. So, the Achilles tendon is rotated 90 degrees on its insertion. However, the direction of pull was still parallel with the longitudinal axis of the Achilles tendon. It had no impact on the results because all the specimens were aligned with the same technique. Fifth, in this study, the length of the loop distance of the Krackow suture has proven to be stronger, but the optimal loop distance has not yet been determined for a range of more than 10 mm. So, further research is needed to assess biomechanical strength for loop distances more than 10 mm. Sixth, the cadaver Achilles tendon certainly does not have the biochemical healing ability, as occurs in living human tendon. Intrinsic factors and extrinsic factors can influence the tendon healing process. Too much tendon suture can also cause fibrotic tissue to form, thereby increasing stiffness in the ankle joint. Therefore, further research is needed to assess biomechanical of the Krackow suture fixation strength of living human tendon with loop distance variance of 5 mm, 7.5 mm, and 10 mm to strengthen the results of this study.

CONCLUSIONS

Based on the results, this research concludes that the 10 mm loop distance of the Krackow suture results in a significantly stronger suture fixation than the 7.5 mm and 5 mm loop distance for Achilles tendon rupture in cadaver.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

REFERENCES

- Lantto I, Heikkinen J, Flinkkila T, Ohtonen P, Leppilahti J. Epidemiology of Achilles tendon ruptures: increasing incidence over a 33-year period. *Scandinavian journal of medicine & science in sports*. 2015;25(1):e133-8.
- Maffulli N, Via AG, Oliva F. Achilles Tendon Rupture. In: Volpi P, editor. *Arthroscopy and Sport Injuries: Applications in High-level Athletes*. Switzerland: Springer International Publishing 2016. p. 77-81.
- Sheth U, Wasserstein D, Jenkinson R, Moineddin R, Kreder H, Jaglal SB. The Epidemiology and Trends in Management of Acute Achilles Tendon Ruptures in Ontario, Canada. *The Bone & Joint Journal*. 2017;99-B(1):78-86.
- Lemme NJ, Li NY, DeFroda SF, Kleiner J, Owens BD. Epidemiology of Achilles Tendon Ruptures in the United States: Athletic and Nonathletic Injuries From 2012 to 2016. *The Orthopaedic Journal of Sports Medicine*. 2018;6(11):1-7.
- Vafek EC, Plate JF, Friedman E, Mannava S, Scott AT, Danelson KA. The effect of strain and age on the mechanical properties of rat Achilles tendons. *Muscles, ligaments and tendons journal*. 2017;7(3):548-53.
- Gulati V, Jaggard M, Al-Nammari SS, Uzoigwe C, Gulati P, Ismail N, et al. Management of achilles tendon injury: A current concepts systematic review. *World journal of orthopedics*. 2015;6(4):380-6.
- Alan NG, Jacobson KL. Achilles Tendon Trauma. In: South-erland JT, editor. *McGlamry Comprehensive Textbook of Foot and Ankle Surgery*. Fourth Edition ed. Philadelphia: Wolters Kluwer Health | Lippincott Williams & Wilkins; 2013. p. 1580-99.
- Maffulli G, Buono AD, Richards P, Oliva F, Maffulli N. Conservative, minimally invasive and open surgical repair for management of acute ruptures of the Achilles tendon: a clinical and functional retrospective study. *Muscles, ligaments and tendons journal*. 2017;7(1):46-52.
- Bunnell S. Primary repair of severed tendons the use of stainless steel wire. *The American Journal of Surgery*. 1940;47(2):502-16.
- Kessler I, Nissim F. Primary Repair without Immobilization of Flexor Tendon Division within the Digital Sheath: An Experimental and Clinical Study. *Acta orthopaedica Scandinavica*. 1969;40(5):587-601.
- Sebastin SJ, Ho A, Karjalainen T, Chung KC. History and evolution of the Kessler repair. *The Journal of hand surgery*. 2013;38(3):552-61.
- Krackow KA, Thomas SC, Jones LC. A new stitch for ligament-tendon fixation. Brief note. *The Journal of bone and joint surgery American volume*. 1986;68(5):764-6.
- Krackow KA, Thomas SC, Jones LC. Ligament-tendon fixation: analysis of a new stitch and comparison with standard techniques. *Orthopedics*. 1988;11(6):909-17.
- Hapa O, Erduran M, Havitçioğlu H, Çeçen B, Akşahin E, Güler S, et al. Strength of Different Krackow Stitch Configurations Using High-strength Suture. *The Journal of Foot and Ankle Surgery*. 2013;52(4):448-50.
- Watson TW, Jurist KA, Yang KH, Shen KL. The strength of Achilles tendon repair: an in vitro study of the biomechanical behavior in human cadaver tendons. *Foot & ankle international*. 1995;16(4):191-5.
- McCoy BW, Haddad SL. The Strength of Achilles Tendon Repair: A Comparison of Three Suture Techniques in Human Cadaver Tendons. *Foot & ankle international*. 2010;31(8):701-5.
- Lee SJ, Sileo MJ, Kremenic IJ, Orishimo K, Ben-Avi S, Nicholas SJ, et al. Cyclic loading of 3 Achilles tendon repairs simulating early postoperative forces. *The American journal of sports medicine*. 2009;37(4):786-90.
- McKeon BP, Heming JF, Fulkerson J, Langeland R. The Krackow stitch: a biomechanical evaluation of changing the number of loops versus the number of sutures. *Arthroscopy : the journal of arthroscopic & related surgery : official publication of the Arthroscopy Association of North America and the International Arthroscopy Association*. 2006;22(1):33-7.
- C. Ortiz, E. Wagner, P. Mocoçain, G. Labarca, A. Keller, A. Del Buono, et al. Biomechanical comparison of four methods of repair of the Achilles tendon. *The Journal of Bone and Joint Surgery*. 2012;94-B(5):663-7.
- Khan RJ, Fick D, Keogh A, Crawford J, Brammar T, Parker M. Treatment of acute achilles tendon ruptures. A meta-analysis of randomized, controlled trials. *The Journal of bone and joint surgery American volume*. 2005;87(10):2202-10.
- Khan RJK, Carey Smith RL. Surgical interventions for treating acute Achilles tendon ruptures. *Cochrane Database of Systematic Reviews*. 2010(9).
- Bhandari M, Guyatt G, Siddiqui F, Morrow F, Busse J, K. Leighton R, et al. Treatment of Acute Achilles Tendon Ruptures A Systematic Overview and Meta-analysis. *Clinical orthopaedics and related research*. 2002;400:190-200.
- Wilkins R, Bisson LJ. Operative versus non-operative management of acute Achilles tendon ruptures: a quantitative systematic review of randomized controlled trials. *The American journal of sports medicine*. 2012;40(9):2154-60.
- Soroceanu A, Sidhwa F, Aarabi S, Kaufman A, Glazebrook M. Surgical Versus Nonsurgical Treatment of Acute Achilles Tendon Rupture A Meta-Analysis of Randomized Trials2012.
- Eng D, Schepers T, Schep NWL, Carel Goslings J. Rerupture Rate after Early Weightbearing in Operative Versus Conservative Treatment of Achilles Tendon Ruptures: A Meta-Analysis. *The Journal of Foot & Ankle Surgery*. 2013;52:622-8.
- Willits K, Amendola A, Bryant D, Mohtadi NG, Giffin JR, Fowler P, et al. Operative versus non-operative treatment of acute Achilles tendon ruptures: a multicenter randomized trial using accelerated functional rehabilitation. *The Journal of bone and joint surgery American volume*. 2010;92(17):2767-75.
- Twaddle BC, Poon P. Early Motion for Achilles Tendon Ruptures: Is Surgery Important?: A Randomized, Prospective Study. *The American journal of sports medicine*. 2007;35(12):2033-8.
- Ergün S, Alakbarov A, Yılmaz AM, Karademir B, Akgün U. The Effect of Different Suture Materials on Achilles Tendon Metabolism: A Preliminary in vivo Study of mRNA levels in Rabbits. *Muscles, ligaments and tendons journal*. 2019;9(4):470-7.
- Hong C-K, Kuo T-H, Yeh M-L, Jou I-M, Lin C-L, Su W-R. Do Needleless Knots have Similar Strength as the Krackow Suture? An In Vitro Porcine Tendon Study. *Clinical Orthopaedics and Related Research®*. 2017;475(2):552-7.
- Möller M, Movin T, Granhed H, Lind K, Faxén E, Karlsson J. Acute Rupture Of Tendo Achillis A Prospective, Randomised

- Study of Comparison Between Surgical and Non-Surgical Treatment. *The Journal of Bone and Joint Surgery*. 2001;83:843-8.
31. Rawson S, Cartmell S, Wong J. Suture techniques for tendon repair; a comparative review. *Muscles, ligaments and tendons journal*. 2013;3(3):220-8.
 32. Wu YF, Tang JB. Recent developments in flexor tendon repair techniques and factors influencing strength of the tendon repair. *The Journal of hand surgery, European volume*. 2014;39(1):6-19.
 33. Kozono N, Okada T, Takeuchi N, Hanada M, Shimoto T, Iwamoto Y. Asymmetric six-strand core sutures enhance tendon fatigue strength and the optimal asymmetry. *The Journal of hand surgery, European volume*. 2016;41(8):802-8.
 34. Kalanjati VP, Prasetiowati L, Alimsardjono H. The Use of Lower Formalin-Containing Embalming Solution for Anatomy Cadaver Preparation. *Medical Journal of Indonesia*. 2012;21(4):203-7.
 35. Padulo J, Oliva F, Frizziero A, Maffulli N. Muscles, Ligaments and Tendons Journal - Basic principles and recommendations in clinical and field Science Research: 2018 Update. *Muscles, ligaments and tendons journal*. 2018;8(3):305-7.
 36. Andrew A, Chrenshaw J. Surgical Techniques and Approaches. In: Canales ST, Beaty JH, editors. *Campbell's Operative Orthopaedics*. 12th Edition ed. Philadelphia: Mosby; 2013. p. 10-1.
 37. Shia D, Wanga D, Wanga C, Liub A. A Novel, Inexpensive and Easy to Use Tendon Clamp for In Vitro Biomechanical Testing. *Medical Engineering & Physics*. 2012;34:516-20.
 38. Innocenti B, Larrieu J-C, Lambert P, Pianigiani S. Automatic Characterization of Soft Tissues Material Properties During Mechanical Tests *Muscles, ligaments and tendons journal*. 2017;7(4):529-37.
 39. Hidayat D. Perbandingan Tensile Strength Teknik Jahitan Krackow Dengan Teknik Bunnel Dalam Fase Inflamasi Penyembuhan Tendon Pada Penyambungan Ruptur Akut Tendon Achilles Kelinci [Tesis]. Bandung: Universitas Padjadjaran; 2003.
 40. Rawson S, Margetts L, Wong J, Cartmell S. Sutured Tendon Repair; A Multi-Scale Finite Element Model. *Biomech Model Mechanobiol*. 2015;14:123-33.
 41. Cao Y, Zhu B, Xie RG, Tang JB. Influence of Core Suture Purchase Length on Strength of Four-Strand Tendon Repairs. *The Journal of hand surgery*. 2006;31A:107-12.
 42. Kim J, Wit Td, Hovius S, McGrouther D, Walbeehm E. What is the significance of tendon suture purchase? *J Hand Surg Eur*. 2009;34:497-502.
 43. Lee S, Goldstein R, Zingman A, Terranova C, Nasser P, Hausman M. The effects of core suture purchase on the biomechanical characteristics of a multistrand locking flexor tendon repair: a cadaveric study. *The Journal of hand surgery*. 2010;35:1165-71.
 44. Muraoka T, Muramatsu T, Fukunaga T, Kanehisa H. Elastic properties of human Achilles tendon are correlated to muscle strength. *Journal of Applied Physiology*. 2005;99(2):665-9.
 45. Ruan Z, Zhao B, Qi H, Zhang Y, Zhang F, Wu M, et al. Elasticity of healthy Achilles tendon decreases with the increase of age as determined by acoustic radiation force impulse imaging. *International Journal of Clinical and Experimental Medicine*. 2015;8(1):1043-50.
 46. Oliva F, Rugiero C, Giai Via, et al. Achilles tendon ruptures guidelines. *Muscles, Ligaments and Tendon Journal*. 2018;8(3):310-63

Kinesio Tape In Shoulder Rotator Cuff Tendinopathy: A Randomized, Blind Clinical Trial

L. Martins da Silva², G. Maciel Bello², B. Chuaste Flores², L. Silva Dias², P. Camargo², L. F. Mengue², V. Roque², M. dos Santos Júnior², I. Rodrigues¹, G. Zanelatto¹, M. B. Dohnert³, R. B. Daitx¹

¹ Physiotherapy Course, Lutheran University of Brazil, Torres, RS, Brazil

² Lutheran University of Brazil, Torres, RS, Brazil

³ Teaching and Extension Laboratory in Orthopedic Trauma Physiotherapy (LAPEFITO), Gurupi University (UnirG), Gurupi, Torres, Brazil

CORRESPONDING AUTHOR:

Marcelo Baptista Dohnert
1530 Gioás Street
apartment 900 A
Bairro Centro 77410-010
Gurupi, Torres, Brazil
E-mail: mdohnert@hotmail.com

DOI:

10.32098/mltj.03.2020.03

LEVEL OF EVIDENCE: 1B

SUMMARY

Background. Shoulder pain is a very common musculoskeletal disorder that affects many people, with rotator cuff (RC) injury as one of its main causes.

Objective. To analyze the efficacy of KT, both isolated and associated with exercise, on pain and function of patients with shoulder RC injuries.

Method. A randomized, blind clinical trial with sixty (60) participants with RC injuries, randomized into exercise group (EG), in which participants performed an exercise protocol; kinesio tape group (KTG), with application of the elastic bandage; and exercise + kinesio tape group (EKTG), in which participants performed both protocols. We evaluated pain intensity, active and passive mobility, muscle strength, and function.

Results. All intervention groups significantly improved pain, disability, and function. Regarding the latter, EKTG showed significantly greater improvements than EG and KTG ($p < 0.05$). In addition, EKTG improved muscle strength in all evaluated movements. Groups EG and EKTG improved range of motion in all evaluated movements, for both the right and left shoulder.

Conclusions. Exercises were the basis of the treatment of RC injury. When associated with an exercise protocol, kinesio tape (KT) enhanced the effect of exercise in patients with shoulder RC injury. In isolation, KT was effective in reducing pain. Brazilian Clinical Trials Registry (REBEC) RBR-65qh7j.

KEY WORDS

Rotator cuff; kinesio tape; therapeutic exercise.

BACKGROUND

Shoulder pain is a very common musculoskeletal disorder that affects many people.¹ It causes significant disability, and rotator cuff injury is one of its main risk factors (2,3). The prevalence of shoulder pain ranges from 6.9 to 26.0% (1,4). Among the various conditions that may affect people with shoulder pain, subacromial impingement syndrome (SIS) has the highest prevalence and accounts for 36% of shoulder disorders (5). Subacromial impingement syndrome or SIS is a generic term used to define lesions affecting structures in the subacromial space, such as RC tendinosis, partial

RC rupture, and bursitis (1). Rotator cuff (RC) ruptures are a common cause of shoulder dysfunction and can lead to numerous functional deficits, including decreased range of motion (ROM), pain, and muscle weakness (6). Rotator cuff injuries and subacromial impingement are among the most common diagnoses in the shoulder region (7) being one of the most common causes of shoulder dysfunction (8). These events occur mainly in patients aged between 40 and 60 years and may be traumatic or degenerative (9). Their etiology is multifactorial (10,11) and age is a factor that predisposes to tendon degeneration (10,11). When associated with muscle

overuse, repair does not occur properly, resulting in disorganization and tendon thickening (a process called tendinosis) (11).

Initial treatment of RC injuries is conservative and emphasizes rehabilitation programs as opposed to surgical interventions.² Shoulder rehabilitation programs generally focus on strengthening shoulder and shoulder girdle (more specifically, RC) muscles (2). Several studies prove the effectiveness of an eccentric strengthening program for shoulder rotator cuff (12).

Kinesio tape (KT) is used with or without other physiotherapeutic interventions, especially to control pain, inflammation, and improve functional activity in patients with shoulder pain (3,13). It is usually used as an adjunct in the rehabilitation of injuries (3,13). After KT application, there is an increase in interstitial space leading to an increase in blood and lymphatic flow, with consequent decreased pressure on the underlying soft tissues.¹⁴ Kinesio tape may be an interesting option for improving shoulder neuromuscular control, with wide application in clinical practice (15). It has been argued that KT can reduce symptoms and functional limitations by improving proprioceptive feedback (13,16). Moreover, KT has been reported to increase subacromial space in healthy individuals (7,9).

Exercise programs that not only focus on scapular muscle strengthening but also emphasize motor control, including quality of movement, have been advocated for the treatment of individuals with RC injuries and included in shoulder sports injury prevention programs (17). Exercises play a fundamental role in the treatment of RC injuries (16). Studies show that several benefits can be obtained from physiotherapeutic exercises^{18,19} such as active and passive range-of-motion exercises, Codman pendulum exercises, stretching, and isometric and isotonic exercises. Eccentric exercises have been reported in the literature as the most recommended for treating degenerative tendon diseases (20). Eccentric training consists in the contraction of a muscle to control or slow down a load, while the muscle and tendon stretch or remain stretched (21). This technique has been recommended in the treatment of several pathologies such as Achilles tendon tendinopathy, patellar tendinopathy, lateral elbow epicondylitis, and RC (21). Other physiological processes that justify the use of eccentric exercises are increased fibroblast activity, accelerated collagen formation, formation and increase of type I collagen, and collagen organization and alignment (tendon remodeling) (22,23).

This study is justified by the controversy found in the current literature on the effect of KT, either in isolation or associated with kinesiotherapeutic approaches, on general musculoskeletal disorders, especially in the treatment of partial injuries of shoulder rotator cuff.

Here, we evaluate the effects of KT, either in isolation or associated with exercise, on RC injuries. Variables assessed were: pain, joint mobility, muscle strength, and shoulder function in patients with RC injury.

MATERIALS AND METHODS

Study design

This was a randomized, blind clinical trial conducted at the Physiotherapy Clinic School of the Lutheran University of Brazil (Torres-RS) from August 2017 to June 2019. The study was registered in the Brazilian Clinical Trials Registry (REBEC) under the identification number RBR-65qh7j.

Sample calculation

The pain level assessed by VAS was used as the primary outcome to calculate sample size. Based on the study by Tantawy and Kamel (24), we estimated the mean and standard deviation of the initial pain of the study participants to be 4.9 ± 1.8 in the experimental group and 4.8 ± 1.6 in the control group; and the mean and standard deviation of the final pain, i.e., after six weeks of treatment, to be 3.3 ± 1.4 for the eccentric training group and 4.1 ± 1.5 for the traditional training group. Using a study power of 80%, a significance level of 95%, and a sample size ratio of 1: 1: 1 (KT group: exercise group: KT + exercise group), we estimated 16 participants for each group, totaling 48 participants. Believing that losses and refusals would be around 20%, we reached the final number of 20 participants for each group, totaling 60 participants.

Sample randomization

After the initial assessment, eligible participants were randomized through a list of random numbers provided by the EPI-Info[®] software. These participants chose a sealed envelope containing a random number corresponding to one of the intervention groups, previously defined by the list of random numbers. Participants were randomly divided into exercise group (EG), performing only one exercise protocol consisting of seven exercises; Kinesio tape group (KTG), where only the elastic bandage was applied to participants; and exercise + Kinesio tape group (EKTG), which performed both KT and exercise protocols, totaling 60 patients.

Eligibility Criteria

Eligibility criteria are shown in **table I**.

Table I. Study inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
Patients aged 18 to 70 years	Patients with history of shoulder dislocation and positive displacement apprehension test
Having signed the Informed Consent Form (ICF)	Intratendinous rotator cuff calcifications
Subacromial pain (grade 3 or more on VAS) for at least three months	Proximal humeral fracture
Two of three positive impingement tests (Jobe, Hawkins-Kennedy, Neer) performed by an independent expert	Diagnosis of complete tendon rupture in the rotator cuff
	Diagnosis of complete tendon rupture in the rotator cuff
	Previous shoulder surgery
	Adhesive capsulitis
	Type III acronym (Bigliani classification) on x-ray
	Use of corticoid infiltrates within three months prior to the study
	Rheumatic and neurological diseases
	Patients with cognitive impairment that prevents them from understanding the exercises
	Patients with skin hypersensitivity to Kinesio tape or bandage
	Patients undergoing or having undergone physiotherapy treatment in the last 3 months

DATA COLLECTION

The evaluation protocol was performed at three moments. The initial evaluation was performed prior to randomization; the second evaluation after the end of the intervention; and the third (follow-up) at 90 days after the end of the intervention protocol. Evaluations were performed by an independent, previously trained, blind evaluator (who did not know which group the research participant belonged to).

Anthropometric evaluation

Body mass index (BMI) was calculated by measuring body height and weight.

Pain intensity assessment

Pain intensity was measured using the Visual Analogue Scale (VAS). In this scale, the patient is asked about his/her pain level on a scale from zero to ten, where zero means total absence of pain and ten the maximum tolerable pain (25). The intensity of pain at rest was assessed 24 hours prior to evaluation.

Muscle strength assessment

Maximal voluntary isometric contraction (MVIC) was evaluated by manual dynamometry. Muscle strength of the rotators and deltoid was measured using a Chattanooga®

push-pull dynamometer. The peak of maximum voluntary isometric force was measured at the average arc angle of each movement. The average of two of the three measurements taken was used as the maximum voluntary isometric contraction (MVIC), as described by Ling *et al.* (26).

For flexion, abduction, and extension movements, the participant remained standing. The shoulder was oriented at 0°. The dynamometer was positioned above the elbow, and the resistance applied perpendicular to the distal humerus, one centimeter above the lateral epicondyle. The participant was asked to apply a force contrary to the position of the dynamometer. Participants maintained the positions mentioned while the examiner applied the force through the dynamometer with the participant accumulating maximum tension in one to two seconds and maintaining tension for four to five seconds.

External and internal rotation movements were tested with the participant lying supine, shoulder at 90° abduction and 90° elbow flexion. The dynamometer was positioned one centimeter below the wrist, perpendicular to the dorsal distal forearm, and one centimeter proximal to the ulnar styloid. The participant was asked to apply a force contrary to the position of the dynamometer. Two measurements were taken for each strength test, with a 30-second rest between measurements to allow muscle recovery. The average of the two measurements of each strength test was used for data analysis.

Range of motion assessment

Active and passive movements of flexion, abduction, extension, internal and external rotation were measured using a universal goniometer for determining ROM. To assess shoulder joint flexion and extension mobility, the participant remained standing. The goniometer axis was positioned centrally at the glenohumeral joint, with the fixed arm perpendicular to the ground, and the movable arm positioned in line with the axis of the participant's arm. For abduction, the central axis of the goniometer was positioned in the posterior glenohumeral joint. To assess rotation, the participant was placed in the supine position with the shoulder abducted at 90° and the elbow flexed at 90°. The central axis of the goniometer was positioned over the olecranon of the ulna. The fixed arm was positioned perpendicular to the ground, while the movable arm was positioned in line with the patient's forearm.

FUNCTION ASSESSMENT

Data regarding function and disability were collected through function and quality of life questionnaires (UCLA (27), modified) and the Shoulder Pain and Disability Index (SPADI) (28). The University of California, Los Angeles (UCLA) scale assesses pain intensity, shoulder function, active range of motion, muscle strength, and patient satisfaction. The higher the score obtained, the better the patient's functional level. A score of 34-35 points corresponds to excellent results, 28-33 points correspond to good results, 21-27 points correspond to reasonable results, and 0-20 points correspond to bad results. The Constant-Murley score assesses pain and disability in patients with shoulder disorders. It has two subjective parameters (pain and daily living activities) and two objective parameters (range of motion and muscle strength). The scale totals 100 points, of which 35 are allocated to subjective parameters and 65 to objective parameters. Finally, the Shoulder Pain and Disability Index (SPADI) is a quality of life questionnaire designed to assess pain and disability in shoulder disorders.

It consists of thirteen items with two dimensions, one for pain and the other for functional activities. The final questionnaire score obtained separately for each dimension is converted into percentages ranging from zero to one hundred, with the highest score indicating the worst condition of shoulder dysfunction.

EXERCISE PROTOCOL

For participants in the exercise group, the intervention protocol consisted of a program with seven eccentric exercises. The program lasted four weeks and the sessions were held three times a week. As the patient became more familiar with the program and progressed in mobility and strength weekly, the load of each exercise was increased by 0.5 kg per week until reaching 1 kg; from the last week until the end of the intervention protocol, the load was 2 kg. The increase in weekly load was suspended only in case of maintenance of pain on movement or lack of strength to progress further (table II).

KT intervention

Kinesio tape was applied according to the protocol for RC tendinitis/shoulder impingement suggested by Kase (18). For groups KTG and EKTG, KT was applied twice a week for four weeks and was replaced every three days. Each participant used three tapes. The first tape (Y-shaped) was applied over the supraspinatus and infraspinatus muscles, anchored towards their origin (18). The tape was applied using a 15 to 25% tension, with the participant in a contralateral inclination of the cervical spine to the shoulder, and the arm in internal rotation at the back (figure 1). The second tape (also Y-shaped) was applied bypassing the deltoid muscle belly, with a 15 to 25% tension, also towards the origin (18). A portion of the tape bypassed the anterior part of the deltoid, with the arm in horizontal abduction and in external rotation. The other portion of the tape bypassed the posterior aspect of the posterior deltoid, being applied with the arm

Table II. Exercise program.

Exercise 1. Sitting. Performs shoulder/scapular elevation up to 90° (15 repetitions).
Exercise 2. Sitting. Performs internal rotation as if scratching the back (15 repetitions).
Exercise 3. In lateral decubitus, arm supported, elbow at 90°, performs external rotation (15 repetitions).
Exercise 4. In lateral decubitus, arm supported, performs internal rotation (15 repetitions).
Exercise 5. In supine position, performs shoulder/scapular elevation up to 90° (15 repetitions).
Exercise 6. In prone position, with one arm off the examination table, performs extension movement (one-arm rowing exercise) (15 repetitions).
Exercise 7. In orthostasis, performs wall push-ups (15 repetitions).

in horizontal adduction and in internal rotation, as if touching the contralateral hip (**figure 1**) (18). Finally, the third tape (I-shaped) anchored the other two from the coracoid process towards the scapular spine, using a tension of 50 to 75%, with upper limb flexion and slight horizontal adduction (**figure 1**) (18). All participants were monitored for signs of hypersensitivity or allergy to KT. In the presence of these signs, the bandage would be removed and the patient referred for medical evaluation at the health network.

STATISTICAL ANALYSIS

Statistical Package for Social Sciences (SPSS) version 23.0 was used for data analysis. Data were initially expressed as frequency, mean, median and standard deviation. Afterwards, the normality of data distribution was analyzed using the Shapiro-Wilk test.

For intragroup comparisons (EG, KTG, and EKTG) at the various times of the study, parametric data were statistically analyzed by one-way analysis of variance (ANOVA) for repeated measures followed by Bonferroni post-hoc test. For intergroup comparisons, one-way analysis of variance (ANOVA) was used followed by Tukey post-hoc test, both at pre- and postintervention and 90 days after the intervention protocol. For nonparametric variables between groups, the Kruskal-Wallis and Friedman tests were used for intragroup analysis. The significance level established for all statistical tests was $p < 0.05$.

RESULTS

The initial sample consisted of 60 participants randomly assigned to exercise group (EG) ($n=20$), Kinesio tape group (KTG) ($n=20$), and exercise + Kinesio tape group (EKTG)

($n=20$). All participants completed the final evaluation. However, in the follow-up assessment, there were two losses in EG, one for withdrawal and one participant who was referred for surgery. Moreover, EKTG had a loss due to arm fracture. No side effects were observed regarding the use of KT or the progression of the proposed exercises in the study participants. Thus, 57 patients completed the study (**figure 1**).

Table III shows the initial characteristics of the sample. The groups were homogeneous regarding the analyzed variables. All intervention groups improved pain after the intervention and maintained this reduction in the follow-up assessment. There were no significant differences between groups (**figure 3**).

Considering UCLA scores, all groups significantly improved function after the intervention. In the follow-up assessment, EKTG showed a significantly higher score than KTG ($p=0.022$) (**figure 4**).

All groups also significantly improved disability from baseline to endpoint, as assessed by the SPADI-Brazil questionnaire. In the follow-up assessment, EKTG showed a significantly higher score than KTG ($p=0.041$) and EG ($p < 0.024$) (**figure 5**).

The exercise + Kinesio tape group (EKTG) significantly increased right shoulder mobility ($p < 0.05$). For this group, flexion and internal rotation range of motion were significantly higher compared to KTG ($p=0.02$). The exercise group (EG) significantly increased active ROM of flexion, abduction, and external and internal rotation at the end of the protocol ($p < 0.05$). The Kinesio tape group (KTG) improved only abduction and external rotation ROM ($p < 0.05$) (**table IV**).

Participants with left shoulder pain in the EKTG improved all active ROM ($p < 0.05$). For EG participants,



Figure 1. Application of Y-shaped Kinesio tape in the scapula (A); Y-shaped Kinesio tape in the deltoid (B); and I-shaped Kinesio tape for anchorage and scapulohumeral joint positioning (C).

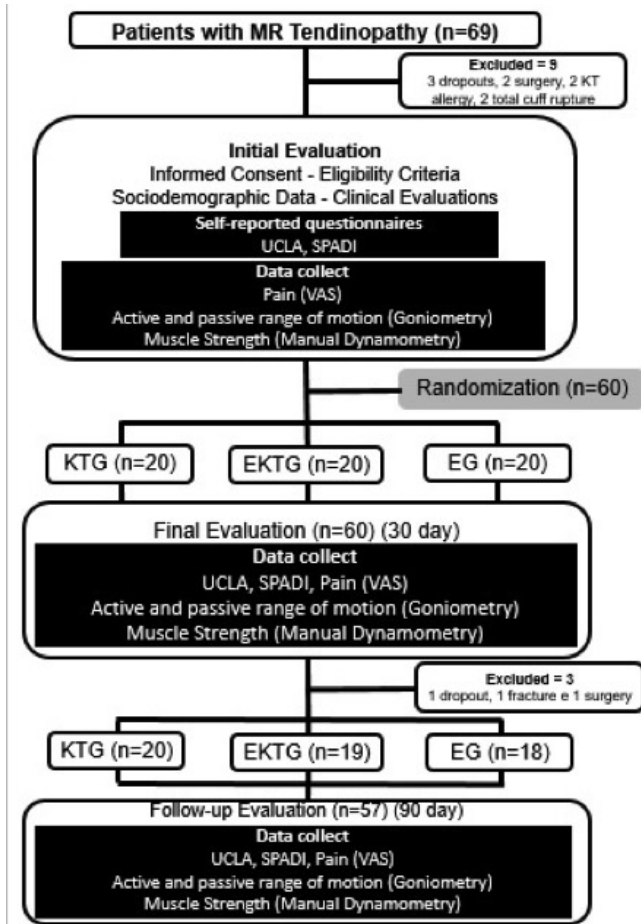


Figure 2. Study flowchart.

only extension ROM did not significantly increase after the intervention. In turn, KTG participants increased only flexion and abduction ROM ($p < 0.05$) (table V). There were no differences between groups in the different evaluation times.

The muscle strength of patients with right shoulder pain, represented by the maximum voluntary isometric contraction (MVIC), did not differ significantly between groups in the different moments of the study. The Kinesio tape group (KTG) significantly improved only flexion and external rotation ROM of the right shoulder ($p < 0.05$) at the end of the protocol. The exercise + Kinesio tape group (EKTG) increased muscle strength in flexion ($p < 0.05$), external rotation ($p < 0.01$), and internal rotation ($p < 0.01$) ROM of the right shoulder at the end of the protocol. However, muscle strength increased significantly for all ROM from baseline to follow-up ($p < 0.05$). Participants in the EG significantly increased strength in flexion, abduc-

tion, and internal rotation ROM of the right shoulder at the end of the intervention ($p < 0.05$). For flexion ROM, this strength gain remained at the follow-up assessment ($p < 0.05$) (table VI).

The muscle strength of patients with left shoulder pain did not differ significantly between groups at the different times of the study. The Kinesio tape group (KTG) significantly increased muscle strength for external rotation only at the follow-up assessment ($p < 0.05$). Participants in the EKTG significantly improved strength from baseline to endpoint only in external rotation ROM of the left shoulder ($p < 0.05$). The exercise group (EG), in turn, did not significantly improve left shoulder ROM (table VII).

Table III. Sociodemographic characteristics of the sample (n=60).

	Intervention Group			p-value
	KTG (n=20)	EKTG (n=20)	EG (n=20)	
Age, years (\pm sd)	48.65 \pm 10.27	46.95 \pm 10.74	49.20 \pm 13.13	0.88 [#]
Gender, n (%)				0.37 [§]
Male	6 (30.0)	8 (40.0)	4 (20.0)	
Female	14 (70.0)	12 (60.0)	16 (80.0)	
Skin color, n (%)				0.15 [§]
White	17 (85.0)	19 (95.0)	8 (100.0)	
Black	3 (15.0)	1 (5.0)	0 (0.0)	
Affected shoulder, n (%)				0.58 [§]
Right	9 (45.0)	10 (50.0)	6 (30.0)	
Left	7 (35.0)	7 (35.0)	7 (35.0)	
Both	4 (20.0)	3 (15.0)	7 (35.0)	
Occupation				0.14 [§]
General services	4 (20.0)	0 (0.0)	1 (5.0)	
Housekeeper	2 (10.0)	6 (30.0)	3 (15.0)	
Bricklayer	2 (10.0)	0 (0.0)	0 (0.0)	
Cook	2 (10.0)	0 (0.0)	0 (0.0)	
Others	10 (50.0)	14 (70.0)	16 (80.0)	
Time of pain, years (\pm sd)	4.95 \pm 5.63	5.00 \pm 8.05	4.88 \pm 8.99	0.98 [#]

Legend: kg: kilogram; cm: centimeters; KTG: Kinesio tape group; EKTG: exercise + Kinesio tape group; EG: exercise group.

One-way ANOVA.

§ Chi-square.

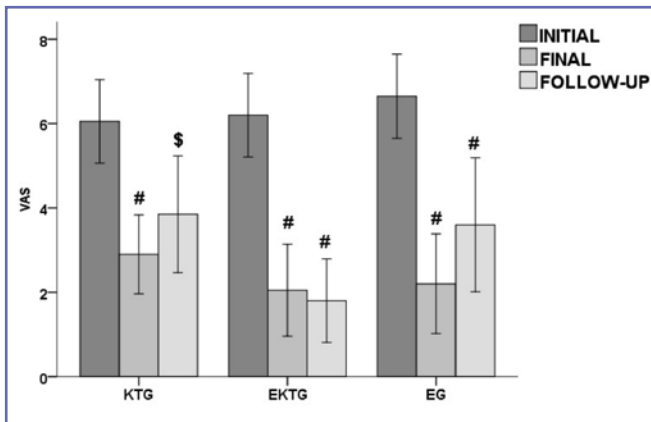


Figure 3. Pain level (VAS) in study groups (n=60)
 # p=0.001 compared to baseline. ANOVA for repeated measures.
 \$ p=0.02 compared to baseline. ANOVA for repeated measures.

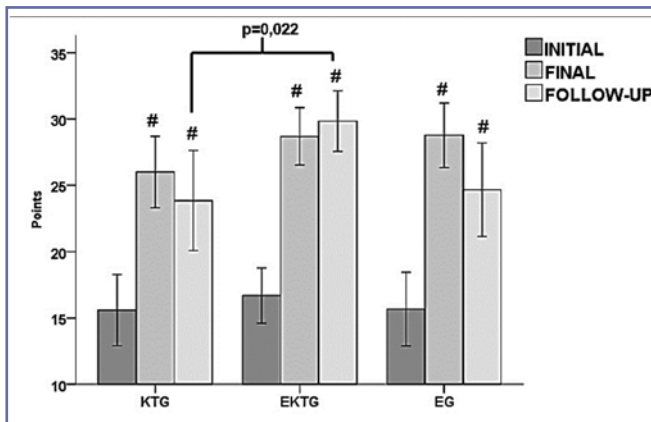


Figure 4. UCLA questionnaire score in study groups (n=60)
 # p=0.001 compared to baseline. ANOVA for repeated measures.

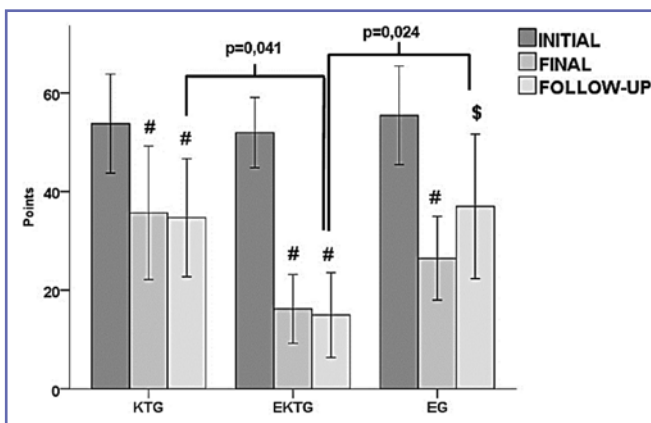


Figure 5. Total SPADI questionnaire score in study groups (n=60).
 # p=0.001 compared to baseline. Student t test.
 \$ p<0.05 compared to KTG. One-way ANOVA.

DISCUSSION

The present study evaluated the efficacy of KT, both isolated and associated with exercise, on pain, disability, mobility, and strength in subjects with RC injury. Oliva *et al.* (29), in their guideline about rotator cuff tears, report that the exercise demonstrates some advantages, in an isolated or associated way in an Individual Rehabilitation Project (PRI) in patients with rotator cuff tears. Pain intensity decreased in all study groups from baseline to endpoint and follow-up. This result is corroborated by the study by Kocyigit *et al.*, (30) who investigated the effect of KT on pain intensity, strength, and mobility in 41 patients with RC injury allocated to KT group (n=21) and placebo KT group (n=20). The authors prescribed a Codman pendulum exercise program with three sets of 10 repetitions per day for all patients (30). The experimental KT group significantly reduced pain intensity (30). The authors concluded that KT immediately improved pain in all patients with RC injury (30). Kinesio tape, in conjunction with other physiotherapy interventions, can facilitate or inhibit muscle function, maintain joint positioning, reduce pain, and provide proprioceptive feedback to achieve and maintain body alignment (31). Increased proprioceptive feedback by sensory stimulation from KT application may strengthen postural control and facilitate the return to previous muscle activity (32). Another probable explanation of the effect of KT on pain modulation relates to the pain gate theory, where it is suggested that the bandage stimulates neuromuscular pathways. This mechanical stimulation provided by KT activates fast conducting fibers performing synapses with inhibitory interneurons, which closes the pain gate, not allowing the passage of nociceptive stimuli (33,34). Micinilli *et al.* approached 21 patients with rotator cuff tendinopathy using real KT and sham KT. Both groups performed the same exercises protocol. The authors observed that KT can facilitate the immediate reduction of pain during rehabilitation, probably due to the mechanism of the pain gate, which KT produces sensory stimulus, which reduces the nociceptive signal (35). Similarly, Aguilar-Ferrandiz *et al.* (36) investigated the effect of KT and placebo KT application in women with chronic venous insufficiency. One hundred and twenty postmenopausal women with mild to moderate chronic venous insufficiency were randomly assigned to GK (n=60) group, who received standardized KT treatment (15 to 50% tension) for gastrocnemius muscle enhancement and ankle functional correction; and placebo KT group (n=60) (36). Pain level and overall health were recorded at baseline and after four weeks of treatment (36). The authors concluded that KT therapy improves pain symptoms and peripheral venous flow and may slightly increase overall health in women with chronic venous insufficiency for up to four weeks after treatment (36).

Table IV. Active range of motion (ROM) of participants with right shoulder pain (n=39).

Variable	Initial	Final	Follow-up	p-value	Initial	Final	Follow-up	p-value	Initial	Final	Follow-up	p-value	
Flexion (m±sd)	141.70 ± 27.14	154.05 ± 24.68 [#]	147.60 ± 24.66	0.057	150.42 ± 21.47	164.21 ± 19.32 ^s	163.58 ± 13.99 [#]	0.006	140.78 ± 29.63	161.67 ± 19.69 ^s	158.72 ± 20.54 ^s	0.000	0.02 †
Abduction (m±sd)	132.65 ± 31.61	149.65 ± 26.24 [#]	141.85 ± 28.44	0.037	144.10 ± 29.30	161.05 ± 22.01 ^s	159.79 ± 18.39 [#]	0.007	135.67 ± 31.33	158.00 ± 18.01 ^s	152.22 ± 22.28 [#]	0.002	0.02 †
Extension (m±sd)	45.74 ± 13.19	48.30 ± 11.80	43.40 ± 10.61	0.114	37.94 ± 7.56	50.16 ± 9.09 ^s	45.26 ± 12.67	0.002	46.22 ± 12.51	50.39 ± 10.65	48.28 ± 13.96	0.250	0.05
Ext. Rot. (m±sd)	64.70 ± 18.00	78.30 ± 11.41 ^s	74.00 ± 17.07	0.008	69.89 ± 4.29	74.47 ± 3.85	81.84 ± 9.25 [#]	0.022	64.72 ± 22.59	84.44 ± 7.83 ^s	79.39 ± 9.68 [#]	0.013	0.08 †
Int. Rot. (m±sd)	64.40 ± 23.76	73.15 ± 10.97	73.25 ± 22.63	0.130	69.78 ± 25.49	82.68 ± 12.14 [#]	83.00 ± 12.00	0.010	72.77 ± 17.59	82.28 ± 8.52 [#]	79.55 ± 10.38	0.023	0.02 †

Legend:

\$ p<0.005 compared to the baseline of the same group. One-way ANOVA for repeated measures.

p<0.05 compared to the baseline of the same group. One-way ANOVA for repeated measures.

† p=0.02 between EKTG and KTG. One-way ANOVA.

Bold data indicate significant values.

Table V. Active range of motion (ROM) of participants with left shoulder pain. (n=35).

Variable	KTG (n=11)				EKTG (n=10)				EG (n=14)				P-value
	Initial	Final	Follow-up	p-value	Initial	Final	Follow-up	p-value	Initial	Final	Follow-up	p-value	
Flexion (m ± sd)	143.45 ± 21.43	159.05 ± 18.14 [#]	161.00 ± 15.39 ^s	0.009	1148.95 ± 21.77	163.94 ± 15.28 ^s	167.74 ± 13.58 ^s	0.001	148.28 ± 23.86	165.78 ± 14.21 ^s	159.28 ± 24.08	0.001	0.95
Abduction (m ± sd)	140.40 ± 28.67	155.00 ± 24.65 [#]	158.25 ± 18.99 [#]	0.010	144.21 ± 24.98	161.47 ± 18.47 ^s	162.00 ± 19.17 [#]	0.001	144.50 ± 30.24	161.78 ± 16.62 ^s	155.17 ± 26.17	0.011	0.88
Extension (m ± sd)	48.15 ± 11.86	50.80 ± 7.90	49.20 ± 10.70	0.234	39.79 ± 8.61	50.63 ± 9.21 ^s	50.00 ± 11.40 ^s	0.000	45.94 ± 10.36	48.78 ± 11.62	50.94 ± 11.05	0.175	0.67
Ext. Rot. (m ± sd)	67.80 ± 16.54	76.45 ± 11.38	74.65 ± 14.56	0.068	69.42 ± 15.40	76.53 ± 15.84	81.84 ± 9.25	0.002	77.33 ± 13.78	88.67 ± 9.89 ^s	86.06 ± 5.99 [#]	0.005	0.31
Int. Rot. (m ± sd)	72.96 ± 20.39	80.90 ± 13.76	79.85 ± 19.79	0.183	75.84 ± 27.69	91.32 ± 18.47 ^s	88.63 ± 8.53	0.000	63.61 ± 23.45	81.89 ± 13.96 ^s	78.11 ± 11.30 [#]	0.007	0.82

\$ p<0.005 compared to the endpoint of the same group. One-way ANOVA for repeated measures.

p<0.05 compared to the baseline of the same group. One-way ANOVA for repeated measures.

Bold data indicate significant values

The UCLA scale showed an increase in functional and quality of life scores from baseline to endpoint in all groups of the present study. For these variables, EKTG demonstrated a better outcome than KTG in the follow-up assessment. Corroborating these results, Şahin *et al.* (37) evaluated 99 patients with RC injury, assigned to nonsteroidal anti-inflam-

matory group (n=33); KT + nonsteroidal anti-inflammatory group (n=33); and subacromial corticosteroid injection + nonsteroidal anti-inflammatory group (n=33). All three groups showed significant improvements after intervention (37). However, the KT + nonsteroidal anti-inflammatory group and the subacromial corticosteroid injection + nonste-

Table VI. Muscle strength (MVIC) of participants with right shoulder pain (n=39).

Variable	KTG (n=13)				EKTG (n=13)				EG (n=13)			
	Initial	Final	Follow-up	p-value	Initial	Final	Follow-up	p-value	Initial	Final	Follow-up	p-value
Flexion (m ± sd)	5.30 ± 2.72	6.17 ± 3.61 [#]	4.97 ± 2.27	0.041	5.39 ± 2.68	7.03 ± 4.14 [#]	6.89 ± 3.94 [#]	0.008	5.5 ± 3.81	7.31 ± 4.60 [§]	6.28 ± 4.39 [#]	0.004
Abduction (m ± sd)	5.47 ± 2.55	6.40 ± 3.56	5.30 ± 2.83	0.132	5.44 ± 2.12	6.95 ± 3.89	6.84 ± 3.80 [#]	0.011	4.89 ± 3.02	6.67 ± 3.98 [#]	6.17 ± 4.30	0.024
External rotation (m ± sd)	6.07 ± 3.81	7.70 ± 4.20 [#]	5.80 ± 3.20	0.024	4.84 ± 2.58	8.47 ± 4.79 [§]	8.68 ± 3.96 [§]	0.000	6.00 ± 4.04	7.64 ± 4.62	6.86 ± 4.40	0.167
Internal rotation (m ± sd)	7.13 ± 3.62	7.97 ± 4.60	7.15 ± 4.69	0.065	5.71 ± 2.63	8.55 ± 4.51 [§]	8.53 ± 5.27 [§]	0.006	6.33 ± 4.01	8.14 ± 4.53 [#]	6.75 ± 4.00	0.050

p<0.05 compared to the baseline of the same group. One-way ANOVA for repeated measures.
 § p<0.01 compared to the endpoint of the same group. One-way ANOVA for repeated measures.
 Bold data indicate significant values.

Table VII. Muscle strength (MVIC) of participants with left shoulder pain (n=35).

Variable	KTG (n=11)				EKTG (n=10)				Initial	Final	Follow-up	p-value
	Initial	Final	Follow-up	p-value	Initial	Final	Follow-up	p-value				
Flexion (m ± sd)	5.58 ± 3.04	6.50 ± 3.63	5.77 ± 3.49	0.067	5.89 ± 2.38	6.66 ± 4.32	7.05 ± 3.58	0.227	5.61 ± 3.22	6.25 ± 4.04	6.08 ± 4.07	0.554
Abduction (m ± sd)	5.92 ± 3.01	7.00 ± 3.40	6.35 ± 3.29	0.069	5.68 ± 2.13	5.69 ± 2.92	7.16 ± 3.33	0.162	4.77 ± 3.05	6.03 ± 3.99	5.81 ± 4.01	0.230
External rotation (m ± sd)	6.72 ± 3.55	8.22 ± 4.51	6.92 ± 4.23 ^{&}	0.022	5.84 ± 2.80	8.08 ± 4.81 [#]	7.74 ± 3.66	0.039	5.50 ± 2.94	7.03 ± 3.87	7.28 ± 4.74	0.113
Internal rotation (m ± sd)	7.58 ± 3.69	8.20 ± 4.54	7.88 ± 4.98	0.367	6.84 ± 4.35	8.34 ± 4.98	9.32 ± 4.94	0.080	6.50 ± 4.14	7.28 ± 3.38	7.75 ± 4.28	0.175

p<0.05 compared to the baseline of the same group. One-way ANOVA for repeated measures.
 § p<0.005 compared to the baseline of the same group. One-way ANOVA for repeated measures.
 & p<0.05 compared to the endpoint. One-way ANOVA for repeated measures.
 Bold data indicate significant values.

roidal anti-inflammatory group showed a greater tendency for improvement in all variables. The authors then concluded that the addition of KT or subacromial corticosteroid injection to treatment with anti-inflammatory drugs seems to have a significant and similar effect in patients with RC injuries (37). Therefore, KT may serve as an alternative treatment when corticosteroid injection is contraindicated (37). The SPADI-Brazil questionnaire showed statistically significant results for functional and quality of life scores in all study groups. However, EKTG showed a statistically significant improvement at follow-up when compared to EG and

KTG. Kaya *et al.* addressed 54 patients diagnosed with SIS allocated to KT + exercise group (n=28) and manual therapy + exercise group (n=26) (38). Shoulder pain and functionality were evaluated (38). After intervention, there were significant differences in both groups in terms of decreased pain and improved disability, demonstrating that exercise is the basis for improvement in these patients (36). However, only the KT + exercise group showed a significant reduction in nighttime pain (38). Simsek *et al.* (2013) conducted a similar study evaluating strength, pain, ROM, and disability on the 5th and 12th day of treatment of 38 subjects with SIS,

assigned to KT + exercise group (n=19) and placebo KT + exercise group (n=19) (39). Both groups improved scores at the 5th and 12th day of evaluations ($p < 0.05$). In comparisons between groups, movement pain and disability score improved significantly in the KT + exercise group on day 5 ($p < 0.01$) (39). Nighttime pain, movement pain, disability score, shoulder external rotation strength, and pain-free shoulder abduction ROM improved significantly in the KT + exercise group on the 12th day ($p < 0.05$) (39). However, passive shoulder flexion ROM increased more in the placebo KT + exercise group on the 12th day ($p < 0.05$) (39). The authors suggest that the reduction of pain and disability in the KT + exercise group occurred due to traction generated by the tension applied along the tape in the area of interest, thus increasing blood flow and producing neurophysiological effects to prevent pain transmission through the mechanism proposed in the pain gate theory (39). Notwithstanding, KT has shown effective short-term results (39). The authors concluded that the addition of KT to the exercise program appeared to be more effective than the exercise program alone for treating SIS (39).

Range of motion (ROM) in the affected shoulder showed that EKTG improved all evaluated movements. Corroborating these findings, Subaşı *et al.* (40) investigated the efficacy of KT compared to subacromial injection of betamethasone and prilocaine associated with a three-month exercise program, including stretching and strengthening exercises in 70 patients with RC injury (40). Participants were randomly assigned to injection group (n=35) and KT group (n=35) (40). Kinesio tape (KT) was applied three times for five consecutive days, with a two-day interval between applications (40). All patients were evaluated at baseline and one and three months after intervention (40). Significant differences were detected in pain and SPADI scores, as well as in ROM measurements in both groups (40). No significant differences were detected between groups, except for the degree of active flexion in favor of the KT group (40). Together with an exercise program, both KT and steroid injection therapy have been found to be effective in treating RC injuries. Thus, KT may be an alternative treatment option in the rehabilitation of RC injuries, especially when a noninvasive technique is required (40).

Muscle strength of the affected right shoulder showed statistically significant differences in KTG only in flexion and external rotation measurements. In turn, EKTG participants showed a statistically significant improvement in all movements. Finally, EG improved all movements at endpoint, except for external rotation. Groups KTG and EKTG improved muscle strength of the affected left shoulder in external rotation only. In contrast, EG did not improve any of the movements. The improvement in muscle strength, most

significant in the KT group, can be justified by the action of KT in facilitating muscle activity and in the immediate increase of muscle strength, which may be due to concentric traction of the fascia (35). In a crossover study, Hsu *et al.* (41) compared the effects of therapeutic KT (Y-shaped tape with 25% tension) and placebo KT (Y-shaped tape without tension) in baseball players with RC injuries in two weekly sessions with three-day intervals (41). Three-dimensional scapular movements and electromyographic (EMG) activities of the upper and lower trapezius and anterior serratus muscle were measured during shoulder elevation with a 2-kg load and were performed three consecutive times to collect electromyographic data (41). Lower trapezius strength was tested before and after each KT application (41). Results showed that the therapeutic KT group significantly increased 30° and 60° posterior scapular inclination during arm elevation (41). Moreover, this group increased lower trapezius muscle activity in the 60-30° arm adduction compared to placebo KT (41). These improvements led to positive changes in scapular movement and muscle performance (41). The results supported the use of KT as an adjunct to the treatment of RC injuries, corroborating the present study, which demonstrated that exercise-associated KT significantly improved all movements tested in the study participants with right shoulder pain. Leong and Fu (42) approached 43 volleyball athletes through three taping protocols, with and without tension. The authors analyzed the subacromial space and observed that athletes with CR tendinopathy demonstrated less reduction in the subacromial space with rigid scapular taping during the early abduction of the arm (42). Tappings applied to the scapula showed no effect in the subacromial space during arm rest at 0° of shoulder abduction (42). However, they showed less space reduction during active shoulder abduction from 0° to 60° in athletes with RC tendinopathy (42). This may indicate better control of the subacromial space during active arm abduction (42). The possible mechanism for the observed changes may be via skin stimulation. To support this assumption, is checked an earlier activation of the scapular muscles during the arm abduction and an increase in the lower trapezius activities during arm elevation (42). Therefore, therapeutic taping can improve neuromotor control of the scapular muscles for better control of the scapula and preserve the subacromial space during abduction of the arm (42). Even if there is some clinical efficacy of applying adhesive tape, there is insufficient evidence of rehabilitation benefits to strongly recommend the application of KT in isolation (35). Other studies that investigate the effect of KT in the treatment of other joints, such as the knee, confirm that KT can support rehabilitation therapy. However, there are no significant effects in terms of recovery (35).

More expressive results were obtained when associating KT with the exercise program, indicating important benefits from the optimization of exercises on the pain and function of these patients. Therefore, it seems to us that KT acts as an important adjunct in the recovery of rotator cuff injuries.

STUDY LIMITATIONS

Our study has some limitations that prevent the extrapolation of results. Initially, the sample - despite being calculated - is small. Moreover, a short follow-up period after the intervention prevents us from observing these results over time. We also chose to analyze the variables only including subjects with affected dominant and nondominant shoulder, which further reduces our sample, being a possible bias for extrapolating the results. The lack of a control or placebo group also hinders the extrapolation of findings. In addition, the lack of use of an isokinetic dynamometer to verify the strength of the study participants may be a limitation. This type of dynamometer has been cited by

some authors to be more reliable than the manual dynamometer, and thus could provide a more effective result for muscle strength.

Research Ethics Committee Opinion No. 2.152.768, Lutheran University of Brazil

ACKNOWLEDGEMENTS

We thank the Group of Studies and Research in Sports and Orthopedic Trauma Rehabilitation, Physiotherapy Course, Lutheran University of Brazil, Torres/RS/Brazil.

CONCLUSIONS

Exercises proved to be the basis of the treatment of rotator cuff injury. Associating KT with exercises significantly improved the function of patients with this type of injury. In isolation, KT was effective in reducing pain and function, but did not alter joint strength and mobility.

REFERENCES

1. Oliveira FCL, Fontenay BP, Bouyer LJ, et al. Effects of Kinesiotaping Added to a Rehabilitation Programme for Patients with Rotator Cuff Tendinopathy: Protocol for a Single-Blind, Randomised Controlled Trial Addressing Symptoms, Functional Limitations and Underlying Deficits. *BMJ*. 2017;7(9):1-10.
2. Edwards A, Chepe J, Jones A, Sheps DM, Beaupré L. Can Clinical Assessment Differentiate Partial Thickness Rotator Cuff Tears from full Thickness Rotator Cuff Tears? A Secondary Analysis. *Disabil Rehabil*. 2019;8:1-8.
3. Saracoglu I, Emuk Y. Does taping in addition to physiotherapy improve the outcomes in subacromial impingement syndrome? A systematic review. *Physiother Theory Pract*. 2017;34(4):251-63.
4. Whittle S, Buchbinder R. In the Clinic. Rotator Cuff Disease. *Ann Intern Med*. 2015;162(1):1-15.
5. Steuri R, Sattelmayer M, Elsig S, et al. Effectiveness of conservative interventions including exercise, manual therapy and medical management in adults with shoulder impingement: A Systematic review and meta-analysis of RCTs. *Br J Sports Med*. 2017;51(18):1340-47.
6. Clement ND, Nie YX, McBurnie JM. Management of degenerative rotator cuff tears: A review and treatment strategy. *Sports Med Arthrosc Rehabil Ther Technol*. 2012;48(4):1-5.
7. McLaren C, Colman Z, Rix A, et al. 2016. The Effectiveness of Scapular Taping on Pain and Function in People with Subacromial Impingement Syndrome: A systematic review. *Int Musculoskelet Med*. 2016; 38(3):81-89.
8. Saraya S, Bakry RE. Ultrasound: Can it replace MRI in the evaluation of the rotator cuff tears? *The Egyptian Journal of Radiology and Nuclear Medicine*. 2016; 47(1): 193–201.
9. Mottram SL. Dynamic Stability of the Scapula. *Man Ther*. 1997; 2(3):123-31.
10. Kibler WB, Sciascia AD. What Went Wrong and What to do About It: Pitfalls In The Treatment Of Shoulder Impingement. In: Duwelius PJ, Azar FM, eds. Instructional course lectures, vol. 57, Rosemont: American Academy of Orthopaedic Surgeons, 2008;47:877-85.
11. Vieira FA, Olawa PJ, Belangero OS, et al. Lesão do Manguito Rotador: Tratamento e Reabilitação. *Perspectivas e Tendências atuais. Rev Bras Ortop*. 2015; 50(6): 647-651.
12. Camargo PR, Avila MA, Albuquerque-Sendin FA, et al. Eccentric training as a new approach for rotator cuff tendinopathy: Review and perspectives. *Word J Ortop*. 2014; 5(5): 634-644.
13. Blume C, Price SW, Jackson ET, et al. Comparison of eccentric and concentric exercise interventions in adults with subacromial impingement syndrome. *Int J Sports Phys Ther*. 2015; 10(4): 441–55
14. Ortega-Castillo M, Medina-Porqueres I. Effectiveness of the eccentric exercise therapy in physically active adults with symptomatic shoulder impingement or lateral epicondylar tendinopathy: A systematic review. *J Sci Med Sport*. 2016; 19: 438–453.
15. Dejaco B, Habets B, Van Loon C, et al. Eccentric versus conventional exercise therapy in patients with rotator cuff tendinopathy: a randomized, single blinded clinical trial. *Knee Surg Sports Traumatol Arthrosc*. 2016; 28: 1-9.
16. Hallgren HB, Holmgren T, Öberg B, Adolfsson L, Johansson K, Adolfsson LE. A specific exercise strategy reduced the need for surgery in subacromial pain patients. *Br J Sports Med*. 2014;48: 1431–1436.

17. Thelen MD, Dauber JA, et al. The Clinical Efficacy of Kinesio Tape for Shoulder Pain: A Randomized, Double-Blinded, Clinical Trial. *J Orthop Sports Phys Ther.* 2008; 38 (7): 389-95.
18. Kase K, Wallis J, Kase T. Clinical therapeutic applications of the Kinesio taping method. USA: Kinesio; 2013.
19. Oliveira FCL, Fontenay BP, Bouyer LJ, et al. Immediate Effects of Kinesiotaping on Acromiohumeral Distance and Shoulder Proprioception in Individuals with Symptomatic Rotator Cuff Tendinopathy. *Clin Biomech.* 2019;1(61):16-21.
20. Seitz A. L, Podlecki L.A, Emily R. Melton E. R, et al. Neuromuscular Adaptions Following a Daily Strengthening Exercise in Individuals with Rotator Cuff Related Shoulder Pain: A Pilot Case-Control Study. *IJSPT.* 2019;1(14):74-87.
21. Kachingwe AF, Phillips B, Sletten E, et al. Comparison of Manual Therapy Techniques with Therapeutic Exercise In the Treatment of Shoulder Impingement: A Randomized Controlled Pilot Trial. *J Man Manip Ther.* 2008; 16(4): 238-47.
22. Wright AA, Wassinger CA, Frank M, et al. Diagnostic Accuracy of Scapular Physical Examination Tests For Shoulder Disorders: A Systematic Review. *Br J Sports Med,* 2013; 47(14):886-92.
23. Karthikeyan S, Griffin DR, Parson N, et al. Microvascular Blood Flow in Normal and Pathologic Rotator Cuffs. *J Shoulder Elbow Surg,* 2015;24(12):1954-60.
24. Tantawy SA, Kamel DM. The effect of kinesio taping with exercise compared with exercise alone on pain, range of motion, and disability of the shoulder in postmastectomy females: a randomized control trial. *J Phys Ther Sci.* 2016;28(12):3300-05.
25. Michener LA, Walsworth MK, Burnet EN. Effectiveness of Rehabilitation for Patients with Subacromial Impingement Syndrome: A Systematic Review. *J Hand Ther.* 2004;17(2):152-64.
26. Ling SM, Conwit RA, Talbot L, et al. Electromyographic patterns suggest changes in motor unit physiology associated with early osteoarthritis of the knee. *Osteoarthr. Cartil.* 2007;15(10):1134-40.
27. Oku EC, Andrade AP, Stadiniky SP, et al. Translation and cultural adaptation of the Modified-University of California at Los Angeles Shoulder Rating Scale to portuguese language. *Rev Bras Reumatol.* 2006;46(4):246-52.
28. Martins J, Napoles BV, Hoffman CB, et al. The Brazilian version of shoulder Pain and Disability Index- translation, cultural adaptation and reliability. *Braz J Phys Ther.* 2010; 14(6): 527-536.
29. Oliva F, Piccirilli E, Bossa M, et al. I.S.Mu.L.T - Rotator Cuff Tears Guidelines. *Muscles Ligaments Tendons J.* 2016;5(4):227-63.
30. Kocyigit F, Acar M, Turkmen MB, et al. Kinesio taping or just taping in shouler subacromial impingement syndrome? A randomized, double-blind, placebo-controlled trial. *Physiother Theory Pract.* 2016;32(7):501-8.
31. Ingwersen KG, Christensen R, Sorensen L, et al. Progressive High-Load Strength Training Compared with General Low-Load Exercises in Patients with Rotator Cuff Tendinopathy: Study Protocol for a Randomised Controlled Trial. *Trials.* 2015;16(27):1-11.
32. Jaraczewska E, Long C. Kinesio Taping in Stroke: Improving Functional Use of The Upper Extremity in Hemiplegia. *Top Stroke Rehabil.* 2006;13(3):31-42.
33. Shakeri H, Keshavarz R, Arab AM, et al. Clinical effectiveness of kinesiological taping on pain and pain free shoulder range of motion in patients with shoulder impingement syndrome: a randomized, double blinded, placebo-controlled trial. *Int J Sports Phys Ther.* 2013;8(6) 800-810.
34. Thelen MD, Dauber JA, et al. The Clinical Efficacy of Kinesio Tape for Shoulder Pain: A Randomized, Double-Blinded, Clinical Trial. *J Orthop Sports Phys Ther.* 2008;38(7):389-95.
35. Miccinilli S, Bravi M, Morrone M, et al. A Triple Application of Kinesio Taping Supports Rehabilitation Program for Rotator Cuff Tendinopathy: a Randomized Controlled Trial. *Ortop Traumatol Rehabil.* 2018; 20(6):499-505.
36. Aguilar-Ferrández ME, Castro-Sánchez AM, Matarán-Peñarocha GA, et al. A Randomized Controlled Trial of a Mixed Kinesio Taping-Compression Technique on Venous Symptoms, Pain, Peripheral Venous Flow, Clinical Severity and Overall Health Status in Postmenopausal Women with Chronic Venous Insufficiency. *Clin Rehabil* 2014;28(1):69-81.
37. Şahin O Ş, Biçer S, Şahin Z, et al. Effectiveness of Kinesiotaping and Subacromial Corticosteroid Injection in Shoulder Impingement Syndrome. *Am J Phys Med Rehabil.* 2016;95(8):553-60.
38. Kaya DO, Baltacı G, Toprak U. et al. The clinical and sonographic effects of kinesiotaping and exercise in comparison with manual therapy and exercise for patients with subacromial impingement syndrome: a preliminary trial. *J. Manipulative Physiol Ther.* 2014;37(6):422-32.
39. Şimşek HH, Balki S, Keklik SS, et al. Does Kinesio Taping in Addition to Exercise Therapy Improve the Outcomes in Subacromial Impingement Syndrome? A Randomized, Double-Blind, Controlled Clinical Trial. *Acta Orthop Traumatol Turc.* 2013;47(2):104-10.
40. Subaşı V, Çakır T, Arıca Z, et al. Comparison of efficacy of kinesiological taping and subacromial injection therapy in subacromial impingement syndrome. *Clin Rheumatol.* 2016;35(3):741-6.
41. Hsu Y, Chen W, Lin H, et al. The effects of taping on scapular kinematics and muscle performance in baseball players with shoulder impingement syndrome. *J Electromyogr Kinesiol.* 2009;19(6):1092-9.
42. Leong HT, Fu SN. The Effects of Rigid Scapular Taping on the Subacromial Space in Athletes With and Without Rotator Cuff Tendinopathy: A Randomized Controlled Study. *J Sport Rehabil.* 2019 Mar 1;28(3):250-255.
43. Padulo J, Oliva F, Frizziero A, Maffulli N. Muscles, Ligaments and Tendons Journal - Basic principles and recommendations in clinical and field Science Research: 2018 update. *MLTJ* 2018; 8(3): 305 – 307.

Influence of Supplements and Drugs used for the Treatment of Musculoskeletal Disorders on Adult Human Tendon-Derived Stem Cells

F. Di Meglio¹, A. M. Sacco¹, I. Belviso¹, V. Romano¹, F. Sirico¹, C. Loiacono¹, S. Palmeri¹, C. Pempinello², S. Montagnani¹, D. Nurzynska¹, C. Castaldo¹

¹ Department of Public Health, University of Naples Federico II, Naples, Italy

² Department of Orthopaedics and Traumatology, San Giovanni Bosco Hospital, Naples, Italy

CORRESPONDING AUTHOR:

Daria Nurzynska
University of Naples Federico II
Department of Public Health
5 S. Pansin Road
80131 Naples, Italy
E-mail: dariaanna.nurzynska@unina.it

DOI:

10.32098/mltj.03.2020.04

LEVEL OF EVIDENCE: Cell research/Lab study

SUMMARY

Background. Recent findings indicate that the connective tissue of tendons hosts cells that can self-renew and are multipotent. Even if these cells seemingly fail to accomplish tendon regeneration in pathological conditions, their identification and characterization represents a milestone in the research and development of new biological therapies for tendinopathies.

Methods. We isolated the adult human tendon-derived stem cells (hTDSCs) from the fragments of patellar or calcaneal tendons and characterized these cells in vitro by immunocytochemistry and histochemistry. Subsequently, the MTT test and Trypan Blue were used for the evaluation of cytotoxicity of the supplements/drugs commonly used for the treatment of musculoskeletal disorders: Curcumin, Hyaluronic Acid, Palmitoylethanolamide, Diclofenac sodium, Triamcinolone acetonide and Thiocolchicoside. **Results.** Cells obtained by outgrowth expressed mesenchymal markers, were clonogenic and differentiated into chondroblasts, osteoblasts and adipocytes. High concentrations of the anti-inflammatory glucocorticoid Triamcinolone and the analgesic fatty-acid amide Palmitoylethanolamide significantly reduced cell viability. Only curcumin had a positive effect on cell survival, both in the normal and oxidative stress conditions.

Conclusions. Adult human tendons possess stem cells. The influence of several drugs or supplements used for the treatment of musculoskeletal disorders should be taken into consideration in order to take the full advantage of the healing properties of stem cells within tendons.

KEY WORDS

Analgesics; curcumin; glucocorticoids; stem cells; tendon.

BACKGROUND

An ambiguous relationship between tissue inflammation and degeneration prompted the use of a term tendinopathy, encompassing tendon inflammation (tendinitis) and tendon degeneration (tendinosis), to describe the clinical condition characterized by pain and impaired strength of a tendon (1). Tendinopathies represent a growing problem in the clinical practice of general practitioners and specialists in sports medicine or orthopaedic surgery (2,3). Pharmacological treatments, surgical interventions, physiotherapy procedures or, more recently, extracorporeal

real shock wave therapy (4) and platelet-rich plasma injections (5) have been used for treating tendinopathy with different results (6), but the development of new effective therapeutic strategies remains hindered by the lack of biological data regarding the pathogenesis of the disease at the cell and tissue level. Notwithstanding the availability of modern molecular and cellular biology tools, our understanding of tendon biology is far from that of other tissues and the exact mechanism underlying degenerative and reparative processes in tendinopathy has not yet been clarified (7).

Recent findings indicate that tendon connective tissue hosts stem cells that have the capacity for self-renewal and multipotential differentiation (8,9,10). Even if these cells seemingly fail to accomplish tissue regeneration in chronic pathological conditions, their identification and characterization can represent a major turning point in the research and development of new biological therapies for tendinopathies (11). Indeed, the stem cells have increasingly attracted the interest of scientists and physicians for their potential application in different fields of medicine, including orthopaedics (12): for one thing, these cells could be propagated and stimulated *in vitro* and injected into the patients' tendons; for another, they could be activated directly in the tendon using selected cytokines and supplements/drugs. Hence, more data on the biology of tendon-derived stem cells (TDSCs) are needed in order to identify the conditions and factors that would enhance their survival and activity in normal and pathological conditions. Accordingly, we isolated and characterized the adult human TDSCs in order to evaluate the cytotoxicity of several supplements/drugs commonly used for the treatment of musculoskeletal disorders on these cells.

MATERIALS AND METHODS

Isolation and culture of hTDSCs

Fragments of normal patellar tendons ($n = 5$) and calcaneal tendons ($n = 5$) were collected from patients undergoing amputation surgery ($n = 10$; 5 males, 5 females; mean age 40 ± 8 years). All patients provided written informed consent for the use of the specimens from their amputated limbs in research and specimens were collected before the disposal of the amputated limbs as medical waste, without patient identifiers, in conformity with the principles outlined in the Declaration of Helsinki and the MLTJ (13).

Samples were minced and obtained pieces were washed in the sterile PBS solution, placed under sterile cover glasses in 35-mm culture plates and cultured in DMEM High Glucose (Sigma-Aldrich, St. Louis, MO, USA) enriched with 10% fetal bovine serum (Sigma-Aldrich), 5% horse serum (Sigma-Aldrich), 0.2 mM glutathione (Sigma-Aldrich), 10 ng/ml b-FGF (Preprotech, Rocky Hill, NJ, USA), erythropoietin 5 UI, porcine gelatine 50 g/ml, penicillin 10,000 U and streptomycin 10 mg/ml (Sigma-Aldrich), at 37°C in 5% CO₂. Plates were observed daily at an inverted phase-contrast microscope (Olympus, Segrate, Italy) and medium was replaced every 3 days. The outgrowth of the cells was documented by digital image acquisition (Colour View III Soft Imaging System, Muenster, Germany). Once

the adherent cells were more than 75% confluent, they were detached with 0.25% trypsin-EDTA (Sigma-Aldrich) and then expanded up to passage P₃.

Cell growth and cloning

In order to assess the growth dynamics of hTDSCs, cells were seeded at the density of 25×10^3 cells/cm² in 35-mm culture plates. After 48 h, 96 h, 144 h and 192 h of culture, cells were stained with Trypan Blu (Lonza, Walkersville, MD, USA) and live cells were counted manually in the cell counting chamber (Hausser Scientific, Horsham, PA, USA). The population doubling time was calculated in the exponential growth phase of the growth curve, according to the formula $PDT = T \times \ln 2 / \ln (N_1/N_0)$, where PDT is the population doubling time, T represents the incubation time, N₀ is the cell number at the beginning of the incubation time, and N₁ is the cell number at the end of the incubation time. Cell cloning test was performed by serial dilution in 96-well plates, according to the manufacturer's protocol (Corning Incorporated, NY, USA).

Immunocytochemistry

For the *in vitro* phenotypic characterization by immunocytochemistry, cells were fixed in 4% paraformaldehyde for 20 minutes and incubated first with 10% donkey serum (Sigma-Aldrich) for 1 hour at room temperature, and then with primary antibodies targeting CD34, CD44, CD45, CD90, CD105 (all from Abcam, Cambridge, UK), Vimentin, Phalloidin (both from Sigma-Aldrich) for 1 hour at 37°C. Subsequently, the matching secondary antibodies conjugated with rhodamine or fluorescein (Jackson ImmunoResearch, Europe, Newmarket, UK) were applied. The stained area of culture dish was mounted with coverglass in Vectashield mounting medium with DAPI (Vector Laboratories, Burlingame, CA). Stained cells were observed, quantified and documented using fluorescence microscope (Nikon, Tokyo, Japan).

Differentiation potential of hTDSCs *in vitro*

Cells were seeded at a density of 2×10^5 cells/cm² in 35-mm Petri dishes in DMEM High Glucose enriched with 10% bovine fetal serum, 10,000 U penicillin and 10 mg/ml streptomycin (all from Sigma-Aldrich). After reaching 75% confluence, hTDSCs were cultured for 14 days in osteogenic, adipogenic, and chondrogenic induction medium, according to the previously validated protocol (14). Adipogenic induction medium consisted of DMEM High Glucose with 10% horse serum (Sigma-Aldrich).

Osteogenic induction medium consisted of serum-free DMEM High Glucose enriched with 10^{-7} M Dexamethasone, 0.2 mM Ascorbic Acid, 10 mM β -Glycerophosphate (all from Sigma-Aldrich). Chondrogenic induction medium consisted of serum-free DMEM High Glucose supplemented with 100 ng/ml TGF- β (Peprotech). Culture medium was changed every 72 hours. To confirm the adipogenic, osteogenic or chondrogenic differentiation, cells were fixed in 4% paraformaldehyde and stained using specific methods: Oil Red O, von Kossa and Alcian blue (all from Bio-Optica, Milan, Italy), respectively. The cells were then observed at a microscope equipped with a digital camera (Leica Microsystems).

hTDSC Metabolic activity and Viability test

The hTDSCs were seeded at the density of 1×10^4 cells/cm² in a 96-well microplate and allowed to proliferate. After 24 hours, cells were treated with one of the supplements: Curcumin, Hyaluronic Acid, Palmitoylethanolamide, or drugs: Diclofenac sodium, Triamcinolone acetonide, Thiocolchicoside (all from Sigma-Aldrich) at different concentrations (15,16) for 72 hours (**table I**). The cytotoxicity of these supplements and drugs was evaluated by the MTT assay, according to the manufacturer's protocol (Sigma-Aldrich). Since the total mitochondrial activity is related to the number of alive cells, this assay can be used to measure the in vitro effects of drugs on cell viability (17). The experiments were performed in triplicates. The absorbance was measured at 570 nm, using the Multiscan EX microplate reader (Thermo Labsystems, Vantaa, Finland).

Next, cells were incubated in the culture medium with the addition of the supplement or drug at the concentration which was associated with the highest metabolic activity in MTT assay and their viability was evaluated using trypan

blue, as previously published (18,19,20,21). Three independent experiments were performed, with the evaluation of the number of alive and dead cells at 48, 72 and 96 hours in normal conditions, and at 4 and 8 hours in the presence of oxidative stress evoked by the addition of 200 μ M hydrogen peroxide. For each in vitro assay, control cells were incubated for the same time in the standard culture medium.

STATISTICAL ANALYSIS

Data were analysed with GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA), using t-student test. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Characteristics of hTDSCs in culture

The outgrowth of hTDSC from the tendon fragments occurred on the sixth day of culture (**figure 1**). At about 21 days, the cells reached 75% confluence and were passaged for the subsequent experiments. As observed at a phase-contrast microscope, hTDSCs in culture were spindle-shaped or star-shaped; cells were expanded up to three passages without altering their morphology.

The estimated normal growth curve of hTDSCs showed a typical sigmoidal pattern, with an adaptation phase, logarithmic phase and plateau phase (**figure 2**). The population doubling time of hTDSCs in vitro was approximately 36 hours. Following serial dilution of hTDSCs suspension, single cells formed colonies after 96 hours of incubation. The observed frequency of colony formation from single cells reached 12%. The formation of clones was monitored for 10 days (**figure 3**). Phenotypic analysis performed by immunofluorescent staining confirmed that isolated cells were CD34, CD44 and CD45 negative (positive cells represented 0.12%, 1.6%, and 0.57% of the population, respectively), and expressed specific mesenchymal surface markers CD90 and CD105 (66.2% and 67.3% of cells, respectively). Furthermore, hTDSC expressed filamentous actin and vimentin (89%) intermediate filaments in the cytoskeleton (**figure 4**).

Differentiation potential of hTDSCs in vitro

The hTDSCs were cultured in specific media to induce their differentiation into osteoblasts, adipocytes or chondroblasts (**figure 5**). After 14 days of culture, the hTDSCs incubated in the osteogenic differentiation medium formed aggregates and changed their shape from flat and elongated to round. The von Kossa stain demonstrated calcium deposits in the

Table I. Concentrations of supplements/drugs used in hTDSCs culture.

Supplement/drug	Concentration		
	Low	Medium	High
Curcumin [μ g/ml]	1	2.5	5
Hyaluronic acid [mg/ml]	0.5	1.25	2.5
Palmitoylethanolamide [mM]	1	10	100
Diclofenac sodium [μ g/ml]	10	100	500
Triamcinolone acetonide [mg/ml]	1	5	10
Thiocolchicoside [μ g/ml]	2.5	5	10

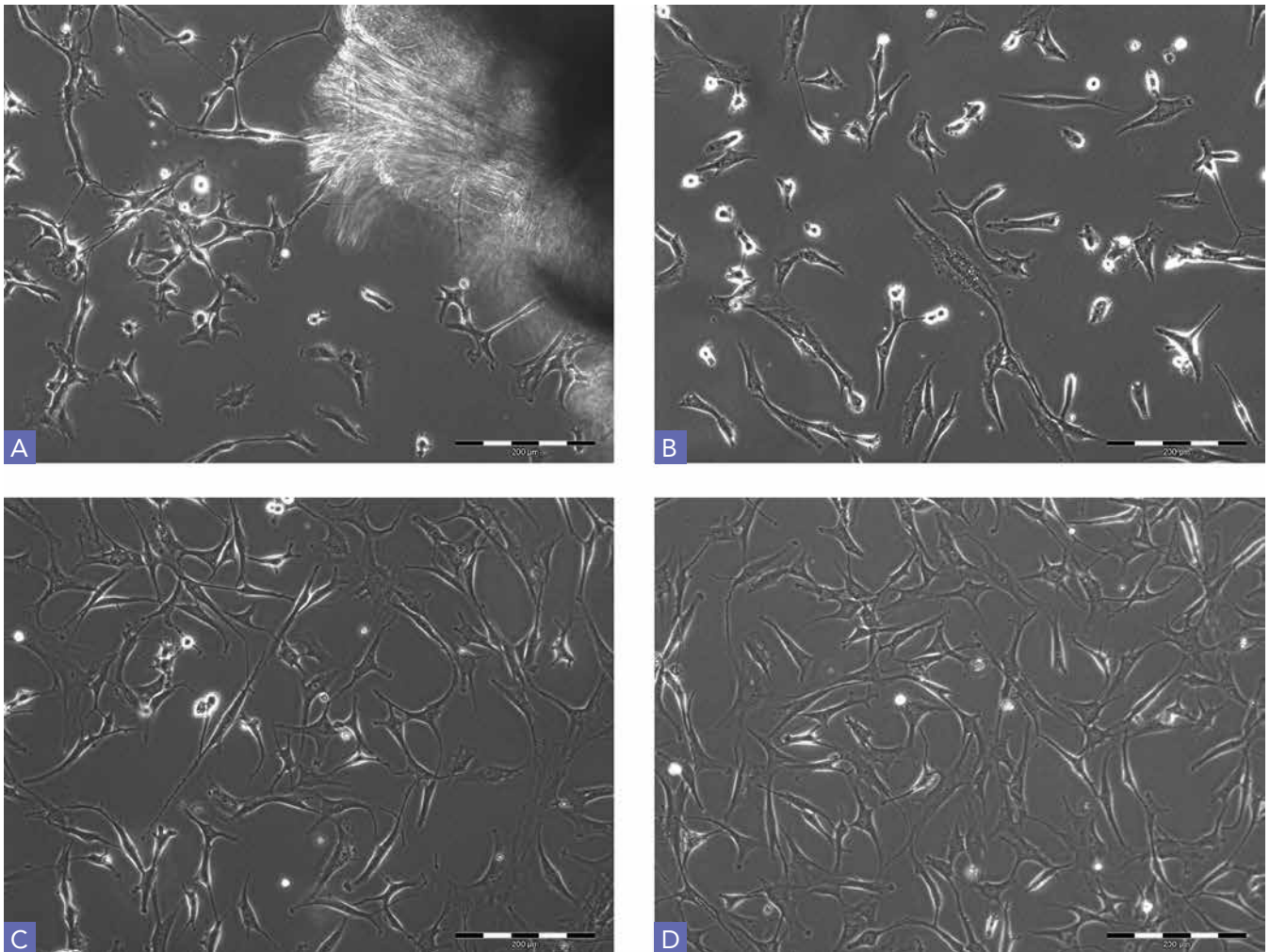


Figure 1. Representative images of hTDSCs in vitro at different passages. (a) P0; (b) P1; (c) P2; (d) P3. The scale bar corresponds to 200 μm.

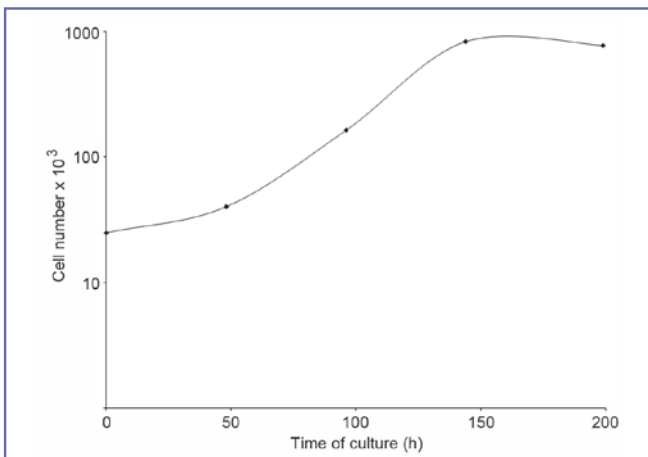


Figure 2. Growth curve of hTDSCs in vitro.

cytoplasm. In the presence of the chondrogenic culture medium, the hTDSCs became smaller, but remained spindle-shaped. Staining with Alcian blue confirmed the synthesis of acid mucopolysaccharides, as typical of chondroblasts. The hTDSC cultured in the adipogenic medium maintained their morphology and accumulated lipids in the cytoplasmic vacuoles.

Viability of hTDSCs in the presence of supplements/drugs

The MTT test was used for the evaluation of toxicity of the supplements/drugs in hTDSCs (**figure 6**). High concentrations of Triamcinolone (10 mg/ml) and Palmitoylethanolamide (100 mM) significantly reduced viability of cells. Only curcumin had a positive effect on hTDSC viability. In

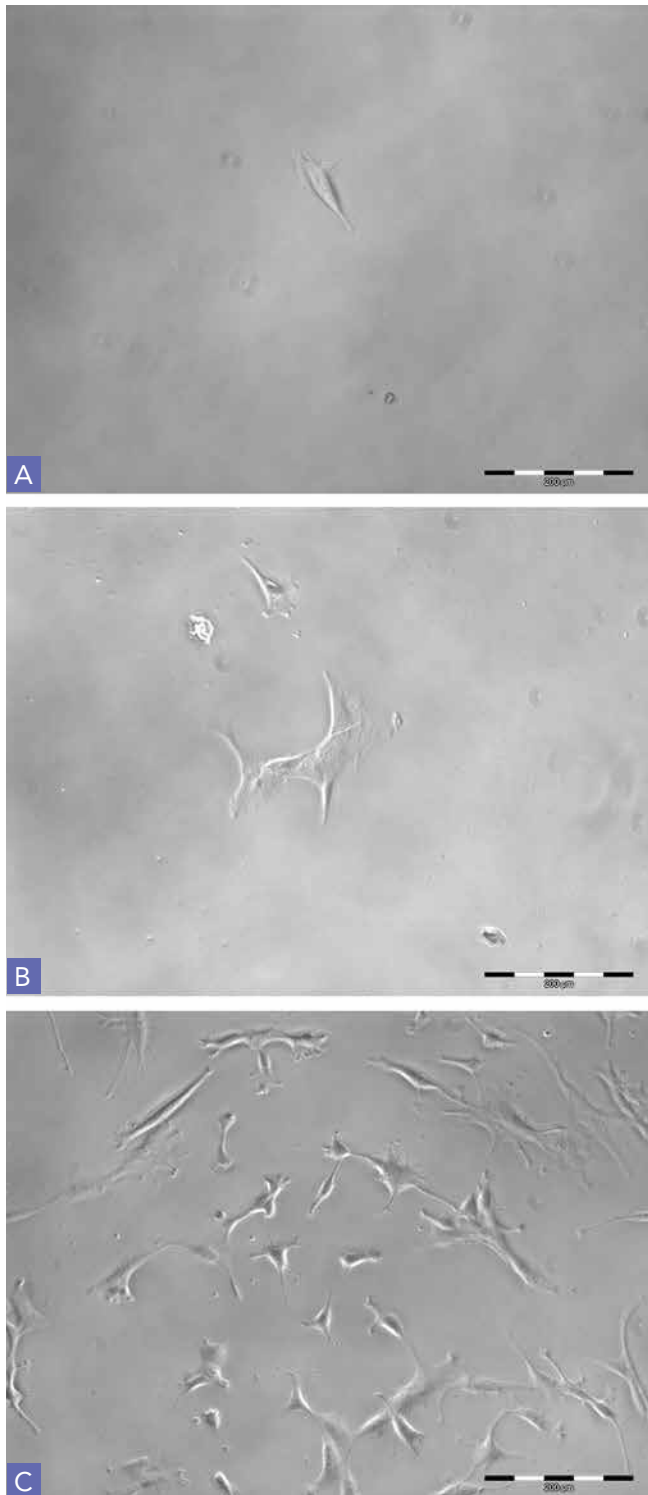


Figure 3. Clonogenic potential of hTDSCs in vitro. Phase contrast image of a clone obtained from a single cell monitored over time: (a) 2 days; (b) 4 days; (c) 10 days. The scale bar equals 200 μm .

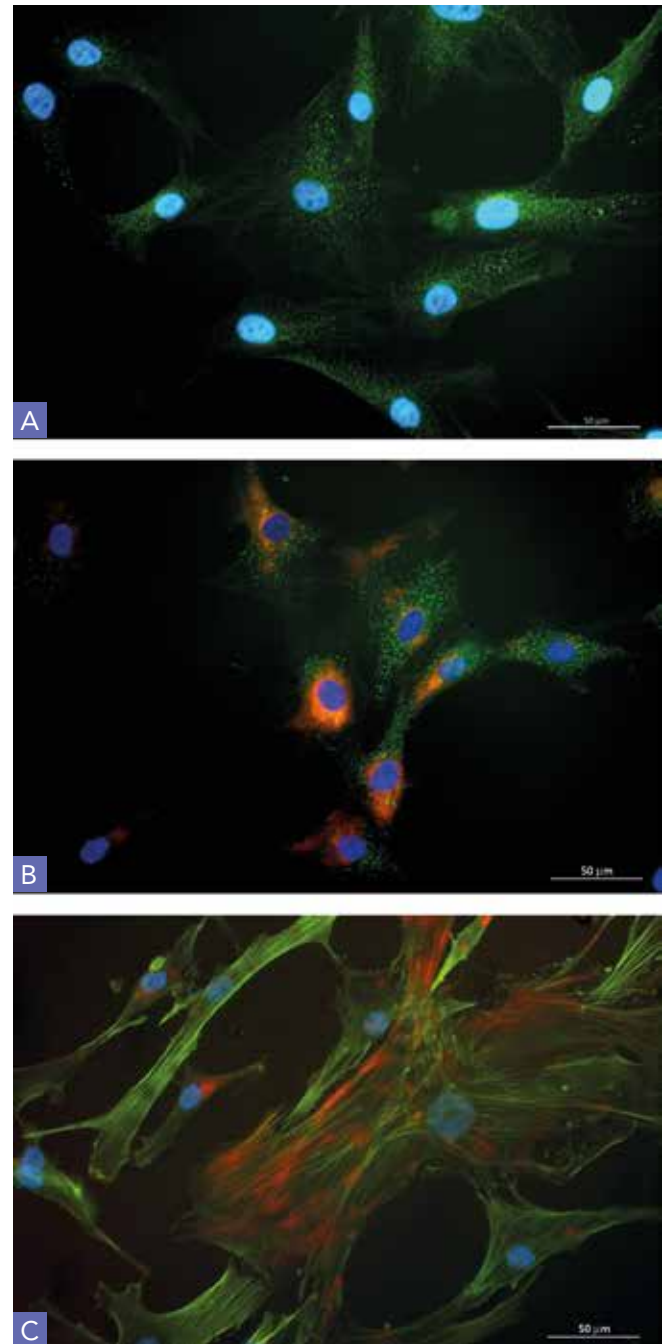


Figure 4. Phenotypic analysis of hTDSCs in vitro. Representative images of immunofluorescent staining, showing the expression of different markers: (a) CD90, green; (b) CD105, green, and vimentin, red; (c) actin, green, and vimentin, red. Nuclei are stained with DAPI, blue. The scale bar equals 50 μm .

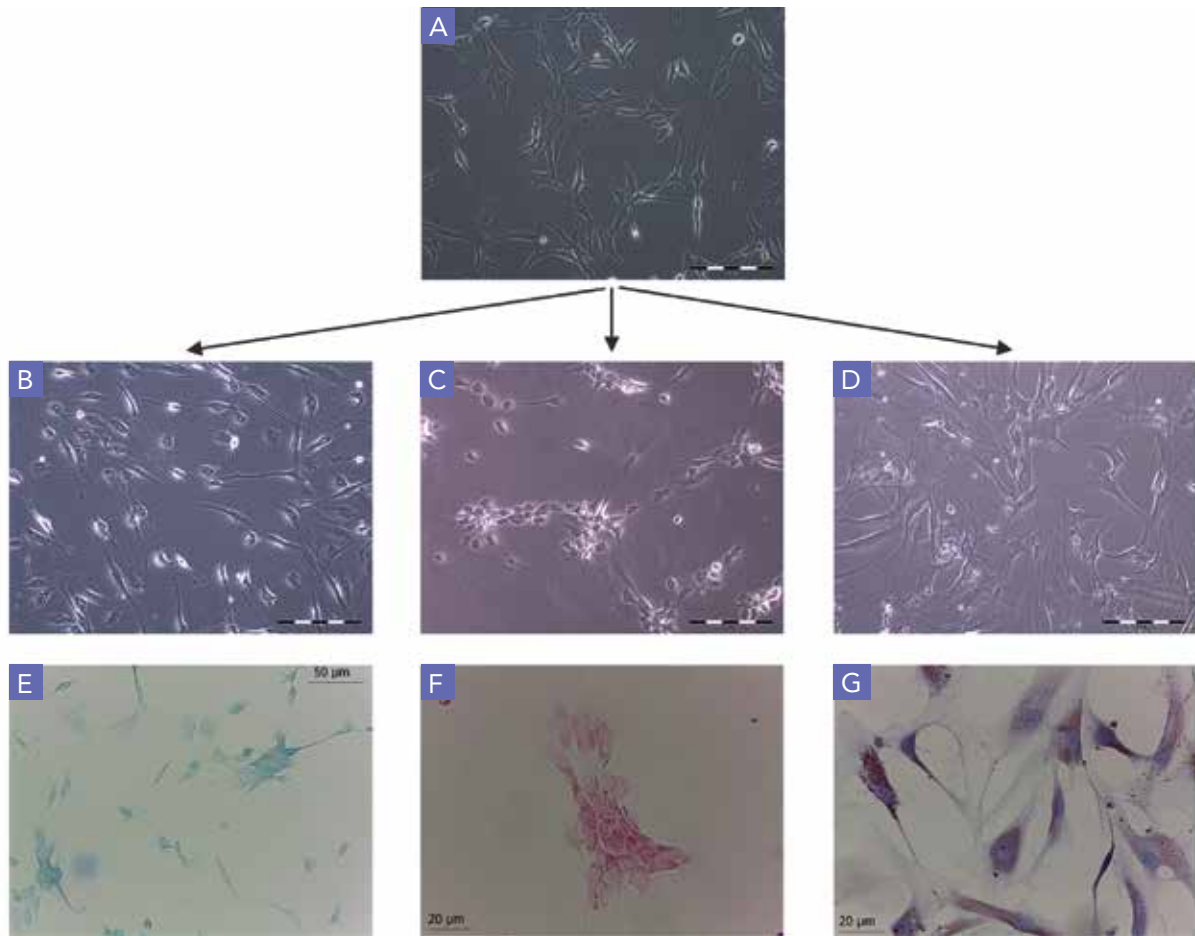


Figure 5. Differentiation potential of hTDSCs in vitro. Cells before (a) and after incubation in the specific differentiation medium: (b) chondrogenic, (c) osteogenic or (d) adipogenic observed at a phase-contrast microscope. Specific histochemical stains confirmed differentiation into (e) chondroblasts (Alcian blue), (f) osteoblasts (von Kossa staining) and (g) adipocytes (Oil Red).

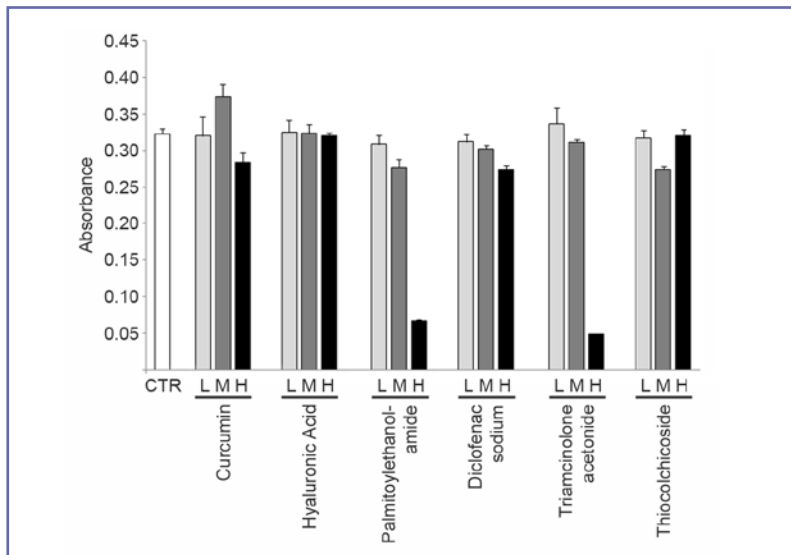


Figure 6. Toxicity of supplements/drugs at different concentrations to hTDSCs in vitro. Cell viability was assessed by MTT assay after 72 hours of incubation. Data are reported as mean \pm SEM (n = 4). H, high concentration; L, low concentration; M, medium concentration.

particular cell metabolic activity, as measured by MTT assay, reached the highest level in the presence of curcumin at the concentration of 2.5 µg/ml.

The effects of 2.5 µg/ml curcumin were further studied by the analysis of cell survival in vitro (figure 7). In normal conditions, the percentage of alive cells was significantly higher at 48 hours in the presence of curcumin than in the untreated control group. Moreover, treatment of hTDSCs with curcumin resulted in significantly higher viability in the presence of oxidative stress induced by hydrogen peroxide, compared to control cells cultured in the standard conditions.

DISCUSSION

The current study showed that stem cells can be easily isolated from the connective tissue of human tendons. The

hTDSCs were isolated from small fragments of tendons and maintained in adherent culture, in standard conditions. The results of the study indicate that these cells express typical mesenchymal markers, have a high proliferation capacity, possess self-renewal properties and have multipotent differentiation potential. Thus, we have successfully isolated a tendon cell population that meets the defining criteria for stem cells (22). Another important finding was that several supplements or drugs used in orthopaedics, physiotherapy and sports medicine can influence hTDSC survival in vitro. Among them, the anti-inflammatory glucocorticoid Triamcinolone and the analgesic fatty-acid amide Palmitoylethanolamide proved to be cytotoxic, while curcumin prevented cell death in vitro.

Our findings may be limited by the lack of in vivo testing of hTDSCs in an animal model of tendinopathy. Indeed, the studies of supplements or drugs often show discrepancy between the effects observed in vitro and in vivo. In both conditions, multiple variables can influence drug concentration and action on target cells. However, several unresolved issues, including the modifications of cell biology in tendinopathy, are best studied at the cell level in vitro (23). It is the preclinical, basic-science research that allows us to fill a gap in knowledge whenever the clinical results are ambiguous or unexpected (as is the case with the use of anti-inflammatory drugs in orthopaedics), and understand the rationale of a new therapeutic strategy before going from bench to bedside (as could be the case with the injection or activation of stem cells in tendons).

The adult tissue-resident and tissue-specific stem/progenitor cells represent a fertile ground for research and offer practical advantages over bone marrow-derived mesenchymal stem cells. The first evidence of their presence in tendons came from Bi *et al.* (9), who estimated that these cells represent 1–4% of the tendon cell population and show a greater proliferative and synthetic capacity of ECM when compared with bone marrow-derived MSCs, which make up only 0.001–0.01% of all cells in the bone marrow aspirate. The hTDSCs reside in the tendon, hence in a microenvironment that makes them more predisposed to differentiate into tenocytes. Al-Ani *et al.* (24) compared TDSCs with bone marrow-derived stem cells in the treatment of ruptured Achilles tendons in rats and concluded that the former had higher regenerative potential. In pathological conditions, however, modification of the local microenvironment composition, known as tissue remodelling, can adversely influence the vitality and the regenerative properties of tendon stem/progenitor cells (25). The isolation and culture of stem cells from adult human tendons offers the possibility of studying and influencing their activity in vitro, in order to favour a better and faster

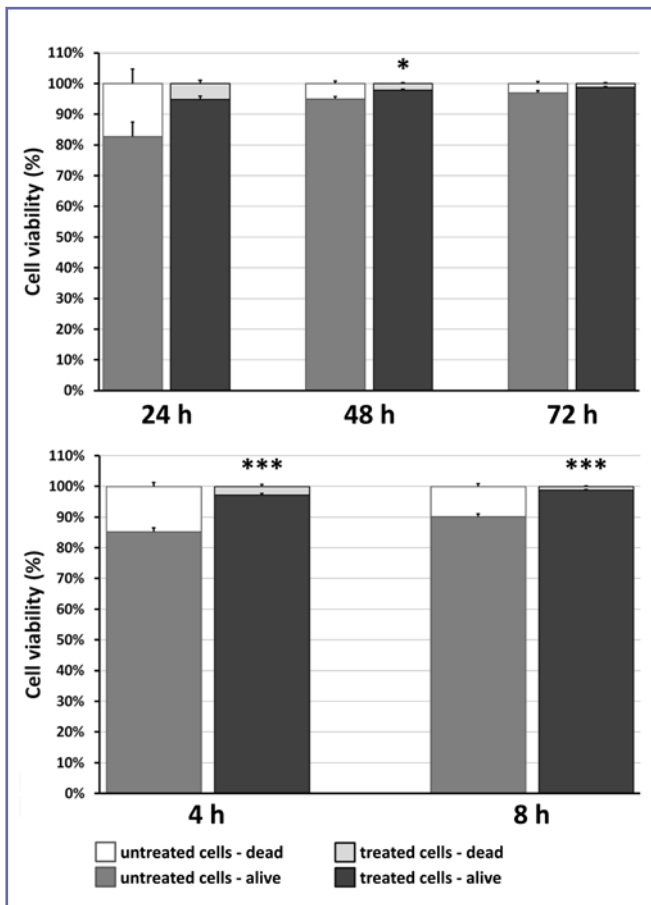


Figure 7. Effect of 2.5 µg/ml curcumin on hTDSC survival in normal conditions (upper panel) and in the presence of oxidative stress (lower panel). Data are reported as mean ± SEM (n = 4). Significant difference from untreated cells at the same time point is indicated as * p < 0.05 or *** p < 0.001.

recovery of the injured tendon through TDSC injection or stimulation *in vivo*.

While the role of endogenous stimuli, such as locally secreted cytokines, that can modulate cell vitality, matrix remodelling or angiogenesis in tissue repair is extensively studied and widely acknowledged, little is known about the exogenous factors, including therapeutics and nutraceuticals (26), that can influence these processes. Having isolated hTDSCs, we addressed the effects of several supplements/drugs, frequently used by patients with tendinopathy, on these cells. The results showed that Palmitoylethanolamide and Triamcinolone acetonide can reduce the viability, while curcuma significantly increases hTDSC viability. To the best of our knowledge, this is the first study that explored the effects of curcumin on these cells. Our findings complement the experiments on rodents (27,28) and support the growing interest of the scientific and lay population in curcumin anti-oxidative and anti-inflammatory properties. With regard to steroids, their injections have become mainstream in the treatment of tendinopathy, even if the evidence of the long-term benefit of such treatment is lacking. Worse still, recent meta-analysis of the effects of local glucocorticoid administration highlighted their deleterious effects on

tendon structure and function (29). Considering that Palmitoylethanolamide and Triamcinolone acetonide had negative effects on hTDSCs, our study further supports the notion of harmful influence of steroids on the connective tissue healing. Of note, the treatments evaluated in our study did not produce significant changes in the marker expression. Nevertheless, further studies are warranted to determine the effects of these supplements/drugs on the staminality and differentiation of hTDSCs.

The major contribution of the present research is that it provides much needed data on the effects of supplements/drugs used for the treatment of musculoskeletal disorders on hTDSCs. In practice, these findings could lead to the improvement of outcome in patients with tendon disease. Considering that hTDSC potentially play an important role in tendon healing, other therapies commonly used in tendinopathy management should be tested on this cell population in order to use the emerging knowledge on tendon healing to the best advantage of our patients.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

REFERENCES

- Lipman K, Wang C, Ting K, Soo C, Zheng Z. Tendinopathy: injury, repair, and current exploration. *Drug Des Devel Ther* 2018;12:591–603.
- Abat F, Alfredson H, Cucchiariini M et al. Current trends in tendinopathy: consensus of the ESSKA basic science committee. Part I: biology, biomechanics, anatomy and an exercise-based approach. *J Exp Orthop* 2017;4:18.
- Loiacono C, Palermi S, Massa B et al. Tendinopathy: Pathophysiology, Therapeutic Options, and Role of Nutraceuticals. A Narrative Literature Review. *Medicina (Kaunas)* 2019;55:447.
- Everhart JS, Cole D, Sojka JH et al. Treatment Options for Patellar Tendinopathy: A Systematic Review. *Arthroscopy* 2017;33:861–872.
- Sirico F, Ricca F, Di Meglio F et al. Local corticosteroid versus autologous blood injections in lateral epicondylitis: meta-analysis of randomized controlled trials. *Eur J Phys Rehabil Med* 2017;53:483–491.
- Guevara-Alvarez A, Schmitt A, Russell RP, Imhoff AB, Buchmann S. Growth factor delivery vehicles for tendon injuries: Mesenchymal stem cells and Platelet Rich Plasma. *Muscles Ligaments Tendons J* 2014;4:378–385.
- Cook JL, Rio E, Purdam CR, Docking SI. Revisiting the continuum model of tendon pathology: what is its merit in clinical practice and research? *Br J Sports Med* 2016;50:1187–1191.
- Rui YF, Lui PP, Li G, Fu SC, Lee YW, Chan KM. Isolation and characterization of multipotent rat tendon-derived stem cells. *Tissue Eng Part A* 2010;16:1549–1558.
- Bi Y, Ehrichou D, Kilts TM et al. Identification of tendon stem/progenitor cells and the role of the extracellular matrix in their niche. *Nat Med* 2007;13:1219–1227.
- Liu Q, Zhu Y, Amadio PC, Moran SL, Gingery A, Zhao C. Isolation and characterization of multipotent turkey tendon-derived stem cells. *Stem Cells Int* 2018;2018:3697971.
- Lui PP, Chan KM. Tendon-Derived Stem Cells (TDSCs): From Basic Science to Potential Roles in Tendon Pathology and Tissue Engineering Applications. *Stem Cell Rev Reports* 2011;7:883–897.
- Saltzman BM, Kuhns BD, Weber AE, Yanke A, Nho SJ. Stem Cells in Orthopedics: A Comprehensive Guide for the General Orthopedist. *Am J Orthop (Belle Mead NJ)* 2016;45:280–326.
- Padulo J, Oliva F, Frizziero A, Maffulli N. Muscles, Ligaments and Tendons Journal – Basic principles and recommendations in clinical and field Science Research: 2018 update. *Muscles Ligaments Tendons J* 2018; 8:305–307.
- Sacco AM, Belviso I, Romano V et al. Diversity of dermal fibroblasts as major determinant of variability in cell reprogramming. *J Cell Mol Med* 2019;23:4256–4268.
- Sherman SL, Khazai RS, James CH, Stoker AM, Flood DL, Cook JL. In vitro toxicity of local anesthetics and corticosteroids on chondrocyte and synoviocyte viability and metabolism. *Cartilage* 2015;6:233–240.
- Fusini F, Bisicchia S, Bottegoni C, Gigante A, Zanchini F, Busilacchi A. Nutraceutical supplement in the management of tendinopathies: a systematic review. *Muscles Ligaments Tendons J*. 2016;6:48–57.

17. van Meerloo J, Kaspers GJ, Cloos J. Cell sensitivity assays: the MTT assay. *Methods Mol Biol* 2011;731:237–245.
18. Di Meglio F, Nurzynska D, Romano V et al. Optimization of human myocardium decellularization method for the construction of implantable patches. *Tissue Eng Part C Methods* 2017;23:525–539.
19. Adan A, Kiraz Y, Baran Y. Cell Proliferation and cytotoxicity assays. *Curr Pharm Biotechnol* 2016;17:1213–1221.
20. Strober W. Trypan Blue exclusion test of cell viability. *Curr Protoc Immunol*. 2015;111:A3.B.1–3.
21. Riss T, Niles A, Moravec R et al. Cytotoxicity assays: in vitro methods to measure dead cells. In: Sittampalam GS, Grossman A, Brimacombe K, et al. *Assay guidance manual*. Bethesda (MD): Eli Lilly & Company and the National Center for Advancing Translational Sciences 2004. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK540958/>.
22. Lui PP. Markers for the identification of tendon-derived stem cells in vitro and tendon stem cells in situ - update and future development. *Stem Cell Res Ther* 2015;6:106.
23. Ruzzini L, Longo UG, Rizzello G, Denaro V. Stem cells and tendinopathy: state of the art from the basic science to clinic application. *Muscles Ligaments Tendons J* 2012;2:235–238.
24. Al-Ani MKh, Xu K, Sun Y, Pan L, Xu Z, Yang L. Study of Bone Marrow Mesenchymal and Tendon-Derived Stem Cells Transplantation on the Regenerating Effect of Achilles Tendon Ruptures in Rats. *Stem Cells Int*. 2015;2015:984146.
25. Liu C, Luo JW, Zhang KK et al. Tendon-derived stem cell differentiation in the degenerative tendon microenvironment. *Stem Cells Int* 2018;2018:2613821.
26. Fusini F, Bisicchia S, Bottegoni C, Gigante A, Zanchini F, Busilacchi A. Nutraceutical supplement in the management of tendinopathies: a systematic review. *Muscles Ligaments Tendons J*. 2016;6:48-57.
27. Jiang D, Gao P, Lin H, Geng H. Curcumin improves tendon healing in rats: a histological, biochemical, and functional evaluation. *Connect Tissue Res* 2016;57:20–27.
28. Güleç A, Türk Y, Aydın BK, Erkoçak ÖF, Safalı S, Ugurluoğlu C. Effect of curcumin on tendon healing: an experimental study in a rat model of Achilles tendon injury. *Int Orthop* 2018;42:1905–1910.
29. Dean BJ, Lostis E, Oakley T, Rombach I, Morrey ME, Carr AJ. The risks and benefits of glucocorticoid treatment for tendinopathy: a systematic review of the effects of local glucocorticoid on tendon. *Semin Arthritis Rheum* 2014;43:570–576.

The Effect of *Elaeagnus Angustifolia* Extract on the Joint Friction and Antioxidant Activity in Knee Non-Traumatic Osteoarthritis Model in Rat

M. Mofid^{1,2}, S. Homayoon Sadraie¹, H. Imani¹, G. Torkaman³, G. Kaka¹, M. Reza Naghii⁴, G. Alishiri⁵

¹ Department of Anatomical Sciences, Faculty of Medicine, Baqiyatallah University of Medical Sciences, Tehran, Iran

² Neuroscience research center, Baqiyatallah University of Medical Sciences, Tehran, Iran

³ Department of Physiotherapy, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

⁴ Department of Nutrition, Health School, Baqiyatallah University of Medical Sciences, Tehran, Iran.

⁵ Department of Rheumatology, Faculty of Medicine, Baqiyatallah University of Medical Sciences, Tehran, Iran

CORRESPONDING AUTHOR:

Giti Torkaman
Tarbiat Modares University
Ale-Ahmad Street
Tehran, Iran
E-mail: torkamg@modares.ac.ir

DOI:

10.32098/mltj.03.2020.05

LEVEL OF EVIDENCE: Animal Study

SUMMARY

Object. The objective was to investigate the effect of *Elaeagnus angustifolia* (*E. angustifolia*) extract on the joint lubrication and the activity of antioxidant in non-traumatic knee osteoarthritis model in rat.

Methods. In this study, 28 male albino Wistar rats were randomly assigned into four groups, each comprised of seven rats: Control (healthy animals); Saline (intra-articular injection of 50 μ L Saline); monoiodoacetate, MIA (intra-articular injection of 50 μ L MIA); and MIA+extract (intra-articular injection of MIA and 500mg/kg daily of *E. angustifolia* extract intraperitoneally for four weeks). Osteoarthritis was induced by injection of 50 μ L solution of 3 mg MIA in rats through infrapatellar ligament. Six weeks after intra-articular injection, Joint friction parameters consisting of cycle number, maximum peak, exponential and linear slope of cycle decay, and coefficient of friction (COF) were measured in knee joint. Activity of the superoxide dismutase (SOD), malondialdehyde (MDA) and glutathione peroxidase (GPx) were determined in blood plasma.

Results. MIA injection reduced the cycle number of joint oscillation compared to the Control group. In the MIA+extract group, cycle number and maximum peak increased significantly compared to the MIA group ($P=0.032$ and $P=0.016$, respectively). *E. angustifolia* extract resulted in the increase of SOD and GPx activity ($P=0.011$ and $P=0.05$, respectively) and the decrease of MDA activity ($P=0.000$) compared to MIA group.

Conclusions. The results showed that *E. angustifolia* extract is effective to improve the antioxidant enzyme activity and subsequently to decrease the joint friction in the non-traumatic knee osteoarthritis model.

KEY WORDS

Elaeagnus angustifolia extract; knee osteoarthritis; joint friction; Superoxide dismutase (SOD); Malondialdehyde (MDA); Glutathione peroxidase (GPx); rat.

INTRODUCTION

Osteoarthritis (OA) is the most common joint disease in adults, and its prevalence increases with age, causing disability in the elderly population (1). OA is a multifactorial disorder of synovial joints, which is characterized by escalated degeneration and loss of articular cartilage(2). Hyaline cartilage in the diarthrodial joints is serving as a low friction,

wear resistant surface for load support, load transfer and joint motion (3).

Proposed mechanisms including hydrodynamic lubrication, boundary lubrication, elastic deformation, and fluid pressurization are responsible for maintaining the low-friction and good lubrication in the diarthrodial joint (4). The excessive friction accelerates cartilage wear after failure of carti-

lage lubrication. Damage of cartilage may be a key factor in the onset of osteoarthritis (5).

The joint coefficient of friction (COF) is thought to increase with osteoarthritis progression, and this increase may occur due to a decrease of lubricants concentration such as lubricin (6).

Lubricin as a glycoprotein, thought to be a boundary lubricant in synovial fluid and articular cartilage (7) is deficient in aspirates of acute post-traumatic knee effusions, which is likely to be result of inflammatory destruction (7).

Friction measurements are important to determine the relative tribological contributions made by synovial fluid or cartilage (8) and/or to assess the efficacy of therapeutic modalities for preventing the development of osteoarthritis (9).

Evidence from both experimental and clinical studies suggests that oxidative stress plays an important role in the pathogenesis of OA (10, 11). It is indicated that the oxidant parameters including total peroxide (TP), lipid hydroperoxide and Oxidative stress index (OSI) increased and antioxidant parameters such as plasma total antioxidant capacity (TAC), thiol level, catalase activity and prolidase activity decreased in patients with osteoarthritis; therefore, these patients may be exposed to a potent oxidative stress (12). Malondialdehyde (MDA) and superoxide dismutase (SOD) have often been treated as matching indicators, of which the SOD level is associated with free radical scavenging ability, whereas MDA reflects the degree of damage caused by free radicals to the cells. When the level of free radicals was high, it was found that SOD failed to effectively remove them, leading to lipid peroxidation and cell damage (13).

The extracts of leaves and flowers of *E. angustifolia* leaves contain phenolic and flavonoid compounds which have antioxidant properties and protect cells from oxidative damages and delays or reduces the risk of many degenerative diseases. It has shown that the amounts of phenolic and flavonoid compounds are higher in leaves than flower of the plant. The explanation might be due to the process of photosynthesis which occurs in the leaves and also the existence of high amount of flavonoid biosynthetic pathway precursors in the leaves compared to any other organs (14). *E. angustifolia* leaves contain various chemical compounds including amino acids, flavonoids, phenolic compounds, polysaccharides and some other essential elements (15). The polysaccharides are important component of *E. angustifolia* leaves with anti-radiation, antioxidant and immune regulatory activities (16). The anti-inflammatory effects of the fruits of this plant on rheumatoid arthritis and osteoarthritis have been reported (17).

Hence, the aim of this study was to investigate the effect of *E. angustifolia* extract on the joint friction and the activity of antioxidants in the non-traumatic OA model in rats.

MATERIALS AND METHODS

This research was carried out according to the internationally valid guidelines and the institutional animal ethics committee in Baqiyatallah University of Medical Sciences. In this study, 28 adult male albino Wistar rats (12-14 weeks age and weighing 200-250 g) were purchased from the laboratory animal center of the university. The rats were kept under standard housing laboratory conditions (room temperature of 23 ± 2 °C with relative humidity of $60\pm 5\%$, and 12 hr/12 hr light/dark cycles) and were fed with a standard laboratory pellets rat chow and water *ad libitum*. One week after arrival and adaptation, rats were randomly divided into four groups. Each group comprised of seven rats and were observed regularly. Control group (consisted of healthy animals that underwent no intervention); Saline group (50 μ L of normal Saline 0.9%, injected into the right knee joint as a placebo group); Monoiodoacetate group (MIA group, received 50 μ L solution containing 3 mg MIA into their right knee joint); and MIA+ *E. angustifolia* extract group (MIA+ extract group, MIA injection and 500mg/kg body weight daily *E. angustifolia* extract injection intraperitoneally for four weeks (12).

Method of osteoarthritis induction

Osteoarthritis was induced by intra-articular injection of MIA. MIA injection is considered to induce osteoarthritis in joint by damaging glycolysis in the joint, resulting in the eventual death of chondrocytes by inhibiting glyceraldehyde-3-phosphate dehydrogenase activity and increasing inflammation (18). First, the rats of MIA and MIA+extract groups were anesthetized by using Ketamine (50 mg/kg) and Xylazine (5 mg/kg). After shaving the knee skin and bending the knee joint, a single dose of 3 mg MIA (Sigma-Aldrich, St. Louis, MO) dissolved in 50 μ L physiological Saline (0.9%) was injected through infrapatellar ligament of the right knee using a 26-gauge needle. In the Saline group, by using a 26-gauge needle, 50 μ L Saline 0.9% solution was injected into the right knee joint capsule through infrapatellar ligament. At 43th day, all animals in four groups were euthanized by overdose of the anesthetic drugs and were sacrificed to measure the joint friction. For blood plasma analyses, blood samples obtained from anaesthetized mice were centrifuged immediately (Megafuge 1.0 Heraeus Sepatech GmbH) at 3000 rpm for 15 min at +4 °C to determine the activity of

superoxide dismutase (SOD), malondialdehyde (MDA) and glutathione peroxidase (GPx).

Method of preparation and injection of *E. angustifolia* extract

The fruits of *E. angustifolia* were collected from mountain area of Niavaran, Tehran, Iran and was identified and approved by Herbarium Department of Pharmacognosy of Shahid Beheshti University of Medical Sciences, Tehran, Iran. The voucher specimen [No. 1257] is preserved in the herbarium of this department for reference.

The aqueous extract was prepared by adding 1000 ml of distilled water to 100 g of fruit powder (seed, flesh, and peel) and the resulting solution was boiled for 8-10 minutes. Then, the mixture was filtered and the solution was completely dehydrated for 8-10 hours in water bath to provide a crude extract with 20% yield. This extract was then weighed and after being dissolved in physiological Saline, and consequently 14 days after induced OA, 500 mg/kg was injected intraperitoneally in animals in the MIA+extract group, daily for four weeks.

Joint friction (biotribological) measurement

The knee joint was resected following transection of the middle of tibia/fibula and femoral shafts. Friction testing was performed immediately after the joint resection by a pendulum friction tester. The knee joint was placed in the free pendulum tester. Pendulum rotation was calculated for flexion–extension in the sagittal plane and other motion out of this plane was ignored (**figure 1**). Through an automated gated mechanism, movement was started from a fix point and free oscillation around the knee joint recorded. Ravanbod *et al.*, 2011, report more technical details on the friction tester(19).

The pendulum load was approximately half of the body weight of animal. Angular changes were recorded to the equilibrium point at a sampling frequency of 30 Hz. Cycle number to reach equilibrium, maximum peak oscillation value, exponential and linear slope of cycles decay were calculated with Labview 7.1 Software (National instrument Labview 7.1 software, Austin, TX, USA). Coefficient of friction (COF) was calculated according to Stanton's equation (19).

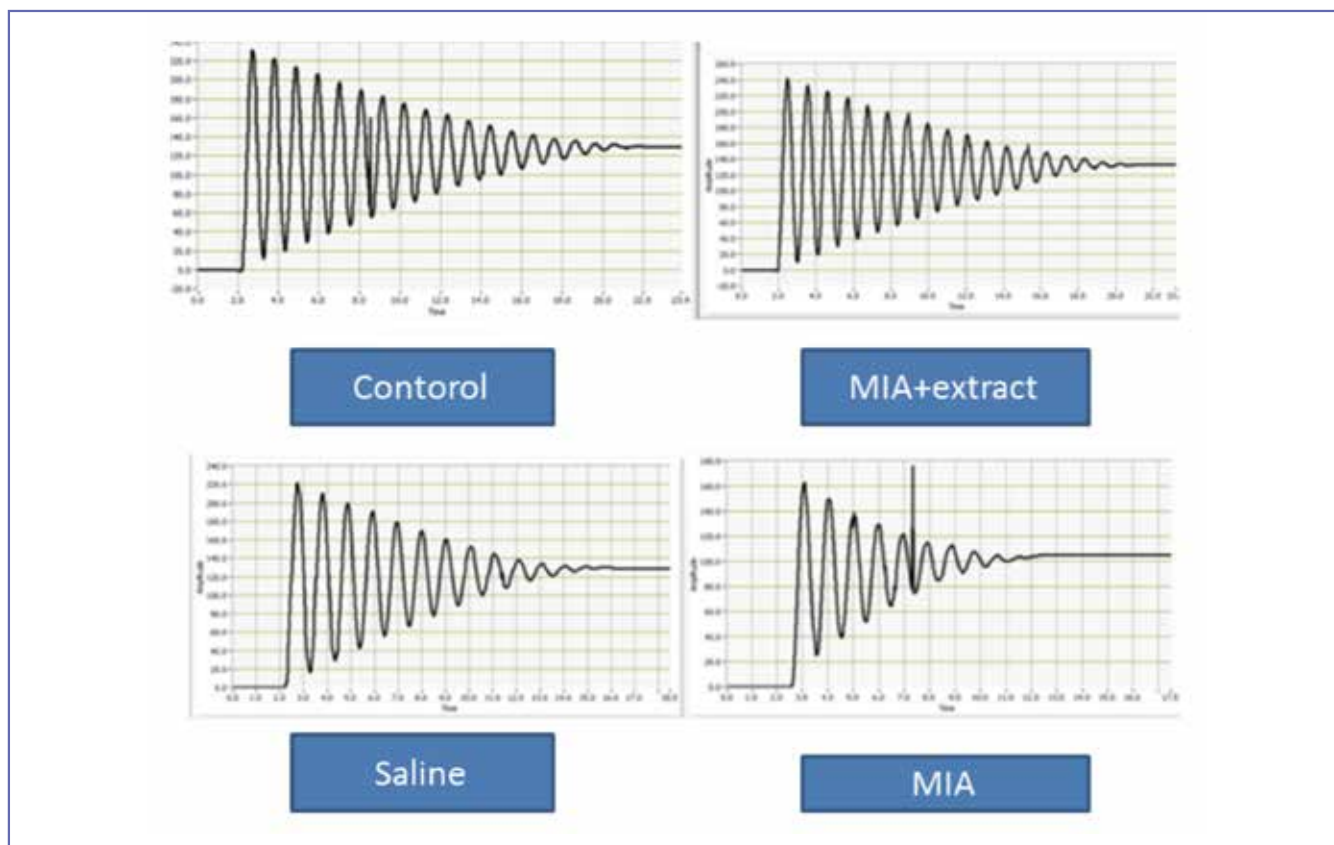


Figure 1. A sample of free oscillation in Control, Saline, MIA, and MIA+extract groups.

Determination of biochemical parameters

SOD activity was determined according to Light spectroscopy method by Winterbourn *et al.* (20).

Glutathione peroxidase activity was determined by glutathione reductase enzyme coupling reaction according to method by Lushchak *et al.*(21). Reduction of glutathione oxide occurs by Gpx reaction with NADPH in the presence of glutathione reductase (GR). In this reaction, NADPH oxidation to NADP⁺ reduces absorption at 340 nm, which is proportional to GPx activity ($H_2O + GS-SG \rightarrow 2GSH + H_2O_2$). Malondialdehyde is the ultimate oxidizing peroxidase product in the body's lipid membrane cells. Thiobarbituric acid assay (TBA) (22) was used to measure MDA level.

STATISTICAL ANALYSIS

The Shapiro-Wilk test was used to determine the normal distribution of data. Whereas data did not follow a normal distribution $P < 0.05$, the non-parametric Kruskal-Wallis test and Mann-Whitney u test was used to analyze and compare the group differences. The statistical significance was defined as $P \leq 0.05$. These statistical analyses were carried out using the SPSS for Windows Statistical Software Package.

RESULTS

The joint friction parameters

The measurements of joint friction parameters are shown in **table I**.

Cycle number

The cycle number to reach equilibrium showed a significant difference through the study groups ($P=0.005$). Cycle number in Saline, MIA, and MIA+extract groups decreased compared to Control group ($P < 0.05$). After using the *E. angustifolia* extract, the cycle number in the MIA+extract

group was significantly higher than MIA group ($P=0.032$). An increase in the cycle number reflects the reduction of joint friction.

Maximum peak

The maximum peak oscillation showed a significant difference among the groups ($P=0.002$). The maximum Peak oscillation decreased significantly in Saline, MIA, and MIA+extract compared to Control group ($P<0.05$). After using the *E. angustifolia* extract, the maximum peak oscillation increased significantly compared to Saline group ($P=0.016$) and MIA group ($P=0.016$). Increasing the maximum peak reflects the facilitated movement in the joint and the reduction of joint friction.

Exponential slope of cycle decay

The exponential slope of cycle decay showed a significant difference among the groups ($P=0.010$). The exponential slope of oscillation showed an increase in all groups compared to Control group, but this increase was only significant in Saline group ($P= 0.008$), and MIA group ($P= 0.008$). After administration of the *E. angustifolia* extract, the exponential slope of the oscillation decreased and its value showed no significant difference compared to Control group. The decrease in the exponential slope of cycle decay indicates that the joint movement is performed with low friction.

Linear slope of cycle decay

The slope of cycle decay showed no significant difference among the groups ($P= 0.062$).

Joint friction coefficient

The friction coefficient calculated by the Stanton formula showed no significant difference among the groups ($P = 0.131$).

Table I. The value of joint friction parameters (Mean±SD)

Variables	Control	Saline	MIA	MIA+extract
Cycle Number	20.60±1.51	12.60±2.88 ^a	12.20±1.48 ^a	15.40±2.40 ^{ab}
Maximum Peak	28.14±1.76	21.38±1.03 ^a	21.35±1.34 ^a	24.07±0.85 ^{abc}
Exponential Slope	0.04±0.007	0.12±0.08 ^a	0.11±0.03 ^a	0.08±0.3
Linear slope	0.008±0.000	0.011±0.002	0.012±0.001	0.010±0.001
Friction coefficient	0.24±0.01	0.31±0.05	0.26±0.15	0.28±0.04

a: Significant decrease compared to Control group; b: Significant increase in the MIA+extract group compared to MIA group; c: Significant increase in the MIA+extract group compared to Saline group.

Biochemical parameters

The measured biochemical variables including the activity of antioxidant enzymes: SOD, GPx and MDA as a lipid peroxidation index are shown in **table II**.

Superoxide dismutase (SOD) activity

The amount of SOD activity was significantly different in the groups ($P=0.001$). The induction of osteoarthritis using MIA reduced the amount of SOD activity. This decrease was significant in both MIA ($P=0.000$) and MIA+extract ($P=0.025$) groups compared to Control group. Using *E. angustifolia* extract increased the amount of SOD activity compared to MIA group ($P=0.011$).

Glutathione peroxidase (GPx) activity

The level of GPx activity revealed significant differences in the groups ($P=0.041$). The GPx activity in the MIA+extract group was significantly higher than Control ($P=0.018$), Saline ($P=0.021$), and MIA ($P=0.05$) groups.

Malondialdehyde (MDA) activity

The level of MDA activity showed significant differences in the groups ($P=0.002$). In MIA group, the level of MDA activity increased compared to Control and Saline groups; this increase was significant in Saline group ($P=0.031$). The level of MDA activity in the MDA+extract group decreased compared to Control ($P=0.002$), Saline ($P=0.005$) and MIA ($P=0.000$) groups.

DISCUSSION

In this study, the effect of *E. angustifolia* extract was investigated on the SOD, GPx, and MDA activity and also the decrease of joint friction in the non-traumatic OA model. Although the measuring of friction is very important to determine of joint function, but it is an aggressive method that is not feasible in human studies. So, in this study we used MIA rat model to investigate the effect of *E. angustifolia* extract on the joint friction. The MIA model in rat, is

well established and resembles the histological and pain-related behavior of human degenerative OA (23). Injection of MIA leads to a decreased number of chondrocytes and subsequent histological and morphological articular alterations similar to human osteoarthritic changes (23).

The results of this study revealed that induction of osteoarthritis by MIA injection reduced the cycle number of joint oscillation compared to Control group. In the MIA+extract group, after four weeks administration of *E. angustifolia* extract, the number cycle increased significantly compared to MIA group. The use of Saline also reduced the cycle number of oscillation, which was previously reported by Caligaris *et al.* (24). After using the *E. angustifolia* extract, the maximum peak oscillation increased compared to the Saline and MIA groups. Administration of *E. angustifolia* extract displayed the decrease of exponential slope of cycle decay, and after treatment, no significant difference observed between MIA+extract group and Control group. Both linear slope of cycle decay and the friction coefficient showed no significant decrease in the groups. Crisco *et al.*, (2007) showed that exponential slope of cycle decay is more accurate than the linear slope of cycle decay for predicting the joint friction coefficient (25). They suggest that the exponential model is capable to predict the friction coefficient accurately, when the viscous damping is quite small. In our study, exponential slope of cycle decay determined the difference of joint coefficient of friction among groups; whereas, both linear slope of cycle decay and the friction coefficient prediction by Stanton formula (based on the linear decay) could not determine any difference among groups.

Dunham *et al.*, (1993) suggested that, "the intra-articular injection of MIA leads to inhibition of glyceraldehyde-3-phosphate dehydrogenase in chondrocytes, which causes interruption of glycolysis, increased cartilage destruction, as well as a decrease of proteoglycan synthesis and ultimately cell death" (18). It is noted that MIA injection causes cartilage destruction (9). Naveen *et al.*, (2014) showed that, two weeks after induced OA by MIA, the ratio of glycosaminoglycan/total protein content and the cartilage stiffness were decreased (26).

The joint friction can be significantly changed in articular diseases even before appearance of clinical or laboratory

Table II. The measured biochemical parameters (Mean±SD)

Variables	Control	Saline	MIA	MIA+extract
SOD(um/l)	0.74±0.05	0.73±0.03 ^b	0.61±0.04 ^a	0.65±0.07 ^{ab}
GPx(u/l)	195.5±62.19	190.3±36.31	235.7±130.4	401.7±186.9 ^{abc}
MDA(umol/l)	4.49±1.19	4.24±1.38	5.71±0.72 ^{ab}	2.36±1.08 ^{abc}

a: Significant difference with Control group, b: significant difference with MIA group, c: significant difference with Saline group

symptoms (27). The interaction of articular cartilage and synovial fluid through the two important mechanisms of boundary lubrication and fluid film produces smooth and low friction movements in the normal synovial joints and causes performing of daily functional activities with minimal friction and good lubrication to prevent the joint cartilage wearing. Some studies have shown that there is a direct correlation between the increase of friction coefficient and the intensity of OA (28-30).

The experimental and clinical studies suggest that oxidative stress may be considered as one of the main etiological factors in the pathogenesis of OA (10, 11, 31). SOD expression is significantly down regulated in OA chondrocytes *in vivo*, but this precedes the development of OA lesions, raising the possibility that alterations in SOD expression are associated with the earliest stages of OA pathogenesis (32). *E. angustifolia* is consisting of various chemical compounds including amino acids, flavonoids, phenolic compounds, polysaccharides and some other essential elements (15). Most studies on *E. angustifolia* is related to its antioxidant capacity of phenolic compounds and antocyanosides. Polyphenols can suppress the oxidative stress produced by reactive oxygen species (ROS) with their hydroxyl groups (33, 34).

Our results showed that the intra-articular injection of MIA caused the decrease of SOD activity level. The level of SOD and GPx activities increased in MIA+extract group. The amount of GPx activity in the MIA+ extract group was significantly higher than Control, Saline, and MIA groups. Moreover, the amount of MDA activity increased in the MIA group compared to the Control and Saline groups. The level of MDA activity decreased significantly after administration of *E. angustifolia* extract in MIA+extract group compared to other groups. It was found that the intra-peritoneal injection of *E. angustifolia* extract was effective in increasing the level of activity of antioxidant enzymes (SOD and GPx) and decreasing the level of MDA activity. It is reported that antioxidants have been effective in altering the oxidative enzymes levels in osteoarthritis (35). Increased MDA and decreased glutathione (GSH), and catalase activities (CAT) were evident in OA patients (11). MDA or lipid peroxidation increased in plasma and synovial fluid of osteoarthritis and rheumatoid arthritis patients (11). Anti-

oxidant enzymes, such as SOD, CAT, GPx are decreased in OA patients, confirming the role of oxidative stress in OA pathogenesis (10, 12).

In the non-traumatic OA model, Maghzi *et al.*, (2015) showed that the oral treatment with *E. angustifolia* extract decreased the Mankin score (glycosaminoglycan index) and cellular degradation in articular cartilage (36).

Based on the effectiveness of *E. angustifolia* extract on the prevention of chondroblast cell death, it may be facilitated the expression of joint lubricants such as glycoproteins and hyaluronic acid and subsequently decrease of joint friction. We could not measure the level of these lubricants in this study and it suggests to consider in future studies. It seems that the prevention of cartilage degradation and the synthesis of lubricant agents after intraperitoneal injection of *E. angustifolia* extract, may be effective for decreasing the joint friction and better lubrication during functional activity, but special histological and biochemical studies should be considered in the future studies.

CONCLUSIONS

Induction of osteoarthritis by MIA injection reduced the cycle number of joint oscillation. Administration of *E. angustifolia* extract decreased the exponential slope of cycle decay, and after treatment this parameter showed no significant difference compared to Control group. *E. angustifolia* extract was effective in increasing the level of activity of antioxidant enzymes (SOD and GPx) and decreasing the level of MDA activity. It seems that *E. angustifolia* extract through its antioxidant effect be able to increase the joint lubrication in the osteoarthritis.

ACKNOWLEDGEMENTS

We would like to express my special thanks of gratitude to Dr Roya Ravanbod (assistant professor of Tarbiat Modares University) to help for performing joint friction test.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

REFERENCES

1. Felson DT, Lawrence RC, Hochberg MC, McAlindon T, Dieppe PA, Minor MA, et al. Osteoarthritis: new insights. Part 2: treatment approaches. *Ann Intern Med* 2000;133(9):726-37.
2. Poole AR GF, Abramson SB. Etiopathogenesis of osteoarthritis. In: *Osteoarthritis. Diagnosis and Medical/Surgical Management*. 4 ed. RW Moskowitz RA, MC Hochberg, JA Buckwalter, VM. Goldberg, editor. Philadelphia Lippincott Williams & Wilkins 2007.
3. Morlock M, Schneider E, Bluhm A, et al. Duration and frequency of every day activities in total hip patients. *J Biomech* 2001;34(7):873-81.

4. Margareta Nordin DirSci VHF. Basic Biomechanics of the Musculoskeletal System. USA: LWW; Fourth, North American 2012.
5. Ballantine GC SG. The effect of lipid depletion on osteoarthritic wear. *Wear*. 2002(253):385-93.
6. Teeple E, Elsaid KA, Fleming BC, Jay GD, Aslani K, Crisco JJ, et al. Coefficients of friction, lubricin, and cartilage damage in the anterior cruciate ligament-deficient guinea pig knee. *J Orthop Res* 2008;26(2):231-7.
7. Jay GD, Elsaid KA, Zack J, et al. Lubricating ability of aspirated synovial fluid from emergency department patients with knee joint synovitis. *J Rheumatol* 2004;31(3):557-64.
8. Tanaka E, Iwabe T, Dalla-Bona DA, et al. The effect of experimental cartilage damage and impairment and restoration of synovial lubrication on friction in the temporomandibular joint. *J Orofac Pain* 2005;19(4):331-6.
9. Kawano T, Miura H, Mawatari T, et al. Mechanical effects of the intraarticular administration of high molecular weight hyaluronic acid plus phospholipid on synovial joint lubrication and prevention of articular cartilage degeneration in experimental osteoarthritis. *Arthritis Rheum* 2003;48(7):1923-9.
10. Ostalowska A, Birkner E, Wiecha M, et al. Lipid peroxidation and antioxidant enzymes in synovial fluid of patients with primary and secondary osteoarthritis of the knee joint. *Osteoarthritis Cartilage* 2006;14(2):139-45.
11. Surapaneni KM, Venkataramana G. Status of lipid peroxidation, glutathione, ascorbic acid, vitamin E and antioxidant enzymes in patients with osteoarthritis. *Indian J Med Sci*. 2007;61(1):9-14.
12. Altindag O, Erel O, Aksoy N, Selek S, Celik H, Karaoglanoglu M. Increased oxidative stress and its relation with collagen metabolism in knee osteoarthritis. *Rheumatol Int* 2007;27(4):339-44.
13. Yang X, Zhao J, He Y, Huangfu X. Screening for characteristic genes in osteoarthritis induced by destabilization of the medial meniscus utilizing bioinformatics approach. *J Musculoskelet Neuronal Interact* 2014;14(3):343-8.
14. Saboonchian F, Jamei R, Hosseini Sarghein S. Phenolic and flavonoid content of *Elaeagnus angustifolia* L. (leaf and flower). *Avicenna J Phytomed* 2014;4(4):231-8.
15. Amiri Tehranizadeh Z, Baratian A, Hosseinzadeh H. Russian olive (*Elaeagnus angustifolia*) as a herbal healer. *Bioimpacts*. 2016;6(3):155-67.
16. Chen Q, Chen J, Du H, Li Q, Chen J, Zhang G, et al. Structural characterization and antioxidant activities of polysaccharides extracted from the pulp of *Elaeagnus angustifolia* L. *Int J Mol Sci*. 2014;15(7):11446-55.
17. Nikniaz Z, Ostadrahimi A, Mahdavi R, Ebrahimi AA, Nikniaz L. Effects of *Elaeagnus angustifolia* L. supplementation on serum levels of inflammatory cytokines and matrix metalloproteinases in females with knee osteoarthritis. *Complement Ther Med* 2014;22(5):864-9.
18. Dunham J, Hoedt-Schmidt S, Kalbhen DA. Prolonged effect of iodoacetate on articular cartilage and its modification by an anti-rheumatic drug. *Int J Exp Pathol* 1993;74(3):283-9.
19. Ravanbod R, Torkaman G, Esteki A. Biotribological and biomechanical changes after experimental haemarthrosis in the rabbit knee. *Haemophilia* 2011;17(1):124-33.
20. Winterbourn CC, Hawkins RE, Brian M, Carrell RW. The estimation of red cell superoxide dismutase activity. *J Lab Clin Med* 1975;85(2):337-41.
21. Lushchak VI, Bagnyukova TV. Temperature increase results in oxidative stress in goldfish tissues. 2. Antioxidant and associated enzymes. *Comp Biochem Physiol C Toxicol Pharmacol* 2006;143(1):36-41.
22. Lapenna D, Ciofani G, Pierdomenico SD, Giamberardino MA, Cucurullo F. Reaction conditions affecting the relationship between thiobarbituric acid reactivity and lipid peroxides in human plasma. *Free Radic Biol Med* 2001;31(3):331-5.
23. Lampropoulou-Adamidou K, Lelovas P, Karadimas EV, et al. Useful animal models for the research of osteoarthritis. *Eur J Orthop Surg Traumatol* 2014;24(3):263-71.
24. Caligaris M, Canal CE, Ahmad CS, et al. Investigation of the frictional response of osteoarthritic human tibiofemoral joints and the potential beneficial tribological effect of healthy synovial fluid. *Osteoarthritis Cartilage* 2009;17(10):1327-32.
25. Crisco JJ, Blume J, Teeple E, et al. Assuming exponential decay by incorporating viscous damping improves the prediction of the coefficient of friction in pendulum tests of whole articular joints. *Proc Inst Mech Eng H* 2007;221(3):325-33.
26. Naveen SV, Ahmad RE, Hui WJ, et al. Histology, glycosaminoglycan level and cartilage stiffness in monoiodoacetate-induced osteoarthritis: comparative analysis with anterior cruciate ligament transection in rat model and human osteoarthritis. *Int J Med Sci* 2014;11(1):97-105.
27. Katta J, Jin Z, Ingham E, Fisher J. Biotribology of articular cartilage--a review of the recent advances. *Med Eng Phys* 2008;30(10):1349-63.
28. Elsaid KA, Jay GD, Warman ML, et al. Association of articular cartilage degradation and loss of boundary-lubricating ability of synovial fluid following injury and inflammatory arthritis. *Arthritis Rheum*. 2005;52(6):1746-55.
29. Jay GD, Fleming BC, Watkins BA, et al. Prevention of cartilage degeneration and restoration of chondroprotection by lubricin tribosupplementation in the rat following anterior cruciate ligament transection. *Arthritis Rheum* 2010;62(8):2382-91.
30. Teeple E, Elsaid KA, Jay GD, et al. Effects of supplemental intra-articular lubricin and hyaluronic acid on the progression of posttraumatic arthritis in the anterior cruciate ligament-deficient rat knee. *Am J Sports Med* 2011;39(1):164-72.
31. Sutipornpalangkul W, Morales NP, Charoencholvanich K, et al. Lipid peroxidation, glutathione, vitamin E, and antioxidant enzymes in synovial fluid from patients with osteoarthritis. *Int J Rheum Dis* 2009;12(4):324-8.
32. Scott JL, Gabrielides C, Davidson RK, et al. Superoxide dismutase downregulation in osteoarthritis progression and end-stage disease. *Ann Rheum Dis* 2010; 69(8):1502-10.
33. Wang Y GT, Zhao C, Zhao P. Changes in total phenolic and flavonoid contents and antioxidant activities of the fruit from *Elaeagnus angustifolia* during an 80-day study period *Agro Food Ind Hi Tech* 2014 (25):7-10.
34. Yalcin G SO. Antioxidant capacity of *Elaeagnus angustifolia* L. and investigation of eosin y as the fluorescent probe in ORAC method *J Food Agric Environ* 2014; (12):51-4

35. Panahi Y, Rahimnia AR, Sharafi M, et al. Curcuminoid treatment for knee osteoarthritis: a randomized double-blind placebo-controlled trial. *Phytother Res* 2014;28(11):1625-31.
36. Maghzi M, Khorsandi L, Orazizadeh M, et al. Histological effects of *Elaeagnus angustifolia* aqueous extract on cartilage degradation in experimental osteoarthritis. *Asian J Phytomed Clin Res* 2015(3): 50-4.

Outcomes after Micronized Fat Adipose Transfer for Glenohumeral Joint Arthritis and Rotator Cuff Pathology: a Case Series of 18 Shoulders

D.M. Robinson¹, C. Eng^{1,2}, M. Mitchkash¹, A.S. Tenforde^{1,3}, J. Borg-Stein^{1,2}

¹ Spaulding Rehabilitation Hospital, Harvard Medical School, Department of Physical Medicine and Rehabilitation, Charlestown, Massachusetts

² Spaulding Rehabilitation Hospital, Harvard Medical School, Department of Physical Medicine and Rehabilitation, Spaulding Wellesley Outpatient Care Center, Newton Wellesley Hospital, Newton, Massachusetts

³ Spaulding Rehabilitation Hospital, Harvard Medical School, Department of Physical Medicine and Rehabilitation, Spaulding National Running Center, Cambridge, Massachusetts

CORRESPONDING AUTHOR:

David Robinson
Spaulding Rehabilitation Hospital
Harvard Medical School
Department of Physical Medicine and Rehabilitation
300 1st Avenue
Charlestown, Massachusetts 02129
E-mail: drobinson22@partners.org;
k09dr02@gmail.com

DOI:

10.32098/mltj.03.2020.06

LEVEL OF EVIDENCE: 4

SUMMARY

Background. This study aimed to evaluate the safety and clinical outcomes of patients treated with micro-fragmented adipose tissue for shoulder pain secondary to glenohumeral osteoarthritis and rotator cuff pathology.

Methods. 16 patients (18 shoulders) who had failed previous conservative therapies and received a single injection of micro-fragmented adipose tissue for shoulder pathology. Outcomes including pain, disability, and safety were assessed at minimum of six months.

Results. Significant improvements in visual analog scale scores ($p < 0.001$) and pain disability index scores ($p = 0.02$) with no major adverse events were observed at six months.

Conclusions. Micro-fragmented adipose tissue may be helpful to improve pain and function in a subset of patients with chronic glenohumeral osteoarthritis and rotator cuff tears. No major complications were identified in our case series.

KEY WORDS

Medicinal signaling cells; MFAT; orthobiologics; regenerative medicine; shoulder.

INTRODUCTION

Shoulder pain is a common debilitating condition with an estimated annual prevalence ranging from 4.7-46.7% (1). In 2008, nearly 19 million adults (>8% of the US adult population) reported chronic shoulder pain (2). Rotator cuff disorders and glenohumeral osteoarthritis are two of the most common etiologies (3,4). Patients frequently continue to suffer with pain despite treatment, with 40-50% having continuous or recurrent pain complaints at one year (5). Direct costs in 2000 for treating shoulder pain in the United States totaled \$7 billion (3).

Early in the clinical course of shoulder disorders typical treatments offered include activity modification, physical therapy, oral anti-inflammatories and corticosteroid injections (4). The long-term use of corticosteroid is becoming

increasingly controversial, as there are concerns regarding tendon and chondral toxicity (6). Until recently, the next step in treatment was often surgical with rotator cuff repair or shoulder arthroplasty. Not all patients are candidates for repair secondary to a variety of patient and lesion factors, and post-operative recovery for arthroplasty has significant downtime (7,8). Newer injection options such as platelet rich plasma and mesenchymal signaling cells (MSCs), the latter of which are typically obtained from autologous bone marrow or adipose tissue are now being increasingly used (9). MSCs are cells with the perceived capability to differentiate into cells that regenerate tissue functionality following injury (10). Numerous mechanisms have been proposed to explain how MSCs may support tissue repair and relieve pain. Secretion of cytokines and growth factors through a

paracrine mechanism likely plays a large role (11). This paracrine activity is thought to stimulate angiogenesis and have anti-inflammatory properties (12). Secondary to its abundant vasculature, adipose is an excellent medium for MSC harvest. The procurement procedure consists of a minimally invasive harvest with higher cell concentration per unit volume compared to bone marrow concentrate (13). Complex regulations limit utility of culture expansion techniques and enzymatic digestion to obtain stromal vascular fraction. Mechanical treatment with micro-fragmentation provides an effective means of obtaining minimally manipulated adipose tissue known as micro-fragmented adipose tissue (MFAT) for therapeutic use (14).

Limited literature is available on the outcomes of patients treated with MFAT for shoulder pain. Continued clinical use necessitates continued analysis of outcomes of the procedure. In the present study, we report the functional and pain outcomes of 16 patients (18 shoulders) treated with MFAT injections for glenohumeral osteoarthritis and rotator cuff tears.

METHODS

Participants

All patients with glenohumeral osteoarthritis or rotator cuff tears treated with adipose-derived stromal cells from November 2017 to May 2019 at a single outpatient sports medicine clinic were evaluated for inclusion. Diagnosis was obtained through history, examination, and imaging.

Inclusion criteria were: (1) primary diagnosis of glenohumeral osteoarthritis or rotator cuff tear, (2) pathology present on magnetic resonance imaging or plain radiography, (3) pain duration greater than 6 months, and (4) failed conservative management with any combination of physical therapy, corticosteroid injection, visco-supplementation, platelet rich plasma, or arthroscopy. Exclusion criteria were corticosteroid injection within 3 months of MFAT procedure, malignancy, active infection, or auto-immune arthritis.

Sixteen patients (18 shoulders) met criteria and were analyzed. Seventeen shoulders had pathology confirmed through MRI, and in one case severe glenohumeral osteoarthritis was confirmed with plain radiography.

Procedure

The Lipogems® procedural kit was used in all patients. This kit provides the necessary resources for the harvest, processing, and transfer of MFAT. Mild mechanical forces are employed to reduce adipose tissue cluster size and eliminate proinflammatory oil and blood residues (15). The resultant

product is neither enzymatically treated nor culture expanded. All injections were performed by two experienced physiatrists using ultrasound guidance (CE, JBS). The following standardized procedural protocol was used:

Harvest site was determined in the standing position. The patient was subsequently placed supine or prone for flank and thigh harvest sites, respectively, and the skin overlying the harvest site was cleansed with chlorhexidine. After injection of local anesthetic, an 18-gauge needle tip was used to create a small incision. The site was infiltrated with tumescent anesthesia (500 mL Normal Saline, 1 mL of 1:1000 Epinephrine, 50 mL of 1% Lidocaine/25mL of 2% Lidocaine, 5 mL of 8.4% Sodium Bicarbonate) using the 17-gauge Lipogems® blunt anesthesia tip connected to a 60 mL syringe. The cannula was advanced laterally to medially to disperse the tumescent anesthesia. At least 20 minutes post completion of tumescent anesthesia, the lipoaspiration was started. Fat was harvested using a 13-gauge lipoaspirate cannula connected to a 20 mL VacLok syringe. Care was taken to avoid any air being introduced to the syringe. The lipoaspirate syringes were placed in a sterile cup to decant and subsequently transferred to the Lipogems® device for processing. The final product was placed in 3mL syringes for the treatment. As the lipoaspirate was being washed, excess tumescent was expressed from the harvest site and the puncture site was dressed in steri-strips and a sterile gauze, which was then covered by Tegaderm. Tape was placed along the harvest site to minimize swelling, bruising, and post-procedure pain. Ultrasound guidance with a curvilinear probe for the glenohumeral joint and linear probe for rotator cuff lesions was used for tissue transfer with an in-plane needle approach. Under sterile conditions, the final adipose aspirate was injected into the target site using a 18g 3.5-inch needle. Average MFAT injectate volume was 4.9 ± 1.4 mL for glenohumeral arthritis and 1.4 ± 0.6 mL for rotator cuff pathology. After completion of the procedure, the patient was monitored for 15-20 minutes and provided the post-procedure instructions, which were reviewed in-person.

Assessments

Approval was obtained from our institution's quality improvement advisory board; IRB approval was thereby waived by the institution. This study meets the ethical standards of the journal (16). Data was collected during routine clinic follow-up visits. The following outcomes were collected: visual analog scale (VAS), pain disability index (PDI), and percent global improvement. The primary outcome was VAS and PDI score changes from baseline at 6-month follow-up. All patients presented for their 6-month follow-up, with most having additional visits before and after.

The VAS is an 11-point scale with 0 being no pain and 10 being worst possible pain. The PDI is comprised of 7 questions assessing the impact of chronic pain on activities such as recreation, self-care, and occupation. Scores range from 0 (no disability from pain) to 70 (worst disability from pain) (17). Percent global improvement scores ranged from 0% (no improvement) to 100% (maximum possible improvement).

STATISTICAL ANALYSIS

Descriptive statistics were used to evaluate variable frequencies. Within participant changes were evaluated using paired two-tailed Student's t-tests to calculate differences between baseline, 6-month, and greater than one-year VAS and PDI scores. Mean and standard deviations or percentages are reported for the full cohort. Threshold of significance was set at $P < 0.05$. A minimal clinically important difference

(MCID) value of 1.4-point change was used for VAS and 9.5-point change for PDI (18,19). Descriptive values of proportion of participants meeting MCID are presented.

RESULTS

Demographics and clinical characteristics are depicted in **table I**. Most patients' (72%) procedure was in the shoulder of the dominant arm and on average duration of symptoms was approaching 6 years. Follow-up was obtained in all participants for minimum of 6 months (average follow-up was 12.1 ± 5.6 months). Diagnostic imaging identified severe glenohumeral joint osteoarthritis and partial supraspinatus tears as the most prevalent pathologies, seen in 50% and 33% of shoulders, respectively (**table II**).

At baseline, mean VAS was 6.1 ± 2.1 and mean PDI was 21.4 ± 9.2 . There was significant improvement between baseline and 6-month follow up VAS ($p < 0.001$) and PDI ($p = 0.02$) (**figures 1,2**). For the 10 shoulders with greater than one-year follow-up, significant improvement in VAS ($p < 0.001$) and PDI ($p = 0.03$) was observed. At 6 months, the MCID was met for 14 (78%) of shoulders for VAS and 9 (50%) of shoulders for PDI. At final follow-up, global improvement averaged 70% (range 20-100%). One minor complication was reported of transient contact dermatitis from the dressing applied to the harvest site. No other complications were observed or reported.

Table I. Demographic and Clinical Characteristics (N = 16 Patients). Values are mean and standard deviation or percentages. BMI: body mass index; PRP: platelet-rich plasma; MRI: magnetic resonance imaging.

N = 16 Patients	
Age	65.1 \pm 9.6
BMI	28.5 \pm 6.1
Laterality	
Right	11 (68.7%)
Left	3 (18.8%)
Bilateral	2 (12.5%)
Dominant Arm	
Right	16 (100%)
Symptom Duration (m)	65.7 \pm 59.9
Follow-up (m)	12.1 \pm 5.6
Sex	
Male	9 (56%)
Female	7 (44%)
Previous Treatments	
Physical Therapy	16 (100%)
Steroid Injection	15 (94%)
PRP Injection	10 (63%)
Imaging	
Plain Radiograph	16 (100%)
MRI	15 (94%)

Table II. Pathology on Imaging (N = 18 Shoulders).

N = 18 Shoulders		
Glenohumeral Osteoarthritis		
Moderate	4	22,20%
Severe	9	50%
Rotator Cuff Pathology		
Supraspinatus		
Tendinosis	2	11,10%
Partial Tear	6	33,30%
Full tear	2	11,10%
Acromioclavicular Osteoarthritis	5	27,80%
Infraspinatus		
Tendinosis	2	11,10%
Partial Tear	2	11,10%
Subscapularis		
Partial Tear	1	5,60%

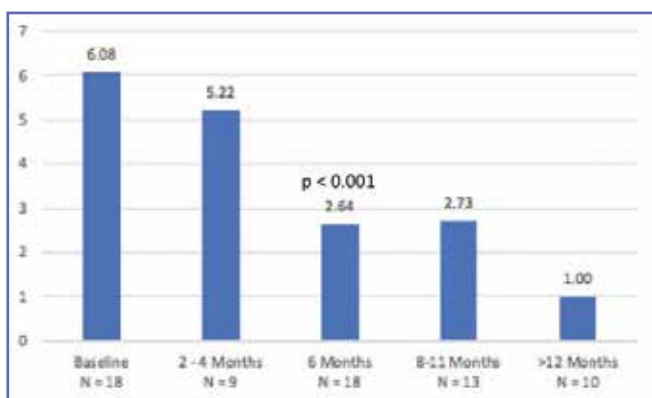


Figure 1. Visual Analog Scale Score Changes. Depicted are mean scores with corresponding number of patients for each follow-up time point.

DISCUSSION

Recalcitrant shoulder pain from osteoarthritis and rotator cuff pathology remains a common problem encountered in clinical practice. In this population of 15 patients, a majority reported significant improvements in pain and/or disability at six months using MCID criteria and no major adverse events were reported. These findings are notable as all patients had failed multiple prior conservative interventions, were not candidates or not ready for surgery, and reported pain and functional limitations for many months. The findings suggest MFAT may be successful in a portion of patients with shoulder pain from GHJ arthritis or RTC disease, with benefits measured 6 months from procedure. Current literature on MFAT use for shoulder pain remains limited. Striano *et al.* reported results on 18 patients with chronic shoulder pain treated with MFAT (20). Patients were followed for one year and significant improvement was noted for pain reduction and functional improvement using the Numeric Pain Scale and American Shoulder and Elbow Surgeons Score. Pain scores decreased from a baseline of 7.5 to 3.6 at one year. In the present study, we observed similar improvement in pain and disability. While statistical analyses could not be completed for after 6 months in our study, seven patients had follow-up times greater than 10 months with average point decreases of 4.2 and 4.6 in VAS and PDI, respectively, from baseline. A case report of a T10 complete paraplegic wheelchair user with chronic shoulder pain found improved pain and functional outcomes one-year post Lipogems[®] procedure (21). This allowed him to maintain independence and resume activities of daily living. Two studies report stromal vascular fraction (SVF) outcomes on shoulder pain via arthroscopy. Jo *et al.* treated 19 patients with varying doses of culture expanded SVF at the time

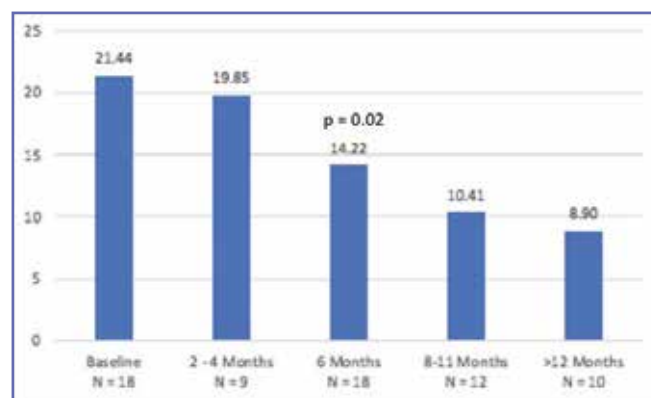


Figure 2. Pain Disability Index Score Changes. Depicted are mean scores with corresponding number of patients for each follow-up time point.

of an arthroscopic examination (22). Significant improvement was found at six months for the mid and high dose groups. The SVF used in this study was obtained through enzymatic digestion. Notably, culture-expanded cells are not approved for use in the United States as they violate the Federal Drug Administration's minimal manipulation regulations for MSCs. Kim *et al.* compared outcomes of surgical repair alone for rotator cuff tears against repair coupled with injection of adipose-derived MSCs loaded in fibrin glue. They found decreased re-tear rates in the surgery plus adipose-derived MSC group, but no significant differences in pain outcomes (23).

Multiple mechanisms have been proposed for how adipose-derived signaling cells may improve pain and function for shoulder pathology. Immunomodulatory and anti-inflammatory properties secondary to a paracrine secretion of growth factors and cytokines likely contributes (11). In MFAT, these trophic properties can be attributed to the undifferentiated cells which are isolated from adult harvested adipose tissue (24). Additionally, tendon needling when treating rotator cuff tears invariably occurs and can have pain relieving properties (25).

While the findings are encouraging, results of this large case series should be interpreted with caution. Case series retrospective design is limited by sample size, low level of evidence (IV) and absence of a control group which precludes comparing outcomes amongst other treatment options. Selection bias of participants may influence results (including choice of procedure and out of pocket expense). MFAT is not a covered benefit under most commercial and government insurers in the United States, and out of pocket expense varies depending on which particular MFAT kit is used as well as overhead and demographics of the practice location. Often this amounts to a couple thousand US dollars.

Notably no patients studied had received visco-supplementation with hyaluronic acid (HA) prior to their MFAT procedure. *In vitro* studies have demonstrated HA to potentially enhance tendon cell viability and proliferation (26). Additionally, in a review of 11 clinical studies, HA was found to improve pain and function in shoulders with rotator cuff tears (27). While often not covered by insurers in the United States, HA is typically a cheaper treatment option that can be considered prior to MFAT. The degree of osteoarthritis and rotator cuff tear severity may also influence results and was not controlled for in this study. Follow-up was obtained for a subset of patients to 6 months, and clinical results are often reported at time points 12 months after the procedure (28,29). However, this report adds to the limited literature

using Lipogems® and suggests further investigations using rigorous methodology (randomization, blinding, and larger sample sizes with more homogenous degree of pathology) would help understand the benefits of this procedure. In conclusion, our results suggest MFAT may be helpful to improve pain and function in a subset of patients with chronic glenohumeral osteoarthritis and rotator cuff tears. No major complications were identified in our case series. Additional studies ideally prospective with a control group are needed for further evaluation of this treatment.

CONFLICTS OF INTERESTS

The author declare that they have no conflict of interests.

REFERENCES

1. Luime JJ, Koes BW, Hendriksen IJM, et al. Prevalence and incidence of shoulder pain in the general population; a systematic review. *Scandinavian Journal of Rheumatology* 2004; 33: 73–81.
2. Black EM, Higgins LD, Warner JJP. Value-based shoulder surgery: Practicing outcomes-driven, cost-conscious care. *Journal of Shoulder and Elbow Surgery* 2013; 22: 1000–9.
3. Meislin RJ, Sperling JW, Stitik TP. Persistent shoulder pain: epidemiology, pathophysiology, and diagnosis. *American journal of orthopedics (Belle Mead, N.J.)* 2005; 34: 5–9.
4. Oliva F, Piccirilli E, Bossa M, et al. I.S.Mu.L.T - Rotator cuff tears guidelines. *Muscles Ligaments Tendons J* 2015 Oct 1 [cited 2020 Feb 10];5(4):227–63. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/26958532>.
5. Cadogan A, Laslett M, Hing WA, McNair PJ, Coates MH. A prospective study of shoulder pain in primary care: Prevalence of imaged pathology and response to guided diagnostic blocks. *BMC Musculoskeletal Disord* 2011;12.
6. Dean BJF, Lostis E, Oakley T, Rombach I, Morrey ME, Carr AJ. The risks and benefits of glucocorticoid treatment for tendinopathy: A systematic review of the effects of local glucocorticoid on tendon. Vol. 43, *Seminars in Arthritis and Rheumatism* 2014: 570–6.
7. Petrillo S, Longo UG, Papalia R, Denaro V. Reverse shoulder arthroplasty for massive irreparable rotator cuff tears and cuff tear arthropathy: a systematic review. *Musculoskelet Surg* [Internet]. 2017 Aug [cited 2019 Sep 16];101(2):105–12. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/28444541>.
8. Wilcox RB, Arslanian LE, Millett P. Rehabilitation following total shoulder arthroplasty. *J Orthop Sports Phys Ther* 2005 Dec [cited 2019 Sep 16];35(12):821–36. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16848103>.
9. Borg-Stein J, Osoria HL, Hayano T. Regenerative Sports Medicine: Past, Present, and Future (Adapted From the PASSOR Legacy Award Presentation; AAPMR; October 2016). *PM R* 2018 [cited 2019 Sep 7];10(10):1083–105. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/30031963>.
10. DeChellis DM, Cortazzo MH. Regenerative medicine in the field of pain medicine: Prolotherapy, platelet-rich plasma therapy, and stem cell therapy-Theory and evidence. *Tech Reg Anesth Pain Manag* 2011 Apr;15(2):74–80.
11. Freitag J, Bates D, Boyd R, Shah K, Barnard A, Huguenin L, et al. Mesenchymal stem cell therapy in the treatment of osteoarthritis: Reparative pathways, safety and efficacy - A review. *BMC Musculoskeletal Disorders BioMed Central Ltd* 2016; 17.
12. Hudetz D, Borić I, Rod E, et al. Early results of intra-articular micro-fragmented lipoaspirate treatment in patients with late stages knee osteoarthritis: a prospective study. *Croat Med J* 2019 Jun 13;60(3):227–36.
13. Strioga M, Viswanathan S, Darinskas A, Slaby O, Michalek J. Same or not the same? comparison of adipose tissue-derived versus bone marrow-derived mesenchymal stem and stromal cells. Vol. 21, *Stem Cells and Development* 2012; 2724–52.
14. Bianchi F, Maioli M, Leonardi E, Olivi E, Pasquinelli G, Valente S, et al. A new nonenzymatic method and device to obtain a fat tissue derivative highly enriched in pericyte-like elements by mild mechanical forces from human lipoaspirates. *Cell Transplant* 2013;22(11):2063–77.
15. Tremolada C, Colombo V, Ventura C. Adipose Tissue and Mesenchymal Stem Cells: State of the Art and Lipogems® Technology Development. Vol. 2, *Current Stem Cell Reports*. Springer International Publishing 2016; 304–12.
16. Padulo J, Oliva F, Frizziero A, Maffulli N. Muscles, ligaments and tendons journal – Basic principles and recommendations in clinical and field science research: 2016 update. *Muscles Ligaments Tendons J* 2016 Jan 1;6(1):1–5.
17. Jerome A, Gross RT. Pain disability index: construct and discriminant validity. *Arch Phys Med Rehabil* 1991 Oct [cited 2019 Sep 9];72(11):920–2. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/1929812>.
18. Tashjian RZ, Deloach J, Porucznik CA, Powell AP. Minimal clinically important differences (MCID) and patient acceptable symptomatic state (PASS) for visual analog scales (VAS) measuring pain in patients treated for rotator cuff disease. *J Shoulder Elb Surg* 2009 Nov;18(6):927–32.
19. Soer R, Reneman MF, Vroomen PCAJ, Stegeman P, Coppes MH. Responsiveness and minimal clinically important change

- of the pain disability index in patients with chronic back pain. *Spine (Phila Pa 1976)* 2012 Apr 15;37(8):711–5.
20. RD Striano GMNBKA. Refractory shoulder pain with osteoarthritis, and rotator cuff tear, treated with micro fragmented adipose tissue. *Orthop Spine Sport Med* 2018;2:014.
 21. Cherian C, Malanga GA, Hogaboom N, Pollack MA, Dyson-Hudson TA. Autologous, micro-fragmented adipose tissue as a treatment for chronic shoulder pain in a wheelchair using individual with spinal cord injury: a case report. *Spinal Cord Ser Cases* 2019 Dec;5(1).
 22. Jo CH, Chai JW, Jeong EC, Oh S, Kim PS, Yoon JY, et al. Intratendinous Injection of Autologous Adipose Tissue-Derived Mesenchymal Stem Cells for the Treatment of Rotator Cuff Disease: A First-In-Human Trial. *Stem Cells* 2018 Sep [cited 2019 Sep 9];36(9):1441–50. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/29790618>.
 23. Kim YS, Sung CH, Chung SH, Kwak SJ, Koh YG. Does an Injection of Adipose-Derived Mesenchymal Stem Cells Loaded in Fibrin Glue Influence Rotator Cuff Repair Outcomes? A Clinical and Magnetic Resonance Imaging Study. *Am J Sports Med* 2017 Jul 1;45(9):2010–8.
 24. Bembo F, Eraud J, Philandrianos C, Bertrand B, Silvestre A, Veran J, et al. Combined use of platelet rich plasma & micro-fat in sport and race horses with degenerative joint disease: Preliminary clinical study in eight horses. *Muscles Ligaments Tendons J* 2016 Apr 1;6(2):198–204.
 25. Krey D, Borchers J, McCamey K. Tendon needling for treatment of tendinopathy: A systematic review. *Phys Sportsmed* 2015 Feb [cited 2019 Sep 9];43(1):80–6. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/25613418>.
 26. Gallorini M, Berardi AC, Berardocco M, et al. Hyaluronic acid increases tendon derived cell viability and proliferation in vitro: comparative study of two different hyaluronic acid preparations by molecular weight. *Muscles Ligaments Tendons J* [Internet]. 2017 [cited 2020 Feb 10];7(2):208–14. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/29264330>.
 27. Osti L, Buda M, del Buono A, Osti R, Massari L. Clinical evidence in the treatment of rotator cuff tears with hyaluronic acid. Vol. 5, *Muscles, Ligaments and Tendons Journal*. CIC Edizioni Internazionali s.r.l.; 2015;270–5.
 28. Jo CH, Chai JW, Jeong EC, Oh S, Shin JS, Shim H, et al. Intra-articular Injection of Mesenchymal Stem Cells for the Treatment of Osteoarthritis of the Knee: A 2-Year Follow-up Study. *Am J Sports Med* [Internet]. 2017 Oct [cited 2019 Sep 16];45(12):2774–83. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/28746812>.
 29. Cattaneo G, De Caro A, Napoli F, Chiapale D, Trada P, Camera A. Micro-fragmented adipose tissue injection associated with arthroscopic procedures in patients with symptomatic knee osteoarthritis. *BMC Musculoskelet Disord* 2018 May 30 [cited 2019 Sep 16];19(1):176. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/29848328>.

Ultrasound Tissue Characteristics of Diabetic Muscles and Tendons: Associations with Strength and Laboratory Blood Tests

C.Y. Kuo¹, W. N. Lee², S. S. M. Fong³, P. Chaiyawat⁴, G. Bashford⁵, Tiffany T.F. Shih^{6,7}, L. M. Chuang^{8,9}, H. K. Wang^{1,10}

¹ School and Graduate Institute of Physical Therapy, College of Medicine, National Taiwan University, Taipei, Taiwan

² Department of Electrical and Electronic Engineering and Biomedical Engineering Programme, The University of Hong Kong, Hong Kong, China

³ School of Public Health, The University of Hong Kong, Pokfulam, Hong Kong, China

⁴ Faculty of Physical Therapy, Mahidol University, Nakhon Pathom, Thailand

⁵ Department of Biological Systems Engineering, University of Nebraska–Lincoln, Lincoln, Nebraska, USA

⁶ Department of Radiology, College of Medicine, National Taiwan University, Taipei, Taiwan

⁷ Department of Medical Imaging, National Taiwan University Hospital, National Taiwan University, Taipei, Taiwan.

⁸ College of Medicine, National Taiwan University, Taipei, Taiwan

⁹ Division of Endocrinology and Metabolism, Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan

¹⁰ Department of Physical Medicine and Rehabilitation, National Taiwan University Hospital, National Taiwan University, Taipei, Taiwan

CORRESPONDING AUTHOR:

Hsing-Kuo Wang
Medicine, National Taiwan University
Sports Physiotherapy Laboratory
School and Graduate Institute
of Physical Therapy
College of Medicine
Xuzhou Road, Zhongzheng District
Taipei, Taiwan, 100
E-mail: hkwang@ntu.edu.tw

DOI:

10.32098/mltj.03.2020.07

LEVEL OF EVIDENCE: 3B

SUMMARY

Background. This study sought to compare the ultrasound tissue characteristics of the muscles (including muscle thickness, echo intensity, and stiffness) and tendons (including thickness, stiffness, and peak spatial frequency radius (PSFR)) of participants with or without diabetes mellitus. Moreover, the study sought to determine any relationships between the muscle stiffness and strength or tendon PSFR and the results of blood tests, including the glycation or lipid profiles, of the diabetics.

Methods. Twenty-three participants with type 2 diabetes mellitus and nineteen controls without a history of diabetes were recruited.

Results. The diabetic muscles exhibited less thickness ($P=0.024$), greater echo intensity ($P=0.033$ and 0.002), and lower muscle stiffness ($P=0.015$ and 0.009) than the control muscles. Furthermore, the diabetic tendons exhibited a lower PSFR (P ranged between 0.037 and <0.001). There were correlations between the resting stiffness of the gastrocnemius muscle and the height of heel lifting ($r=0.450$, $P=0.031$), between the PSFR in the patellar tendon and the hemoglobin A1c level ($r= -0.539$, $P=0.017$), and between the PSFR in the Achilles tendon and the high-density lipoprotein cholesterol level ($r=0.545$, $P=0.019$).

Conclusions. The diabetic muscles and tendons exhibited morphomechanical changes associated with force capacity or markers of insulin resistance. Clinical applications of musculoskeletal ultrasound techniques to diabetics include using them to design exercise strategies and for microstructural screening.

KEY WORDS

Diabetes; musculoskeletal ultrasound; tissue characteristics; muscle; tendon.

BACKGROUND

Diabetes mellitus (DM) is a major cause of mortality and functional disability worldwide, and the complications resulting from it include microvascular diseases and musculoskeletal disorders (1). Long-term hyperglycemia and impaired lipid metabolism are considered to be among the causes of the diabetic myotendinous disorders, which may be accompanied by morphological (structural) changes, dysfunction, and pain (2). In a magnetic resonance imaging study focusing on morphology, diabetic calf muscles were found to exhibit atrophy with fat infiltration, loss of muscle mass, increased fatigability, and a greater reliance on glycolytic metabolism in comparison to the muscles of controls without diabetes (3). In a study observing the microstructure and morphology of the Achilles tendons of patients with long-term DM using an electron microscope, it was reported that such tendons have reduced fiber diameter, increased collagen fiber bulk density, and abnormal fiber morphology (4). These findings themselves suggest possible mechanisms underlying the findings of other reports indicating that (a) the hyperechoic appearance of diabetic muscles in ultrasound imaging correctly predicted diabetes in 70 of 79 patients (5); (b) long-term diabetes is associated with a high prevalence of chronic Achilles tendinopathy, including hypoechoogenicity, enthesal thickening, and enthesophytes (2,6); and (c) diabetic tendons demonstrate inferior elasticity (Young modulus), maximum load, and stiffness levels (7,8). Theoretically, these respective muscle morphological and tendon microstructural changes are the causes of diabetic muscle force compromise, in addition to being evidence of diabetic complications in tissues with low reparative capacity. However, the studies referenced above left unanswered questions about (a) whether atrophy with fat infiltration in diabetic muscles leads to a reduction of muscle stiffness, which is an elemental determinant of force development; (b) whether abnormal morphology and disorganization of the collagen fibrils in the diabetic tendon could reflect on the primary fiber bundle, the basic unit of transmitting and resisting tensile stresses in the tendon (9); and (c) whether the aforementioned morphomechanical changes are associated with force production within the myotendinous complex or the profiles of blood biomarkers representing the pathology of diabetes. Further studies are thus required to answer the above questions and establish clinical protocols for periodic evaluations to monitor diabetes-related myotendinous deteriorations.

High-resolution B mode ultrasonography and elastography have been determined to be reliable techniques for measuring the echo intensity (EI), mechanical stiffness (elastic modulus), and morphologies of skeletal muscle. Skeletal muscle stiffness is determined using shear-wave elastogra-

phy according to the tension produced by muscle contraction and mechanical properties along the muscle fibers (10). In addition, changes in the physical characteristics of the microstructure of the tissue can be quantified by detecting the energy behavior of scattering of the B-mode image structure with spectral analyses of the waveform data of ultrasonic radiofrequency signals, such as a high proportion of energy at low frequencies as the scatter size increases (11). Other studies have shown the microstructural changes that occur in tendinopathy via the low values of peak spatial frequency radius (PSFR), which consists of the distance from the origin to the spatial frequency peak of greatest amplitude on the 2-D fast Fourier transform spectrum, exhibited in symptomatic subjects (12). It was inferred that, the ultrasound wavelength ranged between 0.32 mm (320 μm) and 0.13 mm (130 μm), with a 5-12 MHz probe being able to detect the scattering from the structures forming a primary bundle or subfascicle (15-400 μm in diameter), which was mainly composed of collagen fibrils (with diameters of 30-300 nm) and small collagen fibers (with diameters of 1-300 μm) (9). Collectively, DM-associated deteriorations in muscle mechanical properties and tendon microstructures respectively represent the compromised capacity of muscle force production and the pathologies of diabetes and can be assessed by elastographic and ultrasonic tissue characterization using spectrum analysis. However, profiles of the above characteristics have not been reported or completely analyzed in past studies.

The aim of the present study, therefore, was two-fold. First, to measure and compare the ultrasound tissue characteristics of the muscles (including muscle thickness, EI, and stiffness) and tendons (including thickness, stiffness, and PSFR) of participants with DM who demonstrated negative results in peripheral vascular and nerve screening testing to those of participants without DM. In addition, the study sought to determine any relationships between the muscle stiffness and strength or tendon PSFR and the results of blood tests, including the glycation or lipid profiles, in the participants with DM. We hypothesized that there would be differences in the aforementioned ultrasonic tissue characteristics of the myotendinous complex of the lower extremities in the DM patients when compared to controls. Furthermore, it was hypothesized that, in the DM group, the stiffness in certain muscles (namely, the vastus lateralis and medial gastrocnemius) and the PSFR in the tendons (namely, the patellar and Achilles tendons) would be associated, respectively, with muscle strength results and blood test results, including hemoglobin A1c (HbA1c) and high-density lipoprotein cholesterol (HDL-C) levels. The HbA1c and HDL-C levels were chosen because abnormalities in serum lipid profiles and HbA1c levels represent markers of insulin resistance

and the risk for micro- and macroangiopathies in known type 2 diabetic patients (13).

MATERIALS AND METHODS

Subjects and study design

This study utilized a cross-sectional design that compared a group of diabetic participants with another group of participants who were matched in terms of physical characteristics but did not have a history of DM. This study was approved by the institutional review board of National Taiwan University Hospital (reference no. 201703035RINC) and meets the ethical standards of the journal (14). All of the eligible diabetic and control participants were recruited from an outpatient clinic of a university-affiliated hospital. Written informed consent was obtained from all the participants prior to participation, and details that might disclose the identity of the participants have been omitted. All the diabetic participants were between 30 and 80 years old and were recruited based on the 2018 criteria of the American Diabetes Association (15) and their negative results on peripheral vascular or nerve screening tests, including their ankle-brachial index results (< 0.9 or > 1.3), Semmes Weinstein monofilament examination results (any one of 4 sites was insensate on each foot), and 128-Hz tuning fork vibration test results (perceptions - 5 seconds). The participants in the control group who were matched in terms of physical characteristics to the DM group were recruited from the hospital department of volunteer and charity workers. The inclusion criteria for the control participants included no history of DM. Any of the potential participants were excluded if they had risk factors for structural weakness in the myotendinous complex, including a chronic kidney injury treated with hemodialysis, hyperuricemia, rheumatoid arthritis, systemic lupus erythematosus, osteogenesis imperfecta, musculoskeletal tuberculosis, parathyroidism, hypothyroidism, or alkaptonuria.

Experimental protocol

The participants were asked to change into a hospital gown if their clothes prevented ultrasonographic measurements of the rectus femoris, vastus lateralis, quadriceps tendon, patellar tendon, medial gastrocnemius, or Achilles tendon. They were instructed to rest for 10 minutes and fill out a brief questionnaire including questions regarding their DM history. Ultrasound image acquisition was conducted in the medical imaging department of the university hospital with an Aixplorer® system (Supersonic Imaging, Aix-en-Provence, France) coupled with an SL15-4 probe.

The Young's modulus, grayscale image features, thickness, and PSFR of the aforementioned muscles or tendons were measured in a longitudinal view. The elasticity measurements using a round region of interest (ROI) with an approximately 4-7 mm diameter were recorded with the mean resting Young's modulus within the ROI on the same aforementioned muscles and tendons. During each elasticity measurement, the ultrasound transducer was kept stationary for 10s during the acquisition of the image (**figure 1 A-F**). To standardize and optimize the image quality and to avoid variability in the B-mode images, the gain was adjusted to a default set of control settings (46-48%), and the focus area was increased to maximum and kept consistent across all the participants to adjust for differences in muscle size among the participants. Muscle thickness assessed by B-mode ultrasound was measured as the distance between the superficial and deep aponeuroses at the mid-line of the image, while the tendon thickness was determined by the maximum anteroposterior diameter of the tendons. The measurements for the rectus femoris and vastus lateralis muscles (on the lower third of the thigh), quadriceps tendon, and patellar tendon were conducted with the participant positioned in a supine position with the knee flexed at 60°, with this position maintained by pillows placed under the tested knee. Ultrasound measurements of the quadriceps and patellar tendons were respectively conducted at 1.5 cm above the superior border of the patella and in the area around the middle portion of the patellar tendon. For measurements of the proximal third of the gastrocnemius medialis muscle (i.e., the region between the lateral malleolus of the ankle and the lateral condyle of the knee) and the mid-portion of the Achilles tendon (i.e., the region 2-4 cm proximal to the calcaneal insertion), each subject lay prone (face down) on an examination bed with both ankles hanging over the edge of the bed, with the foot being placed at an angle of 90° to the tibia.

B-mode ultrasound images were stored as jpeg files and transferred to a computer for processing regarding the muscle EI and tendon PSFR. The mean pixel intensity (i.e., the EI) of the selected muscle regions was obtained by gray-scale analysis using the standard histogram function in Image-J (ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA) without any bone, aponeurosis, or surrounding fascia. The mean EI values for the different regions of muscle were expressed in values ranging between 0 and 256 (0: black; 256: white). The measurements of tendon PSFR were conducted using a protocol similar to that used by Kulig et al. (12), including the use of custom image analysis programs written in MATLAB (Mathworks, Natick, MA, USA). A quadrilateral-shaped ROI enclosing a maximum tendon area corresponding to the mid-substance

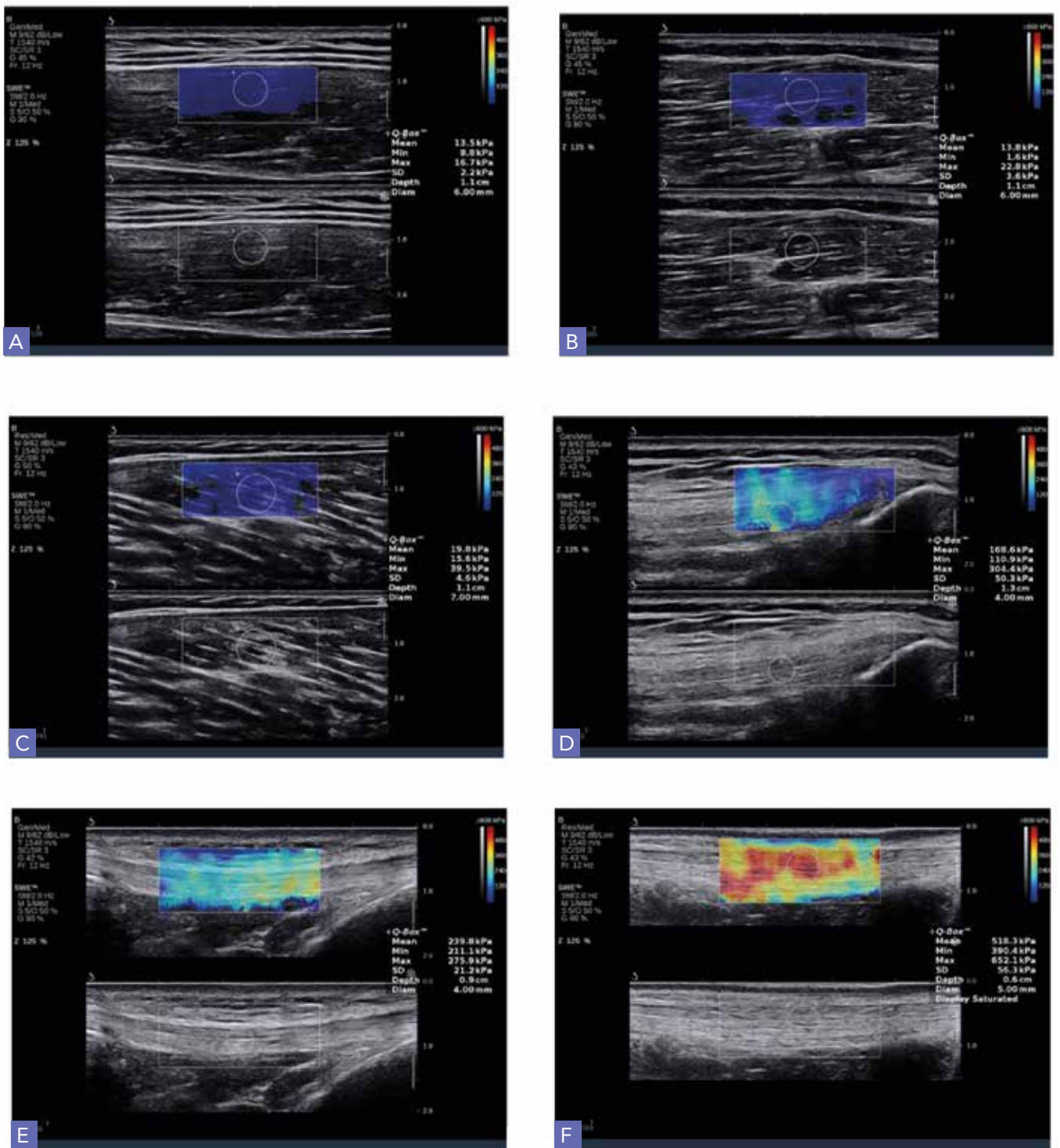


Figure 1. The measurements of and Young's modulus (shear-wave velocity) of the rectus femoris muscle (A), vastus lateralis muscle (B), medial gastrocnemius muscle (C), quadriceps tendon (D), patellar tendon (E), and Achilles tendon (F).

of the tendon without the distal and proximal image (i.e., the distal and proximal tendon curvature) was selected for each PSFR measurement (12) (**figure 2 A-C**). Within the ROI, the PSFR in every 2 mm-square kernel (32 x 32 pixel) circumscribed by the ROI was analyzed and averaged for each image (12).

After the ultrasonographic measurements, the participants were assessed in two strength testing sessions, the first of which involved the knee extension exercise and the second of which involved the heel raising exercise. There was a five-minutes interval of rest between the two sessions. During the testing sessions, each participant was first instructed to perform the maximum isometric knee extension in a sitting position and then the one-legged heel raising in a standing position using his or her bodyweight. The knee testing was completed with a customized wheelchair with an affixed load cell attached to the ankle cuff just above the medial malleolus, with the lumbar-hip and knee joints respectively positioned at 100-110° and 60° extensions (with 0° corresponding to the complete extension). Both upper limbs of each participant were kept crossed in front of the participant's chest. In the heel raising testing, each participant was asked to stand on the tested leg and instructed to perform maximal heel lifting. The participant was allowed to touch a wall with an index finger at the shoulder level to maintain a steady one-leg standing position. The maximal force for the knee extension exercise and height for the heel raising exercise were recorded.

Laboratory tests

Results of blood tests (including tests of HbA1c, triacylglycerol (TG), total cholesterol, low-density cholesterol (LDL), and HDL-C levels) were collected over a period of approximately 6 months. The HbA1c and HDL-C levels were enrolled for the correlation analyses.

STATISTICAL ANALYSIS

The Shapiro-Wilk test was used to determine if the data for all the variables were normally distributed. The Mann-Whitney U test was used to analyze the differences between the diabetic tendons and non-diabetic tendons. Spearman's rank correlation coefficients were calculated to determine whether, respectively, the muscle Young's modulus and force production capacities (i.e., the knee extension torque and the heel lifting height) and the tendon microstructural characteristics and laboratory blood test results (HbA1c and HDL-C levels) were related. All of the analyses were performed using SPSS 22.0 for Windows (SPSS Inc, Chicago, IL, USA), with the α level set at 0.05.

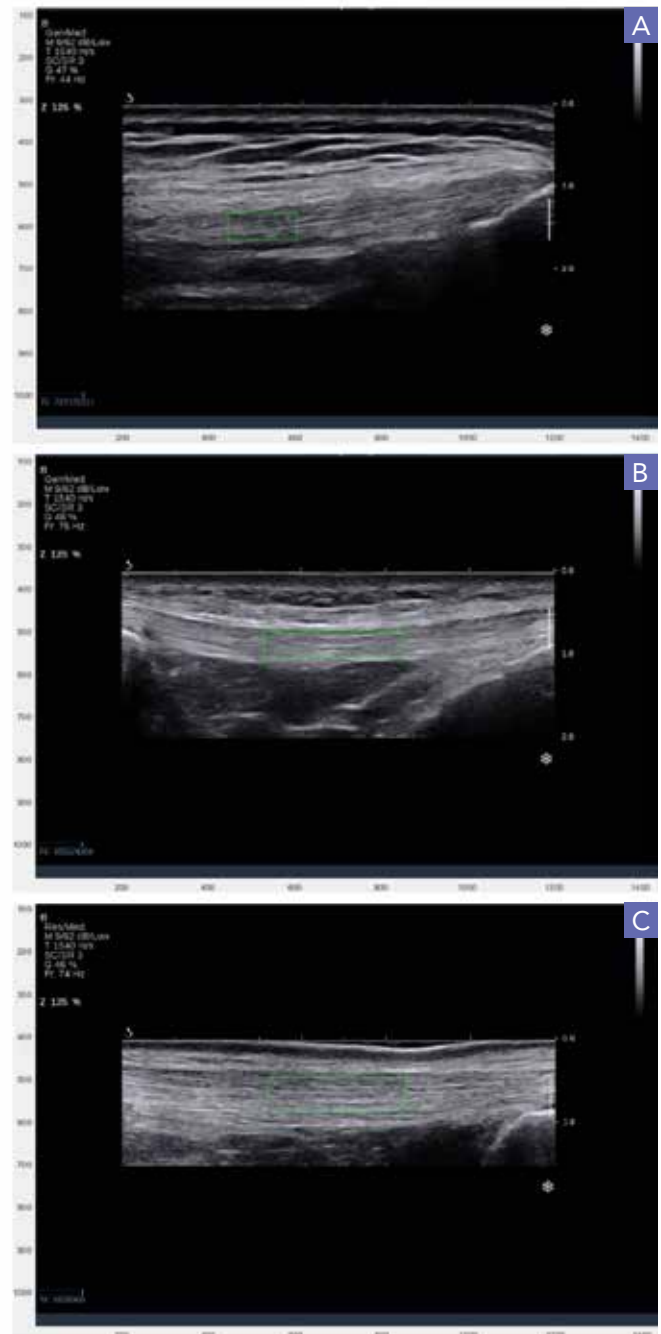


Figure 2. The measurements of peak spatial frequency radius (PSFR) for the quadriceps (A), patellar (B), and Achilles (C) tendons.

RESULTS

Participants

Twenty-three participants with type 2 DM and nineteen physically matched controls without a history of diabetes were recruited. The characteristics of the participants are summarized in Table 1. None of the diabetic participants had shown complications or signs of high risks of peripheral artery disease or neuropathy, such as the loss of the sensation of touch (**table I**). There were no significant differences in age or gender between the diabetic and control groups (both $P>0.05$), while the height of heel lifting was found to be greater in the controls ($P<0.001$). The blood laboratory test results for the diabetic participants ranged between normal and moderate hypertriglyceridemia (TGs between 150–499 mg/dL), between normal and acceptable LDL levels (100–129 mg/dl), and between low (HDL <50 mg/dl) and normal HDL levels (16). The investigated diabetic muscles exhibited less thickness ($P=0.024$), greater EI ($P=0.033$ and 0.002), and lower muscle stiffness ($P=0.015$ and 0.009) when compared to the control muscles. In addition, the diabetic tendons exhibited a lower PSFR (P ranged between 0.037 and <0.001) (**table II**); however, this was not consistent with the thickness and material stiffness results observed (all $P>0.05$) (**table**

III). There were correlations between the resting stiffness of the gastrocnemius muscle and the height of heel lifting ($r=0.490$, $P=0.021$), between the PSFR in the patellar tendon and the HbA1c level ($r= -0.539$, $P=0.017$), and between the PSFR in the Achilles tendon and the HDL-C level ($r=0.545$, $P=0.012$).

DISCUSSION

The results of this study verified our hypotheses and demonstrated that diabetes has effects on the morphomechanical properties of the muscles and tendons, as well as their associations with, respectively, plantarflexor muscle strength and blood sugar/lipid levels. These findings expand the existing knowledge of how long-term hyperglycemia and impaired sugar/lipid metabolism impact the mechanical properties and micro-structure, respectively, in muscles and tendons. They also provide further information regarding possible mechanisms of diabetic muscle weakness and tendinopathy genesis in type 2 DM patients. Moreover, they highlight exercise strategies for early detected or well-controlled diabetes patients without significant signs of peripheral vascular disease and neuropathy aimed at preventing functional disability and tendon pain. In addition, they also indicate the potential contributions of ultrasound techniques in

Table I. Physical characteristics, laboratory test results, and muscle strength levels of the participants with and without diabetes mellitus.

	Diabetic group (N=23)	Non-diabetic group (N=19)	P value
Age (in years)	65 (51-70)	65 (45-70)	0.551
Gender (M/F)	12/11	10/9	0.363
History (in years)	9.5 (3.0-23.0)	NA	
HAb1C (%)	7.3 (5.9-10.5)	NA	
TG (mg/dL)	86.0 (37.0-509.0)	NA	
T-CHO (mg/dL)	164.0 (124.0-242.0)	NA	
LDL-C (mg/dL)	94.0 (51.0-132.0)	NA	
HDL-C (mg/dL)	49.5 (33.0-66.0)	NA	
Ankle-brachial index - right side	1.2 (1.1-1.3)	NA	
Ankle-brachial index - left side	1.2 (1.1-1.3)	NA	
Semmes-Weinstein test - right foot	0.0 (0.0-0.0)	NA	
Semmes-Weinstein test - left foot	0.0 (0.0-0.0)	NA	
Vibration test - right foot	7.0 (6.0-8.0)	NA	
Vibration test - left foot	7.0 (6.0-8.0)	NA	
Knee isometric extension (kg)	21.0 (8.96-45.7)	22.6 (16.2-34.2)	0.336
Heel raising height (cm)	10.0 (6.0-12.5)	11.8 (7.2-14.1)	<0.001

Abbreviations: NA, not available. Results are presented as median values, with the range between the minimum and maximum values in the parentheses.

Table II. Ultrasonic tissue characteristics of muscles in the participants with and without diabetes mellitus.

	Diabetic group (N=23)	Non-diabetic group (N=19)	P value
Muscle thickness (cm)			
Rectus femoris	1.17 (0.86-1.76)	1.08 (0.75-1.46)	0.225
Vastus lateralis	1.52 (1.15-1.98)	1.48 (0.89-2.31)	0.781
Medial gastrocnemius	1.31 (0.95-1.91)	1.59 (0.93-2.23)	0.024*
Echo intensity (A. U.)			
Rectus femoris	75.54 (41.29-123.47)	64.93 (29.66-114.10)	0.033*
Vastus lateralis	84.65 (50.80-122.00)	66.6 (38.65-116.63)	0.067
Medial gastrocnemius	80.14 (34.29-113.44)	50.69 (28.35-109.53)	0.002*
Mechanical stiffness (kilopascal)			
Rectus femoris	12.10 (8.20-65.60)	14.90 (9.70-68.00)	0.197
Vastus lateralis	12.00 (8.50-21.20)	14.70 (9.6-31.9)	0.015*
Medial gastrocnemius	16.30 (8.70-28.40)	18.50 (10.70-54.3)	0.009*

Results are presented as median values, with the range between the minimum and maximum values in the parentheses. * means a significant difference. A. U. means arbitrary unit.

Table III. Ultrasonic tissue characteristics of tendons in the participants with and without diabetes mellitus.

	Diabetic group (N=23)	Non-diabetic group (N=19)	P value
Thickness (cm)			
Quadriceps tendon	0.58 (0.43-0.72)	0.51 (0.40-0.66)	0.558
Patellar tendon (M)	0.38 (0.29-0.54)	0.33 (0.30-0.44)	0.068
Achilles tendon (M)	0.49 (0.28-0.66)	0.51 (0.32-0.64)	0.980
PSFR (A. U.)			
Quadriceps tendon	1.25 (0.70-2.33)	1.48 (0.83-2.72)	0.037*
Patellar tendon (M)	1.47 (0.83-2.58)	2.08 (0.83-3.08)	0.003*
Achilles tendon (M)	1.21 (0.70-2.45)	2.08 (1.33-2.95)	<0.001*
Mechanical stiffness (kilopascal)			
Quadriceps tendon	294.0 (179.4-508.4)	233.6 (108.7-446.6)	0.071
Patellar tendon (M)	188.3 (57.9-341.3)	163.7 (69.9-334.1)	0.503
Achilles tendon (M)	373.0 (166.30-532.90)	298.0 (121.7-475.4)	0.126

Results are presented as median values, with the range between the minimum and maximum values in the parentheses. * means a significant difference. M means the middle portion of the tendon. A. U. means arbitrary unit.

assessing muscle tissue characteristics and screening tendon pathogenesis in patients with metabolic disorders.

In the present study, the ultrasonic tissue characteristic results for diabetic muscles at rest indicated that in type 2 DM patients without peripheral vascular and neuropathic signs, the main deterioration of skeletal muscles in the lower extremities included decreases of muscle mass (reduced thickness), the accumulation of intramuscular fat (increased EI), and the reduction of the mechanical proper-

ty (Young's modulus or shear-wave velocity). These results are consistent with previous studies that reported that (a) leg muscle thickness levels are reduced in diabetic patients without neuropathic signs (17) and (b) there is excessive fat infiltration in leg skeletal muscles in individuals with obesity and DM (18). Since the muscle fiber bundles are composites of fibers and extracellular matrix (ECM) that bear the majority of passive muscle loads (19), the reduced shear-wave velocity or Young's modulus in muscles in this

study may be related to reduced contractile and biochemical properties of muscle fibers (20); adverse remodeling of the ECM in diabetic muscle, including irregular organization and decreased matrix metalloproteinase (MMP) activation (21); and increases of intramuscular fat (18) in diabetes. The reduced muscle Young's modulus found in this study has three clinical implications: (a) insulin resistance and diabetes are detrimental to the mechanical properties of muscle fiber and ECM, muscle composition, and force production, an implication which is supported by the correlation between the calf muscle Young's modulus and the maximal heel lifting height in the present study; (b) the adaptive capacity of diabetic muscles for exercise or physical training may be reduced due to the diminished Young's modulus, which jeopardizes the force transmission, and the sensitivity of the muscle to mechanical stimulation (22); and (c) based on our findings showing that the medial gastrocnemius muscle has all of the aforementioned deterioration characteristics in diabetic muscles, the calf (distal) muscles of patients may develop diabetic myopathy earlier than the hip or thigh (proximal) muscles, and therefore, progressive strengthening and stretching exercises for leg muscles are strongly recommended for early detected or well-controlled diabetes. This exercise strategy is aimed at preventing muscle dysfunction and tendon pain. The benefits of exercises may include the promotion of ECM production and stiffness through mechanical stimulation (23). Although exercise might be beneficial to the turnover of ECM structure and could potentially lead to an attenuation of ECM accumulation as seen during chronic metabolic disease in humans (24), there have been few studies to illuminate differences in the effects of active and passive training on the muscle ECM. It is thus suggested that elastography be utilized in future studies to periodically measure the effects of exercise on the muscle ECM, to monitor deterioration in the muscle tissue, and to estimate the capacity of muscle force generation in diabetic patients.

The ultrasound results in this study regarding the microstructure (PSFR) of tendon tissue indicated that the diabetic tendons showed altered structural and composition characteristics in terms of packing density and alignment of the collagen bundles and fibrils consisting of changes of sizes, density, spatial alignments, and distribution (organization) (12). These results echo those of a previous study that reported increased packing density of collagen fibrils, decreases in fibrillar diameter, and abnormal fibril morphology consisting of twisted, curved, overlapping, and otherwise highly disorganized fibrils observed by electron microscopy in the Achilles tendons of diabetic patients (4). Combined with the results regarding correlations between the PSFR and the HbA1c or HDL-C levels found in this study, these morpho-

logic abnormalities of the collagen bundles and fibrils in diabetic tendons appear to be the result of nonenzymatic glycation and the production of inflammatory cytokines expressed over the years in those suffering from DM (4,25). These microstructural changes are suggested to be the causes of reduced tendon flexibility, tendon thickening, and shortening of the Achilles tendon-gastrocnemius complex, as well as tendinopathy (2,4). The ultrasound characteristics of the quadriceps, patellar, and Achilles tendons found in this study indicated the early detection of tendon microstructural changes in diabetes, and suggest that microstructural abnormalities may begin prior to the occurrence of symptoms or macrostructural changes in diabetic tendons, such as tendon thickening and reduced tendon stiffness (2). Potential applications of the technology used in the current study thus include screening for the risk of tendinopathy in diabetic patients while they are still asymptomatic. The results of this study support the conclusions that diabetic muscles and tendons exhibit early mechanical or structural changes, including reduced muscle Young's modulus and PSFR in the tendons, and that the mechanical or structural changes in the Achilles tendon-gastrocnemius complex are associated with force capacity or markers of insulin resistance.

LIMITATIONS

This study limitations include the specific diabetic population studied, such that the results must be interpreted with caution with respect to using well-controlled diabetes as a reference for the tendon system. In addition, this study did not analyze the effects of commonly prescribed drugs (including statins) that may affect the collagen structure and PSFR in patients with DM. Moreover, our results may not apply to diabetes with poor control or peripheral neurovascular disease.

Clinical implication

Based on our study results, which were gathered from participants who had no peripheral neurovasculopathy, strengthening and stretching exercises for the calf muscles are strongly recommended for prophylactic programs aimed at the prevention of lower extremity dysfunction. It is suggested that elastography and the scattering pattern approach be used in future studies to reveal the training effects of calf exercises and to verify the central role of mechanical and morphological plasticity in functional recovery in diabetic participants.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

REFERENCES

1. Merashli M, Chowdhury TA, Jawad AS. Musculoskeletal manifestations of diabetes mellitus. *QJM* 2015;108:853-857.
2. Oliva F, Piccirilli E, Berardi AC, Frizziero A, Tarantino U, Maffulli N. Hormones and tendinopathies: the current evidence. *Br Med Bull* 2016;117:39-58.
3. Karampinos DC, Baum T, Nardo L, Alizai H, Yu H, Carbalido-Gamio J, Yap SP, Shimakawa A, Link TM, Majumdar S. Characterization of the regional distribution of skeletal muscle adipose tissue in type 2 diabetes using chemical shift-based water/fat separation. *J Magn Reson Imaging* 2012;35:899-907.
4. Grant WP, Sullivan R, Sonenshine DE, Adam M, Slusser JH, Carson KA, Vinik AI. Electron microscopic investigation of the effects of diabetes mellitus on the Achilles tendon. *J Foot Ankle Surg* 1997;36:272-278.
5. Soliman SB, Rosen KA, Williams PC, Spicer PJ, Williams LK, Rao SD, van Holsbeeck MT. The hyperechoic appearance of the deltoid muscle on shoulder ultrasound imaging as a predictor of diabetes and prediabetes. *J Ultrasound Med* 2020;39:323-329.
6. Ursini F, Arturi F, D'Angelo S, Amara L, Nicolosi K, Russo E, Naty S, Bruno C, De Sarro G, Olivieri I, Grembiale RD. High prevalence of Achilles tendon enthesopathic changes in patients with type 2 diabetes without peripheral neuropathy. *J Am Podiatr Med Assoc* 2017;107:99-105.
7. Coombes BK, Tucker K, Hug F, Scott A, Geytenbeek M, Cox ER, Gajanand T, Coombes JS. Relationships between cardiovascular disease risk factors and Achilles tendon structural and mechanical properties in people with Type 2 Diabetes. *Muscles Ligaments Tendons J* 2019;9:395-404.
8. Guney A, Vatansever F, Karaman I, Kafadar IH, Oner M, Turk CY. Biomechanical properties of Achilles tendon in diabetic vs. non-diabetic patients. *Exp Clin Endocrinol Diabetes* 2015;123:428-432.
9. Kannus P. Structure of the tendon connective tissue. *Scand J Med Sci Sports* 2000;10:312-320.
10. Eby SF, Song P, Chen S, Chen Q, Greenleaf JF, An KN. Validation of shear wave elastography in skeletal muscle. *J Biomech* 2013;46:2381-2387.
11. Stetson P, Sommer G. Ultrasonic characterization of tissues via backscatter frequency dependence. *Ultrasound Med Biol* 1997;23:989-996.
12. Bashford GR, Tomsen N, Arya S, Burnfield JM, Kulig K. Tendinopathy discrimination by use of spatial frequency parameters in ultrasound B-mode images. *IEEE Trans Med Imaging* 2008;27:608-615.
13. Davis PJ, Liu M, Sherman S, Natarajan S, Alemi F, Jensen A, Avramovic S, Schwartz MD, Hayes RB. HbA1c, lipid profiles and risk of incident type 2 Diabetes in United States Veterans. *PLoS One* 2018;13:e0203484.
14. Padulo J., Oliva F, Frizziero A., Maffulli N. Muscles, Ligaments and Tendons Journal – Basic principles and recommendations in clinical and field Science Research: 2018 update. *Muscles Ligaments Tendons J* 2018; 8:305-307.
15. American Diabetes Association. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2018. *Diabetes Care* 2018;41:S13-S27.
16. Haffner SM; American Diabetes Association. Dyslipidemia management in adults with diabetes. *Diabetes Care* 2004;27:S68-71.
17. Severinsen K, Obel A, Jakobsen J, Andersen H. Atrophy of foot muscles in diabetic patients can be detected with ultrasonography. *Diabetes Care* 2007;30:3053-3057.
18. Hilton TN, Tuttle LJ, Bohnert KL, Mueller MJ, Sinacore DR. Excessive adipose tissue infiltration in skeletal muscle in individuals with obesity, diabetes mellitus, and peripheral neuropathy: association with performance and function. *Phys Ther*. 2008;88:1336-1344.
19. Gillies AR, Lieber RL. Structure and function of the skeletal muscle extracellular matrix. *Muscle Nerve* 2011;44:318-331.
20. Stephenson GM, O'Callaghan A, Stephenson DG. Single-fiber study of contractile and biochemical properties of skeletal muscles in streptozotocin-induced diabetic rats. *Diabetes* 1994;43:622-628.
21. Ahmad K, Lee EJ, Moon JS, Park SY, Choi I. Multifaceted Interweaving Between Extracellular Matrix, Insulin Resistance, and Skeletal Muscle. *Cells* 2018;7: pii: E148.
22. Wisdom KM, Delp SL, Kuhl E. Use it or lose it: multiscale skeletal muscle adaptation to mechanical stimuli. *Biomech Model Mechanobiol* 2015;14:195-215.
23. Bezerra MA, da Silva Nery C, de Castro Silveira PV, et al. Previous physical exercise slows down the complications from experimental diabetes in the calcaneal tendon. *Muscles Ligaments Tendons J* 2016;19;6(1):97-103.
24. Martinez-Huenchullan S, McLennan SV, Verhoeven A, Twigg SM, Tam CS. The emerging role of skeletal muscle extracellular matrix remodeling in obesity and exercise. *Obes Rev* 2017;18:776-790.
25. Yang Y, Lu H, Qu J. Tendon pathology in hypercholesterolaemia patients: Epidemiology, pathogenesis and management. *J Orthop Translat* 2018;16:14-22.

Influence of Blood Flow Restriction Level on Muscle Fatigue During an Intermittent Isometric Exercise Taken to Failure

M. S. Cerqueira¹, R. Pereira², G. Nunes de Mesquita¹, T. Rocha¹, A. Galvão de Moura Filho¹

¹ Department of Physical Therapy, Federal University of Pernambuco, Pernambuco, Brazil

² Department of Biological Sciences, State University of Southwest Bahia, Bahia, Brazil

CORRESPONDING AUTHOR:

Mikhail Santos Cerqueira
Department of Physical Therapy
Federal University of Pernambuco
University City
50670-901 Recife
Pernambuco, Brazil
E-mail: mikalsantosc@hotmail.com

DOI:

10.32098/mltj.03.2020.08

LEVEL OF EVIDENCE: 1B

SUMMARY

Background. Low-intensity exercise with blood flow restriction (BFR) may improve muscle mass and strength but an early neuromuscular fatigue may occur. Since the mechanisms of fatigue during BFR exercise is not yet fully understood we examined, concomitantly, the coefficient of variation of force (CVf) and surface electromyographic signal (sEMG) during an intermittent isometric handgrip exercise (IIHE) taken to failure.

Methods. 12 males completed an IIHE to failure with three blood flow conditions: total BFR (TR), partial BFR (PR) and free blood flow (FF). At each condition, three moments of the task were identified: beginning, midway and failure. The CVf and amplitude of rectified (arEMG) and median frequency (MDF) from the sEMG were analyzed.

Results. FF and PR conditions presented similar increase throughout the entire task in CVf (FF=1.73±0.88%; PR=1.61±0.63%) and arEMG (FF=1.48±0.63%; PR=1.40±0.45%), and similar decrease in MDF (FF=0.88±0.16%; PR=0.88±0.14%). Differently, during the task with TR there was a significant increase in CVf (1.45±0.79%) and arEMG (1.27±0.40%) and decrease in MDF (0.85±0.19%) only at the failure. The comparisons between conditions showed that the CVf, arEMG, and MDF were significantly lower ($p<0.05$) at TR condition, when compared to the FF.

Conclusions. Simultaneous analysis of CVf and sEMG indicates that FF and PR conditions induce similar neuromuscular fatigue during an IIHE, while the adjustments to maintain the task are different in TR. Additionally, it does not seem necessary to add a partial BFR to increase muscle excitation during an exercise taken to failure.

KEY WORDS

Vascular occlusion; electromyography; motor control; median frequency; muscle activation; volitional fatigue.

BACKGROUND

Neuromuscular fatigue can be defined as a condition in which muscles fail to maintain a required effort (1). The mechanisms of exercise-induced muscle fatigue are related to the intensity, duration, type of muscle action and blood flow restriction (BFR) level (2,3). Partial BFR, which have been widely investigated owing to its potential to improve muscle mass and strength at low-intensity training (4) is recognized as a condition that induces early neuromuscular fatigue (2). Poor arterial flow and venous stasis caused by insufflations of a pressure cuff in the proximal end of

the active muscles can lead to muscle fatigue and trigger a number of mechanisms responsible for BFR training adaptations (5).

The maintenance of force output (obtained from strain-gauge/force transducer) during a fatiguing contraction is regulated by mechanisms as the recruitment of new motor units and the modulation of firing rates of active motor units (6). Some parameters related to the muscle features that have been used to investigate the control of force throughout fatiguing tasks are: (i) coefficient of variation of force (CVf), that is influenced by the alterations in motor

unit recruitment and/or discharge timing along the fatigue progresses; (ii) amplitude of the surface electromyographic signal (sEMG), that represents muscle excitation; (iii) median frequency (MDF) of sEMG spectrum, that is related to action potential conduction velocity (6,7).

Increased muscle excitation appears to be of importance for strength gains (8) and hypertrophy (9), however the capacity of partial BFR to increase muscle recruitment is controversial (5). While some studies have shown higher neuromuscular excitation (10) others found no difference in the amplitude of sEMG during BFR exercise compared to free flow exercise with load-matched (11,12). In addition, none of these studies investigated how different BFR levels influence neuromuscular activity during an isometric task taken to failure. Not least, it has been shown that MDF reduces during exercise with total BFR compared to free blood flow exercise (7), but the behavior of MDF during partial BFR exercise until failure is unclear.

Despite the potential relevance of CVf and sEMG assessments to verify how the neuromuscular activity/control is affected by BFR during a fatiguing task, no previous studies have simultaneously measured these outcomes. Additionally, considering that characteristics as blood pressure and limb circumference influences the restriction pressure (13), the arbitrary pressure generally applied (7,11), may create a difference in the hypoxic intramuscular environment and hamper the understanding of mechanisms involved in the exercise-induced fatigue with different BFR levels. Therefore, this study aimed to compare the effects of different and individualized BFR levels on parameters of neuromuscular fatigue (CVf, sEMG amplitude and sEMG median frequency) during an isometric intermittent exercise taken to failure.

MATERIALS AND METHODS

Participants

Twelve healthy men (characteristics show in **table I**) classified as physically active or very active were included. They were right handed with no neuromuscular and cardiovascular disorders, and were naive to the all procedures. In the 24 hours antecedent to the experimental procedure participants avoided strenuous handgrip exercise and alcohol/caffeine consumption. The same physical activity routine and diet were kept during the study period. Participants were informed of all experimental procedures as well as any potential risks associated with the study before giving written informed consent. This study is in accordance with Declaration of Helsinki and has also been previously approved by the Research Ethics Committee of the Feder-

Table I. Values are mean±standard deviation, minimum and maximum from subject characteristics (n=12).

Variable	Mean ±SD	Minimum	Maximum
Age (years)	21±1.71	19	25
Height (cm)	177 ±6	170	187
Body mass (kg)	78.58±9.50	61.50	91.80
BMI (kg/m ²)	25.03±1.98	21.28	28.47
SBP (mmHg)	120.15±11.60	100	140
DBP (mmHg)	76.92±7.51	70	90
TRP (mmHg)	126.92±10.52	110	145
PRP (mmHg)	63.46±5.26	55	72.5

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TRP, total restriction pressure; PRP, partial restriction pressure.

al University of Pernambuco under the protocol number CAAE: 36832814.9.0000.5208. This trial was prospectively registered (Clinical Trials No. NCT02384161) and conducted ethically according to international standards of the journal as described by Padulo *et al.* (14).

Experimental procedures

Volunteers were submitted to three sessions of intermittent isometric handgrip exercise (IIHE) with three blood flow levels: total BFR (TR), partial BFR (PR) and free blood flow (FF). The interval between two successive interventions ranged from 72h (minimum interval) and 1 week (maximum interval). Thus, in this randomized crossover trial, all volunteers carried out the same IIHE sustained to failure with TR, PR and FF.

Through computer-generated random number tables (<http://www.randomization.com/>) the order of BFR level was allocated by an investigator who was not involved in the recruitment, intervention or assessment of subjects. Opaque sealed envelopes were used to conceal the allocation. To blind all procedures, the researcher responsible for maximum voluntary isometric contraction (MVIC) assessment and IIHE (researcher 1) did not know volunteer allocation. Evaluators determining (researcher 2) and applying/controlling restriction pressure (researcher 3) did not participate in the randomization. The volunteers were not informed about the BFR level applied and were instructed not to report the perception of pressure, keeping the researcher 1 blinded.

Initially, eligibility criteria were evaluated and anthropometric data were recorded. In the following day, blood pressure and the pressure necessary to total restriction (TRP – total restriction pressure) of brachial artery flow was determined, and the volunteers were familiarized with MVIC and IIHE.

Then, 72h after the familiarization, volunteers began the IIHE with different BFR conditions. Thenceforth, each IIHE session was identical, except for the BFR level. The volunteers were positioned in dorsal decubitus, with shoulder abducted at 90°, elbow fully extended and forearm supinated for all TRP and MVIC evaluations and during the IIHE.

Total restriction pressure determination

TRP of the brachial artery flow is a measure that shows good reproducibility and was determined according to the previous study (13). Briefly, the brachial artery blood flow of the dominant arm was detected by an ultrasound model SonoAceR3 (Samsung Medison – South Korea), by Power Doppler Technique – with 12 MHz linear transducers placed in the flexor crease of the elbow. Visual and auditory signals indicated the presence of pulse during the cuff inflation (Aneroid sphygmomanometer Premium, Duque de Caxias - RJ; width cuff 14 cm). The TRP was determined as the lowest pressure necessary to occlude completely the blood flow, and the partial occlusion pressure was set as the value corresponding to 50% of TOP. The cuff pressure in the FF condition was set as the pressure sufficient only to adjust to the arm (~10 mmHg), maintaining the forearm blood flow free. The same cuff used to determine the TRP (positioned at the dominant arm, just below the axilla near the insertion of the deltoid muscle) was inflated immediately before and deflated immediately after the end of IIHE.

Force evaluation e fatiguing exercise

Three MVIC of handgrip muscles were evaluated without any BFR and as previously described². The recordings extracted from a custom-made strain gauge-based force transducer (DM 100, Miotec, Porto Alegre, RS, Brazil) were sampled at 2 kHz. The highest handgrip force of the three MVIC attempts was used to calculate the target force (45% of MVIC) throughout the IIHE. Five minutes after the third MVIC, the volunteers started the IIHE, carrying out successive isometric handgrip contractions, each of one sustained for 10 seconds, followed by 5-seconds resting, until the failure. Volunteers were instructed to reach and maintain the target as brief and accurately as possible during active phase and relax (force=0) during the resting. The failure was defined as the incapacity to reach and sustain a force greater than 30% of MVIC for 5 seconds or more in three consecutive contractions (15). The periods of action and resting were controlled by a metronome-timed beep sound. Throughout the entire task, the force was displayed by projection on the ceiling located approximately two meters

away from the volunteer. Volunteers received strong verbal encouragement from researcher 1 during the MVIC assessments and along all the IIHE.

Surface EMG recordings

sEMG were obtained using four channel modules (Miotool 400, Miotec Equipment, Biomedical, Brazil) with a total amplifier gain of 1000, a common mode rejection ratio of 110 dB, sampled at 2.0 kHz and band-pass filtered (5–500 Hz). A 14-bit converter digitalized the analog signals with a sampling frequency of anti-aliasing 1.0 kHz for each channel. Pre-amplified (x100) bipolar superficial and circular adhesive electrodes of Ag/AgCl (teardrop shape and diameter 3 cm) were used with interelectrode (center-to-center) distance of 20 mm. The skin was shaved and cleaned with alcohol, and anatomical landmarks (proximal third of the forearm, between the styloid processes of the radius and the humeral medial epicondyle) were determined to place electrodes over the flexor digitorum superficialis forearm and flexor carpi radialis (16). The reference electrode (Ag/AgCl, oval shaped, 5x3.8 cm diameter) was placed just below the sternal notch. Since the surface electrodes mainly detect the EMG signal of the muscle beneath, some activity from nearby muscles could also be picked up. Thus, for this reason, and because various forearm muscles flexing the carpus and the fingers, we ascribed the signal from the electrodes over the cited muscles as forearm flexor muscles (17). All described procedures followed the recommendation of SENIAM (Surface ElectroMyoGraphy for Non-Invasive Assessment of Muscles) project (16).

DATA ANALYSIS

Force and sEMG signals were recorded continuously and analyzed simultaneously at the beginning, midway and failure moments of the task. The first contraction was excluded by generally be atypical owing to the task adaptation, thus the next contraction was considered the first valid contraction (beginning), that was used to normalize the others time point measures. Task failure was defined as the incapacity to keep the force of at least 30% of MVIC in two consecutive contractions. Then, the last valid contraction was defined as the contraction immediately before the first of the two consecutive failures contractions. The midway of the task was defined as the contraction that divided in half the total number of valid repetitions. The epochs were identified based on force signals from visual inspection, and EMG was segmented based on force onsets-offsets. A specific algorithm was applied to analyze 6 seconds from each contraction, which was lasted for 10 seconds. For then, the first and

the last 2 seconds were excluded. This procedure guarantee that ~60% of the action phase were analyzed, excluding the adjusting of force to reach the target force, at the first 2 seconds, and the last two seconds were the motor control was more imprecise, since the finish of active phase is close. The force data was band passed filtered between 0.05 and 15 Hz (Butterworth, 4th order) and detrended before motor output variability measures were calculated. The within-trial variability was measured using the standard deviation (SD) of the detrended filtered force and as the CVf (SD of force/mean force) x100], as done by Pereira et al. (2012) (17). sEMG was studied in the time [amplitude of rectified sEMG (arEMG)] and frequency (median frequency [MDF] of sEMG) domains. For arEMG analysis the sEMG was band passed filtered between 5 and 500 Hz (Butterworth, 4th order), and then, full-wave rectified. The Fast Fourier transform (FFT - Hamming window processing) was applied to the raw sEMG to identify the MDF of the power spectrum periodogram. The data from CVf, arEMG and MDF obtained at midway and failure moments were normalized by the beginning of the task. Then, the results are reported as a percentage (%) of the beginning.

STATISTICAL ANALYSIS

The data distributions of normality were evaluated by the Kolmogorov–Smirnov test. A two-way ANOVA (3 blood flow levels x 3 moments throughout the task) was used to compare the CVf, arEMG and MDF. Significant ANOVA results were followed by appropriate post hoc tests with Bonferroni corrections. The level of significance was set to $p < 0.05$ and the analyses were performed with the SPSS17.0 statistical package (SPSS Inc., Chicago, IL, USA). Data are reported as means±SD in the text and tables and means±SE (standard error) in the figures. Only the normalized data (midway or failure /beginning) were considered in the statistical analysis. The non-normalized data obtained before (to verify similarity of initial values between the evaluation days) and during exercise are shown in **table II**. The number of subjects included was determined based upon previous BFR investigations that observed similar outcome measures (7,12).

RESULTS

The analyses of CVf exhibited a significant main effect for measures (F-value = 30.7, $p < 0.001$). The post-hoc comparisons indicated that FF and PR conditions exhibited a similar behavior increasing significantly at the midway of the task (FF=1.94±0.78%; PR=1.76±0.64%) and at the failure (FF=2.26±0.97%; PR=2.07±0.44%), while in the TR

Table II. Non-normalized data obtained before and during exercise (n=12). Values are mean±standard deviation.

BFR level	Initial values			Beginning			Midway			Failure			
	MVIC (N)	CVf (%)	arEMG (µV)	MDF (Hz)	CVf (%)	arEMG (µV)	MDF (Hz)	CVf (%)	arEMG (µV)	MDF (Hz)	CVf (%)	arEMG (µV)	MDF (Hz)
TR	445.33±75.30	0.13±0.11	2781.54±1472.4	119.7±15.3	0.03±0.01	1384.00±959.94	110.03±17.61	0.04±0.02	1619.23±1050.42	96.03±22.5	0.07±0.04	1975.4±1195.83	73.24±21.53
PR	447.17±79.43	0.22±0.17	3038.60±1760.49	116.5±16.9	0.03±0.01	1639.90±1046.48	103.1±18.28	0.05±0.02	2413.76±1371.55	88.54±16.85	0.06±0.02	2663.75±1794.72	80.08±19.1
FF	448.08±74.72	0.16±0.07	3142.92±1597.15	117.5±18.9	0.03±0.01	1553.18±843.91	104.49±17.32	0.06±0.02	2366.79±1265.40	84.31±18.05	0.07±0.02	2595.88±1675.72	84.96±22.97

BFR, blood flow restriction; TR, total restriction; PR, partial restriction; FF, free flow; MVIC, maximum voluntary isometric contraction; arEMG, amplitude of rectified EMG amplitude; CVf, coefficient of variation of force; MDF, median frequency; N, Newtons.

condition the CVf increased significantly ($2.09 \pm 0.97\%$) only at the task failure. Additionally, the CVf was significantly greater in FF ($1.94 \pm 0.78\%$) condition at midway of the task, when compared to the TR ($1.26 \pm 0.58\%$) (figure 1). The arEMG also exhibited a significant main effect for measures (F-value = 24.3, $p < 0.001$), with FF (midway = $1.66 \pm 0.55\%$; task failure = $1.76 \pm 0.76\%$) and PR (midway = $1.56 \pm 0.36\%$; task failure = $1.65 \pm 0.49\%$) conditions increasing significantly at midway and task failure moments, when compared to the beginning (figure 2). However, at the TR, the arEMG increased significantly only at the failure ($1.57 \pm 0.45\%$), when compared to the beginning. As observed for CVf, arEMG was significantly greater in the FF ($1.66 \pm 0.55\%$) condition at midway of the task, when compared to TR ($1.23 \pm 0.34\%$). For the MDF analyses (figure 3), it was also observed a significant main effect for measures (F-value = 35.6, $p < 0.001$). The FF and PR conditions exhibited similar behavior decreasing significantly at the midway (FF= $0.82 \pm 0.16\%$; PR= $0.86 \pm 0.12\%$) and at failure (FF= $0.81 \pm 0.16\%$; PR= $0.78 \pm 0.15\%$) moments, while the TR decreased significantly only at the failure ($0.66 \pm 0.13\%$). Additionally, MDF was also significantly smaller in FF condition (FF= $0.81 \pm 0.16\%$) at the failure, when compared to TR ($0.66 \pm 0.13\%$).

Regarding to the reliability of the measures, intra-reliability for CV values ranged from 3.7 to 4.7% for CIVM, 8.7 to 11.7% for MDF, and 10.4 to 12.9% for arEMG. Inter-reliability for CV values were 4.4% for CIVM, 10.8% for MDF and 26.1% for arEMG.

DISCUSSION

Our main findings were: (i) throughout the task, the FF and PR conditions induced similar increases in CVf and arEMG, as well as similar decreases in MDF; (ii) the TR condition exhibited significant increases in CVf and arEMG, as well as decreases in MDF, only at the failure; (iii) CVf, arEMG and MDF were significantly lower in the TR condition, only when compared to FF.

The CVf is a motor output measure, indicating that force during voluntary actions fluctuates around a target (17), however, CVf have not been studied during BFR exercises. CVf along a submaximal fatiguing task is influenced by decreased cortical excitability and motoneuron discharge, inhibition by Renshaw cells, and type III and IV afferents or decreased excitation of Ia afferents (18). Our results indicated that the motor output was similar between PR and FF conditions, increasing constantly throughout the entire task, while the TR induces a significant increase only at the failure. In our study, the typical metabolites accumulation imposed by the BFR was not sufficient to induce differ-

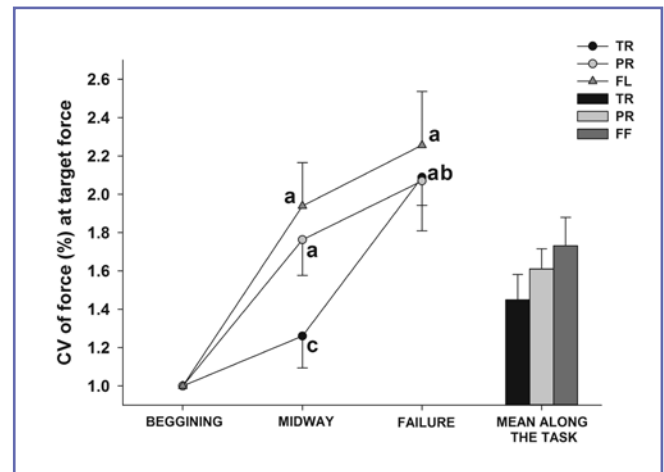


Figure 1. Mean \pm SE of CV of force (%) at the beginning, midway and failure, as well as the mean along the task. Data are presented for total restriction (TR), partial restriction (PR) and free flow (FF). a: significantly different from the beginning of task ($p < 0.05$); b: significantly different from beginning and midway of task in the TR condition ($p < 0.05$); c: significantly different from FF condition at the midway of task ($p < 0.05$).

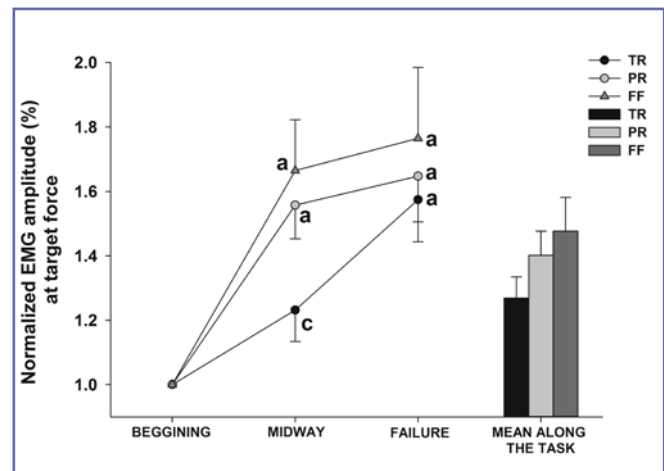


Figure 2. Mean \pm SE of normalized EMG amplitude (%) at the beginning, midway and failure, as well as the mean along the task. Data are presented for total restriction (TR), partial restriction (PR) and free flow (FF). a: significantly different from the beginning of task ($p < 0.05$); c: significantly different from free flow (FF) condition at the midway of task ($p < 0.05$).

ent sensorimotor integration between PR and FF conditions. On the other hand, TR induces divergent sensorimotor integration from the FF condition, since the CVf did not increase at the midway of the task. A sudden increase in CVf may indicate a facilitation of force, which may be

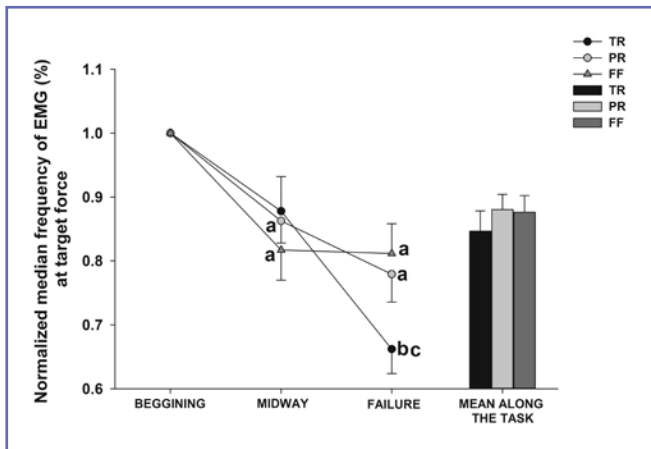


Figure 3. Mean \pm SE of normalized median frequency of EMG signal (%) at the beginning, midway and failure, as well as the mean along the task. Data are presented for total restriction (TR), partial restriction (PR) and free flow (FF). a: significantly different from the beginning of task ($p < 0.05$); b: significantly different from beginning and midway of task in the TR condition ($p < 0.05$); c: significantly different from free flow (FF) condition at the failure ($p < 0.05$).

sustained by augmented motor unit recruitment, increases and decreases in rate coding, alterations in motor control, and neural and post activation potentiation (18). Thus, the behavior adopted during the TR condition could indicate a strategy to maintain the task, delaying the motor unit recruitment increases, probably as a way of saving energy in a situation of extreme oxygen shortage. Interestingly the PR, despite the imposed blood flow limitation, was not sufficient to change the neuromuscular strategy than FF condition, suggesting that differences in the long term exercise adaptations induced by a PR could be related to the molecular level, than to the neuromuscular level.

The results from sEMG corroborate our hypothesis raised from the CVf. In other words, if an increasing in the descending drive along the task is necessary to maintain the force, an increase in sEMG amplitude, as well as a decrease in MDF should be expected, owing to an increasing in discharge rates of active motor units and/or adding motor units recruitment (19). In fact, we identified a significant and similar increase in arEMG throughout the task in the FF and PR. This is in agreement with previous results indicating that increases in sEMG amplitude has been associated to the increases in CVf (19). Accordingly, it is suggested, that the sudden increases in CVf can be partially explained by the recruitment of additional motor units to compensate the decline in force capacity of the already activated motor units by recruiting additional higher threshold motor units

(20). In fact, sudden increases of arEMG in the TR condition at the failure could be attributable to enhanced excitatory descending drive onto the motoneuron pool, as also suggested by the more rapid increases in CVf (21).

Although PR has been shown to augment arEMG over FF with load-matched protocols (10,22), we found a similar increase in arEMG during FF and PR conditions. Our findings agree with previous studies^{11,12} that showed no difference between FF and PR conditions when low-intensity exercise was conducted until failure. Thus, the combined findings of these previous investigations and the current study findings constitute robust evidence indicating that, when taken a submaximal task to failure, PR induces similar neuromuscular strategies than FF condition.

On the other hand, it has been suggested that higher occlusion pressure increases neural compression and energetic demand, resulting in the elevated neuromuscular excitation commonly observed with BFR exercise (7,23). However, we observed an attenuated arEMG rise at the midway during the task with TR. This disagreement may be due to the fact that in previous studies the exercise did not reach failure and was carried out at smaller load (20% of 1-RM or MVIC). Another explanation for this attenuated rise of arEMG in TR condition is that the metabolite accumulation (sensed by type III and IV muscle afferents) may inhibit alpha motor neurons and/or the descending neural drive, mitigating the motor unit recruitment and reducing muscle excitation (24).

As observed for CVf and arEMG, the behavior of MDF in FF and PR conditions was similar, with a drastic decrease from the beginning to the midway of the task and sustained until the failure. In TR condition there was a little decrease at midway of the task with an abrupt and significant reduction at failure, when compared to the beginning and midway of the task. Again, as observed for CVf and arEMG, significant difference was observed only between FF and TR conditions, but now, at the failure moment. These results agree with previous results that did not observed differences in spectral parameters of the sEMG during low-intensity exercise with PR or FF (25). In addition, the greater MDF decrease in the TR condition, when compared to FF, was also observed in previous studies (7,22). It is important to note that higher decrements of median frequency are related to higher force fluctuations (26), indicating once again the consistency of our results among all evaluated variables. Under fatigue conditions, the motor units synchronization and fibers conduction velocity impairment cause spectral shift towards low-frequency regions (27). Thus, at first glance it may appear that the greater MDF decrease at task failure, as observed during the TR condition, indicates greater fatigue, which could be supported by the statement

that higher occlusion pressures may cause greater magnitude of muscle fatigue (28). It is noteworthy, however, that despite precipitate the muscle failure, the same exercise protocol with TR allows a faster force recovery after the task failure, when compared to FF and PR (2). It is reasonable to consider, therefore that TR can cause selective fatigue in the oxygen-independent muscle fibers, thus contributing to a depletion of the glycolytic fibers, greater accumulation of H^+ and consequently greater reduction in MDF (7). Then, from the reestablishment of blood flow, the partially fresh oxygen-dependent fibers would contribute to the faster return of the force-producing capacity. In FF and PR conditions, however, both glycolytic and oxidative fibers were likely to fatigue, causing a greater magnitude of function impairment as indicated by a slower recovery in the capacity of force production post-failure. In short, considering previous data on force recovery post-failure and the behavior of the CVf and arEMG found in the present study, the sudden MDF reduction at failure in the TR condition does not indicate a greater magnitude of fatigue, but rather a specific strategy to sustain the task for a long time as possible in a metabolically unfavorable condition.

Limitations

The current study is not free of limitations. First, the IIHE protocol used differs from most studies in which dynamic contractions are performed by larger (knee extensors and elbow flexors) muscle groups. However, to add the CVf in the muscle fatigue analysis, we chose to work with the wrist and finger flexors, enabling the maintenance in the target force due to higher motor control of these muscles (17). Moreover, isometric actions minimize some methodological limitations related to the sEMG recorded during dynamic actions (29). Additionally, we did not include a high intensity exercise with free flow condition. Recent systematic review, however, indicate that partial BFR only potentiates strength gains when compared to the low-in-

tensity exercise with FF, but not in comparison to the high intensity exercise (30). Lastly, we do not provide measures of the blood perfusion/oxygenation in the muscle tissue (e.g. near infrared spectroscopy). Considering that 50% of blood restriction used in the present study does not necessarily mean 50% of oxygenation level/perfusion reduction, measurement of muscle perfusion /oxygenation could help explain the absence of difference between FF and PR conditions in the mechanical and electrophysiological variables.

Practical implications

Despite augmented muscle excitation is considered as a potential mechanism underlying strength gains and hypertrophy conducted by low-intensity BFR training the literature involving sEMG parameters is discordant. In this sense, our results summed to previous indicate that, when executed until task failure, the PR induces similar neuromuscular strategies than FF. From a practical viewpoint, this finding supports that subjects with contraindications for tourniquet use may obtain stimulus for muscular adaptation when exercise is taken to failure even without partial BFR.

CONCLUSIONS

Our results indicate, from a simultaneous analysis of CVf, arEMG and MDF during a submaximal intermittent isometric exercise, that the FF and PR require similar neuromuscular strategies to carry out the task until the failure and that TR induce different neuromuscular adjusts to maintain the task. Additionally, we suggest that it seems not necessary to add a partial BFR to increase muscle excitation during an exercise taken to failure.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

REFERENCES

1. Boyas S, Guével A. Neuromuscular fatigue in healthy muscle: underlying factors and adaptation mechanisms. *Ann Phys Rehabil Med* 2011;54(2):88–108. doi:10.1016/j.rehab.2011.01.001
2. Cerqueira MS, Pereira R, Rocha T, et al. Time to failure and neuromuscular response to intermittent isometric exercise at different levels of vascular occlusion: a randomized crossover study. *Int J Appl Exer Physiol* 2017;6(1):55–70. doi:10.22631/ijaep.v6i1.108.
3. Babault N, Desbrosses K, Fabre M-S, Michaut A, Pousson M. Neuromuscular fatigue development during maximal concentric and isometric knee extensions. *J Appl Physiol* 2006;100(3):780–785. doi:10.1152/jappphysiol.00737.2005.
4. Patterson SD, Hughes L, Warmington S, Burr JF, Scott BR, Owens J, et al. Blood Flow Restriction Exercise: Considerations of Methodology, Application, and Safety. *Front Physiol* 2019;10:533. doi:10.3389/FPHYS.2019.00533.
5. Pearson SJ, Hussain SR. A review on the mechanisms of blood-flow restriction resistance training-induced muscle hypertrophy. *Sports Med* 2015;45(2):187–200. doi:10.1007/s40279-014-0264-9.

6. Contessa P, Adam A, De Luca CJ. Motor unit control and force fluctuation during fatigue. *J Appl Physiol* 2009; 107(1):235-43 doi:10.1152/jappphysiol.00035.2009.
7. Moritani T, Sherman WM, Shibata M, Matsumoto T, Shinohara M. Oxygen availability and motor unit activity in humans. *Eur J Appl Physiol Occup Physiol* 1992;64(6):552-6.
8. Shinohara M, Kouzaki M, Yoshihisa T, Fukunaga T. Efficacy of tourniquet ischemia for strength training with low resistance. *Eur J Appl Physiol Occup Physiol* 1998;77(1-2):189-91.
9. Morton RW, Oikawa SY, Wavell CG, Mazara N, McGlory C, Quadrilatero J, et al. Neither load nor systemic hormones determine resistance training-mediated hypertrophy or strength gains in resistance-trained young men. *J Appl Physiol* 2016;121(1):129-138. doi:10.1152/jappphysiol.00154.2016.
10. Killinger B, Lauver JD, Donovan L, Goetschius J. The Effects of Blood Flow Restriction on Muscle Activation and Hypoxia in Individuals with Chronic Ankle Instability. *J Sport Rehabil* 2019;16:1-25 doi:10.1123/jsr.2018-0416.
11. Wernbom M, Järrebring R, Andreasson MA, Augustsson J. Acute effects of blood flow restriction on muscle activity and endurance during fatiguing dynamic knee extensions at low load. *J Strength Cond Res* 2009;23(8):2389-2395. doi:10.1519/JSC.0b013e3181bc1c2a.
12. Cayot TE, Lauver JD, Silette CR, Scheuermann BW. Effects of blood flow restriction duration on muscle activation and microvascular oxygenation during low-volume isometric exercise. *Clin Physiol Funct Imaging* 2016;36(4):298-305. doi:10.1111/cpf.12228.
13. Bezerra de Morais AT, Cerqueira MS, Moreira Sales R, Rocha T, Moura Filho AG. Upper limbs total occlusion pressure assessment: Doppler ultrasound reproducibility and determination of predictive variables. *Clin Physiol Funct Imaging* 2017;37(4):437-441. doi:10.1111/cpf.12330.
14. Padulo J, Oliva F, Frizziero A, Maffulli N. Muscles, Ligaments and Tendons J - Basic principles and recommendations in clinical and field science research: 2016 update. *MLTJ* 2016;6(1):1-5. doi:10.11138/mltj/2016.6.1.001.
15. Cerqueira MS, Pereira R, Mesquita GN, Rocha T, Moura Filho AG. Rate of force development to evaluate the neuromuscular fatigue and recovery after an intermittent isometric handgrip task with different blood flow restriction conditions. *Motriz* 2019;25(1):1-6. doi:10.1590/s1980-6574201900010009.
16. Hermens HJ, Freriks B, Disselhorst-Klug C, Rau G. Development of recommendations for SEMG sensors and sensor placement procedures. *J Electromyogr and Kinesiol* 2000(5);10:361-374. doi:10.1016/S1050-6411(00)00027-4.
17. Pereira R, Freire IV, Cavalcanti CVG, Luz CPN, Neto OP. Hand dominance during constant force isometric contractions: evidence of different cortical drive commands. *Eur J Appl Physiol* 2012(8);112: 2999-3006. doi:10.1007/s00421-011-2278-4.
18. Behm DG. Force maintenance with submaximal fatiguing contractions. *Can J Appl Physiol* 2004;29(3):274-90.
19. Mendez-Villanueva A, Baudry S, Riley ZA, Rudroff T. Influence of rest duration on muscle activation during submaximal intermittent contractions with the elbow flexor muscles. *J Sports Med Phys Fitness* 2009;49(3):255-64.
20. Löscher WN, Cresswell AG, Thorstensson A. Excitatory drive to the α -motoneuron pool during a fatiguing submaximal contraction in man. *J Physiol* 15(0):271-80. doi: 10.1113/jphysiol.1996.sp021214.
21. Carson RG, Riek S, Shahbazzpour N. Central and peripheral mediation of human force sensation following eccentric or concentric contractions. *J Physiol* 2002;15(Pt3):913-25. doi:10.1113/jphysiol.2001.013385.
22. Yasuda T, Brechue WF, Fujita T, Shirakawa J, Sato Y, Abe T. Muscle activation during low-intensity muscle contractions with restricted blood flow. *J Sports Sci.* 2009;27(5):479-89. doi:10.1080/02640410802626567
23. Yasuda T, Brechue WF, Fujita T, Sato Y, Abe T. Muscle activation during low-intensity muscle contraction with varying levels of external limb compression. *J Sports Sci Med* 2008;7(4):467-474.
24. Light AR, Hughen RW, Zhang J, Rainier J, Liu Z, Lee J. Dorsal Root Ganglion Neurons Innervating Skeletal Muscle Respond to Physiological Combinations of Protons, ATP, and Lactate Mediated by ASIC, P2X, and TRPV1. *J Neurophysiol* 2008;100(3):1184-1201. doi:10.1152/jn.01344.2007.
25. Karabulut M, Cramer JT, Abe T, Sato Y, Bemben MG. Neuromuscular fatigue following low-intensity dynamic exercise with externally applied vascular restriction. *J Electromyogr and Kinesiol* 2010;20(3):440-447. doi:10.1016/j.jelekin.2009.06.005.
26. Kouzaki M, Shinohara M, Masani K, Fukunaga T. Force fluctuations are modulated by alternate muscle activity of knee extensor synergists during low-level sustained contraction. *J Appl Physiol* 2004;97(6):2121-2131. doi:10.1152/jappphysiol.00418.2004.
27. Bigland-Ritchie B, Donovan EF, Roussos CS. Conduction velocity and EMG power spectrum changes in fatigue of sustained maximal efforts. *J Appl Physiol* 1981;51(5):1300-1305. doi:10.1152/jappl.1981.51.5.1300.
28. Fatela P, Reis JF, Mendonca G V, Avela J, Mil-Homens P. Acute effects of exercise under different levels of blood-flow restriction on muscle activation and fatigue. *Eur J Appl Physiol* 2016;116(5):985-95. doi:10.1007/s00421-016-3359-1.
29. González-Izal M, Malanda A, Gorostiaga E, Izquierdo M. Electromyographic models to assess muscle fatigue. *J Electromyogr and Kinesiol* 2012;22(4):501-512. doi:10.1016/j.jelekin.2012.02.019.
30. Centner C, Wiegel P, Gollhofer A, König D. Effects of Blood Flow Restriction Training on Muscular Strength and Hypertrophy in Older Individuals: A Systematic Review and Meta-Analysis. *Sports Med* 2019;49(1):95-108. doi:10.1007/s40279-018-0994-1.

In Vivo Assessment of the Tensor Vastus Intermedius Cross-sectional Area Using Ultrasonography

C. Sahinis, E. Kellis, A. Ellinoudis, K. Dafkou

Laboratory of Neuromechanics, Department of Physical Education and Sport Sciences at Serres, Aristotle University of Thessalonica, Serres, Greece

CORRESPONDING AUTHOR:

Eleftherios Kellis
Laboratory of Neuromechanics
Aristotle University of Thessalonica
TEFAA Serres
Serres, Greece, 62100
E-mail: ekellis@phed-sr.auth.gr

DOI:

10.32098/mltj.03.2020.09

LEVEL OF EVIDENCE: 2B

SUMMARY

Background. There is growing evidence that the quadriceps muscle group includes a fifth head, the tensor of vastus intermedius (TVI). The purpose of this study was to quantify the anatomic cross-sectional area (CSA) of the TVI using ultrasound.

Methods. Ultrasonography scans were taken from 21 young males and females at rest, at 0° (= full extension), 45° and at 90° of knee flexion and they were repeated a week after to establish reliability. Measurements of the CSA were obtained from the most proximal and the most distal part of the muscle belly and from three different parts toward the course of the tendon.

Results. US evaluation of the CSA displayed high reliability with an intraclass coefficient (ICC_{3,3}) ranging from 0.85 to 0.99 and a standard error of measurement ranging from 0.0019 to 0,2789 mm². Analysis of variance indicated that the TVI muscle belly was larger proximally than distally and it was smaller at full extension compared with greater flexion angles ($p < 0.05$). The tendon CSA was greater proximally than middle and distal measurement sites ($p < 0.05$) but it did not differ between various knee flexion angles.

Conclusions. Future studies on the quadriceps muscle function and morphology should include examination of the TVI.

KEY WORDS

Quadriceps; architecture; ultrasound; tensor of vastus intermedius; muscle function.

INTRODUCTION

The quadriceps femoris muscle is considered a dominant extensor of the knee joint and a flexor of the hip joint. Traditionally the quadriceps is described as a muscle that is composed of the rectus femoris (RF), the vastus medialis (VM), the vastus intermedius (VI) and the vastus lateralis (VL). In particular, the RF originates from the anterior inferior spine of ilium, the VM originates from the linea aspera, the VL from the greater trochanter and the linea aspera of femur while the VI from the anterior and lateral shaft of femur (1–4). However, literature reports the presence of a fifth head, the tensor of vastus intermedius (TVI) which is located between the VL and the VI (5,6). The function of an additional muscle can alter the mechanics of the patella and it may have an impact on the extensor apparatus of knee. There are a few studies that examined the morphology of the TVI (5–10); Early cadaveric studies have identified the TVI

in 29% (11) or 36% of the specimens (10). Golland *et al.* (11). found that the TVI originates from the anterior aspect of the upper femoral shaft while Willan *et al.* (10) reported that the muscle is located in the distal area between the VL and the VI but the insertion of the TVI was different in all cases. Consequently, this muscle has not been considered as a basic part of the quadriceps in the general population. Recent studies, however, identified the TVI in all limbs (5–7). Firstly, Grob *et al.* (5) reported that the TVI originates from the anterolateral aspect of the greater trochanter, it is then combined with an aponeurosis merging separately into the quadriceps tendon and then inserting into the medial aspect of the patella (5,7). Further, TVI was supplied by independent muscular and vascular branches of the femoral nerve and lateral circumflex femoral artery (5,7). Four different types of insertion in the patella across subjects were identified (5). The authors commented that the failure

to recognize this muscle is likely related to its association with the VL and the VI (5). In another study, Grob *et al.* (7) found that the TVI attaches distally to the quadriceps tendon. Moreover, Raveendranath *et al.* (9) identified the TVI in thirty-six cadaveric lower limbs. They reported that the length (distance between most proximal and most distal points at which muscle fiber can be seen) of the TVI was 145.40 ± 37.55 mm and the aponeurosis (most distal point of muscle fiber to the superior aspect of patella) was 193.55 ± 42.32 mm² (9).

The appearance of the TVI has also been examined using diagnostic imaging techniques (6,8). Grob *et al.* (8) confirmed the anatomic descriptions of the TVI in magnetic resonance images (MRI). Rajasekaran and Hall (6) identified the TVI using ultrasound (US) image in all participants. The muscle belly and tendon were identified in the transverse plane. Based on these results, it was suggested that failure to recognize the TVI, may provide an erroneous interpretation of US images of the quadriceps femoris (6). However, in this study, no quantification of the dimensions and the architecture of the identified muscle were presented.

Quantification of the cross-sectional area (CSA) of the muscle could provide a more precise measure for its morphology than a simple description of its anatomical position. Further, the CSA provides an indication of the force potential of the muscle (12,13) although the relationship between CSA and force is not always linear (14–16). Quantification of the morphometry of the TVI may assist in better identification of this specific muscle using US as well as in explanation of the functional role of the muscle in vivo. For example, the clinical significance of this muscle is not fully understood but given its oblique course it may have a role in stabilizing the patella (5). Since the TVI has a proximal muscle belly and a long distal tendon, its morphology may also differ along its length. Therefore the purpose of the present study was firstly, to identify and then to evaluate the reliability of the US examination of the anatomic CSA of the TVI, second, to compare the CSA between different locations along the muscle and the tendon and finally, to examine whether TVI morphology differs between three different flexion angles of the knee joint.

METHODS

Participants

A total of 21 subjects volunteered to participate in this study after signing written informed consent. There were 17 males (age 21.72 ± 1.83 years; mass 78 ± 6.11 kg; height 181 ± 8.23 cm; femur length 42.5 ± 3.19 cm) and 4 females (age 21.3 ± 0.8 years; body mass 60.21 ± 3.65 kg; height 170 ± 5.2

cm; femur length 40.4 ± 1.51 cm). The participants were healthy university students and they had no injury of the lower limbs. The participants gave their informed written consent to the experimental procedure, which was complied with the rules of the local scientific board and met the ethical standards of the journal (17).

Design

All participants underwent US examination of the TVI in three different knee angles (0° , 45° , 90°) for the measurement of the CSA of muscle. The measurements of the CSA of the TVI were obtained from the proximal and the distal portion of the muscle belly and from three different locations along the course of the tendinous portion of the TVI of the dominant leg. The measurements were performed from the same investigator. The participants were re-tested in a separate session, 6-7 days after the initial measurement session to establish the reliability of the measurements. All US examinations were performed in the morning.

Ultrasonographic assessment

Procedure

Tissue movement was recorded using an US device (GE LOGIQ 400 CL PRO, GE Medical Systems, U.K) with a linear array probe of 10 MHz wave frequency and a length of 6 cm. The image signal was stored in digital form through an analogue to digital converter (Canopus, Model ADVC 100, Grass Valley Inc., USA) at a rate of 25 Hz. Tests were performed with the subject lying in the supine position with the hip joint in neutral position and the knee joint in 0° , 45° , 90° in a physiotherapy bed. The hip joint was remained in a neutral position during the examination. A twin-axis goniometer (Model TSD 130B, Biopac Systems, Inc., Goleta, Calif., USA) was used to record the knee joint angular position during the examination.

The participants were asked to relax their quadriceps muscles during US examination. In vivo examination of muscle architecture requires precise identification of the scanned muscle path on the surface of the skin. This is particularly important when examining the TVI muscle because the muscle path is not a straight line; instead, the muscle arises from the anterolateral aspect of the greater trochanter and then it is combined with an aponeurosis merging separately into the quadriceps tendon and then inserting into the medial aspect of the patella. First, we measured the length of the femur in all participants as the distance between the greater trochanter (GT) to the outer femoral condyle with a measuring tape. After starting from

GT, the US probe moved sequentially along the length of the femur towards the patella for the identification of the muscle belly and of the tendinous part of the TVI. From the recorded US video images, the TVI was identified and markers were then placed upon the skin, to indicate the start (proximally) and the end (distally) of the TVI along the length of the femur. These markers ensured consistent identification of the TVI in repeated scans. The muscle belly of the TVI is best identified when the transducer is placed in the transversal plane in the anterior thigh approximately 10 cm below the greater trochanter (GT) (figure 1). The TVI was identified as the thin muscle located between the VL and the VI (figure 1). Afterward, we measured the distance from the GT to the origin of the TVI and also we measured the length of the TVI as the distance from the origin of the muscle to the patella with a measurement tape. Consequently, we used a skin marker to mark upon the skin the 10% (proximal part) and the 90% (distal part) of the muscle belly and the 10% (proximal part) and the 90% (distal part) of the tendinous portion. These markers ensured that we consistently measured the same part of the muscle belly and the tendinous portion of

the TVI in repeated scans. Subsequently, transversal-axis images from the four different parts towards the length of the muscle were obtained. This procedure was performed 0° (full knee extension), 45° and 90° of knee flexion of the dominant leg.

ANALYSIS

The muscle belly and the tendinous part of the TVI was identified on the captured US image (figure 1). Identification of the CSA was performed by manually digitizing the identified contours using an image-based software (ImageJ version: 1.52a, National Institutes of Health, USA). The TVI was identified between the VL and the VI.

Firstly, we measured the CSA of the most proximal and the most distal end of the muscle belly. Second, we took three measurements of the tendinous part: one measurement from the most proximal part of the tendon, a second measurement from the middle area of the tendon (approximately 50% from most proximal area of the tendinous part to the patella) and a third measurement from the distal area of the tendon (just before the tendon of the TVI was

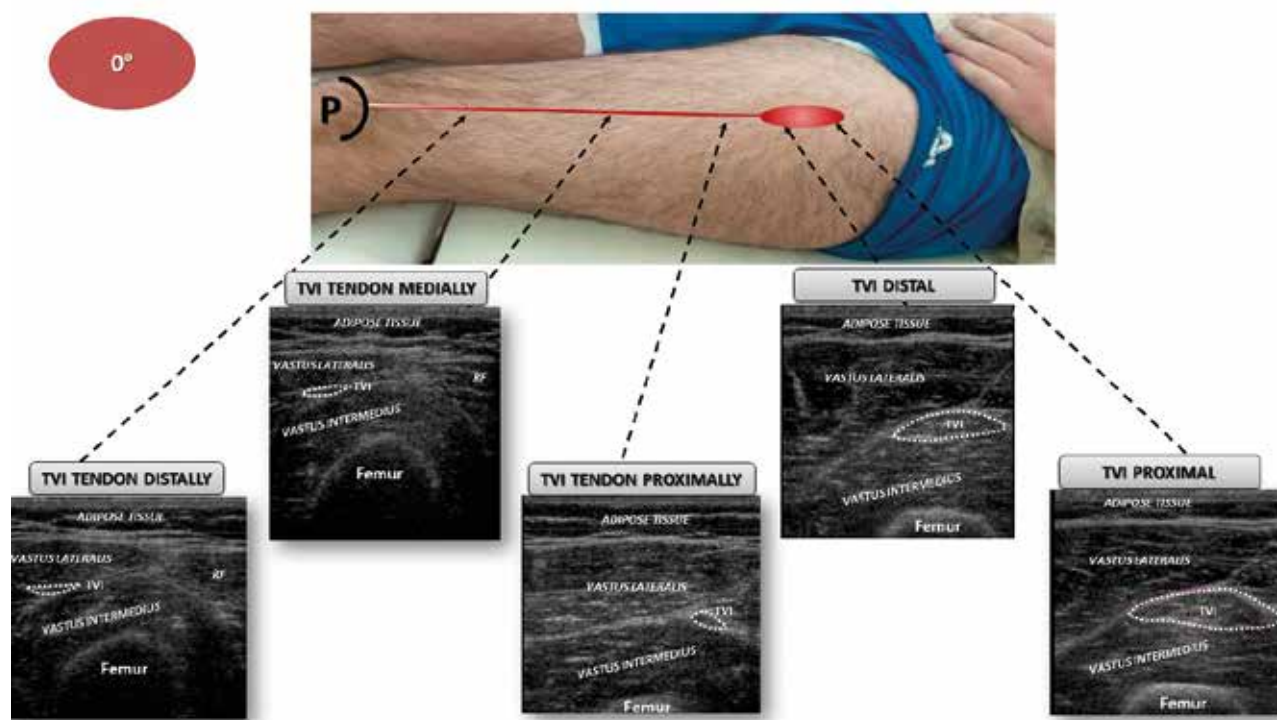


Figure 1. Illustration of ultrasound recording procedure. Following identification of the two visible ends of the tensor vastus intermedius (TVI), cross-sectional images were obtained from the muscle belly (proximally and distally) and the from the tendon (proximal, middle, distal). The procedure was repeated at knee flexion angles of 0°, 45° and 90° (not illustrated). The cross-sectional area of the TVI is drawn with dotted lines.

blended with the tendon of the RF, the VL, the VI and the VM). Three measurements in each area were taken and the average value was calculated.

STATISTICAL ANALYSIS

Reliability

To examine the test-retest reliability, we calculated the intraclass correlation coefficient ($ICC_{3,3}$) with 95% confidence interval (CI: 95%) based on the average of 3 measurements per measurement site, per session. An ICC value ≤ 0.50 was considered as low, 0.50 to 0.75 was considered as moderate, ≥ 0.75 was considered as good and ≥ 0.90 was considered as excellent. The agreement between repeated measurements was examined using Bland-Altman analysis, which includes the Bias and the limits of agreement (LoA) (18). The upper and lower LoA was calculated as $1.96 \times SD$ representing a measure of random error between measurement sessions. In addition, the standard error of measurement (SEM) was calculated using the following formula:

$$SEM = SD \sqrt{(1 - ICC)}$$

where SD is the standard deviation of the differences between test and retest values.

Measurement site and knee flexion angle

A Kolmogorov-Smirnov test indicated that the data were normally distributed. Analysis of variance (ANOVA) tests were used to compare muscle CSA in two measurement sites (proximal, distal) between the three knee flexion angles (0° , 45° , 90°). A separate ANOVA was used to examine the

effects of measurement site (proximal, middle, distal) and knee flexion angle on tendinous CSA. In case of significant F-ratios, simple effects were applied to identify significance between means. If significant, a post-hoc analysis Tukey test was applied to determine significant differences between various pairs of means. The level of significance was set at $p < .05$.

RESULTS

Reliability

The results from the reliability analysis are presented in **tables I** and **II** for the muscle and tendon part, respectively. The ICC values ranged from 0.86 to 0.97 for the muscle belly and from 0.85 to 0.99 for the tendon of the TVI. In absolute terms, the SEM values ranged from 0.0720 mm² to 0.2789 mm² at the muscle belly and from 0.0019 mm² to 0.0827 mm² for the tendon. Overall the systematic error was low ranging from -0.0423 mm² to 0.0827 mm² for the muscle belly and from -0.0153 mm² to 0.0061 mm² for the tendon.

Effects of measurement site and knee flexion angle

The CSA values for the muscle belly and tendon are presented in **figure 2**. The ANOVA showed a non-statistically significant site by knee flexion angle interaction effect on CSA values ($F_{2,40} = 0.93$, $p > 0.05$). There was, however, a statistically significant main effect of the measurement site, as CSA was greater proximally than distally ($F_{1,20} = 160.81$, $p < 0.05$). There was also a significant main effect of knee flexion angle on CSA ($F_{2,40} = 17.41$, $p < 0.05$). Post-hoc Tukey tests indicated that CSA was smaller at 90° of knee flexion compared to 45° and 0° of knee flexion ($p < 0.05$).

Table I. Reliability values for the proximal and the distal muscle belly cross-section area of the tensor of vastus intermedius.

Knee flexion angle	Test (mm ²)	R-test (mm ²)	ICC _{2,1}	SEM (mm ²)	Bias \pm Lower LoA	Upper LoA
0°						
Proximal	1.320 \pm 0.163	1.319 \pm 0.179	0.97	0.0898	0.0008 \pm -0.0228	0.0244
Distal	1.229 \pm 0.144	1.180 \pm 0.180	0.94	0.2284	0.0403 \pm -0.0022	0.0827
45°						
Proximal	1.248 \pm 0.100	1.224 \pm 0.113	0.86	0.2789	0.0239 \pm -0.0100	0.0578
Distal	1.122 \pm 0.106	1.133 \pm 0.121	0.90	0.2152	-0.0113 \pm -0.0423	0.0197
90°						
Proximal	1.182 \pm 0.130	1.186 \pm 0.130	0.97	0.0720	-0.0037 \pm -0.0226	0.0153
Distal	1.068 \pm 0.120	1.080 \pm 0.111	0.94	0.1295	-0.0111 \pm -0.0352	0.0129

Measures of reliability: ICC: Intraclass Correlation Coefficient, SEM= standard error of measurement, Bias \pm LoA= 95% Limits of agreement.

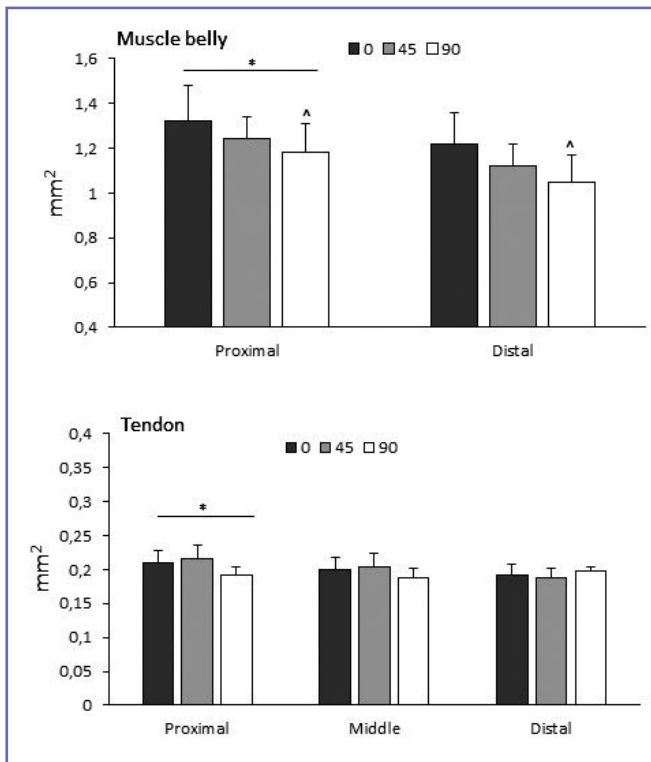


Figure 2. Mean group values of cross-sectional area recorded from the most proximal and most distal position of the muscle belly (upper graph) and tendon (lower graph) at three angles of knee flexion (0°, 45°, 90°). Error bars indicate standard deviation (*significant different compared with the distal position, ^ significantly different compared with the values at knee flexion angles of 45° and 90° $p < 0.05$).

Tendon CSA values are presented in **figure 2**. The ANOVA yielded non-statistically significant interaction and a knee flexion angle main effect ($p > 0.05$). The main effect for measurement site was significant, as CSA was greater proximally than the medial and distal CSA value ($F_{1,20} = 160.81$, $p < 0.05$).

DISCUSSION

The main findings of this study were first, that TVI anatomical CSA was identifiable using US in all individuals, second that the US evaluation of the CSA displayed high test-retest reliability, third, that muscle and tendon belly CSA was larger proximally than distally and, finally, increasing knee flexion angle resulted in a greater CSA of the muscle belly but it did not have an influence on tendon CSA.

In the present study, the TVI was easily identifiable in all participants. This is in agreement with recent cadaver-

ic studies (5–7) as well as studies using diagnostic imaging techniques (6,8). Similar to a previous US study (6), we found that the TVI has a muscle belly which is located proximally and a long distal tendon which coursing obliquely to insert in the middle aspect of the patella (**figure 1**). In addition, the distal part of the tendon is traceable in US images, thus allowing further examination of the TVI morphology along its length. In contrast, these results do not confirm previous anatomical studies which did not identify the TVI in all examined limbs (10,11). We assume that the failure to recognize the TVI by previous studies was down to the large variation of the origin of the TVI. From the results of the present study, it appears that assessment of the TVI should be part of the US quadriceps evaluation.

To our knowledge, this is the first study which examined the anatomical CSA of the TVI determined via US imaging. There are many studies which examined the physiological CSA of the quadriceps femoris (19–25). For example, Blazevitch *et al.* (26) has reported that the CSA of the quadriceps ranges from 6.14 cm² (VM) to 29.3 cm² (VL). These values are much greater than those reported for the TVI (**figure 2**). The limited field of view of the US imaging device which was used in the present study does not allow quantification and comparison of the TVI CSA relative to the other muscles. Although the anatomical CSA does not have a linear relationship with force generation capacity of the muscle (21), the low CSA values indicates that the TVI relative contribution to the force generated capacity of the quadriceps muscle group is low.

The CSA was greater proximally than the most distal position of the muscle belly (**figure 2**). Similarly, the tendon CSA was greater proximally than the CSA obtained from the middle and distal cross-sections (**figure 2**). This is in line with previous studies which showed that CSA of the quadriceps muscle components varies along muscle length (26–29). In particular, Narici *et al.* (27) reported that the portion of femur length where the muscle reached the maximal cross-sectional area was different between the quadriceps. Two studies have (26,28) found a greater CSA of the VL, VI and RF proximally than distally. In contrast, Horsman *et al.* (28) reported that the CSA of the VM was larger proximally than distally but Blazevitch *et al.* (26) indicated the VM was larger distally than proximally.

The complex architecture between adjacent muscles is not an uncommon phenomenon because the same inter-muscular variation in architecture has been reported for the quadriceps (22) and the hamstrings (30–32). The relatively large muscle belly of the TVI which is located proximally (**figures 1,2**) indicates, that the quadriceps maximum CSA is likely to be recorded in the proximal 1/3 of its length. Within the limitations of our study, it appears that the TVI CSA

is maximally proximally to the hip and this may be taken into consideration when assessment of the CSA of the whole muscle group is necessary.

The CSA was smaller at 90° of knee flexion compared to other knee angles while knee flexion angle did not have an effect on tendon CSA (**figure 2**). The effect of knee flexion angle on TVI CSA has not been previously investigated. One may suggest that the reduction in the recorded CSA in the US image may be due to the lengthening of the quadriceps musculature as the knee flexes. However, Myers *et al.*³³ indicated that the anatomical CSA of the RF increased from full extension to 90° of knee flexion which is in contrast to the present results. This could be due to differences in architecture between those two muscles, but clearly, more research is necessary to confirm this suggestion.

The results of this study indicated high test-retest reliability of the CSA for both the muscle belly (**table I**) and the tendon (**table II**). To our knowledge, the reliability of US evaluation of the TVI has not been previously reported. Reeves *et al.* reported that US imaging is a valid and a reliable method for the measurement of the CSA (34). Our results are greater than those reported by Delaney *et al.* (35) who reported ICC values which ranged from 0.67 to 0.99 for CSA of the RF. Similarly, high inter-rater reliability (ICC = 0.94) for the RF CSA and a low SEM (1.07 cm²) have also been reported (36). Finally, Lima *et al.* (37) found high reliability of the RF anatomical muscle CSA with ICCs ranging between 0.87 and 0.99. As far as the tendon CSA is concerned, the high ICCs for the TVI tendon CSA are in agreement with previous studies on US evaluation of the patellar tendon CSA (38,39). For example, Gellhorn *et al.* (38) reported intra-rater reliability ICCs ranging from 0.87

to 0.96 while Murtagh *et al.* (39) even greater ICCs (0.89 and 0.99). In contrast, Ekizos *et al.* (40) reported low reliability of the patellar tendon CSA which was attributed to the low clarity and the unclear visibility of tissue boundaries in the US images. Subsequently, US imaging constitutes a promising, non-invasive tool to evaluate the architecture of the muscle belly and of the tendon of TVI muscle in vivo.

A limitation of this study is that the tendinous part of the TVI as it inserts into the patella was not examined. Preliminary measurements indicated that the distal part of the TVI tendon was not easily identifiable as it is relatively small in size and it is blended with the tendons of the VL, the VI, the VM, and the RF. Another limitation was that the activation of the muscle during the test was not recorded. Although the participants were asked to relax as much as possible in each testing position, it is possible that muscle activation was not minimal. Furthermore, in the present study, we measured the anatomical CSA instead of the physiological CSA. The physiological CSA is a better predictor of force generation capacity than the anatomical CSA (41,42). Evaluation of the physiological CSA based on US measurements requires determination of the angle of pennation (21). In the case of the TVI, however, evaluation of pennation in longitudinal US images is difficult because the TVI is located between the VL and VI and the orientation of its fascicles relative to its aponeurosis is difficult to visualize.

CONCLUSIONS

The TVI was easily identified using in-vivo US. The muscle belly is located proximally along its length and it displays a greater CSA proximally than distally. The long distal tendon

Table 2. Reliability values for the tendinous-part cross-section area of the tensor of vastus intermedius.

Knee flexion angle	Test (mm ²)	R-test(mm ²)	ICC _{2,1}	SEM (mm ²)	Bias ± Lower LoA	Upper LoA
0°						
Proximally	0.210 ± 0.018	0.208 ± 0.016	0.96	0.0135	0.0017 ± -0.0014	0.0048
Medially	0.199 ± 0.018	0.199 ± 0.017	0.99	0.0019	-0.0003 ± -0.0012	0.0005
Distally	0.192 ± 0.016	0.193 ± 0.015	0.90	0.0299	-0.0013 ± -0.0057	0.0030
45°						
Proximally	0.216 ± 0.019	0.215 ± 0.019	0.95	0.0181	0.0007 ± -0.0029	0.0044
Medially	0.204 ± 0.019	0.202 ± 0.019	0.94	0.0221	0.0020 ± -0.0021	0.0061
Distally	0.187 ± 0.015	0.192 ± 0.014	0.86	0.0472	-0.0048 ± -0.00105	0.0010
90°						
Proximally	0.210 ± 0.012	0.213 ± 0.012	0.85	0.0581	-0.0085 ± -0.0153	-0.017
Medially	0.207 ± 0.015	0.212 ± 0.013	0.88	0.0313	-0.0709 ± -0.0120	-0.038
Distally	0.197 ± 0.007	0.194 ± 0.009	0.90	0.0331	-0.0058 ± 0.0106	-0.010

Measures of reliability: ICC: Intraclass Correlation Coefficient, SEM= standard error of measurement, Bias ± LoA= 95% Limits of agreement.

of the TVI has similar CSA along its most proximal first half of this length, while the distal part of the tendon is difficult to identify. Quantification of TVI CSA displayed high test-retest reliability and a low SEM. Consequently, future studies on the quadriceps muscle function and morphology should include examination of the TVI.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

REFERENCES

1. Brand RA. A Model of Lower Extremity Muscular Anatomy. *J Biomech Eng.* 1982;104:304.
2. Martini F, Nath JL, Bartholomew EF. *Fundamentals of anatomy & physiology.* Benjamin Cummings 2012.
3. Saladin KS, McFarland R, Gan CA, Cushman HN. *Essentials of anatomy & physiology.*
4. Becker I, Woodley SJ, Baxter GD. Gross morphology of the vastus lateralis muscle: An anatomical review. *Clin Anat* 2009;22:436-450.
5. Grob K, Ackland T, Kuster MS, Manestar M, Filgueira L. A newly discovered muscle: The tensor of the vastus intermedius. *Clin Anat* 2016;29:256-263.
6. Rajasekaran S, Hall MM. Sonographic Appearance of the Tensor of the Vastus Intermedius. *PM R* 2016;8:1020-1023.
7. Grob K, Manestar M, Filgueira L, Ackland T, Gilbey H, Kuster MS. New insight in the architecture of the quadriceps tendon. *J Exp Orthop* 2016;3.
8. Grob K, Manestar M, Gascho D, Ackland T, Gilbey H, Fretz C, et al. Magnetic resonance imaging of the tensor vastus intermedius: A topographic study based on anatomical dissections. *Clin Anat* 2017;30:1096-1102.
9. Veeramani R, Gnanasekaran D. Morphometric study of tensor of vastus intermedius in South Indian population. *Anat Cell Biol* 2017;50:7.
10. Willan PL, Mahon M, Golland JA. Morphological variations of the human vastus lateralis muscle. *J Anat* 1990;168:235-239.
11. Golland JA, Mahon M WP. Anatomical variations in human quadriceps femoris muscles. *J Anat* 1986;263-264.
12. Ikai M, Fukunaga T. Calculation of muscle strength per unit cross-sectional area of human muscle by means of ultrasonic measurement. *Int Zeitschrift Angew Physiol Einschließlich Arbeitsphysiologisch Arbeitsphysiologie* 1968;26:26-32.
13. Moss BM, Refsnes PE, Abildgaard A, Nicolaysen K, Jensen J. Effects of maximal effort strength training with different loads on dynamic strength, cross-sectional area, load-power and load-velocity relationships. *Eur J Appl Physiol* 1997;75:193-199.
14. Young A, Stroke M, Crowe M. Size and strength of the quadriceps muscles of old and young women. *Eur J Clin Invest* 1984;14:282-287.
15. Masuda K, Kikuhara N, Takahashi H, Yamanaka K. The relationship between muscle cross-sectional area and strength in various isokinetic movements among soccer players. *J Sports Sci* 2003;21:851-858.
16. Jones EJ, Bishop PA, Woods AK, Green JM. Cross-Sectional Area and Muscular Strength. *Sport Med* 2008;38:987-994.
17. Padulo J, Oliva F, Frizziero A, Maffulli N. *Muscles, Ligaments and Tendons Journal - Basic principles and recommendations in clinical and field Science Research: 2016 Update.* *Muscles Ligaments Tendons J* 2016;6:1-5.
18. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986;i:307-310.
19. Wickiewicz TL, Roy RR, Powell PL, Edgerton VR. Muscle Architecture of the Human Lower Limb. *Clin Orthop Relat Res* 1983;179:275;283.
20. Friederich JA, Brand RA. Muscle fiber architecture in the human lower limb. *J Biomech* 1990;23:91-95.
21. Lieber RL, Fridén J. Functional and clinical significance of skeletal muscle architecture. *Muscle Nerve* 2000;23:1647-1666.
22. Blazeovich AJ, Gill ND, Zhou S. Intra- and intermuscular variation in human quadriceps femoris architecture assessed in vivo. *J Anat* 2006;209:289.
23. Klein Horsman MD, Koopman HFJM, van der Helm FCT, Prosé LP, Veeger HEJ. Morphological muscle and joint parameters for musculoskeletal modelling of the lower extremity. *Clin Biomech* 2007;22:239-247.
24. Ward SR, Eng CM, Smallwood LH, Lieber RL. Are current measurements of lower extremity muscle architecture accurate? *Clin Orthop Relat Res* 2009;467:1074-1082.
25. Arnold EM, Ward SR, Lieber RL, Delp SL. A Model of the Lower Limb for Analysis of Human Movement. *Ann Biomed Eng* 2010;38:269-279.
26. Blazeovich AJ, Cannavan D, Coleman DR, Horne S. Influence of concentric and eccentric resistance training on architectural adaptation in human quadriceps muscles. *J Appl Physiol* 2007;103:1565-1575.
27. Narici M V, Roi GS, Landoni L, Minetti AE, Cerretelli P. Changes in force, cross-sectional area and neural activation during strength training and detraining of the human quadriceps. *Eur J Appl Physiol Occup Physiol* 1989;59:310-319.
28. Klein Horsman MD, Koopman HFJM, van der Helm FCT, Prosé LP, Veeger HEJ. Morphological muscle and joint parameters for musculoskeletal modelling of the lower extremity. *Clin Biomech* 2007;22:239-247.
29. Ema R, Wakahara T, Miyamoto N, Kanehisa H, Kawakami Y. Inhomogeneous architectural changes of the quadriceps femoris induced by resistance training. *Eur J Appl Physiol* 2013;113:2691-2703.
30. Kellis E, Galanis N, Natsis K, Kapetanios G. Muscle architecture variations along the human semitendinosus and biceps femoris (long head) length. *J Electromyogr Kinesiol* 2010;20:1237-1243.
31. Kellis E, Galanis N, Kapetanios G, Natsis K. Architectural differences between the hamstring muscles. *J Electromyogr Kinesiol* 2012;22:520-526.
32. Kellis E. Intra- and Inter-Muscular Variations in Hamstring Architecture and Mechanics and Their Implications for Injury: A Narrative Review. *Sport Med* 2018;48:2271-2283.
33. Myers H, Davis A, Lazicki R, Martinez C, Black D, Butler RJ. Sex differences in rectus femoris morphology across different knee flexion positions. *Int J Sports Phys Ther* 2013;8:84-90.

34. Reeves ND, Maganaris CN, Narici M V. Ultrasonographic assessment of human skeletal muscle size. *Eur J Appl Physiol* 2004;91:116-118.
35. Delaney S, Worsley P, Warner M, Taylor M, Stokes M. Assessing contractile ability of the quadriceps muscle using ultrasound imaging. *Muscle Nerve* 2010;42:530-538.
36. Silva CR de S, Costa A dos S, Rocha T, Lima DAM de, Nascimento T do, Moraes SRA de. Quadriceps muscle architecture ultrasonography of individuals with type 2 diabetes: Reliability and applicability. Oyeyemi AL, editor. *PLoS One* 2018;13:e0205724.
37. E Lima KMM, da Matta TT, de Oliveira LF. Reliability of the rectus femoris muscle cross-sectional area measurements by ultrasonography. *Clin Physiol Funct Imaging* 2012;32:221-226.
38. Gellhorn AC, Carlson MJ. Inter-Rater, Intra-Rater, and Inter-Machine Reliability of Quantitative Ultrasound Measurements of the Patellar Tendon. *Ultrasound Med Biol* 2013;39:791-796.
39. Murtagh CF, Stubbs M, Vanrenterghem J, O'Boyle A, Morgans R, Drust B, et al. Patellar tendon properties distinguish elite from non-elite soccer players and are related to peak horizontal but not vertical power. *Eur J Appl Physiol* 2018;118:1737-1749.
40. Ekizos A, Papatzika F, Charcharis G, Bohm S, Mersmann F, Arampatzis A. Ultrasound does not provide reliable results for the measurement of the patellar tendon cross sectional area. *J Electromyogr Kinesiol.* 2013;23:1278-1282.
41. Haxton HA. Absolute muscle force in the ankle flexors of man. *J Physiol.* 1944;103:267-273.
42. Fukunaga T, Roy RR, Shellock FG, Hodgson JA, Day MK, Lee PL *et al.* Physiological cross-sectional area of human leg muscles based on magnetic resonance imaging. *J Orthop Res.* 1992;10:926-934.

Study of the Activation and Oxygenation of Multifidus and Gluteus Medius Muscles During Stretching of the Lower Limb Posterior Chain: Comparison Between Two Different Executions Techniques

L. Calanni¹, F. Combi², L. Rampi¹, M. Negro¹, L. Spairani², G. D'Antona^{1,3}

¹ CRIAMS-Sport Medicine Centre, University of Pavia, Voghera, Italy

² Studio Spairani, Torre d'Isola, Pavia, Italy

³ Department of Public Health Experimental and Forensic Medicine, University of Pavia, Pavia, Italy

CORRESPONDING AUTHOR:

Giuseppe D'Antona
Department of Public Health
Experimental and Forensic Medicine
and Sport Medicine Centre Voghera
13 Foscolo Road
Voghera, Italy, 27058
E-mail: gdantona@unipv.it

DOI:

10.32098/mltj.03.2020.10

LEVEL OF EVIDENCE: 3B

ABBREVIATIONS

HHB: Deoxygenated Hemoglobin
sEMG: surface Electromyography
NIRS: Near Infrared Spectroscopy
TOI: Tissue Oxygen Index
HbO₂: Oxygenated Hemoglobin
THB: Total Hemoglobin

SUMMARY

Background. Ischiocrural (IC) stretching procedures may result in different involvement of the posterior kinetic chain and of the lumbar spine.

Objective. The aim of the study was to evaluate homolateral muscle activation and hemodynamic changes in the Multifidus (MM) and Gluteus medius (GM) muscles in healthy subjects during monolateral IC stretching by using two different execution techniques: technique A (TA: forward bending of the torso, hands in Open Kinetic Chain (OKC), leg stretched) and technique B (TB: forward bending of the torso, leg stretched with knee joint angle of 20°, hands in Closed Kinetic Chain (CKC)).

Methods. A total of 12 recreationally active males (35-24±4.2 years) and 14 recreationally active females (26-22±2.3 years) volunteered to participate in the study and randomly performed TA and TB stretching of 20 s in two subsequent sessions one week apart. During execution, hemodynamic parameters obtained with near infrared spectroscopy (NIRS), namely total hemoglobin (tHb) and tissue oxygen index (TOI%), as indicators of blood flow and oxygen extraction respectively, were detected from homolateral MM, and myoelectric parameters obtained with surface bipolar electromyography (sEMG), namely root mean square (RMS) as indicator of muscle activation, were detected from homolateral GM and MM. Percentage of change ($\Delta\%$) was calculated from the beginning and the end of the 20 s recording divided into 5 s epochs for all the parameters measured.

Results. All along the 20 s procedure, compared to TB, TA stretching produced higher muscle activation both in MM and GM muscles ($p<0.001$), and lower tHB% change combined with higher % oxygen extraction in MM ($p<0.05$).

Conclusions. Stretching of the posterior kinetic chain of the lower limbs with 20° bent homolateral knee and hands in CKC prevents MM and GM overload and may represent a useful technique, alternative to classical stretching, to prevent low back pain.

KEY WORDS

Hamstrings; low back pain; near infrared spectroscopy; electromyography; stretching.

BACKGROUND

Elongation (stretching), involves different types of tissues: connective tissue, contractile proteins, muscle bands, tendons, aponeuroses, ligaments (1) and complex neurological systems regulating afferences and proprioceptive responses (2). Its efficacy, for prevention and re-ed-

ucational purposes, has been extensively documented in literature (3-5). Among the various modalities of stretching, the static technique is the most frequently used and involves the slow elongation of a muscle or a muscular chain maintained to the maximum bearable (6,7) for a time varying from few seconds up to one minute (8).

The posterior kinetic chain (PKC) elasticity of the lower limbs, involving ischiocrural (IC) and triceps sural (TS) districts, is essential for the correct functionality of the lumbar district (LD) (9). There is evidence in that the IC rigidity, decreasing the physiological lumbar lordosis, may cause DL overload thus promoting low back pain (1,10). Based on these observations, the correct elongation of IC is considered a pivotal strategy to prevent and/or treat this clinically relevant condition (11).

The classic, widely adopted, IC stretching procedure requires that the ipsilateral lower limb is fully extended. In this position, IC muscles may prevent retroversion of the pelvis thus avoiding its rotation forward on the femoral heads during flexion of the trunk and subsequently overloading the LD structures and muscles.

In the present study we analyzed the level of Gluteus medius (GM) and Multifidus muscle (MM) activation, by means of surface electromyography (sEMG), and the hemodynamic parameters (total hemoglobin and tissue oxygen index as indicators of blood flow and oxygen extraction respectively), by means of near infrared spectroscopy (NIRS), of the GM and MM during IC stretching executed with TA (hands in OKC and ipsilateral lower limb stretched), and with TB (hands in CKC and ipsilateral lower limb with 20° knee joint angle) techniques in healthy young subjects.

Results obtained demonstrated that TB technique determines higher IC elongation while reducing overload on LD.

MATERIALS AND METHODS

Subjects

26 recreationally healthy young subjects (age 28.5±6.5 years; body weight 66±20 kg, height 171±18 cm), 12 males (age 30±5 years; body weight 73±8 kg, height 176±4 cm) and 14 females (age 24±2 years; body weight 62±9 kg, height 167±7 cm). The subjects were fully informed of the objectives, risks and discomfort associated with the experimental research and provided their informed consent written to participate in this study, before completing a questionnaire to assess their status of health. Exclusion criteria were: joint prostheses, prostheses, artificial implants (hip, knee, ankle and shoulder) or spinal cord plaques, lumbar or sciatica pain in the last three months, arthrodesis of the tibio-tarsica joint, tarsus, metatarsal and phalanges of the feet, paralytic arthritis, recent (less than three months) tear or lesion of the ischiocrural and/or sural triceps muscles, femorotibial arthrosis; recent surgical procedures, and BMI>30 kg/m². The study was conducted at CRIAMS-Sport Medicine Centre Voghera, University of Pavia, Italy

and was approved by the institutional local Review Board. The present study meets the ethical standards of the journal (12).

Experimental procedure

All subjects performed static IC unilateral stretching of the dominant leg using two techniques (technique A, TA and technique B, TB, see below) in two separate sessions 1 week apart, in a randomized fashion. During the same session sEMG and NIRS measurements were made in two subsequent randomized stretching procedures separated by 10 min interval. Experimental procedures were conducted between 9 and 11 a.m. at optimal temperature (22°C) and humidity (50%) environmental conditions.

Technique A (TA)

The subject, sitting on a medical table, performed unilateral static stretching for the hamstrings of the dominant leg, maintained in neutral rotation with the ankle dorsiflexed. The hip was passively flexed, with head extended, to the maximum angle which could be tolerated without stretch pain, with the knee fully extended trying to reach the feet with the hands in open kinetic chain (OKC) and this position was maintained for 20 s. For each subject the final angle was measured after stretching. The final angle was defined as that formed by the tibia and horizontal plane when the knee was passively extended from hip and knee angles at 90° flexion to the maximum extension angle which could be tolerated without stretch pain. During procedure the non-dominant leg was maintained dangling from the edge of the cot in neutral position.

Technique B (TB)

The subject sitting on a medical table, performed unilateral static stretching for the hamstrings of the dominant leg, maintained in neutral rotation with the ankle dorsiflexed. The hip was passively flexed, with head extended to the maximum angle, which could be tolerated without stretch pain. During elongation the knee joint maintained at 20° flexion; during procedure the hands were resting on the surface of the cot in closed kinetic chain (CKC). In each subject position was maintained for 20 s at the bending angle previously measured during TA (or *vice versa*).

sEMG measurement

Analog sEMG signals were captured by applying bipolar disposable circular surface electrodes (Ag/AgCl, OT Bioelettronica Torino, 1 cm diameter) with an interdistance of 2 cm. Surface electrodes were placed on the skin of the dominant leg and ipsilateral low back, parallel to the muscle

fibers, to record muscle activity of the gluteus medius (GM) and lumbar multifidus (MM) muscles. Before positioning skin, shaved if necessary, was gently abraded and cleaned with 75% alcohol to reduce electrical impedance. Electrode handling was in accordance to SENIAM guidelines (13). In GM the electrode was placed in mid-point of a line along the length of the iliac crest (IC), and placed at 34% of the distance from the greater trochanter of the femur (14). In MM the electrode was positioned 1 cm medial from line between posterior superior iliac spine and 1st palpable spinous process, lower electrode border at L4 level (15). To ensure reliability of electrode positioning every investigation was carried out by the same examiners (LC and FC). 4-resolution channel data acquisition system (Quattro Ot-Bioelettronica, Torino), consisting of a signal conditioner with a band-pass filter of 10–500 Hz and amplifier gain of 2000, input impedance 1200 GW, noise level <1 mV, was used to obtain biological signals. All data were processed and exported for analysis by a specific software (Quattro software, OT Bioelettronica, Torino). Captured sEMG activity was converted by an A/D board with a 12-bit resolution input range, sampling frequency of 2024 Hz. Average rectified value (ARV) was computed over 5 s epochs of the recorded raw EMG signal by full-wave rectification to represent the muscle activity.

NIRS

Measurements of deoxyhaemoglobin [HHb], oxyhaemoglobin [HbO₂], and total haemoglobin [HbT] concentrations and the muscle % hemoglobin oxygen saturation (tissue oxygen index, TOI % %) was also calculated as $(\Delta \text{HbO}_2 / (\Delta \text{HHb} + \Delta \text{HbO}_2)) \times 100$ were obtained with a continuous-wave tissue oximeter (NIMO, Nirox srl, Brescia, Italy).

Briefly, based on the assumption that at 975 nm the absorption coefficient is dominant over other chromophores (i.e. HbO₂ and HHb) and that water absorption and that the tissue scattering properties vary linearly with wavelength, the scattering spectrum is calculated at this wavelength thus allowing the estimation of absorption coefficient at 685 and 830 nm thus allowing measurements of absolute HbO₂ and HHb tissue concentrations. In each subject, data obtained by continuous recording were subsequently sub grouped in 5 s epoch intervals, averaged and plotted. At each time point, absolute values obtained from each subject were subsequently averaged and differences of the means obtained in TA and TB were statistically analyzed. To account for the possible influence of the fat layer (previously measured by ultrasound) on NIRS measurements, a real-time correction was applied using an algorithm includ-

ed in the software supplied with the spectrometer (Nimo Data Analysis version 2.0). For NIRS tests, the probe was placed in the same position of the sEMG electrodes only in MM, whereas GM was discarded from the analysis due its physiological curve that avoided correct positioning of the probe during stretching. To prevent artifacts from external lights the NIRS probe was firmly secured with a small velcro strap and covered with dark coating. The probe edges were marked on the skin in order to avoid any downward sliding during movements.

Statistical analysis

Linear regression was applied to the data to calculate the initial value and rate of change of ARV. Normalized rate of change for each variable was calculated as the percentage ratio between rate of change and initial value. Paired and unpaired t-test was used to compare significant differences between recorded parameters in TA vs TB and between genders respectively. The data are reported as mean \pm standard deviation (SD). Statistical analysis was completed using Graphpad 5 (San Diego, California) and the significance level was set at $p \leq 0.05$.

RESULTS

Considering that no significant differences were found between males and females at every time point and for every parameter measured, data were pooled for subsequent statistical analysis.

Average percentage changes of RMS recorded at 5 s intervals between initial and final stretching are reported in **Table I** for MM and **Table II** for GM. Compared to TB, TA stretching produced significant higher muscle activation both in MM and GM muscles at every time point along the 20 s stretching procedure. As regards the hemodynamic parameters recorded in homolateral MM (**Table III**), a slightly change in blood volume ($\Delta\%$ tHb) was observed in TA and TB at each time intervals without significant difference between procedures, whereas a higher oxygen extraction ($\Delta\%$ TOI) was observed in TA compared to TB at 10, 15 and 20 time intervals (**Table III**), thus suggesting that an improved oxygen extraction was maintained to sustain the higher muscle activation during stretching.

DISCUSSION AND CONCLUSION

The objective of this study was to compare two different stretching techniques, TA and TB, of the IC muscles to determine which one least overload LD muscles. To this aim, by combining sEMG and NIRS techniques to study GM and MM activation and oxygenation during 20 s IC

Table I. Root Mean Square (RMS) percentage of change from baseline in homolateral Multifidus muscle at 5 s intervals during TA and TB stretching and percentage difference between TA and TB at each time point.

$\Delta\%$ RMS di Multifidus (TA)			$\Delta\%$ RMS di Multifidus (TB)			$\Delta\%$ RMS Multifidus TA vs TB		
Time (s)	mean	SD	Time (s)	mean	SD	Time (s)	mean	SD
0	0.00	± 0.00	0	0.00	± 0.00	0	0.00	± 0.00
5	9.0	± 25.22	5	5.0	± 18.90	5	44.5	± 40.37
10	12.8	± 34.05	10	-4.0*	± 18.31	10	131.1	± 41.43
15	13.9	± 31.30	15	-13.1**	± 23.51	15	194.6	± 46.75
20	17.4	± 30.69	20	-13.0**	± 31.52	20	174.4	± 55.74

* significantly different from TA ($p < 0.05$). ** significantly different from TA ($p < 0.001$). s, seconds; SD, Standard Deviation.

Table II. Root Mean Square (RMS) percentage of change from baseline in homolateral Gluteus medius at 5 s intervals during TA and TB stretching and percentage difference between TA and TB at each time point.

$\Delta\%$ RMS Gluteus medius (TA)			$\Delta\%$ RMS Gluteus medius (TB)			$\Delta\%$ RMS Gluteus medius (TA vs TB)		
Time (s)	mean	SD	Time (s)	mean	SD	Time (s)	mean	SD
0	0.00	± 0.00	0	0.00	± 0.00	0	0.00	± 0.00
5	12.89	± 29.37	5	9.70	± 22.71	5	24.77	± 39.47
10	13.30	± 30.55	10	1.74	± 26.99	10	86.89	± 44.58
15	13.86	± 28.38	15	-4.64*	± 33.32	15	133.45	± 49.47
20	7.90	± 37.25	20	-8.67	± 32.34	20	209.82	± 55.79

* significantly different from TA ($p < 0.05$). s, seconds; SD, Standard Deviation.

Table III. Total Hb (Hbtot) and TOI percentage of change from baseline in homolateral Multifidus muscle at 5 s intervals during TA and TB stretching and percentage difference between TA and TB at each time point.

$\Delta\%$ Hbtot Multifidus TA			$\Delta\%$ Hbtot Multifidus TB			$\Delta\%$ TA vs TB		
Time (s)	mean	SD	Time (s)	mean	SD	Time (s)	mean	SD
0	0.00	± 0.00	0	0.00	± 0.00	0	0.00	± 0.00
5	23.8	± 9.30	5	21.4	± 8.61	5	9.8	± 2.33
10	21.0	± 7.03	10	16.9	± 6.50	10	19.6	± 1.89
15	18.4	± 5.75	15	17.2	± 5.32	15	6.5	± 1.76
20	17.3	± 4.91	20	15.4	± 4.63	20	10.9	± 1.57
$\Delta\%$ TOI Multifidus TA			$\Delta\%$ TOI Multifidus TB			$\Delta\%$ TA vs TB		
Time (s)	mean	SD	Time (s)	mean	SD	Time (s)	mean	SD
0	0.00	± 0.00	0	0.00	± 0.00	0	0.00	± 0.00
5	10.1	± 3.35	5	9.8	± 3.47	5	2.9	± 1.21
10	9.5	± 2.80	10	6.8	$\pm 2.50^*$	10	28.3	± 1.90
15	8.7	± 2.33	15	6.0	$\pm 2.07^*$	15	31.5	± 5.65
20	8.0	± 1.97	20	4.8	$\pm 1.92^*$	20	39.5	± 1.97

* significantly different from TA ($p < 0.05$). s, seconds; SD, Standard Deviation.

stretching, the main findings were: 1) a higher muscles activation in TA compared to TB at each 5 s epochs of the stretching period; 2) no difference in the percentage change of total hemoglobin and a higher oxygen uptake detected in MM during TA compared to TB; 3) no difference in muscle activation and oxygenation in males compared to females in both stretching procedures.

Shortening or tightness of the hamstrings affects postural alignment and results in possible musculoskeletal pain. The kinematics of the lumbar district and of the Coxo-Femoral joint (CF), also called lumbar-pelvic rhythm, was studied during the flexion of the trunk in some daily activities living (ADL), in people with or without low back pain (16,17). In the bending of the trunk, upon reaching the maximum opening of the lumbar veneer joints ($\approx 60^\circ$) (18), the elasticity of the IC induces a coordinated anterior rotation of the pelvis on the femoral heads (10), thus allowing an increase of the trunk bending without further stress to the DL structures (10,19). In contrast, excessive rigidity of the IC muscles, due to their proximal insertion on the ischial tuberosity, by retaining the pelvis, restricts the muscles rotation forward on the femoral heads, thus causing excessive stress in lengthening of the connective structures of the LD. This condition is associated with higher probability of fatigue of the paravertebral muscles and subsequent onset of low back pain (20). As previously demonstrated, the use of various postures during the IC stretching procedure for may result in different involvement of the PKC and of the lumbar spine (21,22). Our results, for the first time, demonstrate that stretching of the posterior kinetic chain of the lower limbs with 20° bent homolateral knee and hands in CKC prevents MM and GM overload. In fact, a lower muscles activation and contemporary lower oxygen uptake along the 20 s stretching period was observed in TB stretching. The observed differences in muscles activation

and oxygenation in TA compared to TB may be due the unfavorable lever arm with greater stress in the pivotal area of the movement, LD and the sacroiliac joint, to which it may contribute the position of the upper limbs stretched out forward without any support in an open kinetic chain. Importantly, an increased GM activation has been found both in classic IC static and in the dynamic stretching and its possible role as critical factor for lumbar pain has been hypothesized in healthy subjects, following the long lasting maintenance of the erect station (23). Therefore, TB stretching may represent a useful technique, alternative to classical static stretching, to prevent low back pain.

LIMITATIONS OF THE STUDY

This study has limitations that need to be addressed. First, no data are presented regarding GM and MM sEMG adaptation following repeated stretching procedures. Further, IC muscles activation measurement during stretching procedures was not performed. This lack hampers the possibility to unravel the contribution of concurrent involuntary hamstring muscle activity contraction in limiting the hip range of motion in the tested stretching procedures and its role in stretching training (24). Further, changes in blood flow through the muscle tissue has not been measured directly but through the blood volume ($\text{HBO}_2 + \text{HHb}$). Based on the present limitations, future studies should expand the presented data, to confirm whether beneficial lesser LD overload due to TB compared with TA is verifiable following stretch training as well as following a single stretching session.

CONFLICT OF INTERESTS

The authors declare no conflict of interests.

REFERENCES

1. Wiemann K, Hahn K. Influence of strength, stretching and circulatory exercises on flexibility parameter of the human hamstrings. *Int J Sports Med* 1997; 18: 340–46.
2. Sale DG, Quinlan J, Marsh E, McComas, AJ, Belanger AY. Influence of joint position on ankle plantarflexion in humans. *J Appl Physiol* 1982; 52: 1636–42.
3. Shellock FG, Prentice WE. Warming up and stretching for improved physical performance and prevention of sports – related injuries. *Sport Medicine* 1985; 2: 267–78.
4. Porter JL, Wilkinson A. Lumbar hip flexion motion. A comparative study between asymptomatic and chronic low back pain in 18 to 36 year old. *Med Spine* 1997; 22: 1508–13.
5. Witvrouw E, Daneels L, Asselman P, D’Have T, Cambier D. Muscle flexibility as risk factor for developing muscle injuries in male professional soccer players. A prospective study. *Am J Sports Med* 2003; 31: 41–6.
6. Behm DG, Bambury A, Cahill F, Power K. Effect of acute static stretching on force, balance, reaction time, and movement time. *Med Sci Sports* 2004; 36: 1397-1402.
7. Cronin J, Nash M, Whatman C. The acute effects of hamstring stretching and vibration on dynamic knee joint range of motion and jump performance. *Phys Ther Sport* 2008; 9: 89-96.
8. Ebben WP, Carroll RM, Simenz CJ. Strength and conditioning practices of National Hockey League strength and conditioning coaches. *J Strength Cond Res* 2004; 18: 889-97.
9. Nourbakhsh MR, Arab AM. Relationship between mechanical factors and incidence of Low back pain. *J orthop Sports Phys Ther* 2002; 32, 447–60.

10. Esola MA, McClure PV, Fitzgerald GK, Siegler S. Analysis of lumbar spine and hip motion during forward bending in subjects with and without a history of low back pain. *Spine* 1996; 21, 71–8.
11. Worrell TW, Perrin DH, Gansneder BM, Gieck JH. Comparison of isokinetic strength and flexibility measures between hamstring injured and noninjured athletes. *J Orthop Sports Phys Ther* 1991; 13, 118-25.
12. Padulo J, Oliva F, Frizziero A, Maffulli N. Muscle, Ligaments and Tendons Journal-Basic principles and recommendations in clinical and field science research: 2016 update. *MLTJ* 2016; 6: 1-5.
13. Hermens HJ, Freriks B, Disselhorst-Klug C, Rau G. Development of recommendations for SEMG sensors and sensor placement procedures. *J Electromyogr Kinesiol* 2000; 10, 361–374.
14. Rainoldi A, Melchiorri G, Caruso I. A method for positioning electrodes during surface EMG recordings in lower limb muscles. *J Neurosci Methods* 2004; 134, 37–43.
15. Huebner A, Faenger B, Schenk P, Scholle HC, Anders C. Alteration of Surface EMG amplitude levels of five major trunk muscles by defined electrode location displacement. *J Electromyogr Kinesiol* 2015; 25: 214-23.
16. Li Y, McClure PW, Pratt N. The effect of hamstring muscle stretching on standing posture and on lumbar and hip motion during forward bending. *Phys Ther* 1996; 76, 836 –45.
17. Shum GL, Crosbie J, Lee RY. Effect of low back pain on the kinematics and joint coordination of the lumbar spine and hip during sit to stand and stand to sit. *Spine* 2005; 30, 1998–2004.
18. Kapandji AI. *Physiology of the Joints*. London; Churchill Livingstone 6th Edition 2010.
19. Perret C, Poiraudreau S, Fermanian J, Revel M. Pelvic mobility when bending forward in standing position: validity and reliability of two motion analysis devices. *Phys Med Rehabil* 2001; 82: 221–6.
20. Kendall FP, Kendall McCreary E, Provance PG, McIntyre Rodgers M., Romani WA. *Muscles: Testing and Testing and Function with Posture and Pain*. Baltimore and Philadelphia; Lippincott Williams & Wilkins 5th Edition 2005.
21. Herda TJ, Cramer JT, Ryan ED, McHugh MP, Stout JR. Acute effects of static versus dynamic stretching on isometric peak torque, electromyography, and mechanomyography of the biceps femoris muscle. *Strength Cond Res* 2008; 22: 809–17.
22. Boyce D, Brosky JA. Determining the minimal number of cyclic passive stretch repetitions recommended for an acute increase in an indirect measure of hamstring length. *Physioter Theory Pract* 2008; 24, 113–20.
23. Nelson-Wong E, Gregory DE, Winter DA, Callaghan JP. Gluteus medius muscle activation patterns as a predictor of low back pain during standing. *Clin Biomech (Bristol, Avon)* 2008; 23: 545-53.
24. Foo Y, Héroux ME, Chia L, Diong J. Involuntary hamstring muscle activity reduces passive hip range of motion during the straight leg raise test: a stimulation study in healthy people. *BMC Musculoskelet Disord* 2019; 20, 13.

Gastrocnemius Kinesiotaping to Improve Dynamic Balance Performance in Middle-Age Healthy Men: Protocol of Randomized Controlled Trial

K. Mansouri Jamshidi^{1,2}, S. Jalali^{1,2}, T. Roghani¹, G. Sarhan³, H. Azadeh¹, Z. S. Rezaeian¹

¹ Musculoskeletal Research Center and Department of Physical Therapy, Faculty of Rehabilitation Sciences, Isfahan University of Medical Sciences, Isfahan, Iran

² Student Research Committee of Rehabilitation Students (Treata), Faculty of Rehabilitation Sciences, Isfahan University of Medical Sciences, Isfahan, Iran

³ Bahrain Physio WWL, Sanad, Bahrain

CORRESPONDING AUTHOR:

Hamid Azadeh
Musculoskeletal Research Center
Rehabilitation Sciences Research Institute
Department of Physical Therapy
Faculty of Rehabilitation Sciences
Isfahan University of Medical Sciences
Hezar Jarib Avenue
Isfahan, Iran

DOI:

10.32098/mltj.03.2020.11

LEVEL OF EVIDENCE:

Treatment

Kinesio Taping®

Comparators

Sham Taping

Patient population

Healthy middle-aged men

SUMMARY

Background. The adhesive taping is supposed to improve joint stability and proprioception and enhance the physical protection in unpredictable situations.

Hypothesis/purpose. The aim of present study was to compare the effect of facilitatory gastrocnemius taping on dynamic balance and muscle activity during Y-balance test in healthy middle-age men.

Study design. Double-blinded (participant, assessor) randomized control trial.

Methods. 30 healthy middle-age men who were not routinely involved in exercise program were randomly assigned in Kinesio Taping® and sham group. They took part in Y-balance test before, immediately, 20 minutes and 24 hours after taping. Y-balance records and medial and lateral gastrocnemius activity were reported for each test. Between group and within group changes were analyzed using independent T test and repeated measures. Correlation between Y-balance record and gastrocnemius activity were be analyzed using person correlation test.

KEY WORDS

Dynamic balance; Gastrocnemius; kinesiotaping; middle-age.

BACKGROUND

The adhesive taping is supposed to improve joint stability and proprioception and enhance the physical protection in unpredictable situations (1). Elastic adhesive tape, known as Kinesio Tape (KT), was first introduced by Kenzo Kase (2) and got popular fast (3). KT needs approximately 20 minutes to gain best adhesive strength (2). Several studies have investigated the effects of KT on function and muscle strength and activity using functional tests (3,4,5,6,7,8,9) and surface electromyography (SEMG). Practically, KT-induced changes in muscle activity and the functional measures are time related (3,5,8,10) i.e. in healthy subjects positive effects of KT in muscle activity patterns reached highest level within one hour and then reduced within 24 hours and did not changed up to 5 days (4). Still, the net results of KT on SEMG and functional performance

are conflicting: some studies reported that SEMG did not significantly change following inhibitory (1) or facilitatory taping (11,12) in young people. Some authors showed short-term increase in muscle activity following inhibitory gastrocnemius taping (5), while others reported reduced muscle activity of gastrocnemius with inhibitory taping (8). Balance is a critical feature of human posture and movements in daily living. Aging may decrease the dynamic balance capabilities (13,14) and increase falling risk (15); therefore, it may be associated with various musculoskeletal injuries. Since musculoskeletal injuries frequently happen in dynamic activities (16,17) researcher and clinicians are more interested in measuring and screening dynamic balance. The effects of KT on electrical muscle activity and balance have not been well defined in middle-aged subject. Previous studies mostly recruited young adults and athletes under 40

years old (3,4,5,6,7,8); these people did not suffer from age related balance disorders and fear of falling. Middle-aged people constitute the main society economic power (18). In middle-age (40-65 years) the normal regeneration processes starts to deteriorate as a result of aging process (19). These people are at risk of physical injuries while they are physically and economically active. Beside direct costs, these injuries expose the individual, family and the society to huge indirect costs of unhealthy aging including early retirements and repeated sick leaves. Thus, studying middle-aged subjects are valuable in societies like Iran where the population is gradually moving toward elderly (20).

Gastrocnemius is frequently involved in injuries (21,22); because of its rich proprioception supply (23,24,25,26) gastrocnemius is highly regarded in balance training programs. To improve balance in healthy and injured subjects, physical therapists employ various approaches including adhesive taping. Our aim was to compare the immediate and short-term effects of two types of gastrocnemius kinesiotaping (true facilitatory, sham) on SEMG record of the medial and lateral gastrocnemius and dynamic balance in healthy non-athletic middle-aged men. Our hypothesis was that true KT improves SEMG amplitude (in both gastrocnemius heads) and dynamic balance and these effects become more significant over time.

STUDY AIM

The aim of this student noncommercial trial was comparison of the immediate and short-term effect of gastrocnemius facilitatory kinesiotaping on electrical activity of the muscle and dynamic balance in healthy non-athletic middle-aged men with control kinesiotaping

METHODS

Study design

This study is the protocol of a prospective, double blinded (participant, assessor) randomized, controlled clinical trial of parallel design with two groups. The primary outcome was surface electromyographic record of medial and lateral head of gastrocnemius during Y-balance test. The secondary outcome measure was Y-balance test records in anterior, postero-lateral and postero-medial directions.

Approval and registration

Institutional ethics committee approved the whole procedure (Ethics Code: IR.MUI.REC.1395.3.814). The study is funded by Isfahan University of Medical Sciences, Vice

Chancellery of Research and Technology (Budget Code: 395814). The protocol has been registered in Iranian Registry of Clinical Trials (IRCT20150131020888N8).

Participants

The study was conducted at Musculoskeletal Research Center. Participants were healthy, community-based middle-aged men (N=30), that were recruited through advertisements at urban entertainment districts, March-July 2017. Using non-probability convenient sampling, the volunteers who met inclusion/exclusion criteria of the study were identified according to the medical history and the results of a comprehensive interview and clinical and physical examination by the physical therapist. 40 to 65 years old men were included in the study. The exclusion criteria were any musculoskeletal, cardiovascular and chronic problems that required medication, neurological, vestibular, visual and auditory problems (because of problems in communication with the patient), arthritis or other joint-related problems such as osteoarthritis during the past 12 months, previous allergies to adhesive tapes, previous use of Kinesio Tape or knowledge about its effects (to eliminate psychological effects of taping), regular exercise activities within 6 months prior to and/or during the study, pain in back and lower extremities during the selection process and inability to perform the test.

DATA COLLECTION

All the subjects who were qualified to participate in the study were informed about study design in detail. They had 48 hours to decide about participating in the study. They were assured that their credentials will going to be saved private. The subjects who agreed to take part in the study were a requested to sign a formal consent.

Pre-randomization evaluation

Demographic and anthropometric data were collected to make sure that the groups are similar in physical characteristics.

Randomization and allocation concealment

After signing the inform consent and filling personal data sheet, the participants were randomly assigned into either Kinesio Taping® group (N=15) or sham group (N=15) using a coin (simple randomization). A faculty staff that was blind to the study were requested to throw the coin for each subject. The trial was of parallel design (allocation

ration=1). The subjects were told that they are going to wear tapes for 24 hours but they did not have any clue that what method of taping they are going to experience.

Post-randomization evaluations

Lower quarter Y balance test records and SEMG data were measured before taping, immediately and 20 minutes after taping (for determining the immediate effect of the tape) and 24 hours after taping (to determine the short-term effect of the tape). In both Kinesio Taping® and sham group. In healthy subjects, positive effects of KT in muscle activity patterns reached highest level within one hour and then reduced within 24 hours and did not changed up to 5 days (4). Therefore, in present study Lower Quarter Y balance test records and SEMG data will be measured before, immediately, 20 minutes and 24 hours after applying either Kinesio Tape® or sham tape.

TRIAL INTERVENTIONS

Kinesio Taping® group

Trained physical therapist (SJ) that was blind to the study method, applied facilitatory Kinesio Tape® on gastrocnemius of dominant leg. The dominant leg was the leg by which the subject hit a soccer ball when the ball was thrown toward him unexpectedly. For facilitatory Kinesio Tape®, 15-35% stretch was applied to therapeutic zone of an “I” strip as proposed by Kase et al. (2). The participant lay in prone; proximal head of KT was anchored under the knee with no tension (tape application in popliteal fossa is not recommended because of the risk of skin irritation (2)). The ankle was fully dorsiflexed by the therapist passively so that gastrocnemius be completely stretched. The Kinesio Tape® was then continued along muscle length and Achilles tendon with 15-35% tension. The distal one third of the Kinesio Tape® was attached without tension until it ended over the lower surface of heel.

Sham tape group

According to Gómez-Soriano *et al.* (5), only the ineffective KT heads (anchor and end) were attached to the back of the calf without continuity. This way, the participants were blind to the intervention they received. They were informed that there is a 50% chance to receive sham tape but they had no clue that how sham tape looks like. In addition, to make the assessor blind to the study groups, the physical therapist who applied the tapes for either groups covered participant's leg in a disposable cover.

Neither Kinesio Taping® nor sham tape have any side effect. The risk of skin irritation was mentioned and the participants were requested to remove the tape as soon as they feel itching, burning or they find skin rashes around the tape. The subjects did not receive any other intervention during the study in either groups.

In order to make the participants blind to their group, their calf was covered by a disposable sleeve following taping. The same physical therapist (SJ) applied the tapes in both groups. The physical therapist had no role in baseline assessment of inclusion/exclusion criteria, collecting data or analyzing the outcomes. Inclusion/exclusion criteria were assessed before assigning the subjects into study groups (i.e. inevitably the assessor (KMJ) was blinded to study groups). Covered in disposable sleeve, the assessor (KMJ) might not differentiate the study groups. When transferring data into statistic software, the subjects will be coded and the analyzer (KMJ) has no clue to the groups and code.

Blinding strategy

- The physical therapist who were responsible for primary assessment of the volunteers was informed about the inclusion/exclusion criteria while had no information about the study protocol.
- The selected individuals knew that they will be randomly assigned into one of the study groups and there is 50% chance of receiving sham tape. They had no clue that how sham tape is different from Kinesio Tape®.
- A faculty staff assigned the participant into groups using a coin.
- Employed physical therapist, who had no role in subjects' evaluation, taped subjects in both groups.
- The same researcher who was blinded to study groups, evaluated all the subjects at all assessment sessions.

DATA

Lower quarter Y balance test records and SEMG data were collected before taping, immediately and 20 minutes after taping (for determining the immediate effect of the tape) and 24 hours after taping (to determine the short-term effect of the tape) (**table I**).

STATISTICAL ANALYSIS

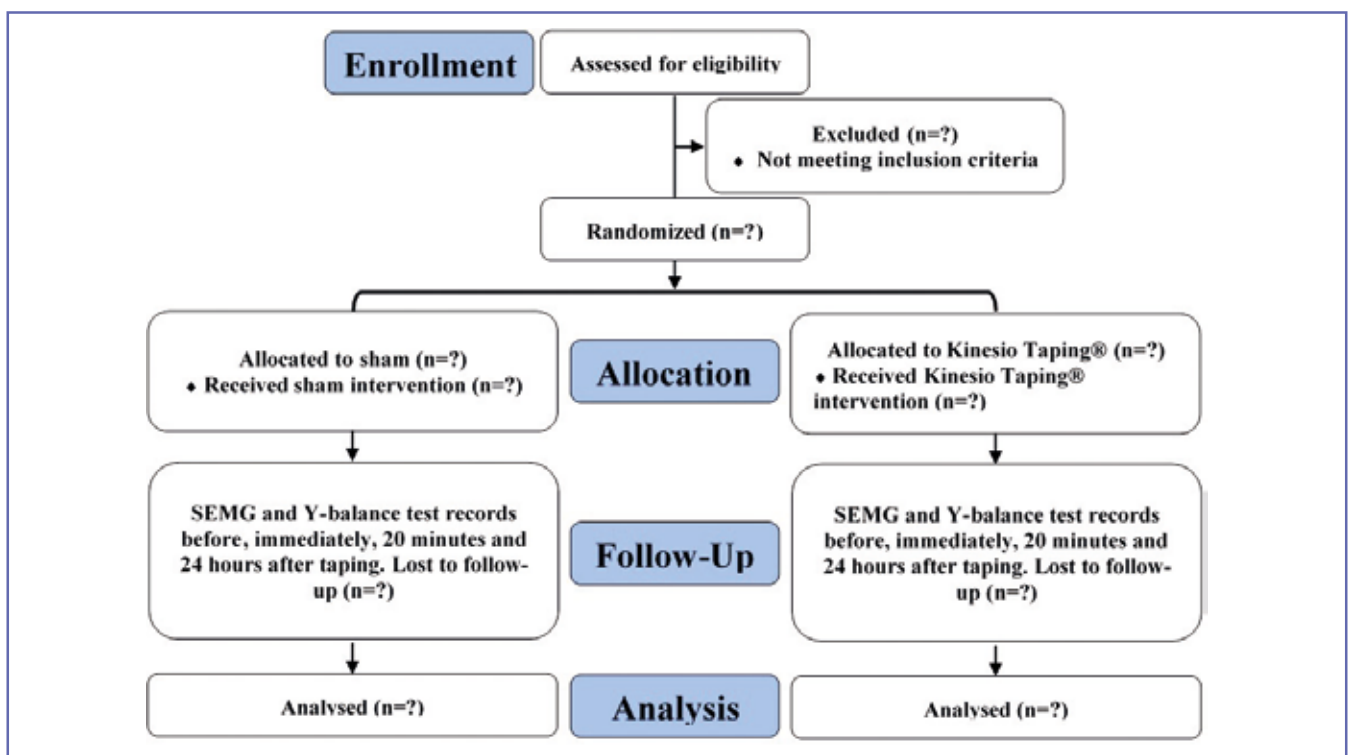
The distribution of the data will be determined using Shapiro-Wilk test. In accordance to the distribution of the variables, data will be analyzed using repeated measures/Wilcoxon signed rank tests in each group. The groups will

Table I. Data to be collected.

Variables	Variable Type	Qualitative	Quantitative	Scale	Measurement
Measurement Time	Independent		Nominal	Before, immediately, 20 minutes and 24 hours after taping	-
Taping Method	Independent		Nominal	Sham/Kinesio Taping	-
Age	Control	Continuous		Year	Questionnaire
Height	Background	Continuous		Meter	Strip Meter
Weight	Background	Continuous		Kilogram	Scale
Body Mass Index	Background	Continuous		Kilogramxm ²	Calculation
Lower Extremity Length	Background	Continuous		Meter	Strip Meter
Dynamic Balance	Dependent	Continuous		Meter	Lower Quarter Y-Balance Test
Amplitude of Muscle Electrical Activity	Dependent	Continuous		Millivolt	SEMG

be compared using Independent T test/Mann-Whitney U test in SPSS software (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY, USA). The χ^2 -test and Fisher's exact test will be used for categorical variables. Correlation between SEMG records and Y test results will be determined using Pearson/Spearman correlation test. Mean difference, partial Eta square, effect size and Cohen's d and their respective 95% CI will

be calculated to determine the clinical relevance of the finding. Statistical significance will be considered at $p < 0.05$. The study power will be calculated using G*Power 3.1.5 freeware (University of Düsseldorf, Düsseldorf, Germany) (27,28). The subjects' adherence and the cause for which people left the study or rejected joining the program will be mentioned in consort flowchart (**figure 1**). Intention to treat analysis will be performed.

**Figure 1.** Schematic diagram(s) of the trial design, procedures, stages and data collection according to CONSORT.

Sample size calculation

There was no similar study to determine the sample size. For pilot studies, 10 subjects in each group is recommended. We considered 15 subjects in either group in present study. Considering 10% attrition rate, 33 subjects were included in the study.

ETHICAL CONSIDERATIONS

Institutional ethics committee approved the whole procedure (Ethics Code: IR.MUI.REC.1395.3.814). The protocol has been registered in Iranian Registry of Clinical Trials (IRCT20150131020888N8). KMJ provided detailed information for the participants about the aim and objectives of the study, the possible complications of taping, measurement times and the data confidentiality. Subjects might stop participating in the study any time they want without penalty. Reporting of this trial is done according to the recommendations of the CONSORT statement (29). The study meets the ethical standards of the journal of Muscle, Ligament, and Tendon Journal (30).

TIMELINE

Ethical approval was obtained in August 2017 from the local medical ethics committee. Subjects were recruited between February-July 2017. All participants completed the study in February 2018. We will finish the statistical analysis by end

REFERENCES

1. McCarthy Persson U, Fleming H.F, Caulfield B. The effect of a vastus lateralis tape on muscle activity during stair climbing. *Man Ther* 2009;14(3):330–337.
2. Kase K A, Wallis J, Kase T, et al. *Clinical Therapeutic Applications of the Kinesio Taping Method - 3rd Edition*. Tokyo, Japan: Ken I kai Co Ltd 2013;12,32.
3. Huang C, Hsieh T, Lu S, et al. Effect of the Kinesio tape to muscle activity and vertical jump performance in healthy inactive people. *Biomed Eng Online* 2011;10(1):70-81.
4. Wilson V, Douris P, Fukuroku T, et al. The immediate and long-term effects of Kinesiotape® on balance and functional performance. *Int J Sports Phys Ther* 2016;11(2):247–53.
5. Gómez-Soriano J, Abián-Vicén J, Aparicio-García C, et al. The effects of Kinesio taping on muscle tone in healthy subjects: A double-blind, placebo-controlled crossover trial. *Man Ther* 2014;19(2):131–136.
6. Nunes G, de Noronha M, Cunha H, et al. Effect of Kinesio Taping on Jumping and Balance in Athletes. *J. Strength Cond Res* 2013;27(11):3183–3189.
7. Lumbroso D, Ziv E, Vered E, et al. The effect of kinesio tape application on hamstring and gastrocnemius muscles in healthy young adults. *J Bodyw Mov Ther* 2014;18(1):130–138.

of July 2019 and after that we will start the elaboration of scientific paper.

DISCUSSION

Middle age men are the main human source in economy.¹⁸ At the same time, gradual progression toward elderly makes them vulnerable to various musculoskeletal injuries.¹⁹ Balance is an essential part of human function (31). On aspect of rehabilitation is planning for interactive aging (31) i.e. helping individuals to step in seniority in more healthier state. KT is one of the non-invasive, low cost, simply applicable interventions. It may be self-administered by a short training and may be used in conjunction with other approaches to improve their efficacy and lasting effect. Several studies have investigated the effect of KT on muscle performance and balance in healthy young people and athletes (3,4,5,6,7,8,9) However, its effects in adults and non-athletes are still questionable. Present study seems to be the first study on the effect of KT on balance and muscle activity in healthy middle-aged men. The design of this protocol is such as to minimize bias and bring the results closer to reality. It can also be the starting point for other studies in this age range and in situations where the balance is compromised.

CONFLICT OF INTERESTS

The authors declare no conflict of interests.

8. Davison E, Andersson C, Ponist B, et al. Inhibitory Effect of the Kinesio Taping® Method on the Gastrocnemius Muscle. *Am J sports Sci Med* 2016;4(2):33–38.
9. Trecroci A, Formenti D, Rossi A, Esposito F, Alberti G. Short-Term Delayed Effects of Kinesio Taping on Sprint Cycling Performance. *J Strength Cond Res* 2019;33(5):1232-1236.
10. Kirmizigil B, Chauchat JR, Yalciner O, Iyigun G, Angin E, Baltaci G. The Effectiveness of Kinesio Taping in Recovering from Delayed Onset Muscle Soreness: A Cross-Over Study. *J Sport Rehabil* 2019;12:1-28.
11. Lins C, Neto F, Amorim A, et al. Kinesio Taping® does not alter neuromuscular performance of femoral quadriceps or lower limb function in healthy subjects: Randomized, blind, controlled, clinical trial. *Man Ther* 2013;18(1):41–45.
12. Mak DN, Au IP, Chan M, Chan ZY, An WW, Zhang JH, Draper D, Cheung RT. Placebo effect of facilitatory Kinesio tape on muscle activity and muscle strength. *Physiother Theory Pract* 2019;35(2):157-162.
13. Lee D, Kang M, Lee T, et al. Relationships among the Y balance test, Berg Balance Scale, and lower limb strength in middle-aged and older females. *Brazilian J Phys Ther* 2015;19(3):227–234.

14. Bouillon LE, Baker JL. Dynamic Balance Differences as Measured by the Star Excursion Balance Test Between Adult-aged and Middle-aged Women. *Sports Health* 2011;3(5):466–469.
15. Talbot L, Musiol R, Witham E, et al. Falls in young, middle-aged and older community dwelling adults: perceived cause, environmental factors and injury. *BMC Public Health* 2005;5(1):86.
16. Cabreira T, Coelho K, Quemelo P. Kinesio Taping effect on postural balance in the elderly. *Fisioter e Pesqui* 2014;21(4):333–338.
17. Malliaropoulos N, Kakoura L, Tsitas K, Christodoulou D, Siozos A, Malliaras P, Maffulli N. Active knee range of motion assessment in elite track and field athletes: Normative values. *Muscle, Ligaments and Tendons Journal (MLTJ)* 2015;5(3), 203-207.
18. Eshagh G. Evaluation of social-economic aspects of elderly in Iran. *ilam uni Publ* 2009;325–348.
19. Thomas D.R. Age-related changes in wound healing. *Drugs Aging* 2001;18(8):607–620.
20. Fakhredin S. Changes in population age in Iran and its effect on social security protection coverage ratio. *Soc Secur Organ* 2014;1–3.
21. Armfield D, Kim J, Towers J, et al. Sports-Related Muscle Injury in the Lower Extremity. *Clin Sports Med* 2006;25(4):803–842.
22. Oberhofer K, Hosseini Nasab SH, Schütz P, Postolka B, Snedeker J, Taylor W, List R. The influence of muscle-tendon forces on ACL loading during jump landing: a systematic review. *Muscles Ligaments Tendons J* 2017;7(1): 125–135.
23. Fitzpatrick R, Taylor J, McCloskey D. Ankle stiffness of standing humans in response to imperceptible perturbation: reflex and task-dependent components. *J Physiol* 1992;454:533–47.
24. Lakie M, Caplan N, Loram I.D. Human balancing of an inverted pendulum with a compliant linkage: neural control by anticipatory intermittent bias. *J Physiol* 2003;551(1):357–370.
25. Loram I.D, Lakie M. Direct measurement of human ankle stiffness during quiet standing: the intrinsic mechanical stiffness is insufficient for stability. *J Physiol* 2002;545(3):1041–53.
26. Loram I.D, Maganaris C.N, Lakie M. Active, non-spring-like muscle movements in human postural sway: how might paradoxical changes in muscle length be produced? *J Physiol* 2005;564(1):281–93.
27. Kiel C. G Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences 2007;39(2):175–191.
28. Erdfelder E. Statistical power analyses using G * Power 3.1: Tests for correlation and regression analyses 2009;41(4):1149–1160.
29. Schulz K, Altman D, Moher D. Consort 2010 statement: updated guidelines for reporting parallel group randomised trials. *BMJ* 2010;340:c332.
30. Padulo J, Oliva F, Frizziero A, Maffulli N. Muscles, Ligaments and Tendons Journal. Basic principles and recommendations in clinical and field Science Research: 2018 update. *MLTJ* 2018; 8(3): 305–7.
31. Ross SE, Guskiewicz KM. Examination of static and dynamic postural stability in individuals with functionally stable and unstable ankles. *Clin J Sport Med* 11/2004;14(6):332–8.

Estimates of Stress Between the Hamstring Muscles

C. Perrin¹, D. Smith², J. Steele^{1,3}

¹ School of Sport, Health and Social Sciences, Solent University, Southampton, UK

² Department of Exercise and Sport Science, Manchester Metropolitan University, Manchester, UK

³ Ukactive Research Institute, London, United Kingdom

CORRESPONDING AUTHOR:

Craig Perrin
School of Sport, Health
and Social Sciences
Solent University
Southampton, UK
E-mail: Craig.Perrin@solent.ac.uk

DOI:

10.32098/mltj.03.2020.12

LEVEL OF EVIDENCE: 5

SUMMARY

Background. The cause of muscle damage and injury is often attributed to large strains. Studies have suggested that strain is the principal cause of muscle damage rather than force.

Results. In this paper we show that force is the principal cause of muscle damage whereas strain is a means of increasing force. The subtle difference has important implications, as many studies use strain as an indicator for injury risk. In addition, we show that the data better supports a theory of stress as the principal cause of injury, rather than force alone, and aligns with both the myofibril and observational data.

Conclusions. The implications of a stress-based model of injury is discussed within the paper.

KEY WORDS

Force; strain; stress; damage; injury; hamstring.

INTRODUCTION

Hamstring injuries can be considered to result from an indirect mechanism (1), though two specific mechanisms of hamstring injury appear to exist and are referred to as stretching and high-speed (forceful) (2). This classification arises from observations in dancers where hamstring strain injuries occur at low forces but long lengths (3) and injuries in sprinters that occur at short lengths but with high forces (4). Thus, these two posited mechanisms can be reduced to either excessive strain or excessive force. The mechanism also determines the muscle susceptible to injury. The semimembranosus (SM) is prone to injury under excessive strain (3) whereas the biceps femoris (BF) is at risk in forceful scenarios, such as sprinting (4). This suggests a unique quality exists dependent on the mechanism that renders each muscle susceptible to injury.

However, the notion of two separate mechanisms for strain injury is at odds with the experimental research in myofibrils, which suggests strain is the principal cause of injury (5,6). In accordance, simulation studies have found the biceps femoris is most lengthened whilst sprinting appearing to explain its susceptibility (7-10). Yet, if lengthening was the determiner of injury, then isometric contractions should not induce damage, but it is reported to do so (10). This is not the case. Likewise, although the biceps femoris is most lengthened during sprinting, its absolute length

is small. At larger absolute strains the semimembranosus is more commonly injured (3). Another variable must exist to explain this discrepancy.

The role of myofiber strain in injury mechanics

The notion of strain causing injury rather than force arises from a seminal study in myofibrils that compared strain magnitudes of 12.5% to 25% with either high or low levels of force, achieved by manipulating the delay between onset and strain (5). Lieber and Friden found that regardless of force, damage was always greater in groups with larger strains, yet similar between muscles exposed to different forces. However, according to Hook's Law (Eq.1) the spring constant ($-k$) means force (F) is proportional to strain (s). Thus, the high strain group intended to have low force could in fact have had greater force than the low strain group intended for high force. This would confound any attempt to attribute injury to strain or force.

It was reported that force was 40% greater in myofibrils in the high force group compared to the low force group but was only reported for myofibrils with a large strain magnitude. No results of force are given for the low strain group. Through Eq. 1, it is possible to estimate the forces in myofibrils of different strains and intended forces from Lieber and Friden. Therefore, the aim of this investigation was to

conduct a secondary analysis of their results to estimate the force in myofibrils of different strains and force (where the high force group correspond to the delayed onset group in Lieber and Friden). It is hypothesised that force in the high strain/low force group will be greater than the low strain/high force group.

METHODS

Myofibril forces for the high strain groups were calculated using data provided by Lieber and Friden. For the high strain/low force group, absolute force was obtained using *WebPlotDigitizer* (11) and Figure 2A of Lieber and Friden, and then converted from grams to newtons using a ratio of 102:1. This showed myofibrils of the low force/high strain group produced 14 N of force. For the high force/low strain group, Lieber and Friden state force is 1.4 times greater than the low force group with 25% strain equalling 20 N of force. Myofibril forces for the low strain groups were calculated using Hook's Law (Eq. 1), which requires the absolute strain and the spring constant. The nominal length for each fibre is 55 mm so absolute strain can be calculated as 25% and 12.5% of this value. The large strain group was calculated to be 13.75 mm and the low strain group this equalled 6.875 mm. Spring constants for myofibrils were calculated using Hook's Law, by dividing force by the absolute strain in each of the 25% strain groups. As myofibrils were homogenous, the spring constants are expected to be the same for groups within high or low force regardless of the strain. For the low force group (k_{ES}), the spring constant was 1.02 N/mm, and for the high force group the (k_{LS}) was 1.45 N/mm. Thus, force in the low strain groups of high and low force were obtained by multiplying the absolute strain of 6.875 mm by their respective spring constant, as per table I. All methods were done in accordance with the ethical standards of the journal (12).

RESULTS

Table 2 shows myofibril forces are greater in in the high strain/low force group compared to the low strain/high force group (14 N and 10 N respectively). The mean differ-

ence in force between timings is ~4.5 N, whereas the mean difference in force between strains is ~8.5 N. The outcomes are displayed in **table II**.

DISCUSSION

Friden and Lieber concluded strain is the principal cause of injury based on finding a significant effect of strain on force reductions whereas onset time (used to manipulate myofibril force) had no significant effect on force. However, the forces within these groups were not reported. The results of this analysis show that at high strains, the group intended to represent low force had 4 N greater force than the group intended for higher force but at low strains. The suggestion that strain is responsible for injury rather than force is incorrect, as strains leads to greater forces. The additional 4 N of force from greater strains is considerable given the peak tetanic tension in myofibrils is ~13N, and suggests that lengthening, on average, increased force by 31% of its peak force than when manipulating the force production through onset time.

As strain created greater forces, then it may seem that Lieber and Friden were correct in their conclusion that lengthening causes injury. Whilst we agree with Lieber and Friden that greater strains lead to greater muscle damage, stating it is not the result of high force is incorrect and not supported by the data. Instead, lengthening is a means of achieving greater forces. Under maximal conditions, and homogenous myofibrils, such as those used by Friden and Lieber, the larger strains will cause greater forces and thus injury. But this relationship does not hold between heterogeneous muscles because of differences in passive tension and active force production. Therefore, identifying force as the cause of injury is a subtle but important difference when comparing muscles.

For example, multiple studies have founded their work on Lieber and Friden and used peak strain to determine the hamstring muscle vulnerable to injury (7-10, 13-15). Yet many of these papers show active force differs between the muscles and therefore do not indicate the muscle most susceptible to injury (7-9). Without understanding the

Table I. Calculations to estimate stress.

Strain		Force	
Relative Strain (%)	Absolute strain (mm)	Low force (N)	High force (N)
25	$S_{25} = 55 \times 0.250$	A = 14	B = A × 1.40
12.5	$S_{12.5} = 55 \times 0.125$	C = $k_{ES} \times S_{12.5}$	D = $k_{LS} \times S_{12.5}$

Table II. Calculated strain and force values

Strain		Force (N)	
Relative Strain (%)	Absolute strain (mm)	Low force group	High force group
25	13.75	14	20
12.5	6.875	7	10

underpinning cause of injury it's possible the protocols put forward for investigation and practice are entrenched on a false understanding. For example, Guex claimed the optimal exercise for hamstring strengthening does not exist with limited strain being one of the reasons (16). This criticism has been echoed for the Nordic hamstring curl also (17). Yet, these conclusions may still be limited to myofibrils as the largest contributor to force when sprinting is the semi-membranosus, and not the commonly injured biceps femoris (7,9,18). Sprint simulations have also combined force and strain through Work to explain the biceps femoris' vulnerability, but this too has failed. Despite force seemingly causing injury in myofibrils, the current outcomes from sprint simulations do not identify the vulnerability of the biceps femoris in this common injury inducing movement. A new perspective is warranted to converge the observed data from simulations to that in myofibril experiments.

Stress as an explanation for injury

It is well established in Newtonian mechanics that materials fracture under excessive tensile stress (19). Stress (σ) is the measure of the internal force (F) acting in a localised area (A), and can be estimated by dividing the muscle force by its cross-sectional area (Eq. 2).

From the perspective that muscle is a biological material, the cause of fracture should not differ. Thus, stress is likely to be the principle cause of injury. In contrast to reported outcomes, this requires not only the forces to be considered but the area of the muscle too. In myofiber research the cross-sectional area (CSA) of homogenous myofibrils is expected to be similar and therefore differences in force would be proportional to stress, such that the force induced by strain would appear as the determining factor. As muscles differ in CSA, this does not hold true.

Stress as the cause of injury can explain why the myotendinous junction (MTJ) is a prevalent location for injury as the area lessens as the muscle tapers to the free tendon (20). This has been alluded to by Storey *et al.*, but an explicit investigation has not been performed. Earlier studies have investigated the aponeurosis size in relation to eccentric strength ($r = 0.24$; $p > 0.2$) but not regarding injury incidence (21).

The semimembranosus is the largest producer of force whilst sprinting, but a large CSA would reduce the stress. Conversely, a small CSA in biceps femoris would increase its stress and propensity for injury. The aim of this study was to identify whether peak stress is greatest in the biceps femoris by approximating the stress at each hamstring's MTJ during sprinting using previous simulations and morphology data. The hypothesis was that peak MTJ stress will be greatest in the biceps femoris.

METHODS

According to Eq. 2, the peak stress at each hamstring's MTJ can be derived by their respective dividing peak force by the CSA of each MTJ. Estimates of each muscles MTJ CSA was obtained from the results of Storey (2016). A two-way 95% CI for each MTJ CSA was estimated from the data provided by Storey. The MTJ area data was obtained from different participants to those used in simulations which may reduce the validity of these estimates; however, aponeurosis area is not related to muscle size or area (Evangelidis *et al.* 2015) and so the average MTJ areas from Storey are a fair approximation of the participant populations used in the simulation studies.

The peak force for each hamstring muscle was obtained by combining the results of simulation research that used data from sprinting. Sprint data was used as this is a common and ubiquitous action during injury (22) and appears to produce the greatest hamstring activity (23). Sprint simulations were identified through prior knowledge and confirmed via a PubMed search using the terms *sprint**, *Simulation*, and *hamstring* connected with the 'AND' Boolean operator. To be included, simulation studies had to identify the peak force for all 3 biarticular hamstrings (SM, semitendinosus [ST], and BF) whilst performing high-speed sprinting.

To account for the between study variability in peak force estimates, a random effects meta-analysis (24) was performed using R statistical software and peak force from each simulation to derive an estimate of the mean and its 95% confidence interval (CI) for peak force in each muscle. The point estimates for the mean and bounds of the 95% CIs for peak force and MTJ CSA were combined using Eq. 2 to obtain an estimate of the mean peak stress in each hamstring muscle and the bounds for the 95% CI.

RESULTS

Three studies were retrieved for analysis of force (7-9) with a combined total of 38 participants (28 males and 10 females; Age: 24; stature: 177 cm; mass: 73 kg). For the morphology data, 5 male cadavers (10 limbs) were used (mean age: 75 years; Storey 2016).

Table III includes the random effects 95% CIs for peak force among the 3 simulation studies and the confidence interval for MTJ area for each muscle. On average, the biceps femoris experiences 4.24 N·cm² more stress than the SM and 7.36 N·cm² more than the ST. The variability in the biceps femoris stress estimate is also greater than the SM and ST. This is highlighted at the upper bounds of the stress estimate where the difference between BF and SM increased to 9.73 N·cm.

Table III. Estimates of 95% confidence intervals for peak force, MTJ area, and MTJ stress.

	Muscle	Lower bound	Mean	Upper bound
Peak force* (N)	BF	861	1408.4	1960.7
	SM	1470	2289	3108
	ST	340.2	450.1	559.3
MTJ Area (cm ²)	BF	39.4	45.0	50.6
	SM	62.1	84.6	107.1
	ST	16.2	18.8	21.4
MTJ Stress (N·cm ²)	BF	21.85	31.30	38.75
	SM	23.67	27.06	29.02
	ST	21.00	23.94	26.14

Note: *Absolute peak force has been calculated for a 70 kg person.

DISCUSSION

The aim of this investigation was to estimate whether stress could identify the biceps femoris vulnerability to injury during high-speed running. The findings presented here show the biceps femoris experiences the greatest MTJ stress. Prior to this study, strain was the only outcome to identify the biceps femoris vulnerability, but it is implausible for strain alone to be responsible for injury. Thus, stress is the first variable to align with both simulated and myofibril research. Chumanov's *et al.* (2011) simulation found the ST had greater peak force than the BF. However, the BF in this study had a comparatively small force contribution which seems to occur from the notably low BF excitation during simulation that does not align with electromyographic data (8). As a result, the BF stress in this study are likely underestimated and may be greater in reality.

Stress as the cause of injury has fundamental implications towards our understanding of injury mechanics. Previously, lengthening was believed to be the fundamental cause of injury (5,8), but as demonstrated, lengthening likely causes injury because it results in greater forces. The notion of stress rather than strain as the muscle damaging factor aligns with earlier findings that showed muscle damage after shortening muscle actions (25). A phenomenon not possible according to the lengthening model. Nonetheless, strain may have an additive effect to injury risk separate from the increased passive tension. The volume of the MTJ does not change during lengthening therefore an increase would cause a decrease in MTJ area and a subsequent increase in stress (19). Although not the principal cause, the greater strain in the BF whilst sprinting may compound its vulnerability to injury here. Estimates of maxima hamstring muscle CSA show the SM undergoes the smallest reduction (2.3%) compared to the BF which reduces the most (8.6%) (26).

Therefore, stress at peak lengths for the biceps femoris are expected to be considerably larger than estimated here, as MTJ measurement used in this analysis were measured *ex vivo* and not lengthened.

More critical implications exist for our understanding of injury prevention. The maximum force in a local area that a material can withstand could be the principal factor in preventing injury. Understanding the factors that determine this threshold would be of great value for optimising injury prevention programmes and screening. For example, the protective role of structures binding actin to the extracellular matrix has been shown in mice studies. Mice over-expressing the α 7BX2 integrin (a common isoform of a muscular integrin) display reduced membrane damage after downhill running ($p < 0.05$) suggesting the increase in integrin and actin to laminin connections increase structural soundness (27). Adding micro dystrophin to mdx mice also reduces muscle damage (28). Likewise, the ACTN3 R577X polymorphism (alpha actinin 3 which binds to z discs) is associated with greater shear modulus in the hamstrings, suggesting larger stress and thus risk (29). Whilst the polymorphism was not associated to injury, the stress value for the modulus was calculated at the muscle belly and not the MTJ (29).

Reduced ability to produce eccentric force is associated with hamstring injury (30,31) and accordingly strength training has been shown to reduce hamstring injury incidence (32). Yet, if stress is the principal factor causing injury, then increasing strength should increase the risk of injury. One explanation is that strength training increases collagen in the MTJ, likely to manage to the new capacity to produce force (33). In addition, eccentric strengthening may be important for reducing fascicle strain (24) and thus reducing the passive force component from strain.

There are inevitable limitations with the approach used in this investigation. Using the peak force data from simulations for comparison assumes that the force distribution is equivalent within each muscle. This is unlikely considering fascicle strain appears non-linear (34), but lengthening is concentrated towards the MTJ adding validity to use of peak force in MTJ stress estimates.

From a practitioner perspective, it may be more effective to utilise supramaximal eccentric exercise at short muscle lengths to expose muscles to high stress without reducing MTJ CSA and causing excessive stresses. This progression would seemingly minimise muscle damage (and the possible acute injury risk) and improve adherence to prevention programmes, where longer length training could impair adherence due to soreness (35). Future research should investigate whether muscle damage can be limited by grad-

ually progressing eccentric exercise to longer lengths whilst still inducing adaptations.

CONCLUSIONS

Until now, simulation studies have failed to identify a cause of injury that aligns with the understanding of injury in myofibril research. Using the data of simulations, this study has shown that stress is greatest for the biceps femoris whilst sprinting and may explain its susceptibility to injury, particularly at the MTJ. Future simulation studies should include stress as an outcome to calculate more valid estimates for each hamstring muscle to confirm these findings.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

REFERENCES

- Maffulli N, Oliva F, Frizziero A, Nanni G, Barazzuol M, Via Ag, Et Al. Ismuilt Guidelines for Muscle Injuries. *Muscles, Ligaments and Tendons Journal*. 2013 Oct;3(4):241-9.
- Askling C, Saartok T, Thorstensson A. Type of Acute Hamstring Strain Affects Flexibility, Strength, And Time to Return to Pre-Injury Level. *British Journal of Sports Medicine*. 2006;40(1):40-4.
- Askling C, Tengvar M, Saartok T, Thorstensson A. Acute First-Time Hamstring Strains During Slow-Speed Stretching. *The American Journal of Sports Medicine*. 2007;35(10):1716-24.
- Askling C, Tengvar M, Saartok T, Thorstensson A. Acute First-Time Hamstring Strains During High-Speed Running. *The American Journal of Sports Medicine*. 2007;35(2):197-206.
- Lieber RL, Fridén J. Mechanisms of Muscle Injury Gleaned from Animal Models. *American Journal of Physical Medicine & Rehabilitation*. 2002 Nov;81(Supplement):S70-9.
- Patel T, Das R, Fridén J, Lutz G, Lieber R. Sarcomere Strain and Heterogeneity Correlate with Injury to Frog Skeletal Muscle Fiber Bundles. *Journal of Applied Physiology*. 2004;97(5):1803-13.
- Schache A, Dorn T, Blanch P, Brown N, Pandy M. Mechanics of The Human Hamstring Muscles During Sprinting. *Medicine and Science in Sports and Exercise*. 2012;44(4):647-58.
- Chumanov E, Heiderscheid B, Thelen D. Hamstring Musculotendon Dynamics During Stance and Swing Phases of High-Speed Running. *Medicine and Science in Sports and Exercise*. 2011;43(3):525-32.
- Chumanov E, Heiderscheid B, Thelen D. The Effect of Speed and Influence of Individual Muscles on Hamstring Mechanics During the Swing Phase of Sprinting. *J Biomech*. 2007;40(16):3555-62.
- Thelen D, Chumanov E, Hoerth D, Best T, Swanson S, Li L, Et Al. Hamstring Muscle Kinematics During Treadmill Sprinting. *Medicine and Science in Sports and Exercise*. 2005;37(1):108-14.
- Rohatgi A. *Webplotdigitizer*. 2019;4.2.
- Padulo J, Oliva F, Frizziero A, Maffulli N. *Muscles, Ligaments and Tendons Journal - Basic principles and recommendations in clinical and field Science Research: 2018 update*. *MLTJ* 2018; 8(3): 305 – 307.
- Yu B, Queen Rm, Abbey An, Liu Y, Moorman Ct, Garrett We. Hamstring Muscle Kinematics and Activation During Over-ground Sprinting. *J Biomech*. 2008;41(15):3121-6.
- Higashihara A, Nagano Y, Takahashi K, Fukubayashi T. Effects of Forward Trunk Lean on Hamstring Muscle Kinematics During Sprinting. *Journal of Sports Sciences*. 2015 Aug 9;33(13):1366-75.
- Higashihara A, Nagano Y, Ono T, Fukubayashi T. Relationship Between the Peak Time of Hamstring Stretch And Activation During Sprinting. *European Journal of Sport Science*. 2016;16(1):36-41.
- Guex K, Millet G. Conceptual Framework for Strengthening Exercises to Prevent Hamstring Strains. *Sports Med*. 2013 Dec;43(12):1207-15.
- Milanese S, Eston R. Hamstring Injuries and Australian Rules Football: Over-Reliance on Nordic Hamstring Exercises as A Preventive Measure? *Open Access Journal of Sports Medicine*. 2019 Jul 1;10:99-105.
- Chumanov Es, Heiderscheid Bc, Thelen Dg. Hamstring Musculotendon Dynamics During Stance and Swing Phases of High-Speed Running. *Medicine and Science in Sports and Exercise*. 2011;43(3):525-32.
- Engineering Materials 1* [Homepage on The Internet]. Oxford: Butterworth-Heinemann. 2012.
- Storey R, Meikle G, Stringer M, Woodley S. Proximal Hamstring Morphology and Morphometry in Men: An Anatomic and Mri Investigation. *Scandinavian Journal of Medicine & Science in Sports*. 2016;26(12):1480-9.
- Evangelidis P, Massey G, Pain M, Folland J. Biceps Femoris Aponeurosis Size: A Potential Risk Factor for Strain Injury? *Medicine & Science in Sports & Exercise*. 2015;47(7):1383-9.

22. Opar Da, Williams Md, Shield Aj. Hamstring Strain Injuries: Factors That Lead to Injury and Re-Injury. *Sports Med.* 2012 Mar 1;42(3):209-26.
23. Van Dt, Solheim Jab, Bencke J. Comparison of Hamstring Muscle Activation During High-Speed Running and Various Hamstring Strengthening Exercises. *International Journal of Sports Physical Therapy.* 2017;12(5):718-27. Available From: [Http://Dx.Doi.Org/10.26603/Ijspt20170718](http://Dx.Doi.Org/10.26603/Ijspt20170718).
24. Viechtbauer W. Conducting Meta-Analyses in R with The Metafor Package. *Journal of Statistical Software.* 2010;36(3):1-48.
25. Gibala M, Macdougall J, Tarnopolsky M, Stauber W, Elorriaga A. Changes in Human Skeletal Muscle Ultrastructure and Force Production After Acute Resistance Exercise. *Journal of Applied Physiology.* 1995;78(2):702-8.
26. Dai Nakaizumi, Hitoshi Asai, Pleiades Tiharu Inaoka, Naoki Ohno, Tosiaki Miyati. Measurement of The Cross-Sectional Area of The Hamstring Muscles During Initial And Stretch Positions With Gravity Magnetic Resonance Imaging. *Journal of Physical Therapy Science.* 2019;31(3):267-72.
27. Boppart M, Burkin D, Kaufman S. Alpha7beta1-Integrin Regulates Mechanotransduction And Prevents Skeletal Muscle Injury. *Am J Physiol Cell Physiol.* 2006;290(6):1660.
28. Banks G, Combs A, Chamberlain J. Molecular and Cellular Adaptations to Chronic Myotendinous Strain Injury in Mdx Mice Expressing A Truncated Dystrophin. *Hum Mol Genet.* 2008;17(24):3975-86.
29. Miyamoto N, Miyamoto-Mikami E, Hirata K, Kimura N, Fuku N. Association Analysis of The Actn3 R577x Polymorphism with Passive Muscle Stiffness and Muscle Strain Injury. *Scandinavian Journal of Medicine & Science in Sports.* 2018;28(3):1209-14.
30. Timmins R, Bourne M, Shield A, Williams M, Lorenzen C, Opar D. Short Biceps Femoris Fascicles and Eccentric Knee Flexor Weakness Increase the Risk of Hamstring Injury in Elite Football (Soccer): A Prospective Cohort Study. *British Journal of Sports Medicine.* 2016;50(24):1524-35.
31. Lee J, Mok K, Chan H, Yung P, Chan K. Eccentric Hamstring Strength Deficit and Poor Hamstring-To-Quadriceps Ratio Are Risk Factors for Hamstring Strain Injury in Football: A Prospective Study Of 146 Professional Players. *J Sci Med Sport.* 2018 [Cited Feb 27, 2019];21(8):789-93.
32. Van Der Horst N, Smits D, Petersen J, Goedhart E, Backx F. The Preventive Effect of The Nordic Hamstring Exercise on Hamstring Injuries in Amateur Soccer Players: A Randomized Controlled Trial. *American Journal of Sports Medicine.* 2015;43(6):1316-23. Available From: [Https://Www.Narcis.Nl/Publication/Recordid/Oai:Dspace.Library.Uu.Nl:1874%2f331854](https://www.Narcis.Nl/Publication/Recordid/Oai:Dspace.Library.Uu.Nl:1874%2f331854).
33. Jakobsen J, Mackey A, Knudsen A, Koch M, Kjær M, Krosgaard M. Composition and Adaptation of Human Myotendinous Junction and Neighboring Muscle Fibers To Heavy Resistance Training. *Scandinavian Journal of Medicine & Science in Sports.* 2017;27(12):1547-59.
34. Fiorentino N, Rehorn M, Chumanov E, Thelen D, Blemker S. Computational Models Predict Larger Muscle Tissue Strains at Faster Sprinting Speeds. *Medicine & Science In Sports & Exercise.* 2014;46(4):776-86.
35. Mjølunes R, Arnason A, Østhagen T, Raastad T, Bahr R. A 10-Week Randomized Trial Comparing Eccentric Vs. Concentric Hamstring Strength Training in Well-Trained Soccer Players. *Scandinavian Journal of Medicine & Science in Sports.* 2004;14(5):311-7.

Combined Treadmill Running and Insulin Therapy Favors the Stabilization of Glycemic Metabolic Parameters and Avoids Increased Achilles Tendon Rigidity in Diabetic Rats

A. C. Nobre de Lacerda Brito¹, W. Alves Martins¹, P. C. da Silva Queiroz¹, S. E. Veríssimo Santos¹, T. Meireles Santos², R. F. de Souza^{3,4}, D. Dutra Pereira³, M. T. Gonzaga Santos⁴, A. C. Falcão Esteves¹, S. R. Arruda de Moraes¹

¹ Laboratory of Neuromuscular Plasticity, Department of Anatomy, Center of Biosciences, Federal University of Pernambuco, Recife, Brazil

² Department of Physical Education, Federal University of Pernambuco, Recife, Brazil

³ Department of Physiology and Pharmacology, Center of Biosciences, Federal University of Pernambuco, Recife, Brazil

⁴ Department of Physical Education, Federal University of Sergipe - UFS, São Cristovão, Sergipe, Brazil

CORRESPONDING AUTHOR:

Ana Camila Nobre de Lacerda Brito
Federal University of Pernambuco
Neuromuscular Plasticity Laboratory
Department of Anatomy
Avenida Professor Moraes Rego, 1235,
Recife 50670-901
Pernambuco, Brazil
E-mail: britoanacamila@gmail.com

DOI:

10.32098/mltj.03.2020.14

LEVEL OF EVIDENCE: 2B

SUMMARY

Background. Hyperglycemia reduces tendon homeostasis. Effects of physical exercise on diabetic rats have been widely studied; however, the effects of a combined physical and insulin therapy on biomechanical properties of the Achilles tendon (AT) remain unclear. Therefore, the objective of this study was to evaluate the effects of the combination of moderate-intensity exercise on a treadmill and insulin therapy on metabolism, physical conditioning, and biomechanics of AT in diabetic rats.

Methods. Forty-eight Wistar rats were divided into six groups: Sedentary Control-SCG, Treadmill Control-TCG, Sedentary Diabetic-SDG, Sedentary Insulin Diabetic-SIDG, Treadmill Diabetic-TDG, and Treadmill Insulin Diabetic-TIDG. Diabetic animals were induced with streptozotocin diluted in sodium citrate buffer (50 mg/Kg; 10 mM; pH 4.5; intraperitoneally). All groups were subjected to the maximal effort test for pre (MET₁) and post (MET₂) maximal speed determination. The exercise protocol was administered for 5 weeks (1 h/day, 5 days/week). Blood glucose levels and biomechanical properties of AT (e.g., traction) were evaluated.

Results. Increased glycemia was observed in SDG (p<0.001; p=0.003), SIDG (p=0.009; p=0.037), and TDG (p=0.002; p=0.009); however, compared with SCG and TCG, TIDG showed no significant differences. Maximal force was reduced in TIDG (p=0.009) and SIDG (p = 0.002) and increased in SCG and TDG compared with in SIDG (p=0.024). The elastic modulus was reduced in TCG compared with in SCG (p = 0.011) and increased in SDG (p<0.001), SIDG (p=0.019), and TDG (p=0.006) compared with in TCG.

Conclusions. The combined physical exercise and insulin therapy favors the stabilization of glycemic metabolic parameters and avoids increased tendon rigidity in diabetic rats.

KEY WORDS

Achilles tendon; diabetes mellitus; maximal effort test.

BACKGROUND

Regular physical exercise concomitant with hyperglycemic control has been widely investigated for the treatment of type 1 diabetes mellitus (DM1) (1,2). Therefore, insulin therapy (IST) is commonly used to control hyperglycemia and other complications of diabetes, such as diabetic ketoacidosis and hyperosmolar states (2).

Insulin therapy is indicated for DM1 cases (3) because high glycemic indexes are associated with cardiometabolic (4) and musculoskeletal disorders (4-7), including tendon damage such as diabetic tendinopathy (5,8).

Evidence suggests that hyperglycemia reduces tendon homeostasis as a consequence of a series of changes, including vascular hyperplasia, disorganized collagen fibers, decrease collagen production, increased advanced glycation end products (AGEs) (9-11), and altered viscoelastic properties (12). Such changes may increase tendon stiffness, thereby inducing diabetic tendinopathy (8) and causing degeneration and rupture (5).

Meanwhile, when physical exercise was regularly performed, physiological and biomechanical benefits, such as increased collagen concentration, fibrillary density (13), elastic module, maximal tension, cross-sectional energy/area, and maximal force (14) as well as decreased thickness and neovascularization (15), were observed in the Achilles tendon (AT) of patients with calcaneus tendinopathy (15,16).

Recent studies in DM1-induced animals have reported positive effects of moderate-intensity aerobic exercise (running and swimming) on the restoration of the elastic characteristics of AT, preventing the progression of tendon degeneration (17,18). However, these studies did not implement IST during exercise. Assuming that IST can prevent the onset of musculoskeletal chronic complications (18) and optimize motor skills in DM1-induced rats (19), we hypothesized that IST concomitant with physical exercise can boost the biomechanical properties of AT.

Considering that some precautions should be ensured regarding exercise practice in patients with diabetes to appropriately prescribe safe exercise intensity, in the present study, a moderate-intensity treadmill exercise standardization model was adopted using the maximal effort test proposed by Brito et al. (20).

The present study aimed to investigate the effects of a combination of moderate-intensity aerobic exercise on a treadmill and IST on the biomechanical properties of AT in as well as the clinical status and physical fitness of DM1-induced rats.

MATERIALS AND METHODS

Experimental design

To evaluate the effects of the combination of IST and moderate-intensity treadmill exercise on AT in healthy and diabetic rats, this study was divided into four stages: (1) distribution of animals into six groups, induction of experimental diabetes, and onset of IST; (2) adaptation to the treadmill and administration of the first maximal effort test (MET_1); administration of the 5-week protocol of moderate-intensity treadmill exercise and the second maximal effort test (MET_2); material collection and administration of AT traction biomechanical test. The study was performed at the Neuromuscular Plasticity Laboratory of the Anatomical Department and the Biopolymer Laboratory of the Chemical Engineering Department of the Federal University of Pernambuco (UFPE).

Forty-eight male Wistar rats (234.4 ± 28.1 g) were housed in cages at $23 \pm 1^\circ\text{C}$ under a 12-h inverted light/dark cycle. Presence Food (Neovia, São Paulo, Brazil) and water were provided *ad libitum*. Animals that did not present a fasting blood glucose level >200 mg.dL⁻¹ on day 7 after the induction of experimental diabetes, did not continuously run during the adaptation week, and reached a MET_1 maximal speed <10 m.min⁻¹ were excluded. Ethical approval of the study was obtained from the Committee of the Federal University of Pernambuco Bioscience Center (number 23076.050209/2016-10) in accordance with the norms recommended by the Brazilian Committee for Animal Experimentation. The research was conducted ethically according to international standards and as required by the journal (21).

PROCEDURES

Animal distribution into groups and induction of experimental diabetes

At the age of 50 days, animals were randomly assigned to six groups (n = 8 per group): sedentary control group (SCG), treadmill control group (TCG), sedentary diabetic group (SDG), treadmill diabetic group (TDG), sedentary insulin diabetic group (SIDG), and treadmill insulin diabetic group (TIDG). Experimental diabetes was induced in animals in the diabetic groups at the age of 60 days, and after 12 h of fasting, measure body weight (FILIZOLA BP6 Digital Scale) and blood glucose (Accu-Chek Active Kit Glucometer) were measured. Experimental diabetes was induced

via the administration of streptozotocin (50 mg.kg⁻¹; STZ, Sigma Aldrich Co., St. Louis, MO) diluted in sodium citrate buffer (10 mM; pH 4.5; intraperitoneally [i.p.]) (22). Animals in the control groups received an injection of sodium citrate buffer alone (23).

Insulin therapy

From day eight after the induction of experimental diabetes, insulin (Eli Lilly and Company) was subcutaneously administered (24) (2 IU/day/mouse; HUMULIN® N) daily to animals in SIDG and TIDG, 4–6 h after the exercise sessions and throughout the experimental protocol (3).

Treadmill adjustment period, MET, and moderate-intensity treadmill exercise protocol

All animals were subjected to treadmill adaptation nine days after the induction of experimental diabetes. Treadmill adaptation was performed for three days (10 min.day⁻¹; 5 m.min⁻¹; 0° inclination) on the multiple rodent treadmill (AVS Projects). On day 4, MET₁ was administered at an initial speed of 5 m.min⁻¹, with a progressive increase in speed by 5 m.min⁻¹ every 3 min. The maximum intensity for each rat was determined when the animal touched the back wall of the treadmill belt five times within 1 min (19,20,22). After 72 h of MET₁, the adapted protocol (19) was initiated at moderate intensity (20,25) in animals in TCG, TDG, and TIDG over 5 weeks (0° inclination; 1 h/day; 5 days/week). Each session comprised three periods: warm-up, moder-

ate-intensity exercise, and recovery. During the first week, time and speed were progressively increased. To encourage the animals, a low-intensity electrical stimulus (1.5–2.0 mA) located at the end of each lane was used.

Approximately 48 h after the last exercise session, MET₂ was administered. All protocols are detailed in **table I**.

Clinical and metabolic evaluation, material collection, and euthanasia

After the experimental period, weight and blood glucose levels were measured in all animals. This was followed by anesthesia with xylazine solution and ketamine hydrochloride (Anasedan®, 20 mg.kg⁻¹ and Dopalen®, 100 mg.kg⁻¹, respectively; 0.1 mL and 0.1 mL for each 100 g of animal weight, respectively, intraperitoneal). An incision was made on the upper surface of the right paw for the visualization and dissection of the myotendinous complex of the *triceps surae* muscle while preserving the distal fixation of the calcaneal bone and the animal’s paw. The anatomical sample was moistened with saline and sent to the Biopolymer Laboratory at UFPE for biomechanical assay. After the collection of biological material and while still under the effect of anesthesia, animals were euthanized via the intracardiac injection of 1 mL potassium chloride (10%).

Tendon traction mechanical test

Biomechanical testing was performed on the mechanical tensile testing conventional machine (EMIC, Model DL 500,

Table I. Treadmill exercise protocol applied in the diabetic treadmill group (TDG) and diabetic treadmill insulin group (TIDG).

Protocol	Week or Day	Time (minutes)	Training
Adaptation	1st day	10	Velocity: 5m.min ⁻¹
	2nd day	10	
	3rd day	10	
Maximum effort test	4th day	Varies according to animal	Initial velocity of 5 m.min ⁻¹ being increased every 3 min at 5 m.min ⁻¹ until fatigue
Moderate intensity exercise	1st day	20	5min at 30% _{MET1} + 10 min at 50% _{MET1} + 5min at 30% _{MET1}
	2nd day	30	5min at 30% _{MET1} + 20 min at 50% _{MET1} + 5min at 30% _{MET1}
	3rd day	40	5min at 30% _{MET1} + 30 min at 50% _{MET1} + 5min at 30% _{MET1}
	4th day	50	5min at 30% _{MET1} + 40 min at 50% _{MET1} + 5min at 30% _{MET1}
	5th day	55	5min at 30% _{MET1} + 45 min at 50% _{MET1} + 5min at 30% _{MET1}
	2nd week	60	5min at 30% _{MET1} + 50 min at 60% _{MET1} + 5min at 30% _{MET1}
	3rd week	60	
	4th week	60	
	4th week	60	
	5th week	60	

Min - minutes; % - percentage; MET₁: Maximum Speed Test.

Brazil, 500 N). The ellipse formula was applied to measure the tendon cross-sectional area (26), as shown in Equation I. Subsequently, the sample was connected to the machine via two pieces that were previously made for this type of biological material, keeping the sample in the anatomical position. The length of the sample was then measured using a caliper (Vernier Calipers, 0.05 mm), and specimen traction was performed at a speed of 0.1 mm.s⁻¹ to its point of failure. Maximal force, maximal force tension, cross-sectional energy/area, maximal strain, specific strain, and elastic modulus were calculated using the stress vs. strain graph generated by the Tesc® software coupled with the biomechanical testing machine.

$$CSA = (D \times d / 4) \times \pi$$

Where:

- CSA = the cross-sectional area;
- D = the larger longest of the medial region of AT;
- d = the shortest diameter of the medial region of AT;
- and $\pi = 3.1416$

STATISTICAL ANALYSIS

After verifying the data assumptions for parametric analysis using Kolmogorov–Smirnov test, one-way ANOVA was performed, followed by post hoc Bonferroni test, for the

analysis of variables with normal distribution. When normality was not verified, Kruskal–Wallis test was used, followed by pairwise comparison. Parametric data were presented as mean and standard deviation and non-parametric data as median and interquartile range. To assess the effect of size, Cohen’s *d* (27) was used. All analyses were performed using SPSS version 20. A significance level of 5% was adopted.

RESULTS

Of the 32 animals with induced diabetes, two did not exhibit a blood glucose level >200 mg.dL⁻¹ on day 7 after the induction of experimental diabetes and were excluded from the study. At the beginning of the study, all animals presented homogeneous parameters of body weight ($p = 0.367$) and glycaemia ($p = 0.094$). At the end of the exercise protocol, blood glucose levels and body weights in TIDG were similar to those in SCG ($p = 1.000$) and TCG ($p = 1.000$). However, compared with the nondiabetic groups (SCG and TCG), the diabetic groups (SDG, SIDG, and TDG) exhibited increased glycemic index ($p < 0.05$). Reduction in body weight was observed only in SDG (SDG vs. SCG: ↓ 24.03%, $p < 0.001$, $d = 2.34$ and SDG vs. TCG: ↓ 22.99%, $p = 0.003$, $d = 2.41$). Conversely, compared with the nondiabetic groups, the diabetic groups with IST alone or combined with treadmill exercise showed similarity in body weight ($p > 0.05$; **figure 1**). Increased blood glucose was observed in

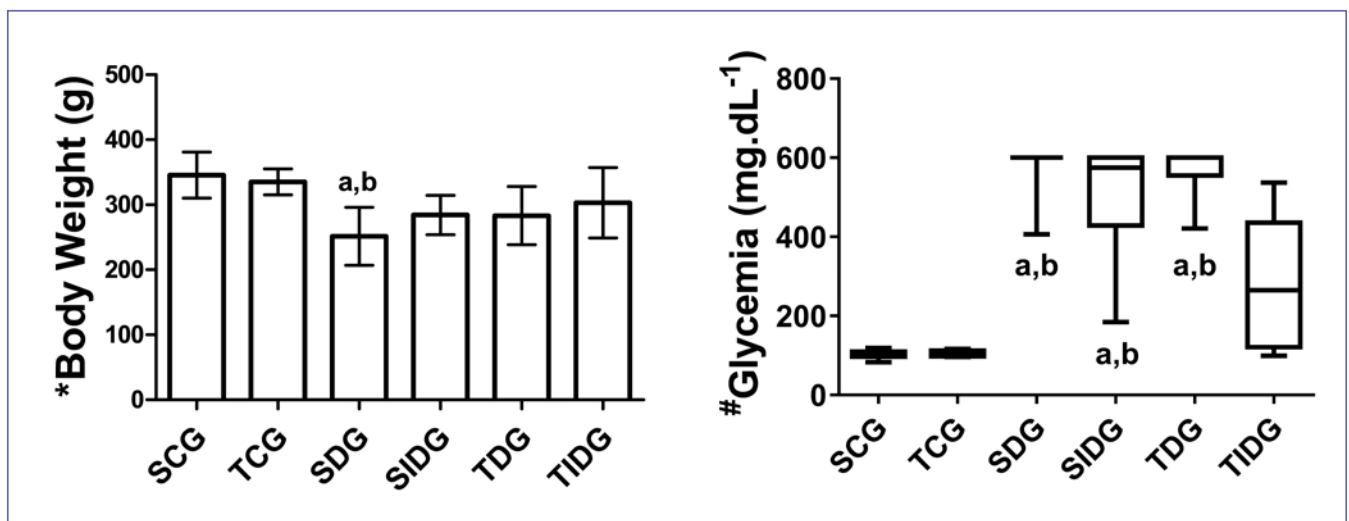


Figure 1. Body weight and glycemic indexes after five weeks of moderate treadmill exercise. SCG - Sedentary Control Group; TCG - Treadmill Control Group; SDG - Sedentary Diabetic Group; SIDG - Sedentary Insulin Diabetic Group; TDG - Treadmill Diabetic Group; TIDG - Treadmill Insulin Diabetic Group. Body weight values are expressed as mean ± standard deviation. (*) Anova with post hoc of Bonferroni, whereas final glycemia values are expressed in median and interquartile range (#) Kruskal Wallis with paired comparison to determine statistical difference between groups ($p < 0.05$). Where (a) represents the difference in relation to SCG, (b) difference in relation to TCG.

all diabetic groups (SDG, SIDG, TDG, and TIDG) ($p < 0.05$) (figure 2).

In MET₁, no differences in maximal speed were observed among the evaluated groups. In MET₂, maximal speed was increased in TIDG compared with that in SDG ($\uparrow 17.03\%$, $p = 0.002$, $d = 2.97$) and SIDG ($\uparrow 23.82\%$, $p = 0.001$, $d =$

2.35) and decreased in SDG ($\downarrow 11.11\%$, $p = 0.020$, $d = 3.88$) and SIDG ($\downarrow 22.86\%$, $p = 0.011$, $d = 2.42$) compared with that in TCG (figure 3).

Regarding the biomechanical properties of AT, maximal force was reduced in TIDG ($\downarrow 31.51\%$, $p = 0.009$, $d = 1.88$) and SIDG ($\downarrow 32.23\%$, $P = 0.002$, $d = 1.84$) compared with

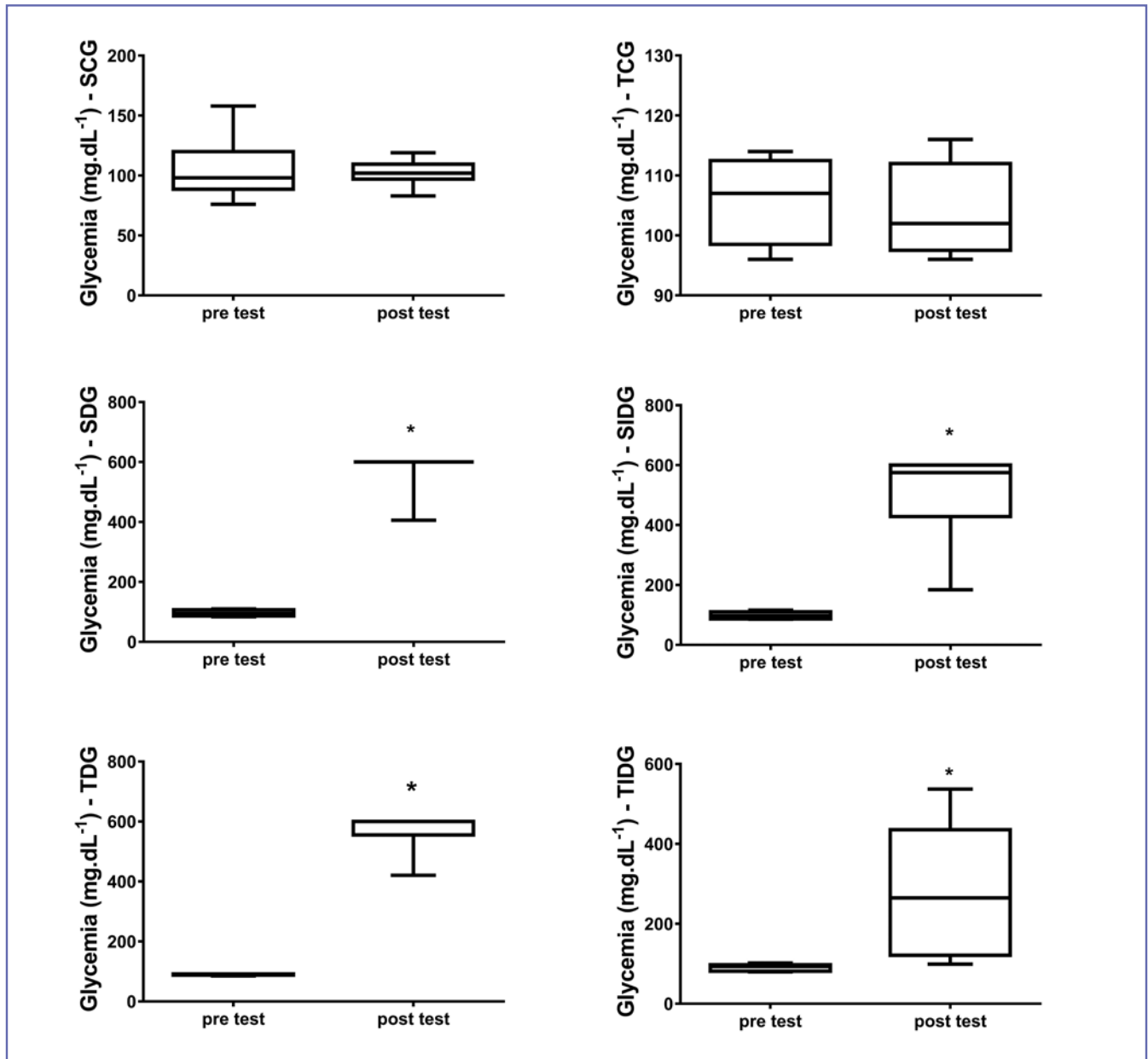


Figure 2. Evolution of Glycemia (mg.dL⁻¹) before and after five weeks of moderate treadmill exercise in each group. SCG - Sedentary Control Group; TCG - Treadmill Control Group; SDG - Sedentary Diabetic Group; SIDG - Sedentary Insulin Diabetic Group; TDG - Treadmill Diabetic Group; TIDG - Treadmill Insulin Diabetic Group. Body weight values are expressed as median and interquartile range. Wilcoxon test ($p < 0.05$).

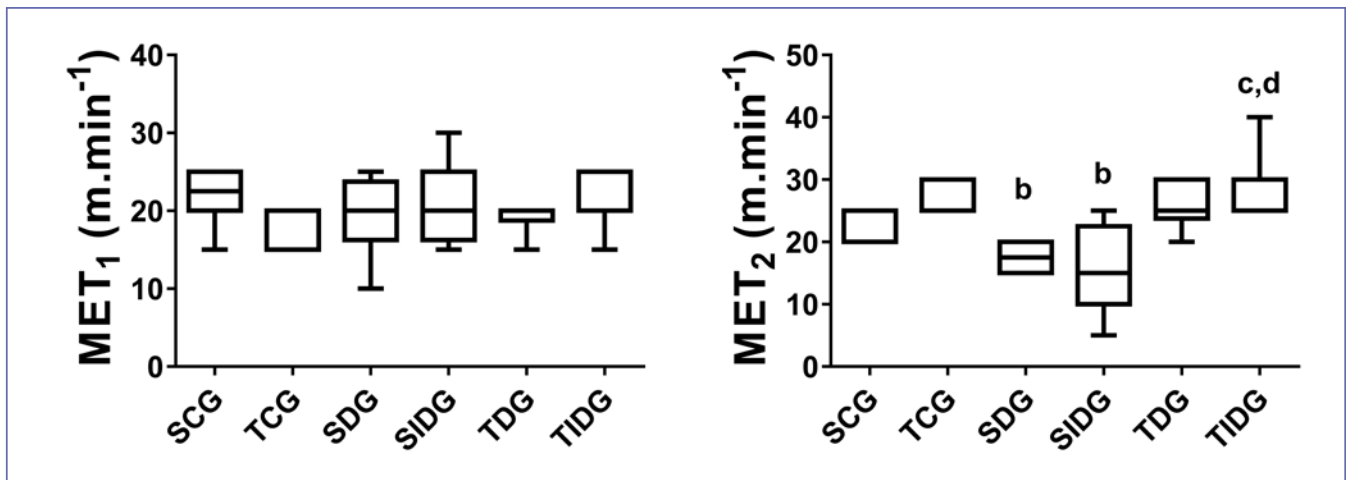


Figure 3. Maximum speed values ($m \cdot min^{-1}$) achieved by the animals in the maximal stress tests before and after five weeks of moderate treadmill Exercise. MET1 - Maximum speed reached by animals in the maximum exercise test performed before the exercise protocol; MET2 - Maximum speed reached by the animals in the maximum exercise test performed after the exercise protocol; SCG - Sedentary Control Group; TCG - Treadmill Control Group; SDG - Sedentary Diabetic Group; SIDG - Sedentary Insulin Diabetic Group; TDG - Treadmill Diabetic Group; TIDG - Treadmill Insulin Diabetic Group. Values are expressed as median and interquartile range. Kruskal Wallis ($p < 0.05$) was used with paired comparison to determine statistical difference between groups. Where, (b) difference from TCG, (c) difference from SDG, (d) difference from SIDG.

that in SCG and increased in TDG compared with that in SIDG ($\uparrow 47.78\%$, $p = 0.024$, $d = 1.40$). Moreover, elastic modulus was decreased in TCG compared with that in SCG ($\downarrow 37.79\%$, $p = 0.011$, $d = 1.59$) and increased in SDG ($\uparrow 28.32\%$, $p < 0.001$, $d = 2.06$), SIDG ($\uparrow 24.22\%$, $p = 0.019$, $d = 2.32$), and TDG ($\uparrow 53.23\%$, $p = 0.006$, $d = 1.06$) compared with that in TCG. There was no difference in the other biomechanical parameters of AT among the evaluated groups (table II).

DISCUSSION

In this study, we aimed to investigate the biomechanical properties of AT as well as the clinical status and physical fitness of DM1-induced rats following a combination of IST and moderate-intensity aerobic exercise on a treadmill, with the exercise speed previously verified through the maximum effort test, thus ensuring the biological individuality and taking into account the pathological state of these animals. Given this, IST combined with five weeks of exercise prevented the increase in the elastic modulus of AT, favored glycemic balance, and increased physical fitness. Similarly, the analysis of biomechanical and structural properties of AT indicated that IST without physical exercise may decrease maximal strength, and sedentary rats exhibited increased elastic modulus. Collectively, these results suggest an adaptive exercise response, which is optimized by IST;

this is in accordance with the results of recent studies (3,28), which reported that regardless of IST being potentially relevant to the health of individuals with DM1, complementary associations and other therapies are necessary to counteract the triggered chronic complications.

Physical exercise aids glycemic control because it increases insulin-receptor sensitivity and facilitates glycolytic removal (29). Although the practice of moderate and regular physical activity alone is beneficial for DM1, we found that in combination with IST, it favored glycemic control, which was not observed in untrained diabetic animals. This implies that the induction of experimental diabetes results in hyperglycemia.

Our findings corroborate that even without the introduction of a low-carbohydrate diet, which is part of the adequate dietary planning for DM1 treatment (1), the combination of moderate-intensity aerobic exercise on a treadmill with IST benefits metabolic balance, possibly because of glycogenolysis and muscle energy production from physical exercise (30). Future studies should be conducted considering the results presented herein association with diet manipulation to test the hypothesis of benefits potentiation.

Insulin combined with moderate-intensity exercise on a treadmill increased the physical fitness of DM1 animals, whereas sedentary animals exhibited decreased physical fitness regardless of IST use. Physical inactivity combined with a hyperglycemic state potentiates the chronic compli-

Table II. Biomechanical and structural parameters of the Achilles tendon in Wistar rats after five weeks of treadmill exercise with moderate intensity.

Parameters	SCG (n = 8)	TCG (n = 5)	SDG (n = 8)	SIDG (n = 8)	TDG (n = 6)	TIDG (n = 8)	Kruskal-Wallis
Cross-sectional Area (mm²)	1,26 (1,12-1,48)	1,25 (1,10-1,71)	0,93 (0,81-1,07)	1,10 (1,03-1,30)	1,28 (0,87-1,51)	1,06 (0,91-1,19)	0,074
Length of tendon (mm)	2,39 (1,84-3,21)	1,78 (1,48-2,21)	2,43 (1,86-2,77)	2,40 (1,75-2,93)	2,24 (1,96-2,99)	1,63 (1,37-2,38)	0,187
Maximum Force (N)	33,30 (29,79-35,07)	30,74 (25,89-34,74)	29,97 (25,91-32,80)	25,16 (22,87-29,67) a	32,32 (28,64-34,12) d	26,84 (25,63-29,45) a	0,022
Maximum Tension (MPa)	25,69 (23,17-29,60)	24,59 (15,47-28,58)	32,36 (26,08-38,06)	23,02 (19,22-25,41)	24,89 (22,19-35,86)	25,61 (22,95-28,46)	0,100
Deformation Maximum Strength (mm)	2,69 (2,18-3,19)	2,87 (2,69-3,39)	2,61 (2,03-3,04)	2,43 (1,94-2,80)	2,33 (1,73-2,77)	2,10 (1,84-2,64)	0,108
Specific Deformation (%)	103,39 (88,67-166,73)	173,40 (127,25-214,10)	97,07 (65,95-169,65)	102,80 (92,82-133,03)	93,73 (71,65-114,63)	133,15 (88,91-155,58)	0,175
Energy/Cross-sectional Area (N.mm/mm²)	48,65 (31,26-56,26)	37,97 (24,98-50,52)	49,58 (35,45-58,58)	31,05 (28,30-42,73)	31,55 (26,67-56,77)	33,65 (28,86-39,79)	0,229
Elastic Modulus (MPa)	35,52 (20,36-46,68)	15,17 (11,21-19,41) a	44,76 (26,48-61,27) b	29,04 (25,43-37,33) b	31,48 (25,82-69,03) b	23,16 (17,97-50,96)	0,018

SCG - Sedentary Control Group; TCG - Treadmill Control Group; SDG - Sedentary Diabetic Group; SIDG - Sedentary Insulin Diabetic Group; TDG - Treadmill Diabetic Group; TIDG - Treadmill Insulin Diabetic Group. The values are median and interquartile range. The Kruskal-Wallis test was used, followed by the paired comparison to determine the statistical difference between the groups (p < 0.05). Where (a) represents the difference in relation to SCG, (b) difference in relation to TCG.

cations of musculoskeletal (5,31) and cardiovascular (4,32) systems, which in turn can be mitigated by physical exercise (33,34). Together, that evidence reinforces the clinical benefits of physical exercise for diabetes.

In AT, IST did not counteract the loss of maximal strength, both in sedentary and diabetic animals that exercised on the treadmill. However, this analysis did not consider the intrinsic characteristics of the biomaterial under study, specifically the cross-sectional area and length of AT. However, these properties determine the maximal tension, which was calculated by the ratio of the maximal force supported by the tendon relative to the cross-sectional area and elastic modulus, as quantified based on the maximal tension vs. specific deformation curve (18).

Although IST did not promote any change in maximal stress, the cross-sectional area, maximal and specific deformation, and elastic modulus were increased in the diabetic groups. However, this effect was not observed in groups that received IST combined with physical exercise. These findings corroborate the results of the study by Silva, Santos (31), which showed that sedentary diabetic animals that did

not receive IST showed increased elastic modulus. Although this result can be interpreted as the facilitation of the transmission of muscle energy to the tendon in healthy rats, this increase is related to the disorganization of collagen fibers in diabetic animals, with tendons being more prone to rupture (5,6).

In addition, increased elastic modulus is related to changes in the extracellular matrix components of the tendon because of prolonged hyperglycemia, with reduced proteoglycan levels and increased number of cross-links between collagen fibers being responsible for changes in the viscoelastic properties of the tendon (12,35). In turn, this decreases lubrication and increases slipping among collagen fibrils, consequently increasing tendon rigidity and promoting the development of diabetic tendinopathy (5). Furthermore, chronic hyperglycemia is associated with a significantly increased number of mast cells and vascular hyperplasia in the transverse area of blood vessels in AT as well as with the upregulation of the vascular endothelial growth factor, collagen type 1, inflammatory molecular indicators, such as nuclear factor kappa B (36), neutrophil

infiltration, increased basophilia of the tenocytes, increased nuclear size/rounding (37).

Achilles tendon plays an important biomechanical role, particularly in gait and body stability. The mechanical changes observed in the diabetic groups in this study were characterized by changes in the properties of rodent AT, notably due to hyperglycemia and consequent fragility of this structure (37). However, this condition could be attenuated by regular aerobic exercise of moderate intensity combined with IST.

In summary, the combination of IST and moderate-intensity exercise performed on a treadmill in DMI1-induced rats enhanced the stability of glycemic metabolic parameters, thus avoiding an increase in tendon stiffness, as demonstrated by the elastic modulus values. Therefore,

the present study supports the importance of combining these two therapies in preventing tendon injuries in diabetic patients. In addition, it suggests that new works be developed combining these therapies with adequate food planning.

FUNDINGS

This research was financed in part by the *Cordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES) - Finance Code 001.*

CONFLICTS OF INTERESTS

The authors declare that they have no conflict of interests.

REFERENCES

1. De Angelis K, da Pureza DY, Flores LJ, et al. [Physiological effects of exercise training in patients with type 1 diabetes]. *Arquivos brasileiros de endocrinologia e metabologia*. 2006;50(6):1005-13.
2. Diabetes SBD. Diretrizes da Sociedade Brasileira de Diabetes (2017-2018). Editora Clannad São Paulo; 2017.
3. Malarde L, Gratas-Delamarche A, Le Douairon-Lahaye S, et al. Endurance training and insulin therapy need to be associated to fully exert their respective beneficial effects on oxidant stress and glycemic regulation in diabetic rats. *Free radical research*. 2014;48(4):412-9.
4. Anaruma CP, Ferreira M, Jr., Sponton CH, Delbin MA, Zanescio A. Heart rate variability and plasma biomarkers in patients with type 1 diabetes mellitus: Effect of a bout of aerobic exercise. *Diabetes research and clinical practice*. 2016;111:19-27.
5. de Oliveira RR, de Lira KD, Silveira PV, Coutinho MP, et al. Mechanical properties of achilles tendon in rats induced to experimental diabetes. *Annals of biomedical engineering*. 2011;39(5):1528-34.
6. Gautieri A, Redaelli A, Buehler MJ, Vesentini S. Age- and diabetes-related nonenzymatic crosslinks in collagen fibrils: candidate amino acids involved in Advanced Glycation End-products. *Matrix biology : journal of the International Society for Matrix Biology*. 2014;34:89-95.
7. Patrocínio-Silva TL, Souza AMFd, Goulart RL, Pegorari CF, et al. Low-level laser therapy associated to a resistance training protocol on bone tissue in diabetic rats. *Archives of endocrinology and metabolism*. 2016;60(5):457-64.
8. de Oliveira RR, Lemos A, de Castro Silveira PV, da Silva RJ, de Moraes SR. Alterations of tendons in patients with diabetes mellitus: a systematic review. *Diabetic medicine : a journal of the British Diabetic Association*. 2011;28(8):886-95.
9. de Oliveira RR, Martins CS, Rocha YR, et al. Experimental diabetes induces structural, inflammatory and vascular changes of Achilles tendons. *PloS one*. 2013;8(10):e74942.
10. Li Y, Fessel G, Georgiadis M, Snedeker JG. Advanced glycation end-products diminish tendon collagen fiber sliding. *Matrix biology : journal of the International Society for Matrix Biology*. 2013;32(3-4):169-77.
11. Mohsenifar Z, Feridoni MJ, Bayat M, Masteri Farahani R, Bayat S, Khoshvaghti A. Histological and biomechanical analysis of the effects of streptozotocin-induced type one diabetes mellitus on healing of tenotomised Achilles tendons in rats. *Foot and ankle surgery : official journal of the European Society of Foot and Ankle Surgeons*. 2014;20(3):186-91.
12. Burner T, Gohr C, Mitton-Fitzgerald E, Rosenthal AK. Hyperglycemia reduces proteoglycan levels in tendons. *Connective tissue research*. 2012;53(6):535-41.
13. Enwemeka CS, Maxwell LC, Fernandes G. Ultrastructural morphometry of matrical changes induced by exercise and food restriction in the rat calcaneal tendon. *Tissue & cell*. 1992;24(4):499-510.
14. Bezerra MA, Santos de Lira KD, Coutinho MP, et al. Biomechanical and structural parameters of tendons in rats subjected to swimming exercise. *International journal of sports medicine*. 2013;34(12):1070-3.
15. Beyer R, Kongsgaard M, Hougs Kjaer B, Ohlenschlaeger T, Kjaer M, Magnusson SP. Heavy Slow Resistance Versus Eccentric Training as Treatment for Achilles Tendinopathy: A Randomized Controlled Trial. *The American journal of sports medicine*. 2015;43(7):1704-11.
16. Heinemeier KM, Skovgaard D, Bayer ML, et al. Uphill running improves rat Achilles tendon tissue mechanical properties and alters gene expression without inducing pathological changes. *Journal of applied physiology*. 2012;113(5):827-36.
17. de Oliveira RR, Bezerra MA, de Lira KD, et al. Aerobic physical training restores biomechanical properties of Achilles tendon in rats chemically induced to diabetes mellitus. *Journal of diabetes and its complications*. 2012;26(3):163-8.
18. Bezerra MA, da Silva Nery C, de Castro Silveira PV, et al. Previous physical exercise slows down the complications from experimental diabetes in the calcaneal tendon. *Muscles, ligaments and tendons journal*. 2016;6(1):97-103.

19. de Senna PN, Xavier LL, Bagatini PB, et al. Physical training improves non-spatial memory, locomotor skills and the blood brain barrier in diabetic rats. *Brain research*. 2015;1618:75-82.
20. de Lacerda Brito ACN, Martins WA, da Silva Queiroz PC, et al. Standardization of a Treadmill Exercise Intensity Protocol in Rats with Diabetes Mellitus. *Journal of Exercise Physiology Online*. 2019;22(2).
21. Padulo J, Oliva F, Frizziero A, Maffulli NJMLTJ. Basic principles and recommendations in clinical and field science research: 2018 update. 2018;8(3):305-7.
22. de Senna PN, Ilha J, Baptista PP, et al. Effects of physical exercise on spatial memory and astroglial alterations in the hippocampus of diabetic rats. *Metabolic brain disease*. 2011;26(4):269-79.
23. Carvalho ENd, Carvalho NASd, Ferreira LM. Experimental model of induction of diabetes mellitus in rats. *Acta Cirurgica Brasileira*. 2003;18(SPE):60-4.
24. Erdal N, Gurgul S, Demirel C, Yildiz A. The effect of insulin therapy on biomechanical deterioration of bone in streptozotocin (STZ)-induced type 1 diabetes mellitus in rats. *Diabetes research and clinical practice*. 2012;97(3):461-7.
25. Rodrigues B, Figueroa DM, Mostarda CT, Heeren MV, Irigoyen MC, De Angelis K. Maximal exercise test is a useful method for physical capacity and oxygen consumption determination in streptozotocin-diabetic rats. *Cardiovascular diabetology*. 2007;6:38.
26. Silveira ACM, Nery CADs. Estudo macroscópico e morfo-métrico do tendão do músculo tibial posterior. *Rev bras ortop*. 1999;34(8):475-80.
27. Nakagawa S, Cuthill IC. Effect size, confidence interval and statistical significance: a practical guide for biologists. *Biol Rev Camb Philos Soc*. 2007;82(4):591-605.
28. Jin HY, Lee KA, Park TS. The effect of exercise on the peripheral nerve in streptozotocin (STZ)-induced diabetic rats. *Endocrine*. 2015;48(3):826-33.
29. Lima-Silva AE, Fernandes TC, De-Oliveira FR, Nakamura FY, Gevaerd MdS. Metabolismo do glicogênio muscular durante o exercício físico: mecanismos de regulação. *Revista de Nutrição*. 2007.
30. Hargreaves M. Exercise, muscle, and CHO metabolism. *Scandinavian journal of medicine & science in sports*. 2015;25 Suppl 4:29-33.
31. Silva RPM, Santos ROd, Junior M, et al. Influence of the use of testosterone associated with physical training on some hematologic and physical parameters in older rats with alloxan-induced diabetes. *Archives of endocrinology and metabolism*. 2017;61(1):62-9.
32. Coombes B, Tucker K, Hug F, et al. Relationships between cardiovascular disease risk factors and Achilles tendon structural and mechanical properties in people with Type 2 Diabetes. 2019;9(3).
33. Pontieri FM, Bachion MM. Crenças de pacientes diabéticos acerca da terapia nutricional e sua influência na adesão ao tratamento. *Ciência & saúde coletiva*. 2010;15:151-60.
34. Green S, Kiely C, O'Connor E, et al. Effects of exercise training and sex on dynamic responses of O₂ uptake in type 2 diabetes. 2020(ja).
35. Snedeker JG, Gautieri A. The role of collagen crosslinks in ageing and diabetes-the good, the bad, and the ugly. *Muscles Ligaments Tendons J*. 2014;4(3):303.
36. Oliveira RR, Medina de Mattos R, Magalhaes Rebelo L, et al. Experimental Diabetes Alters the Morphology and Nano-Structure of the Achilles Tendon. *PloS one*. 2017;12(1):e0169513.
37. Boivin GP, Elenes EY, Schultze AK, et al. Biomechanical properties and histology of db/db diabetic mouse Achilles tendon. 2014;4(3):280.

Long-Term Clinical and Radiographic Results of ACL Reconstruction: Retrospective Comparison Between Three Techniques (Hamstrings Autograft, Hamstrings Autograft With Extra-Articular Reconstruction, Bone Patellar Tendon Autograft)

P. Rota¹, E. Monaco², A. Carrozzo², G. Bruni², A. Rota³, A. Ferretti²

¹ Fatebenefratelli S. Pietro Hospital, Rome, Italy

² La Sapienza University of Rome, Sant'Andrea Hospital, Traumatology Sports Center Kilk Kilgour, Rome, Italy

³ Sandro Pertini Hospital, Rome, Italy

CORRESPONDING AUTHOR:

Edoardo Monaco

La Sapienza University of Rome

Sant'Andrea Hospital

Traumatology Sports Center Kilk Kilgour

Rome, Italy

E-mail: edoardomonaco76@gmail.com

DOI:

10.32098/mltj.03.2020.15

LEVEL OF EVIDENCE: 4

SUMMARY

Background. There is no consensus in the current literature on which surgical options render the best long-term results after ACL reconstruction in terms of clinical outcomes and development of radiographic osteoarthritis (AO).

The aim of this study is to investigate clinical and radiological results at long-term follow up after ACL reconstruction using hamstring tendons autograft (Group HT), hamstrings autograft with extra-articular reconstruction (Group HT-ER), and bone patellar tendon bone autograft (Group BPTB).

Methods. All patients were evaluated at final follow-up using Lysholm, International Knee Documentation Committee (IKDC), and Tegner scores. An arthrometric KT-1000 evaluation was also done. Comparative weight bearing radiographs were taken, including a skyline view for patellofemoral joint and analyzed according to Fairbank, Kellgren, and IKDC classification.

Sixty patients were selected for this retrospective study, 20 for each group. The minimum final follow-up was 10 years for each group. All patients were male and involved in sport activities (Tegner pre-injury >7).

Results. Subjective scores improved significantly in all groups, with no significant differences between groups ($P < 0,05$).

The number of patients classified as C or D at the IKDC objective activity score was higher in Group HT (2/20, 10%), than in Group BTB (1/20, 5%) and Group HT-E (0/20, 0%).

In term of failure-rate, there were no difference between the three groups ($P < 0,05$).

Radiologic evaluation showed more arthritic changes in Group-BT in the patello-femoral joint (PMJ).

Conclusions. All the three techniques showed satisfactory results at long term follow up with no differences in term of subjective scores. Finally, there was a statistically significant higher incidence of arthritic changes in PFJ as evaluated with x-ray in the BPTP group respect to HT and HT + ER groups ($p < 0.05$).

KEY WORDS

Anterior cruciate ligament; extra articular reconstruction; hamstring tendon; knee joint; osteoarthritis; pivot-shift.

BACKGROUND

Anterior cruciate ligament (ACL) injury is a common occurrence, especially among young athletes. Restoring knee stability is thought to benefit not only in the short term with knee stabilization and patient return to sport, but also in the long term due to the increased risk of subsequent chondral or meniscal damage in the unstable knee (1).

Graft selection for anterior cruciate ligament reconstruction has been an intense research topic and debate subject for several decades. Hamstring tendon (HT) and patellar tendon (PT) autograft are currently the most utilized grafts in both research and clinical practice (2).

The PT autograft has its advantages due to the bone blocks at both ends of the graft, which facilitate ingrowth of the graft. However, the quadruple HT autograft is both a stiffer and stronger graft choice, with easy-to-manage harvest site morbidity. Even though the quadruple HT autograft is stronger, concerns have been raised about the risk of increased knee laxity over time (3).

Previous studies have reported that there are no differences in terms of rupture frequency between the two autografts (4–6). However, recent register studies have contested these results and indicated a higher rupture frequency in patients undergoing surgery using HT autografts (3–5).

In the last few years studies with long term follow-up showed that the addition of an extraarticular reconstruction (ER) to an HT graft reduces the failure rate in comparison with HT or PT autograft without ER (7–9). However, there is still concern about over constrain of ER on knee kinematics, that may possibly lead to degenerative osteoarthritis (OA). There are few studies with long-term follow-up comparing simultaneously the different grafts with combined ER (10,11).

Patients who have sustained an ACL injury run the risk of developing post-traumatic OA, with the first signs of radiographic joint space narrowing at the age of as young as forty years (12,13). The incidence of radiographic OA ten to twenty years after an ACL injury has been estimated at approximately 50%, with a higher incidence in patients with combined injuries compared with isolated ACL ruptures (3,14).

In terms of the clinical outcomes and developments of radiographic OA, there is no true consensus in the current literature on which surgical option provides the best long-term results after ACL reconstruction.

The primary objective of this retrospective and multicenter study was to compare the effect of ACL reconstruction with HT graft, a combined reconstruction with HT graft and an ER, and ACL reconstruction with PT, regarding knee stability and function at long-term follow-up (minimum ten years). The secondary objective was to determine whether an ACL-reconstructed knee, in the three different groups, has a greater incidence of degenerative changes.

The primary hypothesis was that there is no difference in knee laxity and in clinical outcomes in the ACL-reconstructed knee with three different techniques.

The secondary hypothesis of this study was that there is no difference in the incidence and severity of knee OA in the ACL-reconstructed knee in the three different groups.

METHODS

Patients selection

For this retrospective and multicentric study, three series of patients (group PT, group HT, group HT-ER) that had previously undergone ACL reconstruction were selected.

Group HT and HT + ER were treated in the same facility by the same senior author (A.F.), while the group PT was treated by a different senior author (A.R.) in a different institute. Exclusion criteria for all groups were as follows: female sex, Tegner activity score pre-injury < 7 (all patients were involved in sport activities at time of injury), BMI > 29, older than thirty-five years at the time of surgery, severe associated ligamentous injuries as documented by laxity tests that were positive other than the Lachman and pivot shift tests, cartilage damage (grade 3 or 4 according to Outerbridge classification), previous knee surgery, and time elapsed from injury to surgery longer than 2 years (13).

Group HT included cases where an anatomic intra-articular ACL reconstruction with quadrupled hamstring graft was performed using out-in technique.

Group HT-ER included patients where the same technique for intra-articular reconstruction was used in association with an extra-articular tenodesis (McIntosh as modified by Coker and Arnold).

Group PT included cases where an intraarticular ACL reconstruction with bone patellar tendon graft performed with a transtibial technique.

All patients were involved in high-risk sports activity considered as follows: football, rugby, volleyball, basketball, skiing, or martial arts.

No patients reported rheumatologic disorders or associated malalignment.

All patients agreed to participate in the study and signed an informed consent form in accordance with the Declaration of Helsinki (1964).

The study meets the ethical standards of the journal (15).

Surgical technique

Group HT: Intra-articular reconstruction

An arthroscopically assisted anatomic single-bundle two incision technique using doubled Semitendinosus and

Gracilis tendons autografts was performed. Point of entry of the femoral tunnel was selected at the center of the anatomic femoral footprint of ACL, which was located midway between resident ridge and over the top position. A tibial tunnel was constructed with a standard guide at 65°, while femoral tunnel was drilled through an outside-in technique. The tendons were also passed outside-in and manually tensioned before fixation. The bundles were fixed on the femur using the Swing Bridges device (Citieffe, Bologna, Italy) and on the tibia using the Evolgate device (Citieffe). A tight fit of the graft in the bone tunnel was aimed for in all patients.

Group HT+ER: Combined Reconstruction (McIntosh as Modified by Cocker Arnold in Addition to Intra-articular Reconstruction)

After the IR was fixed, the incision on the lateral side was extended to 10 to 12 cm in a hockey-stick fashion, extending from the Gerdy's tubercle proximally to just inferior to the lateral epicondyle while the knee was flexed to 90°. The proximal extent of this incision parallels the midportion of the iliotibial tract. The Fascia Lata was exposed and incised along its fibers about 3 cm from the posterior border. With 1 cm of the iliotibial tract left intact posteriorly, a 1-cm-wide and 13-cm-long strip of the iliotibial tract was detached proximally, leaving intact its distal attachment on the Gerdy's tubercle. The lateral collateral ligament was identified, and the proximal part of the strip was passed under the ligament; the band was then reflected on itself and sutured under tension with periosteal absorbable stitches to the Gerdy's tubercle while the tibia was held in maximal external rotation.

The strip was also sutured to the fibular collateral ligament for additional stability. A combined reconstruction required an additional surgical time of fifteen minutes.

Group PT: Intra-articular reconstruction with bone patellar tendon graft

The central third of the PT was harvested either through an open approach with a vertical incision. The tendon defect was sutured, and defects in the patella and the proximal tibia were not bone grafted. The bone blocks were sized at 10 or 9 mm. The tibial tunnel was drilled in the native ACL footprint. The femoral bone tunnels were created through transtibial drilling, aiming at the 10:00 to 10:30 clock position. The grafts were fixed on the femur using the Rigid Fix Cross Pin System (DepuySynthes) and on the tibial side using an absorbable screw (Biointrafix ACL fixation System, DepuySynthes). The knee was hyperextended, and firm traction was applied to the autograft before fixation of the tibial interference screw.

Postoperative rehabilitation

There was no difference in the rehabilitation protocol between the three groups: the involved knee was placed in a full extension brace for two weeks postoperatively with weight bearing with crutches as tolerated; daily isometric and isotonic exercises were prescribed. After wards, progressive range-of-motion exercises were encouraged as well as isometric and isotonic exercises. At four weeks postoperatively, full weight bearing without crutches and without brace was permitted. From the second month postoperatively, a heavier muscle-strengthening program was prescribed, and between four and six months a gradual return to athletic and sport specific training was encouraged. From the fifth month postoperatively, as soon as the trainer deemed patient "ready to go," full return to sports was allowed.

Follow up

A minimum of ten years follow-up examination was performed by the same observer who were independent (but not unaware because of the different scar in the three groups) and who were not involved in the initial surgery.

The activity level was assessed using the Tegner activity score(16). In evaluating the subjective functional status, the Lysholm score was used, whereas in evaluating the clinical outcome, the International Knee Documentation Committee (IKDC) rating system was used (17,18). Patients underwent a standardized bilateral knee examination.

Stability testing was performed using the Lachman test, the pivot-shift test, and the KT-1000 arthrometer (Medmetric, San Diego, CA).

Bilateral weight-bearing anteroposterior radiographs in full extension and lateral views were obtained and evaluated using the Fairbank scale, Kellgren-Lawrence scale, and IKDC grading system (18–20). Moreover, a skyline view was recorded to specifically evaluate patellofemoral joints according to the Kellgren score(20). Evaluation was performed by the same independent observer.

STATISTICAL ANALYSIS

Data were expressed in terms of mean and standard deviation of the mean. To evaluate differences between and among groups, One Way ANOVA have been performed. For all tests $p < 0.05$ was considered significant. SPSS version18 was used for the calculations.

RESULTS

A total of sixty patients were included in the study (twenty for each group). All patients were male, involved in sport activities at the time of injury (Tegner pre-injury ≥ 7).

In group HT the mean age at surgery was 28,5 years (range 21 to 35 years). The mean follow-up time was 10 years and 5 months (range 121 to 128 months). A partial medial meniscectomy was performed in two patients, a partial lateral meniscectomy was performed in three patients, and a partial medial and lateral meniscectomy was performed in one patient. A subjective, clinical and radiologic evaluations were performed for all patients.

In group HT+ER the mean age at surgery was 28,7 years (range 19 to 35 years). The mean follow-up time was 10 years 6 months (range 122 to 130 months). A partial medial meniscectomy was performed in one patient, a partial lateral meniscectomy was performed in five patients, and a partial medial and lateral meniscectomy was performed in two patients. A subjective, clinical and radiologic evaluations were performed for all patients.

In group PT the mean age at surgery was 22,5 years (range 17 to 26 years). The mean follow-up time was 14 years 3 months (range 120 to 264 months).

A partial medial meniscectomy was performed in three patients, a partial lateral meniscectomy was performed in six patients, and a partial medial and lateral meniscectomy was performed in three patients. As for the two previous groups, also in this group subjective, clinical and radiologic evaluations were performed for all patients.

No major complication was reported after surgery in all groups. No patients reported rheumatologic disorders or associated malalignment.

Demographic data are summarized in **table I**.

Subjective evaluation

At final follow-up in group HT the mean Lysholm score was 97,74 (standard deviation [SD]= 2,78); IKDC subjective score was 96,77 (standard deviation [SD]= 3,47), and median Tegner activity score was 7 (range 3 to 8).

In group HT+ER the mean Lysholm score was 97 (standard deviation [SD]= 3,91); IKDC subjective score was 95,69

(standard deviation [SD]= 4), and median Tegner activity score was 6 (range 3 to 8).

In group PT the mean Lysholm score was 97,74 (standard deviation [SD]= 6,45); IKDC subjective score was 96,31 (standard deviation [SD]= 6,66), and median Tegner activity score was 9 (range 4 to 10).

No significant statistical differences were detected between the 3 groups in any subjective scores except for the Tegner score, in favor of group PT. ($p < 0.05$)

Objective evaluation

In group HT, with respect to the IKDC objective score, 12/20 (60%) patients were in group A, 6/20 (30%) in group B, 2/20 (10%) in group C; there were no patients in group D.

In group HT+ER, for the IKDC objective scores, 13/20 (65%) patients were in group A, 6/20 (30%) in group B, and 1/20 (5%) in group C; there were no patients in group D.

In group PT, for the IKDC objective scores, 14/20 (70%) patients were in group A, 5/20 (30%) in group B, and 1/20 (5%) in group C; there were no patients in group D.

No significant statistical differences were detected between the three groups ($p > 0,05$).

Post-operative range of motion among the three groups was significantly different: PT group showed higher extension and flexion deficit compared the other two groups (2 patients in group PT [10%], and 0 in the groups HT and HT+ER) (**figure 1**).

Arthrometric evaluation

In group HT, instrumental laxity testing using a KT- 1000 arthrometer showed a mean side-to-side maximum manual (S/S MM) difference of 2,4 mm (SD = 1,56), with sixteen patients (65%) under 3 mm, six patients (30%) between 3 and 5 mm, and one patient (5%) more than 5 mm.

In group HT+ER, KT-1000 arthrometer evaluation showed a mean side-to-side maximum manual difference of 2,2 mm (SD = 1,29), with 15 patients (75%) under 3 mm, 5 patients (25%) between 3 and 5 mm, and no patient more than 5 mm.

In group PT, KT-1000 arthrometer evaluation showed a mean side-to-side maximum manual difference of 2,2 mm

Table I. Demographic data.

Table I	HT group (n=20)	HT+ER group (n=20)	PT group (n=20)
Age at surgery, years	28,5 (range 21-35)	28,7 (range 19-35)	22,5 (range 17-26)
Age at follow-up, years	38,9(range 31-45)	39,2 (range 29-45)	34,9 (range 25- 44)
Mean Follow-up, months	125(range 121 to 128)	126 (range 122 to 130)	171 (range 120 to 264)



Figure 1. Slight loss of flexion and extension in the PT group.

Table II. Clinical results. IKDC, International Knee Documentation Committee; M, mean; SD, standard deviation; S/S MM, side-to-side maximal manual.

Table II	HT group (n=20)	HT+ER group (n=20)	PT group (n=20)
Lysholm, M (SD)	97,7 (2,8)	97 (4)	96,4 (6,4)
IKDC subjective, M (SD)	96,8 (3,5)	95,7 (4)	96,3 (6,7)
Tegner (median)	6	7	9
IKDC objective			
A	12/20 (60%)	13/20 (65%)	14/20 (70%)
B	6/20 (30%)	6/20 (30%)	5/20 (25%)
C	2/20 (10%)	1/20(5%)	1/20 (5%)
D	-	-	-
KT-1000 S/S MM, M (SD)	2,4 (1,6)	2,2 (1,3)	2,2 (1,3)
<3 mm	13 (65%)	15 (75%)	14 (70%)
3-5 mm	6 (30%)	5(25%)	5 (25%)
>5 mm	1 (5%)	-	1(5%)

(SD = 1,30), with fourteen patients (70%) under 3 mm, five patients (25%) between 3 and 5 mm, and one patient (5%) more than 5 mm.

A mean difference was detected in favor of groups HT+ER and PT, but this difference is not statistically significant ($p>0,05$).

Considering as a failure a presence of a side-to-side maximum manual difference of more than 5 mm using KT-1000 arthrometer or a pivot shift test as ++/+++ , any giving way episode during follow-up period, and the revised case, we found 2 cases of failure in group HT and one case in the groups HT+ER and PT ($p>0,05$), with a trend in favor of HT+ER and PT groups. Clinical results are summarized in **table II**.

Radiologic evaluation

Radiologic results are summarized in **table III**, and **tables IV, V** and **VI** present the radiologic results of meniscectomized patients of groups HT, HT+ER and PT, respectively (**figures 2,3,4**).

The number of patients classified as C for IKDC radiographic score was significantly higher in group HT (2/20, 10%) and in the group PT (3/20, 15%) than group HT+ER (0/20, 0%) ($p<0,05$).

The number of patients included in grades II, III, and IV according to Kellgren classification (tibiofemoral joint) in group HT (7/20; 35%) was statistically higher than in group

Table III. Radiologic results. IKDC, International Knee Documentation Committee; K/L Kelgren Lawrence.

Table III	HT group (n=20)	HT+ER group (n=20)	PT group (n=20)
IKDC score			
Group A	12 (60%)	15 (75%)	11 (55%)
Group B	6 (30%)	5 (25%)	6 (30%)
Group C	2 (10%)	-	3 (15%)
Group D	-	-	-
Fairbank classification			
Grade I	10 (50%)	11 (55%)	9 (45%)
Grade II	7(35%)	7 (35%)	9 (45%)
Grade III	3 (15%)	2 (10%)	2 (10%)
Grade IV	-	-	-
K/L Classification			
Grade 0	3 (15%)	2 (10%)	7 (35%)
Grade I	9 (45%)	16 (80%)	5 (25%)
Grade II	4 (20%)	2 (10%)	6 (30%)
Grade III	3 (15%)	-	2 (10%)
Grade IV	1 (5%)	-	-
K/L Patellofemoral			
Grade 0	1 (5%)	7(35%)	6 (30%)
Grade I	11(55%)	10(50%)	3 (15%)
Grade II	6 (30%)	3(15%)	5 (25%)
Grade III	2 (10%)	-	5 (25%)
Grade IV	-	-	1 (5%)

Table IV. Meniscectomy Radiologic results Group HT. IKDC, International Knee Documentation Committee; K/L Kelgren Lawrence.

HT group (n=20)	Meniscectomized (n=5)	Nomeniscectomized (n=15)
IKDC score		
Group A	-	10 (50%)
Group B	2 (10%)	4 (20%)
Group C	1 (5%)	1 (5%)
Group D	2(10%)	-
Fairbank classification		
Grade I	1(5%)	6 (30%)
Grade II	1 (5%)	8 (40%)
Grade III	2 (10%)	1 (5%)
Grade IV	1 (5%)	-
K/L Classification		
Grade 0	-	3 (15%)
Grade I	2 (10%)	6 (30%)
Grade II	-	6 (30%)
Grade III	1 (5%)	1(5%)
Grade IV	1 (5%)	-

Table V. Meniscectomy Radiologic results Group HT+ER. IKDC, International Knee Documentation Committee; K/L Kelgren Lawrence.

HT +ER group (n=20)	Meniscectomized (n=5)	Nomeniscectomized (n=15)
IKDC score		
Group A	4 (20%)	11 (55%)
Group B	1 (5%)	4 (20%)
Group C	-	-
Group D	-	-
Fairbank classification		
Grade I	2 (10%)	8 (40%)
Grade II	3 (15%)	5 (25%)
Grade III	-	2 (10%)
Grade IV	-	-
K/L Classification		
Grade 0	-	2 (10%)
Grade I	5 (25%)	10 (50%)
Grade II	-	3 (15%)
Grade III	-	-
Grade IV	-	-

Table VI. Meniscectomy Radiologic results Group PT. IKDC, International Knee Documentation Committee; K/L Kelgren Lawrence.

PT group (n=20)	Meniscectomized (n=13)	Nomeniscectomized (n=7)
IKDC score		
Group A	2 (10%)	7 (35%)
Group B	5 (25%)	-
Group C	6 (30%)	-
Group D	-	-
Fairbank classification		
Grade I	2 (10%)	7 (35%)
Grade II	8 (40%)	-
Grade III	3 (15%)	-
Grade IV	-	-
K/L Classification		
Grade 0	2 (10%)	7 (35%)
Grade I	1 (5%)	-
Grade II	7 (35%)	-
Grade III	3 (15%)	-
Grade IV	-	-

HT+ER (2/20; 10%) ($p < 0.05$), but not towards the group PT ($p > 0.05$).

The number of patients included in grades II, III, and IV through Kellgren classification (patellofemoral joint) in group PT (11/20; 55%) was statistically higher than in groups HT (8/20; 40%) and HT+ER (3/20; 15%) ($p < 0.05$). There was no statistically significant difference between groups through Fairbank classification.

In group HT, the number of meniscectomized patients categorized as C and D according to the IKDC radiographic score (3/5; 60%), was significantly higher than the number of nonmeniscectomized patients (1/15; 6,67%) ($p < 0.05$).

Furthermore, also the number of meniscectomized patients included in grades III and IV through Fairbank classification (3/5; 60%) was significantly higher than that of nonmeniscectomized patients (1/15; 6,67%) ($p < 0.05$).

In addition, the number of meniscectomized patients included in grades II, III, and IV via Kellgren classification (2/5; 60%) was not statistically different from that of nonmeniscectomized patients (7/15; 46%), ($p > 0.05$).

No statistically significant difference was found in all scales comparing meniscectomized and nonmeniscectomized patients of group HT+ER.

In group PT, the number of meniscectomized patients categorized as C and D according to the IKDC radiographic score was (6/13; 46,15%), while among nonmeniscectomized patients nobody is among these categories, showing statistically significant evidence among the groups ($p < 0.05$).

The number of meniscectomized patients included in grades III and IV through Fairbank classification (3/13; 23,07%) was not statistically different from that of nonmeniscectomized patients. ($p > 0.05$) In addition the number of meniscectomized patients included in grades II, III, and IV via Kellgren classification (10/13; 76,92%) was significantly higher than that of nonmeniscectomized patients (0/7) ($p < 0.05$).

The number of meniscectomized patients categorized as C and D according to the IKDC radiographic score in group HT (3/5; 60%) was higher than in group HT+ER (0/5), and group PT (6/13; 46,15%), showing a statistically significant difference only towards the group HT + ER. ($p < 0.05$)

The number of meniscectomized patients included in grades III and IV according to Fairbank classification in group HT (3/5; 60%) was significantly higher than in group HT+ER (0/5), and group PT (3/13; 23,07%). ($p < 0.05$)

No statistically significant difference was found comparing Kellgren classification (grades II, III and IV), in the different groups.

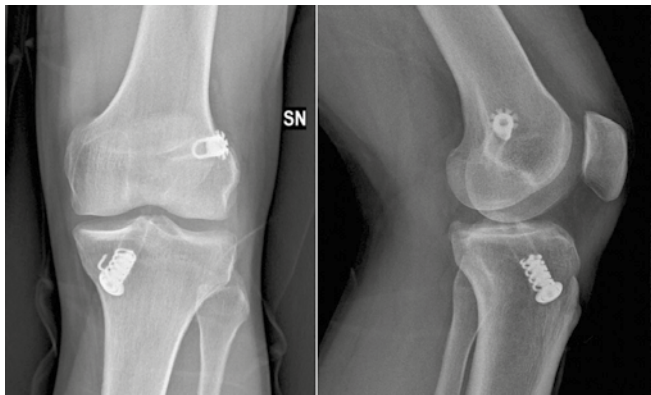


Figure 2. X-rays AP an LL an of ACL reconstructed knee in group HT.

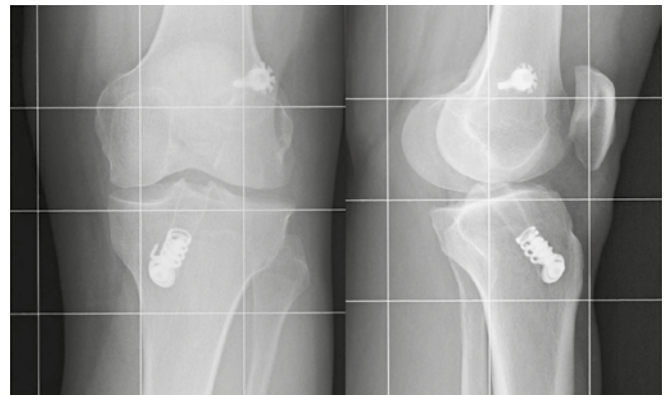


Figure 3. X-rays AP an LL an of ACL reconstructed knee in group HT+ER.



Figure 4. X-rays AP an LL an of ACL reconstructed knee in group PT.

DISCUSSION

This retrospective clinical and radiological study is a comparison between three different techniques for ACL reconstruction (HT, HT+ER and PT) at long-term follow-up (minimum follow-up 10 years.).

The most important finding of this study is that overall results with these surgical techniques were satisfactory and the majority of the patients could return to the same preoperative sports level.

Therefore, the primary hypothesis aforementioned was confirmed: there were no differences in knee laxity and clinical outcomes in ACL reconstructed knee adopting the three different techniques.

Our study has shown a difference comparing post-operative range of motion with two patients showing loss of extension or flexion in PT group, and for these reasons graded as B in the IKDC objective evaluation.

The secondary major finding of this study is the absence of significant differences between the three techniques in radiological evaluation. We only found a difference considering patello-femoral joint degeneration, as evaluated in Kellgren score, with an higher incidence in group PT (11/20 [55%] grades as II, III, IV), in respect to HT and HT+ER (8/20; [40%] and 3/20; [15%] respectively).

The evaluation of long term widening of the femoral and tibial tunnels was not a goal of the present study. As suggested by de Beus et al., correct evaluation of the tunnel widening after ACL reconstruction needs a careful evaluation using a CT scan (21). In this retrospective series of patients, we only performed X – rays at final follow up while we didn't

perform a CT scan evaluation and for this reason, a careful evaluation of the widening of the tunnel wasn't possible. However, we should consider that several studies showed no correlation between tunnel widening and clinical results after ACL reconstruction (21–24). So, we can speculate that microinstability related to tunnel widening should have a negligible effect on the development of OA.

Considering the effectiveness of the three surgical techniques -without any pathology that could influence OA changes- several patients had a meniscectomy at the time of surgery, specifically, 5 meniscectomies in each groups HT, HT+ER, and 13 meniscectomies in group PT. Previous studies well documented the effect of meniscectomy on the development of OA after ACL reconstruction (25,26). In fact, we found higher incidence of post-operative OA in meniscectomized patients, respect to nonmeniscectomized. The effect of meniscal lesions and meniscectomy on the development of OA is well demonstrated in the literature, and we should consider that all the patients of the present study where operated in a chronic phase, where giving away episodes can lead to meniscal or chondral injuries (27). However, early ACL reconstruction to prevent or slow down the onset of degenerative changes and osteoarthritis had not been proven but we can expect a lower rate of meniscal tear in the patient who underwent early ACL reconstruction and subsequently a lower incidence of OA (28). Moreover, we included in this case only patients involved in high – risk sports activities that were operated for an ACL reconstruction and for this reason a comparison with coper patients treated conservatively wasn't possible (29). Another important point highlighted by the authors is the actual risk of over constraint of lateral reconstructions.

Since the eighties, when ER were very popular, this risk was considered. In the last few years, several medium and long term clinical and radiological studies have been reported as well as a level one review paper (8,11,30). All these studies concluded that there is no evidence that adding a lateral tenodesis to an anatomically placed hamstring ACL graft results in either an increased rate of osteoarthritis or in a restricted range of motion or other over constraining related functional impairment.

The lack of difference of OA in 3 groups at final follow-up, seems to be in aligned with Devitt et al. (24).

Another aspect to be considered in a long-term follow-up study comparing difference techniques, is the incidence of failure rate. As previous reported in the literature we considered as a failure a presence of a side-to-side maximum manual difference of more than 5 mm using KT-1000 arthrometer or a pivot shift test as ++/+++, any giving way episode during follow-up period. Even if we did not report a significant difference in the failure rate between the three

groups, results demonstrated a trend with higher incidence in group HT (2/20 10%), in respect to the groups HT+ER, PT (both 1/20 5%). These results seem to be in accordance with other previous studies reporting that HT ACL reconstruction showed a higher risk of failure respect PT and HT+ER (6,11). Moreover, our results suggested that the addition of ER to HT graft reduces the failure rate, even if we had not found a statistically significant difference. However, the protective effect of ER as well as ALL reconstruction has been recently clearly demonstrated by biomechanical and clinical studies (9,31). We should consider that in this case series we only evaluated patients who underwent ACL surgery in a chronic phase, and an extraarticular tenodesis was added to an ACL reconstruction using HT. On the basis of our study, we cannot extrapolate results either on the effect of ALL repair in acute cases or the effect of ALL reconstruction (31). However, Sonnery – Cottet et al. showed similar results in a study where they compared 3 groups: 4HT; B-PT-B an HT + ALL reconstruction (10). They found that the rate of graft failure with HT+ALL grafts was 2.5 times less than with B-PT-B grafts and 3.1 times less than with 4HT grafts. The HT+ALL graft was found also associated with greater odds of returning to preinjury levels of sport when compared with the 4HT graft.

There is a lack in literature evaluating the effect of ER on PT graft. Recently Noyes et al. published a study showing a limited effect in the control of pivot shift phenomenon of an ACL reconstruction with PT with addition of ER with iliotibial band (ITB). The findings of the study of Noyes and coauthors are in agreement with previously published studies performed by the same and other groups of researchers using a robotic apparatus apparently simulating a pivot shift (32). These results are not surprising, because it is well-known that pivot shift is the effect of combined deficiency of two different structures (ACL and secondary restraints) acting as a single unit, as described by Terry et al. (33). In this sense, predictably, in an anatomically well-tensioned reconstructed ACL, a secondary lesion of the ALL (as in the Noyes and co. study) resulted in limited rotational instability and the following extraarticular reconstruction ineffective or, in the worst-case scenario, harmful.

REFERENCES

1. Poehling-Monaghan KL, Salem H, Ross KE, Secrist E, Ciccoti MC, Tjoumakaris F, et al. Long-Term Outcomes in Anterior Cruciate Ligament Reconstruction: A Systematic Review of Patellar Tendon Versus Hamstring Autografts. *Orthopaedic Journal of Sports Medicine*. 2017.
2. Samuelsson K, Andersson D, Ahldén M, Fu FH, Musahl V, Karlsson J. Trends in Surgeon Preferences on Anterior Cruciate

Most of the recent studies concerning biomechanics and clinical evaluation of ER are related to their use along with intraarticular reconstructions with hamstrings. Similarly, the paper of Noyes and coauthors provide an original contribution to this topic as the PT reconstruction they evaluated is still widely used with excellent results.

However, there is a lack in literature of clinical studies evaluated the effect of ER on PT graft at long term follow up. Further studies are needed with the aim better understand possible advantages or risks of a permanent and harmful over constrain of the knee.

LIMITATIONS AND FUTURE RESEARCH

This study has several limitations. First of all, we evaluated a limited number of patients, only twenty for each group were available for radiological and clinical examination at final follow-up. However, patients were homogeneous in three groups in age, sex and participation in sport activity at time of surgery.

Secondly, the retrospective non-randomized design of this study might have influenced the results due to a selection bias. No preoperative X-ray could be analyzed and thus the progression of OA could not be estimated. The results of this study are not generalizable since confounding factors, such as cartilage damage, could not be analyzed in detail. However, we excluded patients with severe chondral lesions at the time of surgery, thus minimizing possible bias due to cartilage damage effect.

Lastly, another limit was that this was a multicentric study-even if all patients were operated by two expert senior authors in the same period. Moreover, different techniques were used for femoral tunnel drilling in the PT and HT groups (transtibial, and an out-in technique respectively), and different fixation device were used. These differences may be negligible with regard to long-term radiographic results.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

4. Barenius B, Nordlander M, Ponzer S, Tidermark J, Eriksson K. Quality of life and clinical outcome after anterior cruciate ligament reconstruction using patellar tendon graft or quadrupled semitendinosus graft: An 8-year follow-up of a randomized controlled trial. *Am J Sports Med.* 2010;
5. Leys T, Salmon L, Waller A, Linklater J, Pinczewski L. Clinical results and risk factors for reinjury 15 years after anterior cruciate ligament reconstruction: A prospective study of hamstring and patellar tendon grafts. *American Journal of Sports Medicine.* 2012.
6. Pinczewski LA, Lyman J, Salmon LJ, Russell VJ, Roe J, Linklater J. A 10-year comparison of anterior cruciate ligament reconstructions with hamstring tendon and patellar tendon autograft: A controlled, prospective trial. *Am J Sports Med.* 2007;
7. Clancy WG, Nelson DA, Reider B, Narechania RG. Anterior cruciate ligament reconstruction using one-third of the patellar ligament, augmented by extra-articular tendon transfers. *J Bone Jt Surg - Ser A.* 1982;
8. Ferretti A, Monaco E, Ponzo A, et al. Combined Intra-articular and Extra-articular Reconstruction in Anterior Cruciate Ligament-Deficient Knee: 25 Years Later. *Arthrosc - J Arthrosc Relat Surg.* 2016;32(10):2039–47.
9. Monaco E, Maestri B, Conteduca F, Mazza D, Iorio C, Ferretti A. Extra-articular ACL Reconstruction and Pivot Shift. *Am J Sports Med.* 2014;42(7):1669–74.
10. Sonnery-Cottet B, Saithna A, Cavalier M, et al. Anterolateral Ligament Reconstruction Is Associated with Significantly Reduced ACL Graft Rupture Rates at a Minimum Follow-up of 2 Years: A Prospective Comparative Study of 502 Patients from the SANTI Study Group. *Am J Sports Med.* 2017;
11. Zaffagnini S, Marcacci M, Lo Presti M, Giordano G, Iacono F, Neri MP. Prospective and randomized evaluation of ACL reconstruction with three techniques: A clinical and radiographic evaluation at 5 years follow-up. *Knee Surgery, Sport Traumatol Arthrosc.* 2006;
12. Roos H, Adalberth T, Dahlberg L, Lohmander LS. Osteoarthritis of the knee after injury to the anterior cruciate ligament or meniscus: the influence of time and age. *Osteoarthr Cartil.* 1995;
13. Outerbridge RE, Dunlop JAY. The problem of chondromalacia patellae. *Clinical Orthopaedics and Related Research.* 1975.
14. Øiestad BE, Holm I, Aune AK, et al. Knee function and prevalence of knee osteoarthritis after anterior cruciate ligament reconstruction: A prospective study with 10 to 15 years of follow-up. *Am J Sports Med.* 2010;
15. Padulo J, Oliva F, Frizziero A, Maffulli N. Basic principles and recommendations in clinical and field science research: 2018 update. *Muscles Ligaments Tendons J.* 2018;
16. Tegner Y, Lysholm J. Rating systems in evaluation of knee ligament injuries. *Clin Orthop Relat Res.* 1985;
17. Lysholm J, Gillquist J. Evaluation of Knee Ligament Surgery Results with Special Emphasis on Use of a Scoring Scale. *Am J Sports Med.* 1982;10(3):150–4.
18. Hefti F, Muller W, Jakob RP, Staubli HU. Evaluation of Knee Ligament Injuries with the IKDC Form. *Knee Surgery, Sport Traumatol Arthrosc.* 1993;1(3–4):226–34.
19. Fairbank TJ. KNEE JOINT CHANGES AFTER MENISCECTOMY. *J Bone Joint Surg Br.* 1948;
20. KELLGREN JH, LAWRENCE JS. Radiological assessment of osteo-arthritis. *Ann Rheum Dis.* 1957;
21. Lanzetti RM, Monaco E, De Carli A, et al. Can an adjustable-loop length suspensory fixation device reduce femoral tunnel enlargement in anterior cruciate ligament reconstruction? A prospective computer tomography study. *Knee.* 2016;
22. Lanzetti RM, Lupariello D, De Carli A, et al. Can the outside-in half-tunnel technique reduce femoral tunnel widening in anterior cruciate ligament reconstruction? A CT study. *Eur J Orthop Surg Traumatol.* 2017;
23. Monaco E, Fabbri M, Redler A, et al. In-out versus out-in technique for ACL reconstruction: a prospective clinical and radiological comparison. *J Orthop Traumatol.* 2017;
24. Monaco E, Fabbri M, Redler A, et al. Anterior cruciate ligament reconstruction is associated with greater tibial tunnel widening when using a bioabsorbable screw compared to an all-inside technique with suspensory fixation. *Knee Surgery, Sport Traumatol Arthrosc.* 2019;
25. Claes S, Hermie L, Verdonk R, Bellemans J, Verdonk P. Is osteoarthritis an inevitable consequence of anterior cruciate ligament reconstruction? A meta-analysis. *Knee Surgery, Sports Traumatology, Arthroscopy.* 2013.
26. Yoo JC, Ahn JH, Lee SH, Yoon YC. Increasing incidence of medial meniscal tears in nonoperatively treated anterior cruciate ligament insufficiency patients documented by serial magnetic resonance imaging studies. *Am J Sports Med.* 2009;
27. Ferretti A, Conteduca F, De Carli A, Fontana M, Mariani PP. Osteoarthritis of the knee after ACL reconstruction. *Int Orthop.* 1991;
28. Rochcongar G, Cucurulo T, Ameline T, et al. Meniscal survival rate after anterior cruciate ligament reconstruction. *Orthop Traumatol Surg Res.* 2015;
29. Maffulli N, Oliva F. Coper Classification Early After ACL Rupture Changes With Progressive Neuromuscular and Strength Training and Is Associated With 2-Year Success: Letter to the Editor. *American Journal of Sports Medicine.* 2019.
30. Devitt BM, Bouguennec N, Barfod KW, Porter T, Webster KE, Feller JA. Combined anterior cruciate ligament reconstruction and lateral extra-articular tenodesis does not result in an increased rate of osteoarthritis: a systematic review and best evidence synthesis. *Knee Surgery, Sports Traumatology, Arthroscopy.* 2017.
31. Monaco E, Ponzo A, Lupariello D, et al. Repair of antero lateral ligament injuries in acute anterior cruciate ligament tears: An in vivo study using navigation. *Muscles Ligaments Tendons J.* 2019;
32. Noyes FR, Huser LE, Jurgensmeier D, Walsh J, Levy MS. Is an Anterolateral Ligament Reconstruction Required in ACL-Reconstructed Knees with Associated Injury to the Anterolateral Structures? A Robotic Analysis of Rotational Knee Stability. *Am J Sports Med.* 2017;
33. Terry GC, Hughston JC, Norwood LA. The anatomy of the ilioapatellar band and iliotibial tract. *Am J Sports Med.* 1986.

Effectiveness of an Ayurvedic Treatment Protocol in Knee Ligament Injuries – An Observatory Report

K.M. Pratap Shankar¹, P. P. Nair², G. Sureshkumar³, G.K. Swamy⁴

^{1,2}Research Office (Ay), NARIP, Cheruthurthy, CCRAS, Ministry of AYUSH, Government of India, New Delhi, India

³ Statistician

⁴ Director, NARIP, Cheruthurthy, CCRAS, Ministry of AYUSH, Govt of India, 679531.

CORRESPONDING AUTHOR:

K.M. Pratap Shankar
Research Office (Ay), NARIP
Cheruthurthy, CCRAS
Ministry of AYUSH, Government of India
679531 New Delhi, India
E-mail: kmpvarma@gmail.com

DOI:

10.32098/mltj.03.2020.16

LEVEL OF EVIDENCE: 4

SUMMARY

Objective. We aimed to document the effectiveness of an Ayurvedic treatment protocol in patients with knee ligament injuries.

Methods. We observed 20 patients with knee ligament injuries ranging from partial to complex meniscal/ligamentous pathological states who underwent an Ayurvedic treatment protocol. Knee Outcome survey (KOOS) and International Knee Documentation Committee (IKDC) scores were assessed as baseline figures along with supportive radiological reports if available such as an MRI and the same scores were used to assess the effectiveness of the Ayurvedic treatment protocol. Person centered stage wise administration of Ayurvedic medicaments and external therapies were carried out for the concerned patients. The outcome measures of change in KOOS and IKDC scores were analyzed pre-treatment [a1], post-treatment [a2] and after a follow-up [a3] of 3 months. Data analysis was carried out using Statistical Package for Social Sciences (SPSS). The ordinal data was subjected to Friedman's test. Post Hoc comparisons were carried out using Wilcoxon test (with Bonferroni correction). Results with p-value < 0.01 were considered significant.

Results. There were statistically significant differences in KOOS and IKDC scores between a1 and a2; & a1 & a3. When analyzed between a2 & a3 in the prescribed parameters, results were statistically insignificant. KOOS-Overall scores with Friedman's test between a1, a2 and a3 were $\chi^2(2) = 28.737, p = 0.000 < 0.01$. Post hoc analysis with Wilcoxon signed-rank tests (at Bonferroni-adjusted significance level) between a1 & a2 gave results $Z = -3.921, p = 0.000 < 0.017$ and between a1 & a3 it was $Z = -3.771, p = 0.000 < 0.017$. Between a2 & a3 the result was $Z = 0.000, p = 1.000 > 0.017$. IKDC score with Friedman's test between a1, a2 & a3 were $\chi^2(2) = 32.430, p = 0.000 < 0.01$. Post hoc analysis with Wilcoxon signed-rank tests, between a1 & a2 was $Z = -3.920, p = 0.000 < 0.017$ and between a1 & a3 was $Z = -3.922, p = 0.000 < 0.017$. The analysis between a2 & a3 gave result $Z = -2.234, p = 0.025 > 0.017$. A variable in knee joint rehabilitation viz. the body mass index (BMI) of the patients did not seem to influence the results. The treatment was found to be comparatively more effective in females and in patients who engaged in moderate labour as well as who led a sedentary lifestyle. Elderly population though of less number in the study, experienced improved joint stability and relief in symptoms statistically as well as clinically.

Conclusions. The preliminary analysis of this observatory report indicates that suggested Ayurvedic treatment protocol is effective in knee ligament injuries, wherein it improves joint stability, reduces the symptoms of pain, swelling, stiffness and rehabilitates the individual towards his daily activities of strenuous/non strenuous origin. The effectiveness observed after the treatment phase sustained across the follow up period of 3 months as well. To substantiate the effectiveness of the prescribed Ayurvedic treatment protocol in decelerating the osteoarthritis onset in a traumatic knee injury requires long term follow-ups. Yet we have documented some positive leads from this report wherein Ayurvedic treatments may be adopted for effective and non-invasive rehabilitation of knee ligament injuries ranging from partial to complex origin and also in decelerating the risk of developing early osteoarthritis.

KEY WORDS

Ayurvedic management; knee ligament injury; rehabilitation.

BACKGROUND

The knee, a compound synovial joint is the largest weight bearing joint which plays a significant role while adopting major routine postures and activities as a part of daily living such as sitting, walking and running. Thus, an injury to the knee adversely affects the quality of life of an individual irrespective of his/her age, gender and occupation. The function and stability of the knee in fact depends on specific muscles, ligaments, cartilage, synovial and other connective tissues (1). The most common knee injury observed in clinical practice is Anterior Cruciate Ligament (ACL) tear which is about 86.5%, followed by Lateral & Medial meniscal injuries which are around 78.24% (2). At times multi ligamentous knee injuries comprising of a wide range of ligaments and intra articular injury patterns are also reported in orthopedic clinics. There are operative methods aiming at anatomical reconstruction and non-operative treatment strategies for rehabilitation and recovery to the pre-injury status and at delaying the post traumatic osteoarthritis onset (3).

A manuscript on multi ligamentous knee injuries, concluded that, the effectiveness of treatments adopted in knee injuries remained controversial due to lack of prospective comparative clinical outcome studies and patient reported outcome. It also suggested the need of individualized rehabilitation protocols towards expected outcomes (4). A literature review on conservative or surgical treatments in anterior cruciate ligament tears observed that, the concept of reduced chances of further meniscal lesions in a surgically reconstructed ACL may not be just because of the intervention but may also result from a decrease in strenuous activities post-surgery (5). It is estimated that 60.2% of sport person does not return to strenuous activities after an anatomical reconstruction of their injured knee (2). The aforementioned literature review concluded that there was not enough evidence to recommend a reconstruction surgery more than a systematic neuromuscular rehabilitation in ligament injuries of knee. Also, whatever be the treatment modality the chances of post traumatic osteoarthritis could also not be denied. Quadriceps weakness, flexion contractures and patella femoral pain etc. post a knee injury hamper the activities of daily living to a significant extent even after adopting surgical correction or neuromuscular rehabilitation (5).

Susruta Samhita, an Ayurvedic treatise details various surgical, parasurgical and other manipulative techniques intended to restore and rehabilitate injured joints and other connective tissues (6). An overview of various forms of bandages and immobilizing techniques is found in this textbook. Current Ayurvedic clinical practices in such joint

pathologies has evolved from these conventional methods and have been upgraded to patient centered quality care intending restoration to the activities of daily living.

We report an Inpatient level observation of 20 patients admitted at National Ayurveda Research Institute for Panchakarma, Cheruthuruthy, Kerala; diagnosed with injuries to the ligaments of knee following which who underwent Ayurvedic treatment protocol. In concerned patients with Ayurvedic treatments that comprised of internal administration of specific medicaments and external therapies we noted significant improvement in the parameters of pain, swelling and functional disability of the knee joint. Patients were able to return to activities of daily living with improved joint stability. This suggests the applicability of Ayurvedic healthcare approaches in joint injuries of sports or non-sports origin. Although Ayurvedic physicians exhibit clinical expertise in rehabilitating acute or subacute traumatic joint disorders, neuromuscular and connective tissue pathologies; minimal effectiveness studies are published in this arena. Ayurvedic therapies also hold substantial scope in the field of Sports Medicine. This observation intends to report the effectiveness of Ayurvedic treatment strategies in ligament injuries of knee.

METHODS

This is an observational report of 20 cases admitted in NARIP, Cheruthuruthy between the time period May to September 2019 diagnosed with knee ligament injuries ranging from partial to complex ones and who underwent Ayurvedic treatment protocol. Information on the knee injury was recorded based on the patient history and supportive radiological report. Personal information such as age, weight, height, and occupation were recorded. Knee Outcome Survey (KOOS) (7) and International Knee Documentation Committee (IKDC)- subjective evaluation score 8 were used to determine the extent of insult with regards to activities of daily living and involvement in sports and recreational activities. Person centered stage wise administration of Ayurvedic medicaments and external therapies were carried out for the concerned patients (**table I**).

The outcome measures of change in the KOOS and IKDC scores were analyzed pre-treatment [a1], post-treatment [a2] and after a follow-up [a3] of 3 months. Data analysis was carried out using Statistical Package for Social Sciences (SPSS). The ordinal data was subjected to Friedman's test. Post Hoc comparisons were carried out using Wilcoxon test (with Bonferroni correction). Results with p-value < 0.01 were considered significant.

Table I. Person centered stage wise management of the symptoms in knee ligament injury.

Sl No.	Stage	Internal Medicine*	External therapy*	Approximate duration of the treatment
01	Acute/Inflammatory phase (Vrana shopha stage)	1. Rasnasaptaka kashaya 2. Yogaraja Guggulu	1. Dasamoola Kashaya Dhara ¹ 2. Lepana ² with Nagaradi choornam 3. Bandana ³ with Murivenna oil	3 – 7 days/ until swelling subside (If swelling is not present, directly stage 2 can be initiated)
02	Post Inflammatory phase (Vrana stage/ Bhagna stage/ Vatahara stage)	1. Dhanvantaram Kashaya 2. Gandha thylam	1. LT bandana 2. Abhyanga ⁴ with Dhanvantaram oil 3. Janu (Knee) Dhara ¹ with Dhanvantaram oil 4. Matra Basti ⁵ with Dhanvantaram Mezhupakam oil	7 days
03	Final stage (Vatahara/ Bhruhmana stage)	1. Dhanvantaram Kashaya 2. Gandha thylam	1. Annalepanam ⁶ 2. LT bandana	7 days
04	Follow up stage (rehabilitative phase)	1. Dhanvantaram Kashaya 2. Gandha thylam	1. LT bandana 2. Quadriceps exercises 3. Diet rich in calcium, Vit.D, Zinc and magnesium	LT bandana is to be done daily for 1month, every alternative days for 2 months, weekly twice for next two months and weekly once in the last month. No.2 & 3 is advised for 6 months

*Details of medicines are attached as supplementary material as Supplementary file with the manuscript

¹Controlled & systematic pouring of herbal decoctions and medicated oils, ²external application of paste, ³bandaging, ⁴massage technique, ⁵enema with medicated oil, ⁶external application of medicated rice.

Selected internal medicines (**table I**) and medicines for external therapies were procured from a GMP certified company.

The external therapies namely Abhyanga (massage technique), Lepana-Annalepana (external applications, **figures 11, 12, 13** & ... \Photos & Videos\Video Rec.2 (Annalepanam).mp4), Kashaya dhara – Taila dhara (controlled & systematic pouring of herbal decoctions and medicated oils **figures 8, 9** & ... \Photos & Videos\Video Rec.1 (Janu dhara).mp4), Matra Basti (enema with medicated oils) and Bandhana (bandaging techniques, **figure 10**) were administered in the Panchakarma theatres of the Institute.

OBSERVATIONS

Demography

Out of 20 patients observed, females and males represented about 45 % and 55 % respectively of the total sample. While considering the nature of work that the concerned patients adopted as a part of daily living; 20% of the patients were

indulged in heavy labour (building workers and the like), 55% of the patients executed moderate labour (such as a home maker) and the remaining 25% had sedentary lifestyles (indulging in long hours of desk work).

Other characteristics considered were variables such as age, height, weight and body mass index (BMI) for which the mean, standard deviation, range and Confidence intervals were analyzed (**table II**).

Type and extent of knee ligament insult

Based on type of ligaments injured; 14 patients were recorded with meniscal tear and the remaining 06 patients presented with cruciate ligament injury (**table III**).

Effectiveness of the Ayurvedic treatment protocol – KOOS and IKDC scores

The effectiveness of the Ayurvedic treatment protocol were assessed in terms of percentage increase in KOOS and IKDC scores recorded post treatment (after treatment and after the follow up period of 3 months, **table IV**)



Figure 11. Method of preparation of Medicated njavara rice paste:

'Njavara or shastika-sali' (*Oryza Sativa L.*) is a traditional medicinal rice grown in Southern part of India which is extensively used in Ayurvedic treatments. The rice is cooked in a decoction prepared with milk and a herb namely bala (*Sidare-tusaLinn*) and applied over the affected area.



Figure 12. Medicated Njavara rice paste.



Figure 13. Massaging both the knee joints with medicated njavara rice paste. Refer to video recording no.2.



Figure 8. Materials required for Janudhara with medicated oil.



Figure 9. Procedure of Janudhara – with medicated oils, Refer to video recording no.1.



Figure 10. Bandaging technique.

Table II. General Characteristics of the sample.

Sample variables	MeanSD	Range	95% CI
Age	38.6512.96	52	(32.97,44.33)
Height	152.26.89	25	(149.18,155.22)
Weight	63.659.09	35	(59.67,67.63)
BMI	27.574.16	18.6	(25.75,29.39)

Table III. Extent of knee ligament insult.

Type of injury	Complete	Percentage	Partial	Percentage	Total
Medial meniscal / Lateral meniscal tear	03	23	10	77	13
Posterior cruciate ligament / Lateral cruciate ligament tear	03	43	04	57	07

Table IV. Summary of Scores.

Score	Pre-treatment Median Score (IQR)	Post-treatment Median Score (IQR)	Follow-Up Median Score (IQR)
KOOS-Pain	49.50(42.00-68.00)	81.00(71.40-91.25)	80.00(76.25-91.25)
KOOS-Symptom	55.50(46.00-69.25)	91.00(71.00-96.00)	93.00(79.00-99.00)
KOOS-ADL	57.50(44.50-96.25)	87.50(69.75-96.25)	86.50(81.25-96.00)
KOOS Sport/ Rec	32.50(11.25-48.75)	72.50(51.25-78.75)	72.50(51.25-85.00)
KOOS-QOL	31.00(19.00-44.00)	63.00(40.25-78.00)	59.50(44.00-83.25)
Overall KOOS Score	49.50 (42.00-68.00)	81.00(71.50-91.25)	80.00(76.25-91.25)
IKDC Score	34.45(26.13-42.80)	70.70(53.73-77.00)	75.85(62.63-88.80)

KOOS score

The outcome measures of change in KOOS Score were analyzed using the prescribed sub parameters namely pain, other symptoms, ADL, sport/rec, quality of life and overall KOOS Score. The scores were measured pre-treatment (a1), post-treatment (a2) and after a follow-up of 3 months (a3) - (**figures 1,2,3,4,5,6**). For each of the study variable, it was observed that the KOOS score differed significantly with respect to pre and post treatment phases, whereas no significant change was observed between the post-treatment and follow-up scores. These results suggested that the scores improved significantly after the treatment phase and the improved scores were maintained at follow-up period of three months.

Friedman's test was conducted on a sample of size $n = 20$ for each of the study variables (**table V**). There was a statistically significant difference in scores measured during the three periods for pain, symptoms, ADL, sports/rec, QoL and overall score.

Further, results of the Post hoc analysis using Wilcoxon signed-rank tests at Bonferroni-adjusted significance level ($p < 0.017$) for the sub parameters between a1 & a2 phase assessments and a1 & a3 showed significant difference at 1% level. However no significant difference was observed in the scores measured during phase a2 & a3 (**table VI**).

These results showed that the scores improved significantly after the treatment phase and were consistent even after a follow-up period of three months. The effect sizes for each of these variables suggested a moderate to high clinical significance.

IKDC scores

As for KOOS, a Friedman's test was conducted to compare the IKDC scores measured at a1 phase, a2 & at a3 (**tables V, figure 7**). There was a statistically significant difference in scores measured during the three periods with $\chi^2(2) = 32.430$, $p = 0.000 < 0.01$. Post hoc analysis with Wilcoxon

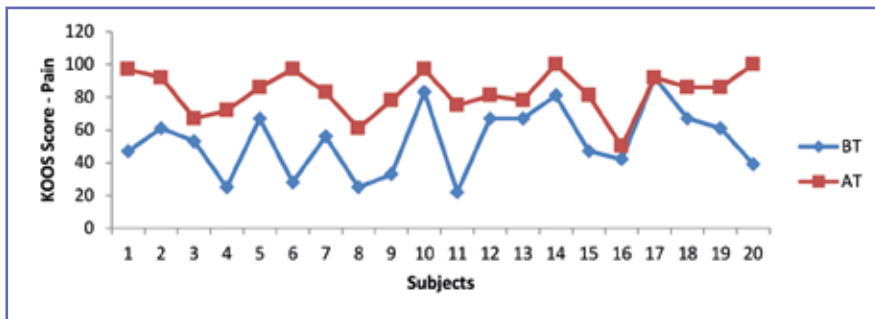


Figure 1. KOOS pre-treatment and post-treatment scores for pain.

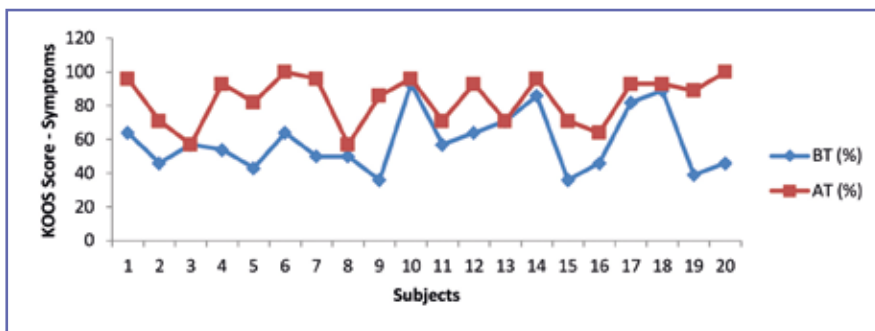


Figure 2. KOOS pre-treatment and post-treatment scores for other symptoms.

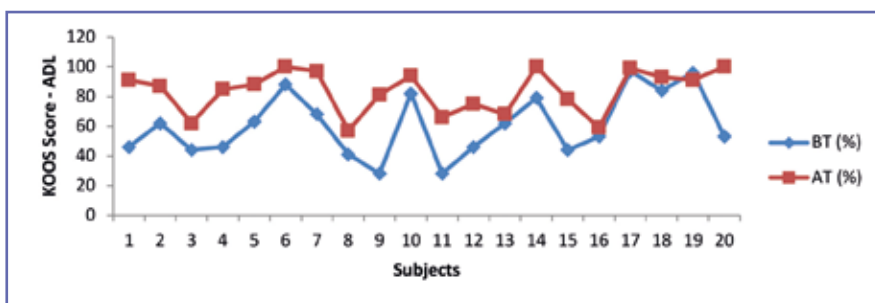


Figure 3. KOOS pre-treatment and post-treatment scores for ADL.

Table V. Friedman’s test results on KOOS parameters and IKDC scores after treatment (n=20).

Score	Chi square Value	P-value
KOOS-Pain	26.083	<0.01*
KOOS-Symptom	27.634	<0.01*
KOOS-ADL	17.636	<0.01*
KOOS Sport/ Rec	26.587	<0.01*
KOOS-QOL	19.541	<0.01*
Overall KOOS Score	28.737	<0.01*
IKDC Score	32.430	<0.01*

*Results significant at 1% level

signed-rank tests at Bonferroni-adjusted significance level (0.017) revealed that IKDC scores for symptoms that measured changes between a1 & a2 scores showed significant results with $Z = -3.920$, $p = 0.000 < 0.017$, with effect size $r = -0.619$ and between a1 & a3 with $Z = -3.922$, $p = 0.000 < 0.017$; with effect size $r = -0.620$. As in KOOS the effect sizes, for each variable suggested a moderate to high clinical significance. No significant difference was observed in the scores measured a2 & a3 with $Z = -2.234$, $p = 0.025 > 0.017$. Like KOOS observations, these results too suggested that the scores improved significantly after the treatment and the improved scores were consistent after a follow-up period of three months (table VI).

Some patients reported at the OPD after a period of 6 months (8/20) to 1 year (9/20). In those selected patients, Wilcoxon test was conducted to determine whether there was a significant difference in the KOOS–IKDC scores observed after such long-term follow-ups (tables 11, 12). The results indicated that scores recorded after treatment showed no significant difference even after a follow-up period of 6 months to 1 year.

Effectiveness of the treatment protocol with respect to sample variables

Age, gender, nature of work and BMI were assessed with respect to KOOS and IKDC scores (tables VII, VIII, IX). Here, the percentage increase in IKDC and KOOS score after the treatment were more evident in elderly people than others. But, as the number of elderly patients was less (05%) compared to the other age groups, this may not be generalized. While considering the gender wise distribution and the effectiveness of the treatment, percentage increase in mean KOOS and IKDC scores were more evident in females.

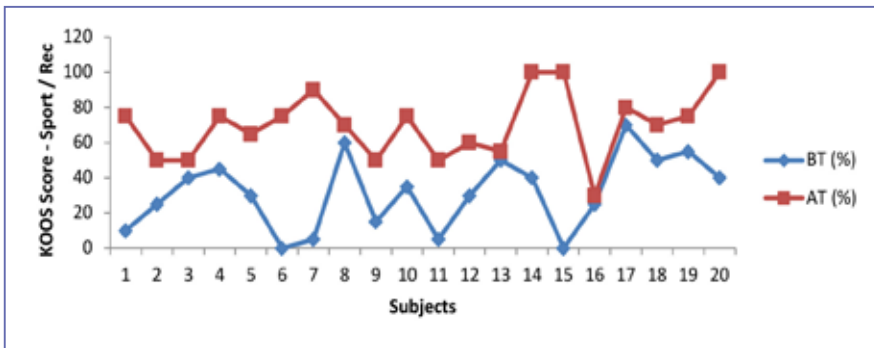


Figure 4. KOOS pre-treatment and post treatment scores for sports/rec.

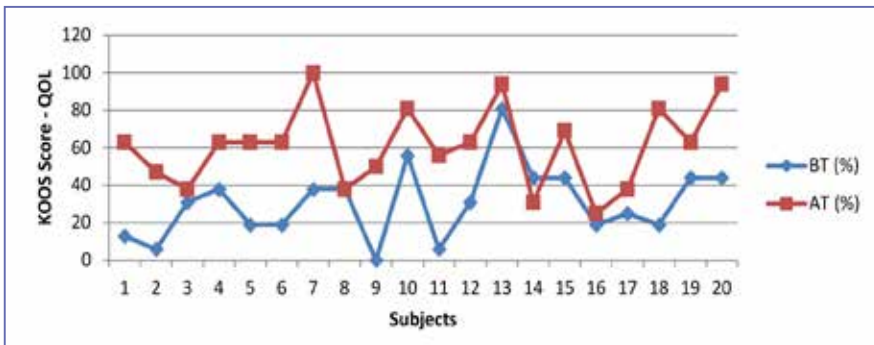


Figure 5. KOOS pre-treatment and post treatment scores for QoL.

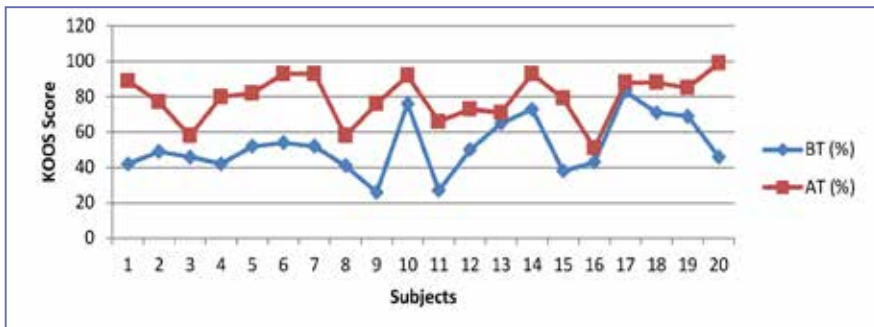


Figure 6. KOOS pre-treatment and post-treatment over all scores.

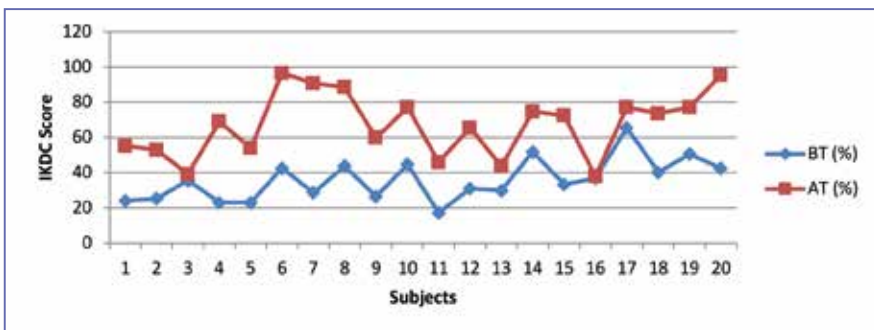


Figure 7. IKDC pre-treatment and post treatment scores.

Further, patients who indulged in moderate labour showed a better response to the treatment protocol in terms of improvement scores on an average, with respect to IKDC, whereas those who lead sedentary lifestyles responded more as far as KOOS scores were considered

While considering the type of ligament injury and the effectiveness of the treatment adopted, it was found that in patients with complete meniscal tear, there was a significant increase in KOOS scores after adopting the treatment protocol. In case of IKDC score, significant improvement in mean percentage score was evident in patients with partial meniscal tear (table X)

Considering the variable body mass index (BMI) and effectiveness of the treatment adopted, measured using KOOS and IKDC scores, there was no significant correlation (Spearman's correlation co-efficient (KOOS)-rho = 0.036, p value = 0.880 > 0.01 and (IKDC) rho = - 0.032, p value = 0.894 > 0.01) between BMI and the improvement in scores.

DISCUSSION

Janu sandhi marma (the knee joint)

Ayurveda is an established complementary healthcare service that originated in India. Because of its person centered diagnostic methods and personalized treatment modalities, it is gaining global attention from various health related communities. WHO is significantly contributing towards upgrading the traditional practices in Ayurvedic Sciences to Evidence Based Medicine considering the increase in demand of Ayurvedic herbals among the global patient community (9). Susruta Samhita is an ancient textbook that was written as early as about 1000 BC, most of which was dedi-

Table VI. Post hoc analysis using Wilcoxon signed-rank tests at Bonferroni-adjusted significance level ($p < 0.017$) for the KOOS sub parameters and IKDC scores.

Score	Phase a1 – a2		Phase a1 – a3		Phase a2 – a3	
	Z Value	P-value & Effect size	Z Value	P-value & Effect size	Z Value	P-value
KOOS-Pain	-3.826	<0.01*; -0.605	-3.463	<0.01*; -0.548	-1.197	>0.01
KOOS-Symptom	-3.724	<0.01; -0.588	-3.812	<0.01*; -0.603	-0.385	>0.01
KOOS-ADL	-3.847	<0.01* -0.608	-3.398	<0.01*; -0.537	-0.071	>0.01
KOOS Sport/ Rec	-3.923	<0.01*; -0.620	-3.717	<0.01*; -0.587	-0.057	>0.01
KOOS-QOL	-3.699	<0.01*; -0.585	-3.219	<0.01*; -0.509	-0.341	>0.01
Overall KOOS Score	-3.921	<0.01*; -0.619	-3.771	<0.01*; -0.596	0.00	>0.01
IKDC Score	-3.920	<0.01*; -0.619	-3.922	<0.01* -0.620	-2.234	>0.01

*Results significant at 1% level

Table VII. Age wise distribution and percentage changes in IKDC and KOOS scores.

Age group	Frequency (in %)	% Increase in Score	
		IKDC	KOOS
Young	20	26.5	33.93
Adults	75	26.8	29.91
Elderly	5	38	46

Table VIII. Gender wise distribution and percentage changes in IKDC and KOOS scores.

Gender	Frequency (in %)	% Increase in Score	
		IKDC	KOOS
Female	45	35.22	35.53
Male	55	20.81	28.23

Table IX. Amount of labour/strenuous activities and percentage changes in IKDC and KOOS scores.

Amount of labour/strenuous activities	Frequency (in %)	% increase in IKDC Score	% increase in KOOS score
Heavy Labour	20	19	29.33
Moderate Labour	55	32.45	31.9
Sedentary / Desk Job	25	23.4	35.88

Table X. Mean percentage score improvement and extent of knee ligament insult.

Mean percentage score improvement	MM / LM Tear		ACL tear	
	Complete	Partial	Complete	Partial
KOOS	38.67	28	22.33	17.25
IKDC	32.60	34.73	24.93	26.47

cated to surgical and parasurgical manipulations. Kshara sutra (medicated seton), kshara karma (caustic alkali), agni karma (cautery), rakta moksha (blood-letting), plastic reconstruction of facial characters with special mention to

ear, nose and lip, manipulations in bhagna (fractures) and sandhimoksha (joint dislocations) such as aanchana (traction), peedana (compression), samkshepa (immobilization) and bandhana (bandaging) have been explained in this

Table XI. Wilcoxon values –KOOS & IKDC post-treatment and at follow-up of 6 month in selected patients.

Score	Comparison of scores measured After treatment and at Follow-up	
	Z Value	P-value
KOOS-Pain	-2.038	0.042>0.01
KOOS-Symptom	-0.524	0.600>0.01
KOOS-ADL	-1.193	0.233>0.01
KOOS Sport/ Rec	-1.355	0.176>0.01
KOOS-QOL	-0.350	0.726>0.01
Overall KOOS Score	-1.183	0.237>0.01
IKDC Score	-1.684	0.092>0.01

textbook. Different types of surgical instruments, suturing materials, usage of twine for ligature, different types of bandaging techniques, dressing materials, splints for fractured bones too are mentioned. Bandaging techniques were specific to the severity of the condition, seasonal variations and the anatomical sites (10) (Sootrasthana 16/ 86-89).

Marmas (vital points) were considered as the conjuncture site of multiple anatomical structures as asthi (bone), sandhi (joints), peshi (muscles) sira (blood vessels) and snayu (ligaments, tendons or anatomical suture lines). There are five different types of marmas of which one is sandhi marma (Joints) (10) (Sharirasthana 6/369-370).

Janu sandhi or the knee joint is considered as a sandhi marma, which when traumatized (janumarma abhighata) results in khanjatva (disability/ weakness) (10) (Sharirasthana 6/372-373). Based on the prognosis of the knee insult, the knee is considered as a vaikalyakara marma. Vaikalyakara marmas are those vital points in the human body which when traumatized results in permanent disability. The specialty of vaikalyakara marma such as the knee joint is that a timely and an appropriate intervention from a good physician shall restore the activities of the tissue afflicted at this site (10) (Sharirasthana 6/370).

Expected effectiveness of procedures

The treatment procedure of “dhara” represents the rhythmic and systematic pouring of medicaments over specific body parts or the entire body surface for a stipulated time. In case of knee ligament injuries, two specific dhara procedures are adopted; the kashaya dhara (pouring of herbal decoctions) and the taila dhara (pouring of medicated oils) (figures 8, 9). Kashaya dhara is selected in an inflammatory phase and taila dhara is appropriate where inflam-

Table XII. Wilcoxon values – KOOS and IKDC post-treatment and at follow-up of 1 year in selected patients.

Score	Comparison of scores measured After treatment and at Follow-up – 1year	
	Z Value	P-value
KOOS-Pain	-0.105	0.917>0.01
KOOS-Symptom	-0.845	0.398>0.01
KOOS-ADL	-0.593	0.553>0.01
KOOS Sport/ Rec	-1.119	0.263>0.01
KOOS-QOL	-1.183	0.237>0.01
Overall KOOS Score	0.508	0.611>0.01
IKDC Score	-1.125	0.260>0.01

matory signs are minimal, i.e., in a degenerative phase. The medicines selected for herbal decoction in Kashaya dhara is Dasamoola (roots of ten different herbs). Dasamoolakashaya is effective in reducing swelling and other signs of inflammation (11) (Guduchyadivarga, 49). An animal study conducted by Parekar et al has proven the analgesic and anti-inflammatory potential of Dasamoola (12). Taila dhara is done with Dhanvantaram oil which is specifically indicated in traumatic injuries is expected to strengthen the musculoskeletal framework (13) (Sharirasthana, 2/47-52).

“Lepana” refers to local application of medicated pastes. Application of paste or the lepa is the first and foremost treatment for inflammation (10) (Sutrasthana, 18/3). Nagaradi choornam (the herbal combination of powdered drugs) along with tamarind leaf juice was used here as lepa. The ingredients in Nagaradi choorna (Suppl. file) possess anti-inflammatory and analgesic properties¹⁴ (ChurnaKalpana). After mixing the medicated powder with the prescribed leaf juice, the mixture is heated and when warm, is applied over the afflicted joint. Tamarind juice possesses anti-inflammatory, analgesic and antinociceptive effects (15) and thus is expected to enhance the therapeutic effects of the herbal drugs.

“Abhyanga” refers to specific massaging techniques with medicated oils. After the acute/inflammatory phase, abhyanga is specifically indicated in fractures and other joint pathologies (16) (Sutrasthana, 3/54-55). Here, abhyanga was done with Dhanwantaram oil (18) (Suppl. file).

Matra-basti is the trans-rectal administration of medicaments especially medicated oils, in predetermined doses. It is expected to reduce the symptoms of pain and stiffness and it also strengthens musculoskeletal systems. Dhanwantaram Mezhukupaka (Mezhukupaka is a special preparation with medicated oils exclusively prepared for transrectal administration) was selected here for the matra basti procedure.

Bandhana refers to unique bandaging techniques that immobilizes the joint and thereby promotes the healing process (10) (Sootrasthana 16-17). Susrutha Samhita has given prime importance to the bandaging techniques in the treatment of injuries. He has given detailed description of various types of bandage materials and fourteen types of bandaging techniques (10) (Sutrasthana, 18/86-89). Here in the management of ligament injuries, bandaging was done initially i.e., in the inflammatory phase with Murivenna oil (Suppl. file). Murivenna is medicated oil which is used for healing contusions, wounds and fractures. The medicines used in the preparation of Murivenna possess anti-inflammatory properties (17).

In the post inflammatory phase, bandaging was done with specific herbal combination named as LT bandhana (**figure 10**); (suppl. file). LT bandhana possess anti-inflammatory, analgesic, antioxidant, tissue regeneration and joint strengthening properties (14, 18, 19). Coconut meat scrap and egg white used in this bandage is rich in protein, trace minerals (Mg, Zn) and vitamins which help in wound healing, tissue building, collagen formation, reduction of inflammation and strengthen the bone, muscles and tendons (20, 21). On bandaging the joint with medicines mixed with egg white and coconut meat scrap, in addition to immobilizing the joint; enough nutrition is also being supplied transdermally which may accelerate the tissue regeneration and strengthen ligaments, muscles, tendons and thereby it improves the joint stability.

Annalepa refers to application of a paste prepared out of cooked 'njavara' medicinal rice variety, over specific body parts. This is a method administered to nourish the joint, enhance joint stability and delay the onset of post traumatic osteoarthritis (**figures 11,12,13**).

The medicinal properties of njavara may be attributed to its anti-oxidant²² and anti-inflammatory activities²³. High thiamine and Ph, K, Na, Ca, Mg contents in the njavara rice indicates the reason why it is found clinically effective in degenerative joint pathologies, muscular atrophies and neuritis.

Ayurvedic intervention and observed results in knee ligament injuries

Ayurvedic interventions show clinically significant improvements in traumatic/degenerative joint pathologies with special mention in delaying osteoarthritis onset. The approach is usually patient centered with individualized treatment guidelines and medicaments. This observatory report is a primary analysis based on patient reported outcomes after Ayurvedic treatments in knee ligament injuries.

Do Kyung Kim (24) discussed regarding the compromised extensor muscle strength and reduced improvement in

females compared to males after reconstruction surgery, making them difficult to rehabilitate (24). But in this report, after the treatment phase and while the follow up period, improved knee indices in form of improved KOOS and IKDC scores were found in female patients compared to male counterparts. This is a positive lead.

While assessing the KOOS-IKDC scores, the treatment protocol gave statistically significant results after the treatment phase and the improved scores were consistent even after the follow up period. Clinically also the patients reported improved joint stability and reduction in symptoms such as pain, swelling and joint stiffness.

Ayurvedic treatment was more significant in improving KOOS and IKDC scores in meniscal injuries than cruciate ligament tears. Interestingly, in patients with partial meniscal injuries, changes in IKDC scores were more significant than KOOS scores. Conversely, in complete meniscal injuries changes in KOOS scores were rather more significant. Also, while considering the amount of labour that the patients engaged in as a part of daily living, those who indulged in moderate labour as homemakers got higher IKDC scores than KOOS scores after treatment. Conversely, those who led a sedentary lifestyle responded to the treatment with improved KOOS scores than IKDC scores. A probable reason for these observations may be the fact that KOOS scores are more concerned with scoring the total disability index with respect to symptoms of meniscal and ligament injuries like pain, swelling and stiffness than functional and stability status. IKDC subjective evaluation score emphasizes functional status with respect to activities of daily living/ indulge in strenuous activities. As patients with meniscal injuries responded more when compared to patients with cruciate ligament tears, it is inferred that Ayurvedic treatments improves the functional status of the joint by reducing the symptoms rather than imparting the functional stability.

Richard F Loesser (25) stated that elderly population were more vulnerable to traumatic knee injuries and if manifested were difficult to rehabilitate due to co morbidities such as sarcopenia and osteopenia²⁵. But in this study, elderly participants reported significant relief in symptoms with improved joint stability after the treatment protocol. The statistical significance reported in this sample is not generalizable as the number of elderly patients was less compared to the other age groups, yet this seems a positive lead in rehabilitating knee injuries with Ayurvedic interventions in elderly.

While considering a significant variable namely the BMI which is related to rehabilitation of the knee injuries (26), knee being an important weight bearing joint; this report found no correlation between BMI of the patients and the

extent of improvement in KOOS and IKDC scores. This is a crucial finding where the results were equally significant in all participants irrespective of their BMI.

Limitations and recommendations

This observatory report lacks radiological evidence after the treatment phase or follow up. A significant confounder namely, the nature of trauma (acute traumatic/ chronic degenerative) with respect to time of initiation of the treatment protocol in patients was not taken into account as these factors were not adequately represented in the sample. But out of curiosity a Mann Whitney U test on improvement in KOOS and IKDC scores across the acute traumatic and chronic degenerative groups yielded insignificant results (KOOS- U= 34, p-value > 0.01; IKDC- U= 35.5, p-value > 0.01) which meant the treatment was effective irrespective of the nature of trauma and time of initiation of treatment. But as afore mentioned, this cannot be generalized due to insufficient representation of this cofounder in the studied sample. Also, long term follow ups are very crucial in knee ligament injuries to collect data on incidence of osteoarthritis, indulgence in strenuous activities, functional indices with respect to activities of daily living etc. Some patients reported to the OPD as a part of long-term follow ups (6 months to 1 year), and the results indicated that the statistical significance noted after treatment sustained even after such long-term follow ups. This is a definite positive lead in knee rehabilitation. Yet it seems incorrect to generalize these finding as all patients did not turn up after such long-term follow ups. This study thus lacks such long term follow up data. Samples

with equal representation of all possible risk factors would help produce a stronger evidence for the findings.

There are effective Ayurvedic treatment strategies and rehabilitation techniques to treat acute, subacute and chronic knee ligament injuries which are of partial or complete origin. This observation gives a primary insight on specific non-invasive Ayurvedic treatments which are commonly practiced in knee ligament injuries that give promising results in knee joint rehabilitation by reducing symptoms of pain, swelling and stiffness and thereby restoration to the activities of daily living. Even such treatments may be adopted following a reconstruction surgery where knee joint stiffness and other symptoms prevail after surgery. Randomized clinical trials may be conducted to compare the efficacies of such person centered alternative medical approaches with standard treatments like reconstruction surgeries. Also, there is significant scope of integrative approaches in effective recovery where Ayurvedic treatment protocols may be added to standard reconstruction surgeries/ non-invasive rehabilitation techniques in injuries of sports or non-sports origin. Appropriate implementation, evaluation and interpretation of clinical findings are fundamental and crucial in case reporting (27). This report shall stand as a significant background to appropriately designed, implemented, evaluated and interpreted clinical trials of different traditional and alternative medical practices in musculo-skeletal disorders/ injuries of varied origin.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

REFERENCES

1. Gupton M, Terryberry RR. Anatomy, Bony Pelvis and Lower Limb, Knee. [Updated 2018 Dec 6]. In: StatPearls. Treasure Island (FL): StatPearls Publishing 2020 Jan.
2. John R, Dhillion MS, Syam K, Prabhakar S, Behera P, and H. Singh. Epidemiological profile of sports-related knee injuries in northern India: An observational study at a tertiary care centre. *J Clin Orthop Trauma* 2016 Jul-Sep; 7(3): 207-211.
3. Lynch AD, Chmielewski T, Bailey L, et al. Current Concepts and Controversies in Rehabilitation After Surgery for Multiple Ligament Knee Injury. *Curr Rev Musculoskelet Med* 2017 Sept; 10(3): 328-345
4. Cox CL, Spindler KP. Multiligamentous Knee Injuries – Surgical Treatment Algorithm. *N Am J Sports Phys Ther* 2008 Nov; 3(4):198-203.
5. Delince P, Ghafil D. Anterior cruciate ligament tears: conservative or surgical treatment? A critical review of the literature. *Knee Surg Sports Traumatol Arthrosc* 2012 Jan;20(1): 48-61.
6. Valiathan MS. The Legacy Of Susruta. Orient Longman 2007; 31.
7. Roos EM, Lohmander LS. The Knee injury and Osteoarthritis Outcome Score (KOOS): from joint injury to osteoarthritis. *Health Qual Life Outcomes* 2003; 1:64.
8. Collins, Devyani Misra, Felson DT, Crossley KM, Roos EM. Measures of Knee Function. *Arthritis Care Res (Hoboken)* 2011 Nov; 63(011): S208-S228.
9. Anand Choudary and Neetu Singh. Contribution of world health organization in the global acceptance of Ayurveda). *J Ayurveda Integr Med* 2011 Oct-Dec; 2(4): 179-186.
10. Yadavji TA, editor. *Susrutha Samhita*. Varanasi: Chaukamba Krishanadas Academy 2008.
11. Pandey GS, editor. *Bhavaprakashanighantu*. Varanasi: Chaukamba Bharati Academy 2004.
12. Parekar RR, Bolegave SS, Marathe PA, Rege NN. Experimental evaluation of analgesic, anti-inflammatory and anti-platelet potential of Dashamoola. *J Ayurveda Integr Med* 2015;6(1):11–18.
13. Vaidya HP, editor. *Vagbhata's Astanga Hrdayam*, Varanasi: Chaukambha Sanskrit Series Office 2002.

14. Sahasrayogam. (A traditional compilation of Ayurvedic formulations practiced by Ayurvedic physicians in Kerala). Alappuzha: Vidyarambam Publications 2016.
15. Pinar Kuru. Tamarindus indica and its health related effects. Asian Pacific Journal of Tropical Biomedicine 2014;4(9):676-681.
16. Astangasangraha. Varanasi: Chaukhambha Orientalia 1998.
17. Lalithamma K. Pharmacopeia. Trivandrum: Govt Ayurveda College Publication dept.
18. Sahu R, Dhongade HJ, Pandey A, Medicinal properties of Nardostachys jatamansi (A Review). Orient J Chem 2016;32(2): 859-866.
19. Saleh-e-In MM, Sultana N, Rahim MM. Chemical composition and pharmacological significance of Anethum Sowa L. Root. BMC Complement Altern Med 17, 127 (2017) <https://doi.org/10.1186/s12906-017-1601-y>.
20. https://www.nutritionvalue.org/Nuts%2C_raw%2C_cocunut_meat_nutritional_value.html.
21. McGee, Harold. On Food and Cooking: The Science and Lore of the Kitchen. New York: Scribner 2004.
22. Rao AS, Reddy SG, Babu PP, Reddy AR. The antioxidant and antiproliferative activities of methanolic extracts from Njavara rice bran. BMC Complement Altern Med 2010 Jan 28; 10:4. Published 2010 Jan 28. doi:10.1186/1472-6882-10-4.
23. Shalini V, Jayalekshmi A, Helen A. Mechanism of anti-inflammatory effect of tricetin, a flavanoid isolated from Njavara rice bran in LPS induced hPBMCs and carrageenan induced rats. Mol Immunol 2015 Aug;66(2)229-39.
24. Do Kyung Kim and Won Hah Park. Compared to male patients, females reported significantly less extensor muscle strength and less improvement 1 year after reconstruction. J Phys Ther Sci 2015 Dec;27 (12): 3847-3849.
25. Richard F Loesser. Ageing changes in the musculoskeletal systems contribute to develop of OA by making the joint more susceptible to joint injuries. Age related changes in musculoskeletal system and developing of osteoarthritis. Clin Geriatric Med 2010 Aug;26(3): 371-386.
26. Zheng H, et al. Body mass index and risk of knee osteoarthritis: systematic review and meta-analysis of prospective studies. BMJ Open 2015 Dec 11; 5 (12): e007568. Doi: 10.1136/bmjopen-2014-007568.
27. Padulo J, Oliva F, Frizziero A, Maffulli N. Muscles, Ligaments and Tendons Journal - Basic principles and recommendations in clinical and field Science Research: 2018 update. MLTJ 2018; 8(3): 305 – 307.

Histology and Type I Collagen, Tenascin and Elastin Expression in Autologous and Allogeneic Anterior Cruciate Ligamentoplasties

I. Guillén-Vicente, J. M. López-Alcorocho, J. Salvador-Marín, E. Rodríguez-Iñigo, G. Díaz-Motola, T. F. Fernández-Jaén, E. Ibáñez, P. Guillén-García

Traumatology and Orthopedic Surgery Department and Research Unit, CEMTRO Clinic, Madrid, Spain

CORRESPONDING AUTHOR:

Juan Manuel López-Alcorocho
Research Unit
CEMTRO Clinic
Ventisquero de la Condesa Street 42
28035 Madrid, Spain
E-mail: jm.lopez@amplifel.com

DOI:

10.32098/mltj.03.2020.17

LEVEL OF EVIDENCE: 4

SUMMARY

Background. Ligamentization has been accepted in Anterior Cruciate Ligament (ACL) surgery. The purpose of this study is to evaluate ligamentization of different allografts and autografts used as plasties through histological and type I collagen (COL1), tenascin (TEN) and elastin (ELA) expression analysis.

Methods. Prospective study of patients who underwent ACL reconstruction with at least 1-year follow-up. Biopsies were taken by arthroscopy and tissue sections were stained with hematoxylin-eosin and Masson's trichrome. Relative COL1, TEN and ELA expression was studied by RT-PCR. Hamstring tendon and intact ACL biopsies were used as controls.

Results. Eleven patients with a mean age of 36.6 ± 12.0 years were included. In 9 cases, ACL reconstruction was carried-out with autologous hamstring tendons while in the 2 remaining patients ACL was reconstructed using an achilles allograft. COL1, TEN and ELA expression of plasties was significantly different to ACL but similar to hamstring tendon. Histological analysis showed a dense fibroconnective tissue with cells similar to tenocytes, which could not be classified neither as a tendon nor as a ligament.

Conclusions. An ACL plasty, independently of its origin (autologous hamstring or allogeneic achilles) does not become a ligament, at least in our study period (5 years) but it does maintain ACL functionality.

KEY WORDS

Anterior cruciate ligament; hamstring autologous graft tendon; achilles allogeneous graft tendon; histological analysis; ligamentization; tenocytes.

BACKGROUND

Therapy for anterior cruciate ligament (ACL) tear includes invasive surgical techniques, in which the ruptured ACL is replaced by autologous (patellar, quadricepsal or hamstring more frequently) or allogeneic tendons (1). The objective of surgery is to restore knee biomechanics by reproducing the mechanical conditions of the native ACL. However, the original biological structures are substituted by different ones: a tendon replaces a ligament.

Tendons and ligaments are elastic connective tissues with similar composition but different arrangement of their components, which is related to the different roles they have to perform in the musculoskeletal system: ligaments connect bones to each other in order to restrict their relative

motions and tendons link muscles to bones (2). Water is the main component for both structures (55-70%) but exists in a higher percentage in tendons (2). Type I collagen, which represents 70-80% of the dry weight (higher in tendons), is responsible for the high tensile strength and hierarchical structure (3,4). Proteoglycans, glycosaminoglycans and glycoproteins are other molecules present in ligaments' and tendons' extracellular matrix (ECM) (4). Fibroblasts (called tenoblasts in the tendon), embedded in the ECM, are the main cell type of tendons and ligaments and synthesize all ECM molecules (2,3). All these components are hierarchically organized and while in tendons, fibers are present as compact parallel bundles, in ligaments they are compactly packed and not arranged in parallel bundles (5).

It is commonly accepted that implanted tendon structures adopt the role of ligaments: However, intra-articular “ligamentization” of the graft does not imply a complete transformation of a tendon graft into a ligament. In literature, a biological transformation process has been described as the graft remodels itself into a viable ACL-like tissue (6-8). Authors refer to an early phase with central graft necrosis and hypocellularity until the fourth week, followed by a proliferation, remodeling and revascularization phase up to the 12th week, followed finally by a restructuring phase which makes the graft resemble the properties of ACL³. Collagen fibers begin to resemble an ACL between 6 and 18 months after reconstruction⁹⁻¹¹. However, although matured tendon graft looks macroscopically similar to intact ACL, several authors have shown differences in biological and mechanical properties as well as in the distribution and diameters of collagen fibers comparing hamstring autograft with intact ACL (7,8,12,13). Some studies in animals have shown a histological structure halfway between tendon and ligament (13). The purpose of this study is to evaluate the features of autologous hamstring and allogeneic achilles tendons used as plasty for the ACL reconstruction through gene expression, histological, cellularity and pathological analysis of the plasty structure compared to normal hamstring tendon and intact ACL.

MATERIAL AND METHODS

Patients

This is a prospective case-series study carried out in patients operated for ACL reconstruction more than 1 year ago and who had been admitted to undergo new surgery in the same knee for other cause different to graft failure. The study was performed in accordance to ethical standards of the Helsinki Declaration of 1964, revised in 2013 and approved by the Institution’s Research Committee. All included patients signed an informed consent (14). Eleven patients accepted to participate in the study and a biopsy of the graft was taken during the new surgery. During exploration all patients showed stable knees. New surgeries consisted in total knee arthroplasty (1 case), arthroscopy after a sport injury (4 cases), meniscectomy (4 cases) and autologous chondrocyte implantation (2 cases). In the surgery, plasty integrity was confirmed in all cases. In order to take the plasty biopsy, synovial membrane that covered the plasty was firstly removed. Then, a 1-2 mm biopsy was taken in the middle third of the plasty with an arthroscopy basket forceps. Eight of these eleven patients accepted for a biopsy from hamstring tendon to be taken at the same surgical act. In the study, 3 more patients who were due to undergo a

knee surgery for meniscal lesions were asked for a biopsy of the ACL to be taken to provide an intact ACL as a control. Biopsies were also taken in the middle third of the ligament following the same protocol described above. At the surgery moment, these patients showed stable knees and no signs of osteoarthritis were observed in any case.

Histological analysis

One portion of each biopsy was stored at -20°C in RNAlater™ Stabilization Solution (ThermoFisher Scientific, Alcobendas, Spain) for gene expression study. The remaining fragment was fixed in formalin and paraffin-embedded. Blocks were cut using a microtome into 4-µm-thick sections. Tissue sections were stained with hematoxylin-eosin and Masson’s trichrome staining to study both tissue architecture and collagen composition as well as fiber disposition.

Gene expression analysis

RNA was isolated after treatment with TRIzol (ThermoFisher Scientific) and chloroform and isopropanol precipitation. One µg RNA was reverse-transcribed using Oligo(dT) (ThermoFisher Scientific), RNasin (Promega Co, Madison, WI) and the SuperScript® Reverse Transcriptase kit (ThermoFisher Scientific), following manufacturers’ instructions. Relative expression of type I collagen (COL1); GenBank accession: NM_000088.3, tenascin-C (TNC); accession: NM_002160.3, and elastin (ELA); accession: NM_000501.3, genes was determined by real-time PCR (RT-PCR) in a StepOnePlus thermocycler (Life Technologies, Alcobendas, Spain) using the expression of the β-actin (ACT) house-keeping gene; accession: NM_001101.3; as standard. Each gene was amplified using the specific primers and probes commercially designed (Taqman Gene Expression Assays, Life Technologies). All amplifications were carried-out using the Taqman Universal PCR Master Mix (Life Technologies), following the manufacturer’s instructions. Each sample was tested in triplicate and all genes were studied in the same PCR run.

STATISTICAL ANALYSIS

Statistical analysis was performed using the IBM SPSS Statistics version 22 software. Qualitative variables were expressed as a count and/or percentage. Normality of quantitative variables was checked with the Shapiro-Wilk test and expressed as the median (minimum – maximum) (non-normal variables) or mean ± standard deviation. Intraclass correlation coefficient (ICC) and 95% confidence interval for ICC were estimated to check consistency of trip-

licate measure corresponding to COL1, TNC and ELA gene expression. Chicchetti and Domenic's criteria¹⁵ were used to interpret reliability of measures for ICC value. According to these authors, consistency was considered poor if ICC value was less than 0.40; fair if it was between 0.40 and 0.59; good if the value was between 0.60 and 0.74 and excellent if ICC value was between 0.75 and 1.00.

With respect to COL1, TNC and ELA relative expression in ACL plasties, hamstring tendon and healthy ACL, if normality could not be demonstrated, expression of each gene were to be expressed as the median (minimum – maximum) of the triplicate and distribution in each sample type (plasty, hamstring tendon and ACL) and then contrasted with Mann-Whitney's U test (2 variables) or the Kruskal-Wallis test, including pairwise post-hoc tests (more than 2 variables). If variables had normal distributions, gene expression were to be expressed as the mean \pm standard deviation. Student's t test (2 variables) or ANOVA were used to contrast gene expression means, including pairwise post-hoc tests carried-out with Tukey's test (more than 2 variables). Gene expression distribution among the different causes second operation was checked with the Kruskal-Wallis test. Spearman's coefficient was used to study the existence of correlation.

Categorical variables were expressed as counts and/or percentage and compared with the Chi-Square Test, if applicable. In all comparisons and parameter estimation, a value of $p < 0.05$ (two-sided) was considered statistically significant.

RESULTS

Eleven patients (11 knees) were included in the study. Nine of them were men and 2 of were women with a mean age of 36.6 ± 12.0 years. Six ligamentoplasties were performed in right knees and 5 in left knees. In 9 cases, ACL reconstruction was carried out with autologous hamstring tendons (semitendinosus and gracilis) while in the 2 remaining patients ACL was reconstructed using an achilles allograft (**figure 1**). All patients practiced non-professional sport (7 soccer, 2 horse riding, 1 ski and 1 paragliding). Elapsed median time between ACL ligamentoplasty and new surgery was 4.0 years (2–30 years). As a control, healthy ACL samples were taken from 3 patients (two men and 1 woman who were 32, 34 and 41) all operated arthroscopically for meniscal lesions.

COL1, TNC and ELA relative expression was measured in triplicate. In **table I**, ICC and 95% confidence interval of ICC is depicted. For the 3 genes ICC value was between 0.40 and 0.59 and thus, the relative expression estimation of each gene by means of the triplicate measures was

considered fairly consistent. COL1, TNC and ELA relative expression in ACL plasties, hamstring tendons and healthy ACL were compared (**figure 2**). For each gene we found that means showed statistically significant difference among the three samples ($p < 0.001$ in each case, ANOVA). Pairwise comparisons gave similar results in the 3 genes: COL1 and TNC expression showed statistically significant higher values in the plasties and hamstring tendons than in healthy ACL while the opposite occurred with ELA expression which was significantly higher in healthy ACL than in plasties and hamstring tendon. In both situations, no statistically significant differences existed between plasty and hamstring tendon gene expression. To study the influence of the environment, gene expression was compared among the causes for the second operation, No significant differences were found between gene expression and cause for second surgery in any of the three studied genes ($p = 0.151$ for COL1, $p = 0.247$ for TNC and $p = 0.248$ for ELA; Kruskal-Wallis test). No correlation was found between COL1, ELA and TEN expression and elapsed time until second surgery either. Finally, we compared gene expression of patients implanted with autologous hamstrings with those implanted with allograft achilles tendon and no differences were found (**figure 3**).

Tissue architecture and collagen fibers disposition studied by hematoxylin-eosin and Masson's trichrome staining showed a dense fibroconnective tissue in all biopsies. In the case of control healthy hamstrings a collagenized matrix with a low-frequency crimp pattern of the collagen fibers is observed. Scattered in the collagen matrix, spindle-shaped cells similar to fibroblasts can be observed. Normal ACL tissue showed a similar fibroconnective tissue but with a high-frequency crimp pattern of the collagen fibers and similar spindle-shaped fibroblasts among collagen fibers. ACL grafts showed a dense fibroconnective tissue with a frequency crimp pattern of the collagen fibers intermediate between normal healthy hamstrings and ACL controls (**figures 4,5**). Spindle-shaped and ovoid fibroblasts disposed throughout collagen fibers were also observed. Although no differences in tissue organization between hamstrings (autologous) and achilles (allogeneic) grafts were observed, cellularity increased in the 5-year evolution grafts (**figure 5**) with respect to those of 2-year evolution (**figure 4**), independently of graft nature. Five-year evolution tissue showed more vascularization than two-year biopsies. No histological differences have been found when the causes for the second surgery were compared. Histologically, in all cases tissue graft showed a tissue organization which could be classified neither as a tendon nor as a ligament (**figures 4,5**).



Figure 1. ACL reconstruction, 8 years later. Arthroscopic view.

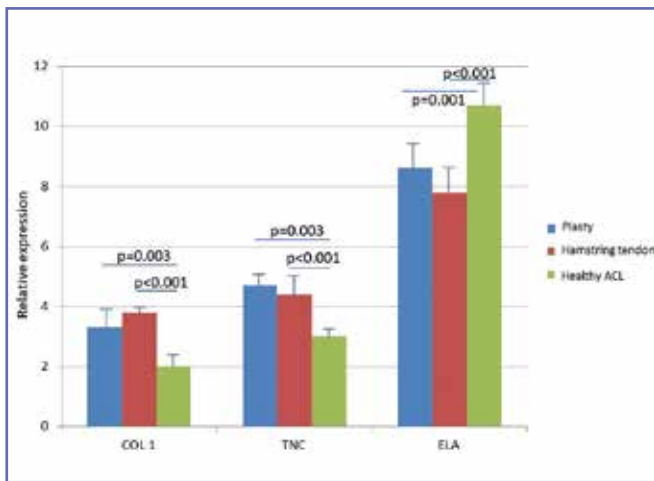


Figure 2. Mean relative Type I Collagen (COL1), tenascin (TNC) and elastin (ELA) expression in plasties, hamstring tendon and healthy ACL. Mean expression of these genes was significantly different among tissues ($p < 0.001$; ANOVA).

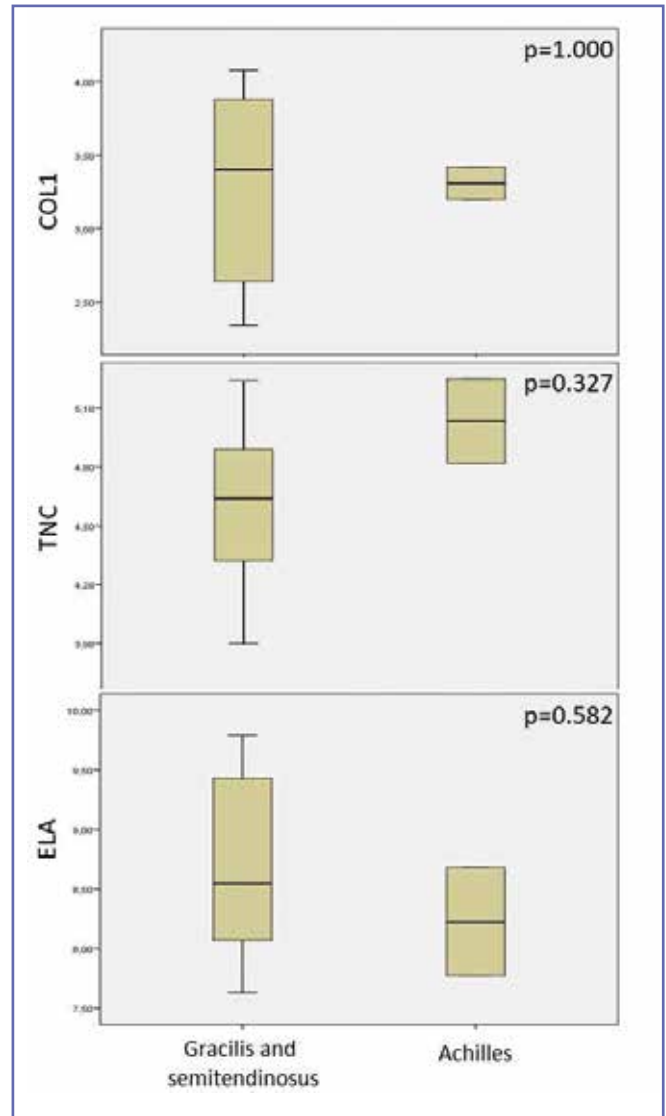


Figure 3. COL1, TNC and ELA expression in autologous hamstrings or allogeneic Achilles tendon plasties. Statistical comparisons were carried-out with the Mann-Whitney's U test.

Table I. Intraclass Correlation Coefficient (ICC) and 95% confidence interval of ICC for relative Type I Collagen (COL1), tenascin-C (TNC) and elastin (ELA) expression.

	COL1	TNC	ELA
ICC (95% Confidence Interval)	0.478 (-0.121 – 0.783)	0.493 (-0.089 – 0.789)	0.548 (0.029 – 0.812)
p-value	0.048	0.041	0.021

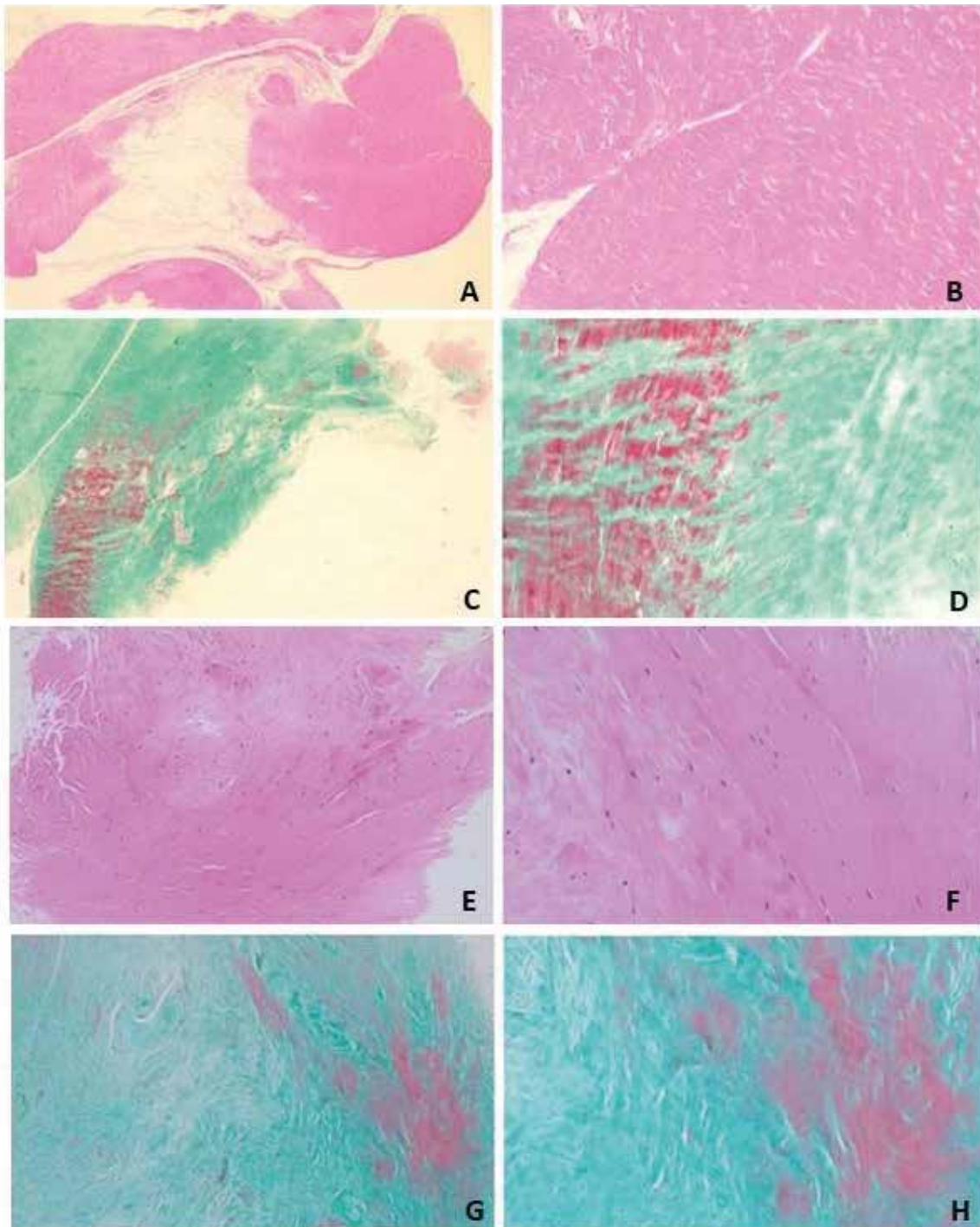


Figure 4. Representative pictures of hematoxilin-Eosin and Masson Trichrome staining of two-year (A-D) and three-year-evolution (E-H) hamstring tendons plasties. Collagenized fibroconnective tissue with the presence of spindle-shaped and ovoid fibroblasts scattered in the collagen matrix and focal smooth muscle fibers is observed. A and E: Hematoxilin-Eosin staining 4x; B and F: Hematoxilin-Eosin staining 20x; C and G: Masson's trichrome 4x; D and H: Masson's trichrome 20x.

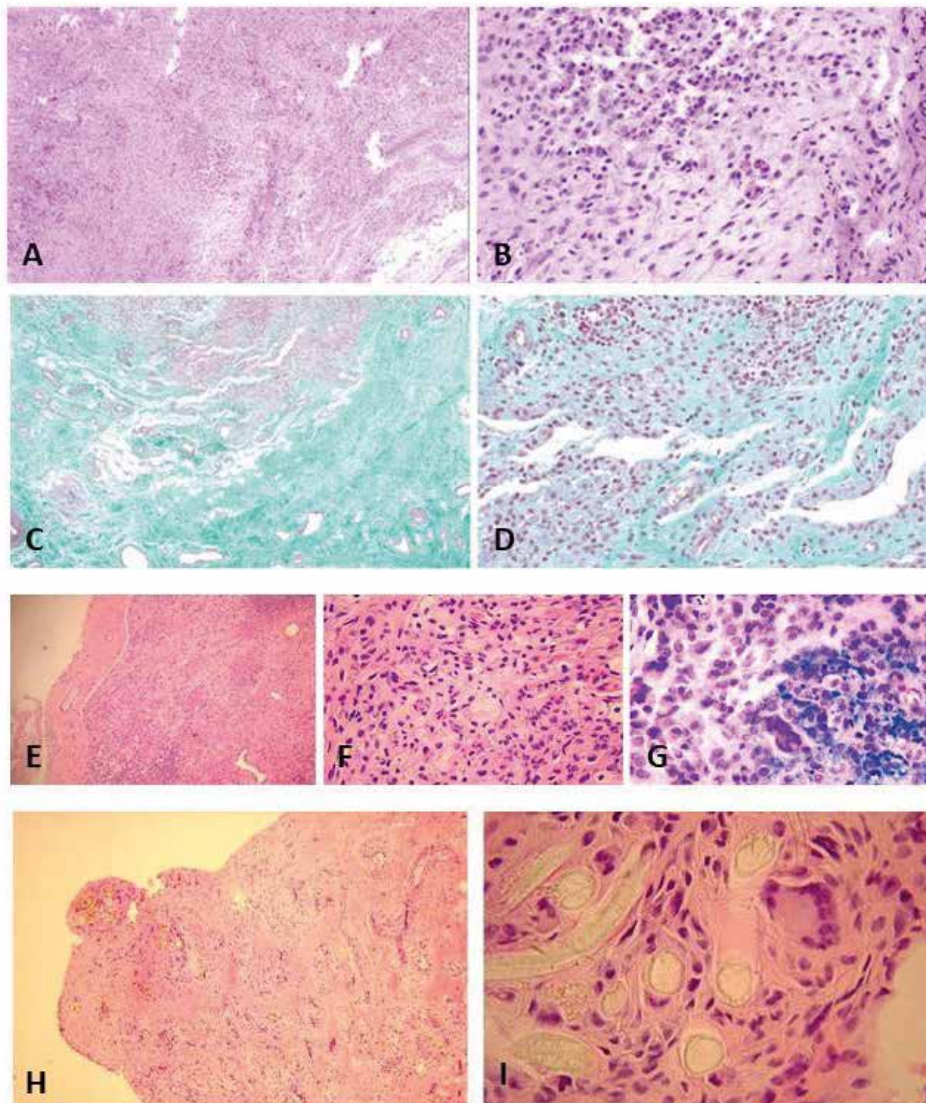


Figure 5. Representative pictures of five-year evolution autologous hamstring (A-D) and allogeneic achilles tendons plasties (E-I). In the hamstring tendon plasty, a collagenized fibroconnective tissue with spindle and ovoid cells and the presence of smooth muscle fibers is observed (A-D). The other two samples corresponded to achilles allograft tendon plasties (E-I) and a collagenized fibroconnective tissue with similar spindle-shaped and ovoid cells is shown. Notice the presence of vascular structures in both autologous and allogeneic grafts. A, E, H: Hematoxilin-Eosin staining 4×; B, F, I: Hematoxilin-Eosin staining 20×; C: Masson's trichrome 4×; D: Masson's trichrome 20×; G: Hematoxilin-Eosin staining 40×.

DISCUSSION

In this work we try to answer the question: is tendon a good substitute for an anterior cruciate ligamentoplasty? Hamstring autologous and Achilles allogeneic tendon plasties did not become a similar tissue to native ACL. Some authors observed tendon-specific biological features which did not exhibit ligamentous histological properties and for this reason the studied tissue resembles a tendon more than a

ligament. Authors agree that the graft will never be completely transformed into normal ACL tissue (7,10,16), although it could exhibit similar biomechanical properties when it comes to restoring knee stability early, with a ratio of between 72% and 100% returning to sport¹⁷. In the presence of adequate surgical techniques and good rehabilitation programmes, failures and re-injury rates are relatively uncommon (3-19%) (18,19), which evidence there is still margin for improvement.

In the present work, we have studied histological features and expression profile of some genes expressed in tendons and ligaments in ACL plasties from two different origins (autologous hamstrings and allogeneic achilles tendons) and with different evolution times, compared to normal hamstring tendon and intact ACL (8). In a systematic revision carried-out by Pauzemberg *et al.* (17), without including studies with allogeneic tendons, authors concluded that surgical technique and specially an anatomically incorrect reconstruction of the ACL could influence postoperative remodeling despite a possible increase of laxity or non-anatomical tension properties. It was not the case in our case-series, since 100% of included patients were operated by other causes.

In order to elucidate whether studied tissue was a tendon or a ligament, COL1, ELA and TEN were chosen due to the different expression pattern in both tissues. In fact, it has been published that COL1 and TEN are more abundant in tendons while ELA presents higher values in ligaments (4,5). Analysis of COL1, ELA and TEN in plasties revealed an expression pattern closer to tendon than to ligament, with higher mean amount of COL1 and TEN mRNA in tendons and plasties than in ligaments and higher mean expression of ELA in ligaments than in tendons and plasties (4,5), independently of plasty nature and the time elapsed between ligamentoplasty and sample harvesting. At least, with respect to the three studied proteins, these results suggest that both autologous and allogeneic plasties continue having the same protein expression profile than tendons. As far as we know, no studies about protein expression of ligamentoplasties in humans have been published. Most studies have been performed in animal models, especially in rabbits (20). Xie *et al.* (21) studied the expression pattern of COL1, COL3, growth factors, angiogenesis-promoting, and nerve-related genes in rabbits, concluding that ligament pattern expression is maintained when ACL remnant is preserved during ACL reconstruction. In our case, tibial fibers remnant are not used in the plasties, which could explain that expression of targeted proteins was more similar to tendon than to ligament.

As stated above, from a histological point of view, tendons and ligaments are structurally quite similar but they differ at the ultrastructural level, being the amount and disposition of large-diameter collagen fibers and collagen fibrils (8) and crimp pattern frequency of the collagen fibers (22,23) the main differences between both tissues. Crimp pattern frequency of the collagen fibers is highly correlated with the presence of myofibroblasts, a specialized cell type involved in crimp formation, whose number is increasing during graft evolution time. On the other hand, Abe *et*

al. (23) have demonstrated that during ACL remodeling, crimp pattern frequency is gradually changing and vascularization is increasing along time. Our plasties had a collagen crimp pattern frequency intermediate between tendon and ligament and higher cellularity and vascularization in 5-year evolution plasties compared with the 2-year ones. Our results support plasty remodeling after ACL replacement by a tendon, becoming a tissue whose features are intermediate between tendon and ligament. Since no differences were found between autologous hamstrings or allogeneic achilles tendon plasties, similar ligamentization process may occur irrespectively of graft nature.

Main weakness of the study are sample size and a relatively short histological determination. Its main strengths are the comparison with healthy hamstring tendon and ACL. This work represents the first attempt to determine protein expression together with histological study of ACL plasties from different origins: autologous hamstring and allogeneic achilles tendons. Furthermore, in the study, plasties from 2 to 5-year evolution have been included. However, a more detailed study, including relative expression of a large set of genes, including those involved in angiogenesis, vascular and nerve regeneration, tissue growth and more detailed histological study including other techniques such as light microscopy with polarization and electron microscopy is now being planned with the aim of ascertaining the mechanisms involved in the process commonly known as ligamentization.

CONCLUSIONS

Taken together, our results suggest that an ACL plasty, independently of its origin (autologous hamstring or allogeneic achilles tendon) does not become a ligament in a period of 5 years, although it is able to maintain ACL functionality and contribute to knee stability. Protein expression and histological analysis reveal that in the course of several years, plasties are gradually remodeled and become an intermediate tissue somewhere between a tendon and a ligament. This tissue shows very similar COL1, ELA and TEN expression patterns to tendons but typical collagen fiber disposition leans more on the side of a ligament, with time lapse being irrelevant.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Mario Wensell for carefully reviewing the manuscript's linguistic. This work has been supported by a grant from the Fundación Dr. Pedro Guillén.

AUTHOR CONTRIBUTIONS

IGV: ligamentoplasties, biopsies harvesting, manuscript reviewing; JMLA: gene expression by RT-PCR, statistical analysis, manuscript written; JSM: analysis of results, manuscript written; ERI: histological analysis, manuscript reviewing; GDM: analysis of results, manuscript reviewing; EI: ligamentoplasties, biopsies harvesting; TFFJ: ligamentoplasties, data analysis, manuscript reviewing; PGG: ligamentoplasties, biopsies harvesting, study planning, manuscript reviewing

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

ETHICS

The authors declare that this research was conducted following basic, ethical aspects and international standards as required by the journal (13).

REFERENCES

1. Marieswaran M, Jain I, Garg B, Shama V, Kalyanasundaram D. A Review on Biomechanics of Anterior Cruciate Ligament and Material for Reconstruction. *Appl Bionics Biomech* 2018; May 13. doi: 10.1155/2018/4657824.
2. Hoffmann A, Gross G. Tendon and ligament engineering in the adult organism: mesenchymal stem cells and gene-therapeutic approaches. *Int Orthop*. 2007;31:791-7.
3. Mienaltowski MJ, Birk DE. Structure, physiology, and biochemistry of collagens. *Adv Exp Med Biol*. 2014;802:5-29.
4. Miller MD, Thompson SR. Miller's review of orthopaedics, 7th Edition. Philadelphia: Elsevier 2012
5. Zitnay JL, Weiss JA. Load transfer, damage, and failure in ligaments and tendons. *J Orthop Res*. 2018;36:3093-104
6. Amiel D, Kleiner JB, Roux RD, Harwood FL, Akeson WH. The phenomenon of "ligamentization": anterior cruciate ligament reconstruction with autogenous patellar tendon. *J Orthop Res*. 1986;4:162-72
7. Zaffagnini S, De Pasquale V, Marchesini Reggiani L, et al. Electron microscopy of the remodelling process in hamstring tendon used as ACL graft. *Knee Surg Sports Traumatol Arthrosc*. 2010;18:1052-58.
8. Dong S, Xie G, Zhang Y, Shen P, Huangfu X, Zhao J. Ligamentization of Autogenous Hamstring Grafts after anterior cruciate ligament reconstruction : Mid-term versus Long-term Results. *Am J Sports Med* 2015;43:1908-17.
9. Weiler A, Peine R, Pashmineh-Azar A, Abel C, Südkamp NP, Hoffmann RF. Tendon healing in a bone tunnel. Part I: biomechanical results after biodegradable interference fit fixation in a model of anterior cruciate ligament reconstruction in sheep. *Arthroscopy* 2002; 18:113-23.
10. Abe S, Kurosaka M, Iguchi T, Yoshiya S, Hirohata K. Light and electron microscopic study of remodeling and maturation process in autogenous graft for anterior cruciate ligament reconstruction. *Arthroscopy*. 1993;9:394-405.
11. Claes S, Verdonk P, Forsyth R, Bellemans J. The "ligamentization" process in anterior cruciate ligament reconstruction: what happens to the human graft? A systematic review of the literature. *Am J Sports Med*. 2011;39:2476-83
12. Janssen RP, Scheffler SU. Intra-articular remodeling of Hamstring tendon grafts after anterior cruciate ligament reconstruction. *Knee Surg Sports Traumatol Arthrosc* 2014;22:2102-8.
13. Giordano M, Falciglia F, Poggiaroni A, Aulisa AG, Savignoni P, Guzzanti V. Histological changes of semitendinosus autograft after anterior cruciate ligament reconstruction in an immature rabbit model. *J Exp Orthop*. 2015;2:17-25.
14. Padulo J, Oliva F, Frizziero A, Maffulli N. Muscles, Ligaments and Tendons Journal - Basic principles and recommendations in clinical and field Science Research: 2016 Update. *Muscles Ligaments Tendons J*. 2016;19:6:1-5
15. Cicchetti, Domenic V. Guidelines, criteria, and rules of thumb for evaluating normed and standardized assessment instruments in psychology. *Psychological Assessment*. 1994;6:284-90.
16. Cho S, Muneta T, Ito S, Yagishita K, Ichinose S. Electron microscopic evaluation of two-bundle anatomically reconstructed anterior cruciate ligament graft. *J Orthop Sci*. 2004;9:296-301.
17. Pauzenberger L, Syré S, Schurz M. "Ligamentization" in Hamstring Tendon Grafts After Anterior Cruciate Ligament Reconstruction: A Systematic Review of the Literature and a Glimpse Into the Future. *Arthroscopy*. 2013;29:1712-21.
18. Hui C, Salmon LJ, Kok A, et al. Fifteen-year outcome of endoscopic anterior cruciate ligament reconstruction with patellar tendon autograft for "isolated" anterior cruciate ligament tear. *Am J Sports Med* 2011;39:89-98.
19. Pinczewski LA, Lyman J, Salmon LJ, et al. A 10-year comparison of anterior cruciate ligament reconstructions with hamstring tendon and patellar tendon autograft: A controlled, prospective trial. *Am J Sports Med* 2007;35: 564-74.
20. Wu B, Qiu Z, Li S, et al. Histological properties of autogenous hamstring grafts after anterior cruciate ligament reconstruction. *Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi*. 2018;32:873-9.
21. Xie GM1, Huang Fu XQ, Zhao JZ. The effect of remnant preservation on patterns of gene expression in a rabbit model of anterior cruciate ligament reconstruction. *J Surg Res*. 2012;176:510-6.
22. Weiss M, Unterhauser FN, Weiler A. Crimp frequency is strongly correlated to myofibroblast density in the human anterior cruciate ligament and its autologous tendon grafts. *Knee Surg Sports Traumatol Arthrosc*. 2012;20:889-95.
23. Abe S, Kurosaka M, Iguchi T, Yoshiya S, Hirohata K. Light and Electron Microscopic Study of Remodeling and Maturation Process in Autogenous Graft for Anterior Cruciate Ligament Reconstruction. *Arthroscopy*. 1993;9:394-405.

Associated Strengthening Exercises to Undenatured Oral Type II Collagen (UC-II). A Randomized Study in Patients Affected by Knee Osteoarthritis

A. P. Costa¹, V. Cunha Teixeira², M. Pereira³, P. Mota Ferreira³, P. A. Kuplich⁴, M. B. Dohnert⁵, J. F. da Silva Guths⁶, R. Boff Daitx⁶

¹ Department of Physiotherapy, Lutheran University of Brazil, Torres (RS), Brazil

² Physiotherapist, Torres (RS), Brazil

³ Physiotherapist, Capão da Canoa (RS), Brazil

⁴ Private Practice, Department of Orthopedics and Traumatology, Santa Luzia Hospital, Capão da Canoa, RS, Brazil

⁵ Research, Teaching and Extension Laboratory in Orthopedic Trauma Physiotherapy (LAPEFITO), Department of Physiotherapy, University of Gurupi - UnirG, Gurupi (TO), Brazil

⁶ Department of Physiotherapy, Lutheran University of Brazil, Torres (RS), Brazil

CORRESPONDING AUTHOR:

Marcelo Baptista Dohnert
Research, Teaching and Extension
Laboratory in Orthopedic
Trauma Physiotherapy (LAPEFITO)
Department of Physiotherapy
University of Gurupi - UnirG
355 Joaquim Batista de Oliveira Street
Vila Alagoana
77.403-170
Gurupi/TO, Brazil
E-mail: mdohnert@hotmail.com

DOI:

10.32098/mltj.03.2020.18

LEVEL OF EVIDENCE: 1B

SUMMARY

Background. Osteoarthritis (OA) is a chronic joint disease characterized by progressive degeneration of articular cartilage. It affects 20-25% of the population older than 45 years. The objective was to evaluate the effect of combining muscle strengthening exercises with the administration of oral type II undenatured collagen formulation (UC-II) in people with knee OA.

Methods. A double-blind, placebo-controlled, randomized controlled trial with 60 patients with knee OA, randomly divided into the following groups: UC-II and physiotherapy group (CPG), placebo UC-II and physiotherapy group (PCPG), and physiotherapy group (PG). For groups using an oral type II undenatured collagen formulation, UC-II, 40 mg/day UC-II was administered for 90 days. Muscle strengthening exercises were associated with neuromuscular electrical stimulation (NMES), being performed three times a week for 30 days. At 30 and 90 days and six months after the intervention, the following were evaluated: pain, quality of life, functional capacity, muscle strength, and joint mobility.

Results. Pain improved in all intervention groups, with no difference between groups. Quadriceps muscle strength increased in the CPG ($p<0.005$) and PG ($p<0.05$), the same being observed for active and passive knee flexion mobility ($p<0.05$). All groups decreased the TUG test execution time after 30 days ($p<0.005$ for the CPG and PG, and $p<0.05$ for the PCPG), but only the CPG and PG maintained the scores at 90-day and six-month assessments ($p<0.005$). Regarding the 6MWT, only the CPG increased the distance covered in all assessments ($p<0.005$). The PG traveled a greater distance than the PCPG at the 90-day assessment ($p<0.05$). The WOMAC score decreased significantly in all intervention groups. The Lequesne score decreased in all groups; however, the CPG and PG showed lower values at 30 days ($p<0.005$).

Conclusions. Muscle strengthening exercises improved pain, mobility, strength, and function in knee OA patients. The association of UC-II seems to have accentuated the effect of exercise on this clinical improvement, especially in the long term.

KEY WORDS

Knee; osteoarthritis; Circuit-Based Exercise; nutraceuticals; collagen type II; muscle strength.

BACKGROUND

Osteoarthritis (OA) is a chronic joint disease characterized by progressive degeneration of articular cartilage accompanied by subchondral bone remodeling and subsequent joint dysfunction (1). Approximately 42% of women and 31% of men over 60 are diagnosed with knee OA (2). Knee joint is the most commonly affected lower limb joint (3,4).

Knee OA treatments can be divided into surgical and nonsurgical (5). Results of pain and function improvement are more related to exercise than to pharmacological treatments (5). Thus, therapeutic exercise is considered a first-line treatment for OA (6). Exercise intervention for patients with knee OA should be broad and include lower limb strengthening, improving flexibility to reduce stiffness, increasing stability and balance, reducing body weight, in addition to a psychosocial intervention (6,7).

Type II collagen is the dominant collagen in articular cartilage (8-11). As collagen is the most prevalent component of the solid matrix of articular cartilage, its supplementation has been considered a key treatment option to prevent progressive cartilage damage over time and accelerate the

healing process after OA onset (11-14). For this purpose, a new nutraceutical has been studied in individuals with knee OA. It is the undenatured type II collagen (UC-II), taken from chicken sternal cartilage (12).

The present study evaluates the effect of combining muscle strengthening exercises with the administration of an oral type II undenatured collagen formulation (UC-II) in patients with knee OA.

MATERIAL AND METHODS

A double-blind, placebo-controlled, randomized controlled trial with 60 patients with grade 1-3 knee OA according to the Kellgren Lawrence radiological classification, conducted from April 2015 to May 2019.

Evaluation protocol

Knee OA was clinically and radiologically diagnosed by an orthopedist of the orthopedics and traumatology service at a regional hospital (**table I**)

Table I. Eligibility criteria.

Inclusion Criteria	Men and women aged 40 to 75 years, with unilateral and/or bilateral grade 1-3 knee OA (according to the Kellgren and Lawrence radiological classification) for more than three months, clinically and radiologically diagnosed by an orthopedist.
	Ambulatory patients.
	Lequesne functional score greater than 4.5.
	Availability during the study period (six months).
	Not participating in any other physical/drug treatment for the knee during the study period.
	Having signed the Informed Consent Form.
Exclusion Criteria	Patients with OA intensity above grade 6 according to the Kellgren and Lawrence classification, as confirmed by an orthopedist.
	Nonambulatory patients.
	History of inflammatory arthropathy, septic arthritis, rheumatoid arthritis, inflammatory joint disease, uric gout, joint fracture, fibromyalgia, collagen genetic disease, neurological disorders, cognitive deficits, history of asthma, type I or II diabetes, psychiatric disorders, and symptomatic heart disease.
	Clinical condition restricting exercise.
	Presence of skin injury.
	Skin disease in the thigh.
	Urinary incontinence.
	Pregnancy.
	Alcoholism.
	Cancer.
	Previous history of corticosteroid joint infiltration or knee viscosupplementation in the last three months.
	History of knee trauma or surgery in the last six months.
	Having three consecutive unexcused absences from physical rehabilitation.

After agreeing to participate in the study, participants were randomized by an independent researcher and referred for a functional physical evaluation performed by a blind assessor previously trained with the evaluation instruments. This professional was not aware of the intervention group to which the participant belonged.

Evaluations were performed prior to randomization, 30, 90 and six months after the end of the intervention protocol with UC-II. The same evaluator throughout the study always performed all evaluations. The Visual Analog Pain Scale (VAS) was used to assess pain. Knee joint mobility was determined by passive and active flexion and extension using a goniometer (brand Carci®). Muscle strength was assessed by manual dynamometry, using a push-pull dynamometer (brand Chattanooga®). The Timed Up & Go test (TUG) and the 6-minute walk test (6MWT) were used to assess functional capacity. Quality of life and level of functionality were assessed using the LEQUESNE scale and the WOMAC questionnaire for knee osteoarthritis.

Sample calculation

Pain was used as the primary outcome of the study for sample calculation. Based on the study of Crowley *et al.* (9), we estimated the mean initial percentage of pain of the study participants to be 100%. The mean final percentage of pain was 60% for subjects in the intervention groups after 90 days, and 85% for subjects in the placebo groups after treatment. Using a study power of 80%, a significance level of 95%, and a sample size ratio of 1:1 for all groups, we reached the estimated number of 13 subjects for each intervention group. Believing that the losses and refusals would be around 50%, we reached the initial number of 20 participants for each of the study groups, totaling 60 participants.

Randomization

Randomization was performed by an independent researcher, who did not participate in intervention and evaluation protocols.

Patients were randomly divided into a collagen + physiotherapy group (CPG), with 20 participants continuously receiving UC-II®, and muscle strengthening exercises; a placebo collagen + physiotherapy group (PCPG), with 20 participants continuously receiving placebo UC-II®, and muscle strengthening exercises; and a physiotherapy group (PG), with 20 participants receiving muscle strengthening exercises.

ETHICAL ASPECTS

This research has been approved by the IRB of the authors affiliated institutions. The study meets the ethical standards of the journal. (15)

UC-II intervention protocol

The randomizing researcher was responsible for providing UC-II or placebo UC-II to the researcher responsible for administering it to the participants. Vials containing the compounds were equally standardized so that the compound could not be identified. Both active and placebo UC-II® were administered for 90 days. Each participant received a vial containing 30 UC-II® capsules every 30 (thirty) days. Each UC-II® capsule contained 40 mg UC-II® standardized to 10 mg of bioactive undenatured type II collagen, identified by specific registration numbers to differentiate them from placebos. Participants in both groups were instructed to take one UC-II® capsule for breakfast for a daily dose of 40 mg UC-II®. Participants in the placebo group received cornstarch capsules. The researcher who administered the nutraceutical intervention, as well as the researcher who administered the physiotherapy and the researcher who performed the evaluations were not aware of which compound was assigned to each participant, only the randomizing researcher had such information.

Physiotherapy intervention protocol

The physiotherapy protocol was carried out for four weeks, with three interventions per week. The protocol was applied by an independent researcher, who was unaware of the administration of the nutraceutical UC-II®.

Prior to protocol implementation, all participants performed a warm-up cycling exercise for five minutes. Afterwards, a closed kinetic chain (CKC) muscle strengthening exercise program was performed through a 45-degree minisquat, associated with the use of neuromuscular electrostimulation (NMES). For NMES administration, a 10-channel electrical stimulator (Neurodyn, Ibramed®) was used with a carrier frequency of 2500 Hz, in the synchronous mode. Electrodes were fixed of the vastus medialis, vastus lateralis, rectus femoris (femoral quadriceps), femoral biceps, and semitendinosus (hamstring) muscles.

After electrode fixation, a five-minute warm-up protocol was initially performed using a 40-Hz stimulus frequency, a four-second contraction (“on”) time, a four-second rise time, a four-second “off” time, and a 12-second relaxation time. Afterwards, a 70-Hz stimulus frequency was used for

another 10 minutes, with the same parameters as above. Finally, a 100-Hz stimulus frequency was used for another 10 minutes for maximum muscle activation, keeping the same parameters.

During the period of muscle stimulation by the electric generator, the patient performed a 30-degree knee flexion minisquat, maintaining this position during the electrical stimulus, returning to full extension at the beginning of the “off” electrostimulation period. During the “off” period, the participant spontaneously performed another active minisquat without the presence of electrical stimulus, returning to the initial position until a new contraction generated by the electrical stimulator.

STATISTICAL ANALYSIS

The Statistical Package for Social Sciences (SPSS) version 23.0 was used for data analysis. Data were initially expressed as frequency, mean, median, and standard deviation. Afterwards, the normality of data distribution was analyzed using the Shapiro-Wilk test.

For intragroup comparative analysis (CPG, PCPG, and PG) at the various times of the study, parametric data were statistically analyzed by one-way analysis of variance (ANOVA) for repeated measures, followed by the Bonferroni post-

hoc test. Moreover, one-way analysis of variance (ANOVA) followed by the Tukey post-hoc test was performed for intergroup analysis, both at preintervention and 30 days, 90 days, and six months after the intervention protocol. For nonparametric variables, the Kruskal Wallis test was used between groups, and the Friedman test was used for analysis within each group. The significance level established for all statistical tests was $p < 0.05$.

RESULTS

Eighty-four (84) patients were initially selected for the study. Of these, 24 participants were excluded prior to randomization because they did not meet the eligibility criteria. Therefore, the initial sample had a total of 60 participants. All participants completed the 30-day and 90-day assessment. In the follow-up assessment, one CPG participant was not found, totaling a final sample of 59 participants (**figure 1**).

The groups were homogeneous for anthropometric characteristics, OA grade classification, pain duration, and affected knee (**table II**).

All intervention groups significantly reduced pain after 30 days of intervention, maintaining this improvement until the follow-up assessment. There were no differences between groups at any time of the study. The CPG reduced pain

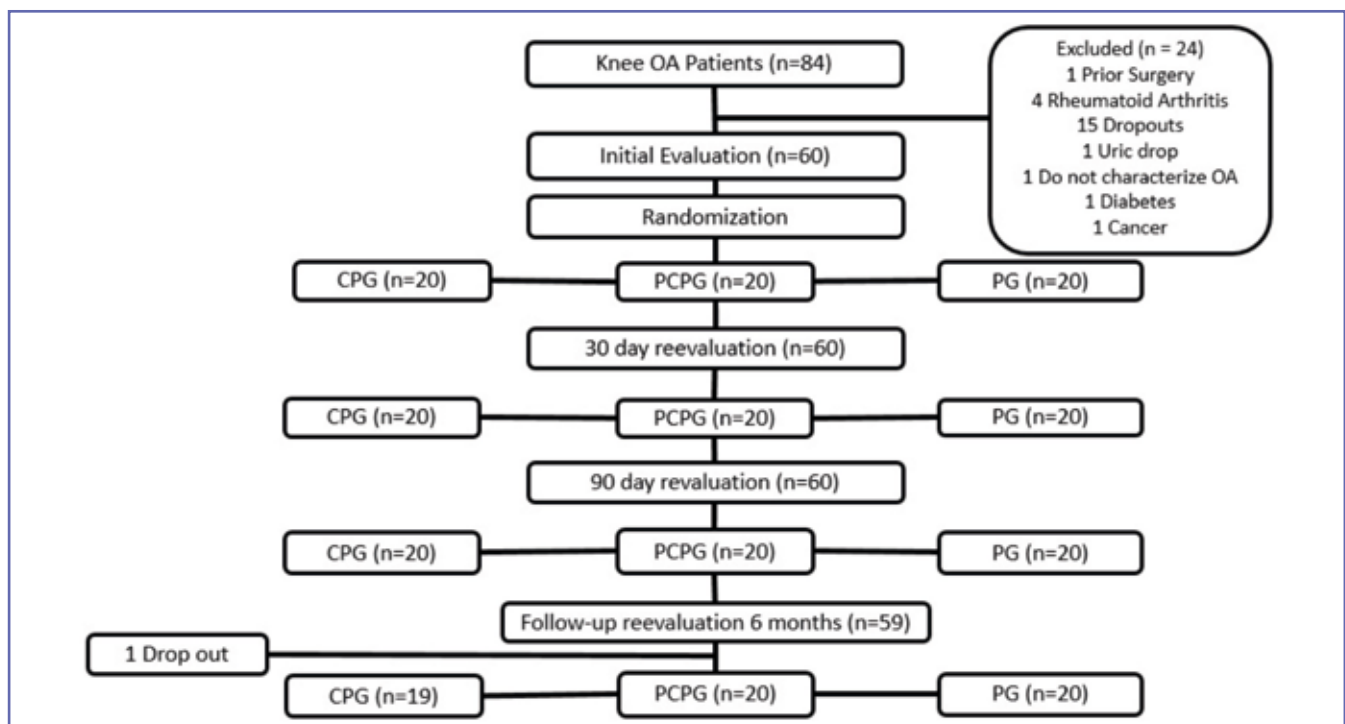


Figure 1. Study flowchart.

Table II. Characterization of the initial study sample (n=60).

Variable	Intervention Group			P
	CPG (n=20)	PCPG (n=20)	PG (n=20)	
Age, years (mean ± SD)	55.45 ± 8.78	57.35 ± 11.44	59.60 ± 8.22	0.397
Gender, M/F	6/14	4/16	11/9	0.057
Skin color, n (%)				0.126
White	20 (100.0)	18 (90.0)	20 (100.0)	
Black	0 (0.0)	2 (10.0)	0 (0.0)	
Occupation				0.186
Retired	5 (25.0)	7 (35.0)	12 (60.0)	
Housekeeper	4 (20.0)	7 (35.0)	0 (0.0)	
Government employee	1 (5.0)	0 (0.0)	0 (0.0)	
Professor	1 (5.0)	0 (0.0)	0 (0.0)	
Other	9 (45.0)	6 (30.0)	8 (40.0)	
Kellgren Lawrence, n (%)				0.880
Grade I	1 (5.0)	2 (10.0)	1 (5.0)	
Grade II	12 (60.0)	10 (50.0)	13 (65.0)	
Grade III	7 (35.0)	8 (40.0)	6 (30.0)	
Affected Knee, n (%)				0.402
Right	2 (10.0)	6 (30.0)	4 (20.0)	
Left	7 (35.0)	5 (25.0)	9 (45.0)	
Bilateral	11 (55.0)	9 (45.0)	7 (35.0)	
Cigarette use, n (%)				0.418
Yes	1 (5.0)	1 (5.0)	3 (15.0)	
No	19 (95.0)	19 (95.0)	17 (85.0)	
Time of pain, years (mean ± SD)	5.62 ± 6.88	7.25 ± 7.59	6.44 ± 6.69	0.507
Weight, kg (mean ± SD)	80.63 ± 13.57	80.67 ± 14.46	80.77 ± 11.22	0.999
Height, cm (mean ± SD)	162.40 ± 7.52	164.10 ± 8.81	163.95 ± 10.12	0.314
BMI, kg/cm ² (mean ± SD)	30.15 ± 4.85	30.34 ± 5.66	30.36 ± 5.75	0.991

#: One-way ANOVA; \$: Chi-square test; CPG = collagen + physiotherapy group; PCPG = placebo collagen + physiotherapy group; PG = physiotherapy group.

from 6.42 ± 1.68 points at baseline to 1.42 ± 1.77 at 30 days ($p=0.0001$), 2.74 ± 2.56 at 90 days ($p=0.0001$ compared to baseline), and 3.26 ± 2.88 after six months ($p=0.0001$ compared to baseline). The PCPG showed an initial pain level of 5.60 ± 2.11 points. At the 30-day assessment, pain was reduced to 2.65 ± 2.62 ($p=0.0001$). At the 90-day assessment, the pain level was 2.75 ± 2.53 ($p=0.0001$ compared to the initial assessment). After six months, the pain level was 3.05 ± 2.82 ($p=0.01$ compared to the initial assessment). Finally, the PG showed an initial pain level of 6.10 ± 1.91 points, which decreased to 1.80 ± 2.28 at the 30-day assessment ($p=0.0001$). At the 90-day assessment, the pain level was 2.35 ± 2.46 ($p=0.0001$ compared to the initial assessment), remaining at 2.30 ± 2.00 after six months ($p=0.0001$ compared to the initial assessment) (**figure 2**).

The PG demonstrated a significantly higher initial right quadriceps strength compared to the CPG. The CPG, however, significantly increased muscle strength at 30 days. The other groups did not improve muscle strength. At the 90-day and six-month assessments, the PG showed significantly greater muscle strength than the CPG and PCPG. Regarding left quadriceps muscle strength, only the CPG and PG increased muscle strength at 30 days, maintaining an improved strength at the 90-day and six-month assessments. The PG showed a significantly higher level of muscle strength than the CPG at the initial evaluation. At 30 days, the PG had a significantly higher level of left quadriceps strength than the PCPG ($p<0.05$). At the 90-day and six-month assessments, the PG showed higher muscle strength than the other groups (**table III**).

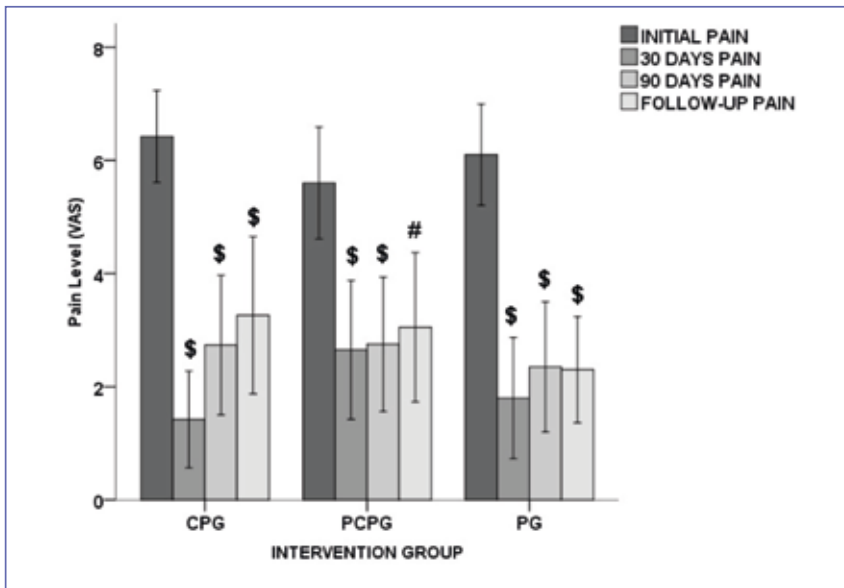


Figure 2. Pain assessment in the study groups.
 # p <0.05 compared to the initial assessment of the same group. ANOVA for repeated measures.
 \$ p <0.005 compared to the initial assessment of the same group. ANOVA for repeated measures.

Table III. Results of quadriceps and hamstring muscle strength (MVIC) in the initial study groups, 30 days, 90 days, and six months after UC-II administration (n=60).

Variable	Intervention Group			P
	CPG (n=20)	PCPG (n=20)	PG (n=20)	
Right quadriceps, kg ± sd				
Initial	17.71 ± 7.05	18.15 ± 7.54	27.60 ± 12.39	0.02 ^b
30 days	24.45 ± 10.59 ^{\$}	22.50 ± 9.39	29.20 ± 12.17	0.14
90 days	23.10 ± 10.24 [#]	21.90 ± 9.19	30.55 ± 11.82	0.02 ^a
Follow-up	23.32 ± 11.29	22.50 ± 10.05	31.05 ± 11.24	0.03 ^a
Left quadriceps, kg ± sd				
Initial	16.33 ± 6.87	19.45 ± 8.35	25.20 ± 11.17	0.01 ^b
30 days	24.15 ± 10.30 ^{\$}	21.95 ± 9.86	30.10 ± 10.98 [#]	0.04 ^c
90 days	23.15 ± 10.95 ^{\$}	22.10 ± 9.42	30.15 ± 10.62 [#]	0.03 ^a
Follow-up	22.95 ± 9.26 ^{\$}	21.95 ± 8.70	30.55 ± 10.92 [#]	0.01 ^a
Right hamstrings, kg ± sd				
Initial	12.41 ± 4.60	12.10 ± 6.18	15.10 ± 6.95	0.30
30 days	14.20 ± 5.30	13.40 ± 5.81	16.15 ± 6.66	0.33
90 days	13.55 ± 5.61	13.50 ± 5.21	16.50 ± 5.24	0.14
Follow-up	14.79 ± 6.49	14.10 ± 8.47	16.20 ± 5.50	0.62
Left hamstrings, kg ± sd				
Initial	11.15 ± 4.85	12.35 ± 6.29	15.00 ± 6.23	0.11
30 days	13.85 ± 5.24	13.80 ± 6.11	17.50 ± 4.82	0.05 ^a
90 days	16.65 ± 15.89	13.85 ± 5.21	16.50 ± 5.24	0.55
Follow-up	13.84 ± 6.21	13.75 ± 5.73	16.05 ± 5.11	0.59

CPG = collagen + physiotherapy group; PCPG = placebo collagen + physiotherapy group; PG = physiotherapy group.

p<0.05 compared to the initial assessment of the same group. ANOVA for repeated measures.

\$ p<0.005 compared to the initial assessment of the same group. ANOVA for repeated measures.

a p<0.05 between the PG and the others. One-way ANOVA.

b p<0.05 between the PG and the CPG. One-way ANOVA.

c p<0.05 between the PG and the PCPG. One-way ANOVA.

Right hamstring muscle strength did not differ significantly between groups and in each group throughout the intervention protocol. For left hamstrings, the PG showed a higher muscle strength than the other groups at the 30-day assessment (**table III**).

There were no differences in active and passive bilateral knee ROM between the study groups. Active flexion range of motion of the right knee increased in the CPG and PG. For the left knee, ROM increased only in the CPG. For passive flexion ROM of the right knee, only the PG increased mobility after 90 days. Passive flexion ROM of the left knee did not change throughout the intervention protocol (**table IV**).

Active extension of both knees did not differ between the groups and throughout the intervention protocol in the study groups (**table IV**).

All intervention groups reduced the TUG execution time at the 30-day assessment, with no differences between groups. However, at the 90-day and six-month assessments, the PCPG did not maintain this improvement. The CPG showed an initial score of 11.53 ± 3.51 seconds, which decreased to 8.91 ± 2.87 at 30 days ($p=0.001$), remaining at 8.99 ± 2.93 at 90 days ($p=0.0001$), and 9.24 ± 3.91 after six months ($p=0.032$). The PG decreased the TUG test execution time from 11.30 ± 3.32 seconds at baseline to 8.39 ± 2.23 at the 30-day assessment ($p=0.0001$), remaining at 8.52 ± 2.47 at the 90-day assessment ($p=0.001$), and 9.08 ± 3.31 at the six-month assessment ($p=0.001$). For the PCPG, the execution time decreased from 11.56 ± 4.26 seconds at baseline to 8.85 ± 2.53 seconds at the 30-day assessment ($p=0.007$). However, the execution time increased to 9.32 ± 3.51 at 90 days, and to 9.93 ± 3.14 at six months (**figure 3**).

The PG significantly increased the distance covered in the 6MWT at 90 days compared to the PCPG. Only the CPG and PG increased the distance covered in the test from the 30-day assessment. The CPG initially covered 301.38 ± 92.21 meters. After 30 days, this distance increased to 356.60 ± 98.92 ($p=0.0001$). At the 90-day assessment, the distance covered was 346.67 ± 99.37 ($p=0.001$ compared to the initial assessment). Six months after the intervention, the distance covered was 372.10 ± 145.04 ($p=0.0001$). The PG covered an initial distance of 333.55 ± 92.98 meters. At the 30-day assessment, the distance covered by this group was 394.55 ± 86.11 ($p=0.0001$). This distance decreased to 390.65 ± 87.79 at the 90-day assessment ($p=0.0001$ compared to the initial assessment), and to 349.00 ± 141.75 at six months ($p=0.001$ compared to the initial assessment). The PCPG showed no significant change in the distance covered on the TUG at different times of assessment ($p>0,05$) (**figure 4**).

Functionality and quality of life, as assessed by the Lequesne Algofunctional Questionnaire, increased for the CPG and

PG compared to the PCPG at the postintervention assessment (30 days). All intervention groups improved functionality scores at the 30-day and 90-day assessments. Only the PCPG did not maintain this improvement at the six-month assessment. The CPG presented an initial Lequesne score of 11.18 ± 3.30 . At the 30-day assessment, the score decreased to 5.18 ± 4.04 points ($p=0.001$). At the 90-day assessment, the score was 5.85 ± 5.10 points ($p=0.0001$ compared to the initial assessment). After six months, the score was 7.08 ± 5.97 ($p=0.013$ compared to the initial assessment). On the other hand, the PG showed an initial Lequesne score of 9.33 ± 2.49 points. After 30 days, the score decreased to 4.00 ± 3.25 ($p=0.0001$). The score was 4.68 ± 4.06 points ($p=0.001$) at the 90-day assessment, and 5.70 ± 3.64 ($p=0.001$) after six months. The PCPG had an initial score of 11.20 ± 3.45 points, which decreased to 7.25 ± 4.66 after 30 days ($p=0.0001$), remaining at 7.73 ± 5.40 after 90 days ($p=0.003$), and 8.75 ± 6.30 six months after the intervention ($p=0.15$) (**figure 5**).

All intervention groups reduced the WOMAC Questionnaire scores, with no differences between them. The CPG had an initial score of 45.25 ± 17.88 points. The score decreased to 16.75 ± 15.91 ($p=0.0001$) at the 30-day assessment, remaining at 22.35 ± 22.17 at the 90-day assessment ($p=0.0001$ compared to the preintervention assessment). Six months after the intervention, the score was 24.11 ± 24.50 ($p=0.0001$ compared to the preintervention assessment). The PCPG showed an initial score of 43.00 ± 20.21 points, which decreased to 22.90 ± 19.99 points at the 30-day assessment ($p=0.0001$), remaining at 23.60 ± 23.75 at the 90-day assessment ($p=0.0001$), and 27.05 ± 24.76 at the follow-up assessment ($p=0.019$ compared to the initial assessment). The PG showed an initial WOMAC score of 34.65 ± 11.93 points. At the 30-day assessment, the score decreased to 11.35 ± 11.45 ($p=0.0001$). At the 90-day assessment, the score was 13.35 ± 12.11 ($p=0.0001$). Six months after the intervention, the score was 12.60 ± 11.37 ($p=0.0001$) (**figure 6**).

DISCUSSION

This study investigated the effect of using the nutraceutical UC-II® combined with a physiotherapy program on symptomatology, joint mobility, muscle strength, and knee joint function in patients with knee OA.

We verified a pain reduction both in the group that administered only UC-II and in the group that performed only muscle strengthening. The association of UC-II with exercises did not demonstrate greater efficacy over pain than each of them administered in isolation. Exercise is strongly recommended for nonsurgical treatment of OA (16). In a

Table IV. Results of active and passive knee joint range of motion (ROM) in the initial study groups, 30 days, 90 days, and six months after UC-II administration (n=40).

Variable	Intervention Group			P
	CPG (n=20)	PCPG (n=20)	PG (n=20)	
Right active flexion, degrees (mean ± SD)				
Initial	117.10 ± 13.71	118.80 ± 17.06	120.80 ± 13.52	0.73
30 days	126.55 ± 9.15#	125.75 ± 15.44	123.10 ± 30.84	0.86
90 days	124.85 ± 13.29	125.25 ± 13.74	129.05 ± 11.21\$	0.53
Follow-up	124.00 ± 14.13	122.00 ± 16.88	128.85 ± 10.58\$	0.29
Left active flexion, degrees (mean ± SD)				
Initial	114.85 ± 14.42	120.30 ± 14.68	120.55 ± 22.15	0.51
30 days	124.20 ± 10.32#	124.80 ± 10.53	130.50 ± 13.73\$	0.18
90 days	124.65 ± 12.21#	124.95 ± 11.70	131.20 ± 14.23	0.20
Follow-up	124.42 ± 12.64#	122.50 ± 14.20	130.35 ± 13.79	0.09
Right passive flexion, degrees (mean ± SD)				
Initial	127.75 ± 12.38	125.60 ± 16.30	128.85 ± 19.77	0.73
30 days	133.60 ± 9.49	132.70 ± 10.78	129.55 ± 32.66	0.81
90 days	132.00 ± 8.94	133.10 ± 11.77	136.65 ± 11.78\$	0.37
Follow-up	130.79 ± 11.33	130.85 ± 15.34	135.90 ± 12.26\$	0.38
Left passive flexion, degrees (mean ± SD)				
Initial	124.05 ± 13.87	125.25 ± 16.30	128.85 ± 16.66	0.65
30 days	132.30 ± 10.78	131.15 ± 11.28	137.10 ± 11.92	0.22
90 days	130.90 ± 12.37	131.75 ± 11.77	136.85 ± 12.47	0.24
Follow-up	130.53 ± 12.52	130.05 ± 13.49	136.80 ± 11.81	0.12
Right active extension, degrees (mean ± SD)				
Initial	- 1.60 ± 3.93	- 1.15 ± 2.64	- 3.65 ± 8.83	0.35
30 days	- 1.05 ± 3.24	- 0.90 ± 3.58	- 0.70 ± 3.13	0.95
90 days	- 0.60 ± 2.26	- 0.10 ± 0.44	- 0.75 ± 3.35	0.66
Follow-up	- 0.53 ± 2.29	- 0.90 ± 3.58	- 0.85 ± 2.85	0.91
Left active extension, degrees (mean ± SD)				
Initial	- 3.90 ± 6.01	- 2.95 ± 6.53	- 2.45 ± 6.24	0.76
30 days	- 1.00 ± 3.08	- 1.15 ± 4.12	- 0.60 ± 2.68	0.87
90 days	- 1.20 ± 3.14	- 0.15 ± 0.68	- 0.75 ± 3.35	0.39
Follow-up	- 1.89 ± 4.58	- 1.05 ± 4.05	- 0.60 ± 2.68	0.38
Right passive extension, degrees ± SD)				
Initial	- 1.00 ± 2.61	- 0.40 ± 1.19	- 2.50 ± 5.74	0.19
30 days	- 1.05 ± 3.24	0.40 ± 1.79	- 0.60 ± 2.68	0.21
90 days	0.00 ± 0.00	0.00 ± 0.00	- 0.50 ± 2.24	0.37
Follow-up	- 0.53 ± 2.29	- 0.20 ± 0.89	- 0.60 ± 2.68	0.81
Left passive extension, degrees ± SD)				
Initial	- 1.95 ± 3.73	- 2.05 ± 5.42	- 1.65 ± 5.10	0.96
30 days	- 0.90 ± 2.79	0.50 ± 2.24	- 0.60 ± 2.68	0.20
90 days	- 0.40 ± 1.79	0.00 ± 0.00	- 0.50 ± 2.24	0.60
Follow-up	- 1.53 ± 3.66	- 0.40 ± 1.79	- 0.60 ± 2.68	0.41

CPG = collagen + physiotherapy group; PCPG = placebo collagen + physiotherapy group; PG = physiotherapy group.

p<0.05 compared to the initial assessment of the same group.

\$ p<0.005 compared to the initial assessment of the same group. ANOVA for repeated measures.

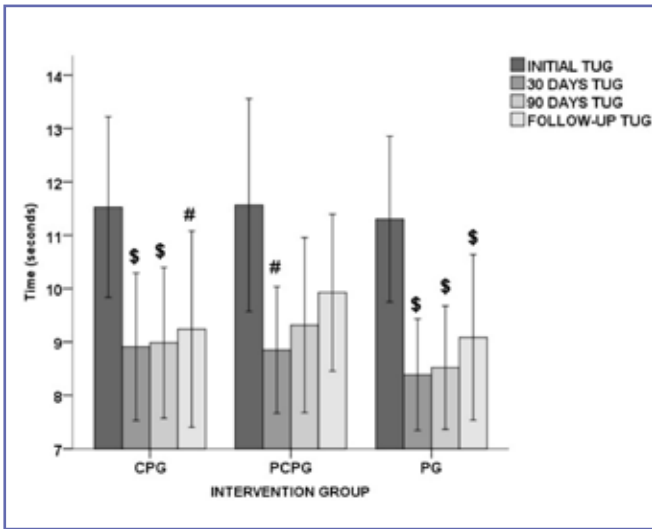


Figure 3. TUG in study groups.
 # $p < 0.05$ compared to the initial assessment of the same group. ANOVA for repeated measures.
 \$ $p < 0.005$ compared to the initial assessment of the same group. ANOVA for repeated measures.

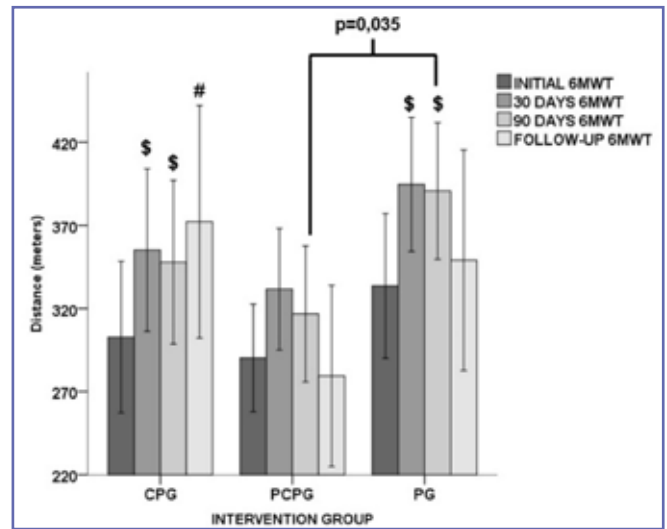


Figure 4. Distance in the 6MWT in study groups. One-way ANOVA.
 \$ $P < 0.005$ compared to the initial assessment of the same group.
 # $p < 0.05$ compared to the initial assessment of the same group. ANOVA for repeated measures.

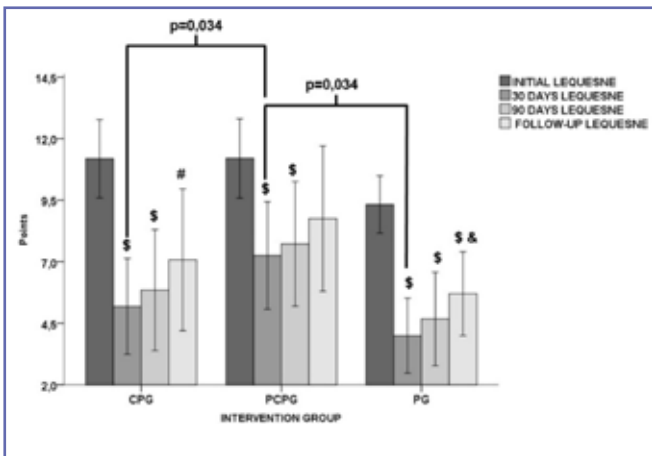


Figure 5. Lequesne score in study groups. One-way ANOVA.
 \$ $P < 0.005$; # $p < 0.05$ compared to the initial assessment of the same group.
 & $p < 0.005$ compared to the 30-day assessment of the same group. ANOVA for repeated measures.

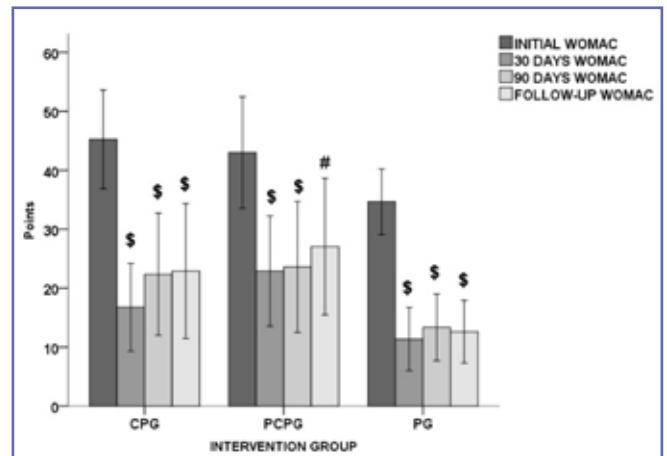


Figure 6. Total WOMAC score in study groups.
 # $p < 0.05$ compared to the initial assessment of the same group. ANOVA for repeated measures.
 \$ $p < 0.001$ compared to the initial assessment of the same group. ANOVA for repeated measures.

study of participants with OA pain exacerbations, strengthening and coordination exercises performed three times a week for 12 weeks reduced pain in 63% of sessions (17). Pain is the main problem in knee OA because it precedes other negative aspects of the subject's life, such as physical

inactivity, which contributes to decreased muscle strength and gait changes (18). Notwithstanding, it is important to understand that there is an inflammatory signaling pathway that leads to pain and loss of muscle strength. Inflammatory cytokines such as IL-1 β and TNF- α lead to the destruction

of the extracellular matrix (ECM) of the cartilage locally in the OA joint (19). In addition, IL-1 β and TNF- α contribute to NF- κ B activation in synovial cells and chondrocytes, both commonly implicated in OA pathogenesis (19). Another important factor that can be attributed to pain improvement after exercise is the facilitation of endorphin release, which makes the patient more tolerant to it (20,21).

The effectiveness of therapeutic ground exercise for people with knee and hip OA has been investigated in terms of pain reduction and disability (22). Nevertheless, few studies have investigated the specificity of the dose, frequency, and intensity of these exercises for knee OA and their effects on pain reduction (22). This omission of essential information on exercise interventions for knee OA is quite problematic and may result in significant clinical uncertainty, variability, and limited implementation, although global recommendations converge to focus on exercises in the therapeutic approach to OA (7). There is a need to specify recommendations and replicate the original studies with close attention to intervention protocols (21). Right quadriceps muscle strength increased by 38% in the CPG after exercise. Left quadriceps strength increased by 48 and 20%, respectively, in the PCG and PG. A 30% increase in knee extensor strength is required to improve pain outcomes (16). In this study, both the group that administered UC-II and the group that performed exercises increased quadriceps strength after interventions. However, the group that administered the exercises showed a quadriceps strength significantly greater than the other intervention groups. Pain reduction after strengthening exercises can be attributed to reversal of quadriceps weakness, which is one of the main conditions present in patients with knee OA (22). Strength training can increase muscle strength and reduce pain at the same time (20).

Stratford, Kennedy and Woodhouse (23) found that the 6MWT and TUG demonstrated two factors consistent with health concepts linked to pain and function of patients with knee and hip OA. Ateef, Kulandaivelan and Tahseen (24) approached 80 people with knee OA and observed that women walked 260.20 meters, while men walked 327.38 meters. Knee pain affects the ability to walk or perform weight-bearing exercises, thus reducing exercise adherence, compromising the goal of improving body composition (25,26). In our study, we found an improvement in the TUG test in the CPG and PG groups in all evaluations, which demonstrates that exercises were largely responsible for the functional improvement of patients with knee OA. Regarding 6MWT, the same groups increased the distance covered. In the present study, there was no significant potential effect of UC-II when associated with muscle strengthening exercise. However, the group that combined the two interventions showed more robust results, especially six months

after the intervention protocol. UC-II has been shown to be able to “turn off” the specifically targeted immune response to articular cartilage (type II) collagen, without presenting adverse effects (27,28).

Osteoarthritis is histologically characterized by changes in chondrocyte cell structure and loss of proteoglycans (29). Other histological features of OA include synovial membrane hyperplasia and thickening, infiltration of inflammatory cells, and fibrosis (29). In advanced stages of the disease, the cartilage shows signs of complete rupture, so that hyaline cartilage is replaced by a scar-like fibrocartilaginous tissue with fibroblast-like cells (29). Nutraceuticals play an important role in the anabolic and catabolic balance of articular cartilage. The association of these with the therapies already commonly postulated increases the therapeutic options in knee OA. Few researches already carried out demonstrated that the treatment of patients with OA with UC-II increases the mobility and functionality of the joints and reduces pain (29). Fusini *et al.* conducted a comprehensive review to analyze the use of nutrients in the treatment of tendinopathies (30). However, the authors have failed to establish a recommendation for the use of these to address tendinopathies (30). Type 1 hydrolyzed collagen can increase the amount of dermatansulfate and decrease levels of hyaluronic acid, especially at high doses (30).

This is the first study to specifically analyze the effect of joint administration of UC-II and muscle strengthening exercises. Thus, patients, physicians, and physiotherapists should have an objective realistic outcome, with a combination of these therapeutic options being the most preferable scenario, particularly considering the available evidence for nonpharmacological management.

LIMITATIONS

We recognize some limitations of the study. This study did not have a control group for an adequate understanding of disease progression. All participants who entered the clinical trial volunteered to participate, indicating motivation to participate in one of the intervention groups. Therefore, the results may not be generalizable to other adults with knee OA. A standard exercise program, specific for strengthening the quadriceps, was used. We believe that other exercise techniques, in conjunction with UC-II, should be performed to find a better therapeutic option for patients with knee OA.

CONCLUSIONS

The results of this study suggest that a 30-day NMES-associated muscle strengthening program was effective in improv-

ing pain, strength, and function in people with knee OA. Combining the nutraceutical UC-II with muscle strengthening exercises seems to have potentiated the effect of muscle strengthening, especially in the long term.

ACKNOWLEDGEMENTS

To the Infinity Pharma® Laboratory in Campinas/SP, Brazil, for the partial funding of this study.

REFERENCES

1. Zhang X, Yang Y, Li X, Zhang H, Gang Y, Bai L. Alterations of autophagy in knee cartilage by treatment with treadmill exercise in a rat osteoarthritis model. *Int J Mol Med* 2019;43(1):336-44.
2. Rabe KG, Matsuse H, Jackson A, Segal NA. Evaluation of the Combined Application of Neuromuscular Electrical Stimulation and Volitional Contractions on Thigh Muscle Strength, Knee Pain, and Physical Performance in Women at Risk for Knee Osteoarthritis: A Randomized Controlled Trial *PM R*. 2018;10(12):1301-10.
3. Hall M, Castelein B, Wittoek R, Calders P, Ginckel A Van. Diet-induced weight loss alone or combined with exercise in overweight or obese people with knee osteoarthritis: a systematic review and meta-analysis. *Semin Arthritis Rheum* 2019; 48(5): 765-777.
4. Van Ginckel A, Hall M, Dobson F, Calders P. Effects of long-term exercise therapy on knee joint structure in people with knee osteoarthritis: A systematic review and meta-analysis. *Semin Arthritis Rheum* 2019; 48(6): 941-949.
5. Kan HS, Chan PK, Chiu KY, Yan CH, Yeung SS, Ng YL, Shiu KW, Ho T. Non-surgical treatment of knee osteoarthritis. *Hong Kong Med J*. 2019; 25(2): 127-133
6. McKay J, Frantzen K, Vercruyssen N, Hafsi K, Opitz T, Davis A, Murrell W. Rehabilitation following regenerative medicine treatment for knee osteoarthritis-current concept review. *J Clinl Orthop Trauma* 2019; 10(1):59-66.
7. Bartholdy C, Nielsen SM, Warming S, Hunter DJ, Christensen R, Henriksen M. Poor replicability of recommended exercise interventions for knee osteoarthritis: a descriptive analysis of evidence informing current guidelines and recommendations. *Osteoarthritis Cartilage* 2019; 27(1):3-22.
8. Quicke JG, Foster NE, Croft PR, Ogollah RO, Holden MA. Change in physical activity level and clinical outcomes in older adults with knee pain: A secondary analysis from a randomised controlled trial. *BMC Musculoskelet Disord* 2018; 19(1):59.
9. Crowley DC, Lau FC, Sharma P, Evans M, Guthrie N, Bagchi M, Bagchi D, Dey DK, Raychaudhuri SP. Safety and efficacy of undenatured type II collagen in the treatment of osteoarthritis of the knee: a clinical trial. *Int J Med Sci* 2009; 6(6):312-21.
10. Lugo JP, Saiyed ZM, Lane NE. Efficacy and tolerability of an undenatured type II collagen supplement in modulating knee osteoarthritis symptoms: a multicenter randomized, double-blind, placebo-controlled study. *BMC Nutrition Journal* 2016; 11(1):1-15.

To the Sea Life® handling pharmacy in Torres/RS, Brazil, represented by the pharmacist-in-charge Josele Milanez Paz. To the Group of Studies and Research in Sports Rehabilitation and Orthopedic Trauma (GEPRETO) of the Lutheran University of Brazil, Torres/RS Campus, Brazil.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

11. Bagi CM, Berryman ER, Teo S, Lane NE. Oral administration of undenatured native chicken type II collagen (UC-II) diminished deterioration of articular cartilage in a rat model of osteoarthritis (OA). *Osteoarthritis Cartilage*. 2017; 25(12):2080-2090.
12. Lewek MD, Rudolph KS, Snyder-Mackler L. Quadriceps femoris muscle weakness and activation failure in patients with symptomatic knee osteoarthritis. *J Orthop Res* 2004; 22(1):110-5.
13. Taş S, Güneri S, Baki A, Yildirim T, Kaymak B, Erden Z. Effects of severity of osteoarthritis on the temporospatial gait parameters in patients with knee osteoarthritis. *Acta Orthop Traumatol Turc* 2014; 48(6):635-41.
14. Lugo JP, Saiyed ZM, Lau FC, Molina JPL, Pakdaman MN, Shamie AN, Udani JK. Undenatured type II collagen (UC-II®) for joint support: A randomized, double-blind, placebo-controlled study in healthy volunteers. *J Int Soc Sports Nutr* 2013;10(1):1.
15. Padulo J, Oliva F, Frizziero A, Maffulli N. Basic principles and recommendations in clinical and field Science Research: 2018 update. *MLTJ* 2018; 8(3): 305 - 307.
16. Wellsandt E, Golightly Y. Exercise in the management of knee and hip osteoarthritis. *Curr Opin Rheumatol* 2018; 30(2):151-9.
17. Bartholdy C, Klokke L, Bandak E, Bliddal H, Henriksen M. A Standardized "Rescue" Exercise Program for Symptomatic Flare-up of Knee Osteoarthritis: Description and Safety Considerations. *J Orthop Sport Phys Ther* 2016; 46(11):942-946.
18. Braghin R de MB, Libardi EC, Junqueira C, Nogueira – Barbosa MH, de Abreu DCC. Exercise on balance and function for knee osteoarthritis: A randomized controlled trial. *J Bodyw Mov Ther* 2018; 22(1):76-82.
19. Krishnasamy P, Hall M, Robbins SR. The role of skeletal muscle in the pathophysiology and management of knee osteoarthritis. *Rheumatol (Oxford)*. 2018; 57(Suppl_4): iv22-iv33.
20. Bokaeian HR, Bakhtiary AH, Mirmohammadkhani M, Moghimi J. Quadriceps strengthening exercises may not change pain and function in knee osteoarthritis. *J Bodyw Mov Ther* 2018; 22(2): 528-533.
21. Marone PA, Lau FC, Gupta RC, Bagchi M, Bagchi D. Safety and toxicological evaluation of undenatured type II collagen. *Toxicol Mech Methods* 2010; 20(4):175-89.
22. Imoto AM, Pardo JP, Brosseau L, Taki J, Desjardins B, Thevenot O, Franco E, Peccin S. Evidence synthesis of types

- and intensity of therapeutic land-based exercises to reduce pain in individuals with knee osteoarthritis. *Rheumatol Int* 2019; 39(7):1159-1179.
23. Stratford PW, Kennedy DM, Woodhouse LJ. Performance Measures Provide Assessments of Pain and Function in People With Advanced Osteoarthritis of the Hip or Knee. *Phys Ther* 2006; 86(11):1489-96.
 24. Ateef M, Kulandaivelan S, Tahseen S. Test-retest Reliability and Correlates of 6-minute Walk Test in Patients. *Indian J Rheumatol* 2016; 11(4): 192-196.
 25. Hall M, Hinman RS, van der Esch M. Is the relationship between increased knee muscle strength and improved physical function following exercise dependent on baseline physical function status? *Arthritis Res Ther* 2017;19(1):271.
 26. Krauss I, Katzmarek U, Rieger MA, Sudeck G. Motives for physical exercise participation as a basis for the development of patient-oriented exercise interventions in osteoarthritis: a cross-sectional study. *Eur J Phys Rehabil Med* 2017; 53(4): 590-602.
 27. Bakilan F, Armagan O, Ozgen M, Tascioglu F, Bolluk O, Alatas O. Effects of native type II collagen treatment on knee osteoarthritis: A Randomized Controlled Trial. *Eurasian J Med* 2016; 48(2):95-101.
 28. Coriolano K, Aiken A, Harrison M, Pukall C, Brouwer B, Groll D. Changes in knee pain, perceived need for surgery, physical function and quality of life after dietary weight loss in obese women diagnosed with knee osteoarthritis. *Osteoarthritis Cartilage*. 2013; 21(4):S261-S312.
 29. Castrogiovanni P, Trovato FM, Loreto C, Nsir H, Szychlinska MA, Musumeci G. Nutraceutical Supplements in the Management and Prevention of Osteoarthritis. *Int J Mol Sci* 2016;17(12): 2042.
 30. Fusini F, Bisicchia S, Bottegoni C, Gigante A, Zanchini F, Busilacchi A. Nutraceutical supplement in the management of tendinopathies: a systematic review. *Muscles Ligaments Tendons J* 2016; 6(1):48-57.

Therapeutic Electrical Stimulation Currents in Chronic Non-specific Low Back Pain: Designing a Systematic Review

S. Izadi Laybidy^{1,2}, T. Shahmahmoodi^{1,2}, A. Rahimi³, F. Bokaei¹, A. A. Pourmomeny¹, Z. S. Rezaeian¹

¹ Musculoskeletal Research Center and Department of Physical Therapy, Faculty of Rehabilitation Sciences, Isfahan University of Medical Sciences, Isfahan, Iran

² Student Research Committee of Rehabilitation Students (Treata), Faculty of Rehabilitation Sciences, Isfahan University of Medical Sciences, Isfahan, Iran

³ Health Information Research Center, Head of Clinical Informationist Research Group, Faculty of Management and Medical Information Sciences, Isfahan University of Medical Sciences, Isfahan, Iran

CORRESPONDING AUTHOR:

Zahra Sadat Rezaeian
Musculoskeletal Research Center
Rehabilitation Sciences Research Institute
Department of Physical Therapy
Faculty of Rehabilitation Sciences
Isfahan University of Medical Sciences
Hezar Jerib Street 2
Isfahan, Iran 8174673461
E-mail: zrezaeian@rehab.mui.ac.ir

DOI:

10.32098/mltj.03.2020.19

LEVEL OF EVIDENCE: 1A

SUMMARY

Introduction. The aim of this study will be to evaluate the effect of electrical stimulation currents on the pain and function in subjects with chronic non-specific low back pain.

Methods. Pubmed, ISI Web of Science, Scopus, Clinical Key, Science Direct, Medline, Embase, PEDro, ProQuest, the Cochrane Library, PROSPERO, and also the MOH Thesis, MOH Articles, Magiran, and SID as the national databases will be searched. Also, Google Scholar search engine will be used. All study types except Qualitative Studies, and Narrative Reviews, *i.e.* Clinical Trials, Cohort, Case-controls, Cross-sectionals, Observational Descriptive, Case Report, Case Series, Ecological Studies, Systematic Reviews, thesis and dissertation, in English and Persian will be retrieved. The publication date should not be after August 2019. To ensure all the publication has been reached, search will be extended to three years before the publication date of first article found for each type of electrical stimulation currents. The search will be limited to human studies of subjects between 18-65, regardless of gender and race. The search strategy will cover PICO. The quality of studies will be determined using Consort, STROBE, NHLBI, PEDro and CASP checklists in expert consensus.

Dissemination. The results of this plan will clarify which electrical stimulation current will improve pain and function in chronic non-specific low back pain. This is valuable in clinical practice to optimize therapeutic planning.

KEY WORDS

Electrical stimulation; current; pain; function; nonspecific chronic low back pain; physical therapy.

INTRODUCTION

Low Back Pain (LBP) is a common health issue all over the world which directly shakes the quality of life, daily activities and social roles (1-3).

Considering the long term systemic complications of medications esp. for some high risk populations such as elderlies and adolescents, nowadays people prefer non-medical strategies (4). Consequently, physical therapy is the non-invasive

approach of choice in the treatment of CLBP (5). Electrical Stimulation (ES) currents may be administered as a part of physical therapy to eliminate pain (6).

ES currents in the CLBP has been studied widely in clinical trials (7-12). The results are controversial due to variations in stimulation parameters, demographic and anthropometric characteristics of the participants, design of the study, the outcome measure, and intervention duration and planning.

It seems that the impact of various types of ES currents on non-specific CLBP has not been reviewed yet. The question is which type of the therapeutic ES currents may help improving pain and function in subjects with chronic non-specific LBP? Present work is the protocol of a systematic review to determine the effect of different ES currents on pain and function in people suffering from chronic non-specific LBP.

OBJECTIVES

To determine the effect of various ES currents on the pain and function of people with non-specific chronic low back pain.

MATERIALS AND METHODS

Trial eligibility criteria

The inclusion/exclusion criteria have been defined to properly cover search strategies and PICO. The detail of the criteria is summarized below.

Study types

All study types *i.e.* Clinical Trials, Cohort, Case-controls, Cross-sectionals, Observational Descriptive, Case Report, Case Series, Ecological Studies, Systematic Reviews and thesis and dissertation will be included. Qualitative Studies and Narrative Reviews will not take into quality assessment.

Participants

People with non-specific CLBP between 18-65 who were treated using ES currents regardless of gender and ethnicity. The study will be entered the review if the participants suffered from back pain for at least three months without known pathology. Studies on nonhuman samples, professional athletes, subjects with acute LBP, or the studies that included LBP cases with less than three months duration of symptoms will be excluded. LBPs of specified origin such as inflammatory diseases, spondylo-arthropathies, disk hernia, spinal canal/foraminal stenosis, visceral pains, fractures and trauma will not be eligible. If the participants complained from referral or radicular symptoms, the study will also be excluded. Pregnant women, children (under 18 years) and elderly (over 65 years) will not be of interest in present protocol.

Interventions

The intervention group shall receive ES currents including: Transcutaneous Electrical Nerve Stimulation (TENS),

Interferential (IF), Diadynamic, High-voltage, Russian currents, Faradic.

Comparators

Studies will be approved to be included only if there was a control group (without treatment), sham group (placebo treatment), healthy group (of matched healthy subjects) or if two or more ES currents were compared.

Outcome measures

Pain and function will be the main outcome measures in this systematic review; pain will be assessed by the Numerical Rating Scale (NRS), Visual Analogue Scale (VAS), Pressure Pain Threshold (PPT), MCGill Pain Questionnaire. Function will be measured by Roland-Morris Disability Questionnaire, Oswestry Disability Index, or clinical/functional tests. Other tools may be also considered according to the included studies.

Studies will be included the experimental (case) group and the control group were established and the related monitoring data were introduced. Two classification variables, continuous variables and variance test should be administered. Within-(pre-post) and between-group measures will be analyzed for clinical trials. For cohorts and case-controls odds ratio will be of interest. Effect size and confidence intervals will be of value in all types included studies.

Additional outcome measures will be considered upon progression of the study. Some of anticipated secondary outcome measures are: anthropometric data (weight, height, BMI), psycho-social and cultural data (literacy level, marital status, economical class), comorbidities (diabetes, cardiac or pulmonary disorders, smoking, alcohol consumption).

Search methods to identify studies

Pubmed, ISI Web of Science, Scopus, Clinical Key, Science Direct, Medline, Embase, PEDro, ProQuest, the Cochrane Library, PROSPERO, and also MOH Thesis, MOH Articles, Magiran and SID as the national databases will be searched. Also Google Scholar search engine will be used.

The review studies will not be included in the final analysis. However, their references will be checked through Cross Reference. The main key words will be electrical stimulation current, pain, function, nonspecific chronic low back pain. The key terms will be updated during the search process. The search strategy will cover the following search query and also PICO.

Nonspecific AND chronic AND (“low back pain” OR (low AND back AND pain) OR “back ache”) AND (“electric

stimulation*" OR (electric AND stimulation) OR "electrical stimulation*" AND (TENS OR "Transcutaneous Electrical Nerve Stimulation" OR Interferential OR Diadynamic OR High Voltage OR Russian OR Faradic) AND ("control group*" OR ((placebo or unrealistic) AND (treatment OR therapy*)) AND Function*) in TITLE/SUMMARY/KEY WORDS.

P: nonspecific AND chronic AND "low back pain" OR (low AND back AND pain) OR "back ache").

I: ("electric stimulation*" OR (electric AND stimulation) OR "electrical stimulation*" AND (TENS OR "Transcutaneous Electrical Nerve Stimulation" OR Interferential OR Diadynamic OR High Voltage OR Russian OR Faradic).

C: "control group*" OR ((placebo or unrealistic) AND (treatment OR therapy)). O: function*.

The search procedure will be repeated every few weeks by two researchers (SIL and TSM) who are blind to other ones' findings. The researchers will also review and retrieve the

reference list of all included articles (Hand Search). In the case of no access to the paper-based or electronic full text of an article, the authors (corresponding or first author) or editor of the publishing journal will be contacted thrice. In the case of unsuccessful tries, the article will be withdrawn from study. The Gray Literature will be search through their specified databases including "http://www.gateway.com/worldwide/", "http://www.proquest.com/", "http://www.irct.ir/", "http://www.trialscentral.com/".

Study selection

The articles that were published until the end of August 2019 (Shahrivar 9th, 1398 Persian Calendar) will be acceptable. To ensure accuracy of the search results, the search will be extended three years before the publication date of the first article found for each type of ES currents. Search results and Reference lists will be imported to the

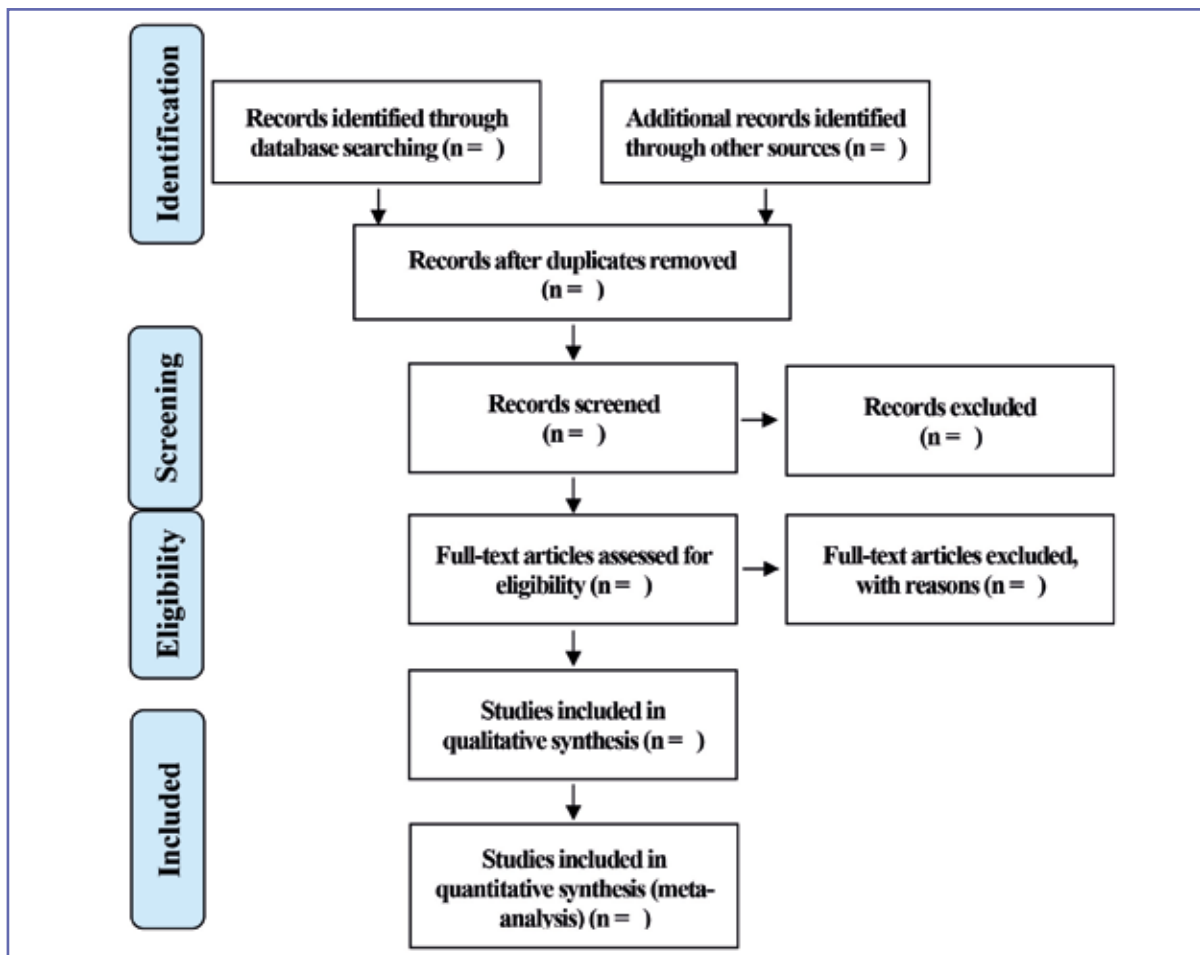


Figure 1. PRISMA 2009 Flow diagram of the articles selection process (14).

citation manager software. Duplicates and unrelated articles will be removed through screening titles and summaries. Then, the full text of the remaining articles will be reviewed in detail. After excluding irrelevant articles, and those that fail according to inclusion criteria, the quality of the remaining articles will be criticized and evaluated. All stages will be performed by two independent researchers (SIL and TSM) under the supervision of ZSR, FB and AR. The principal investigator will supervise the procedure. Any disagreement will be resolved through consensus. The entire process of study selection is summarized in the PRISMA flow diagram

Data extraction

The Search will be done on aforementioned databases. Screening of PubMed title/abstract will be done by two researchers (SIL and TSM) to calculate inter-rater agreement under the supervision of ZSR, AR and FB. The qualitative studies and narrative reviews will not be included in the final analysis while their references will be checked through Cross Referencing. Key terms will be updated along study progression. The references of included articles will be checked manually (Hand Search). If the full text of any article was not found, the corresponding/first author or the editor of the publishing journal will be contacted thrice. If not successful, the article will be withdrawn.

The screening of the title/summaries will be carried out independently by SIL and TSM. The total number of the extracted articles from each database will be reported in a flowchart. Unrelated and duplicate articles will be excluded. All the search results will be saved in a citation manager. This screening process will be repeated again from the beginning every three months to justify fast exclusion phase.

Remaining articles will be criticized through review of full-text article by SIL, ZSR, FB, AR and TSM independently. Decisions will be made regarding inclusion/exclusion criteria. Reasons for the exclusion will be reported.

Data from the approved full texts will be entered into Excel sheet (data extraction) that covers publication details (the author(s), title, publication year, journal, country), participants, study design, sample size, randomization, allocation concealment, blinding, intervention, control intervention, main outcomes, adverse effects, follow-up, withdrawals and results. Necessary information will be obtained through correspondence with the original authors in case. PI (ZSR) supervises the procedure. Disagreements in every step will be clarified through consensus.

Quality assessment

Considering study design, included articles will be qualified in expert consensus using Consort, STROBE, PEDro, CASP and NHLBI checklists. The score for each checklist will be recorded. Articles that gain 50% of total score of one of the checklists will be considered for quantitative analysis. The articles' quality will be ranked as high (75%), medium (50-75%), low (25-50%), poor (< 25%) according to the scores they receive using each single checklist.

PEDro scale will be used to assess the validity of selected studies. The checklist consists of 11 items that may be marked as a "plus" (when the item has been properly addressed in the article text) or "minus" (if the item cannot be localized throughout the text) (14).

The standard STROBE checklist, which has 22 items, evaluates the quality of case-control studies and cohort in two independent scales (15,16). However, some researchers recommend Study Quality Assessment Tools proposed by National Heart, Lung and Blood Institute (NHLBI) as more precise qualification instrument for observational studies (17). Therefore, these articles will also be evaluated using NHLBI recommended checklists.

The CONSORT checklist has been introduced as an international standard and a standardized approach to clinical practice report (18). Studies that earn a score of 50% or more will be included in the study. For precise assessment of the articles, appropriate CONSORT extension may be administered (19).

CASP checklist has been specifically adapted for various study designs including case-control studies, cohort and clinical trials (20). Articles that earn a score of 50% or more will be included in the study.

For comprehensive qualification of all article types that will meet the inclusion criteria of the study, TIDieR checklist will be administered beside the main checklist.

Measure of Treatment Effects

In the case of measuring continuous outcomes *i.e.* pain scales, the mean difference (MD) with a 95% CI will be analyzed, and other form of reports will be covert into MD. For dichotomous data (eg, adverse events), a risk ratio (RR) with a 95% CI and for other binary data an RR value will be calculated.

Missing data

Missing data will be collected through contacting the research team. In case of no adequate reply the available data will be analyzed solely.

Statistical Methods

Data synthesis

If possible, the random-effects or fixed-effects model will be developed for the meta-analysis Using RevMan (Review Manager Software, Version 5.3; Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014, The Cochrane Collaboration, Oxford, England). The RR with the 95% CI for dichotomous data and the MD with the 95% CI for continuous data will be calculated if possible. In the case of acceptable heterogeneity ($I^2 \leq 50\%$), the RR and MD will be calculated in the fixed-effects model; otherwise, the random-effects model will be administered. If quantitative synthesis is not achievable, the results will be reported in the narrative description

Assessment of heterogeneity

Heterogeneity will be determined by the I^2 and χ^2 tests. Considering 50% as the cut-off for statistical analysis of I^2 , the meta-analysis will be indicated in the case of significant heterogeneity of $I^2 > 50\%$, and a subgroup analysis to explore the possible causes will be of value.

Subgroup analysis

Subgroup analysis including the analysis of electrical parameters of each current type, type of control, countries and different outcomes will be performed to assess the heterogeneity between the studies.

Sensitivity analysis

If the heterogeneity persists following subgroup analysis or if there were studies with imperfect results, the sensitivity analysis will be developed by removing the lower quality studies. Then, the meta-analysis will be run again and the results of the two meta-analyses will be compared and discussed according to the sample size, strength of evidence and influence on the pooled effect size.

Assessment of reporting biases

If enough studies were included for qualitative analysis (at least 10 trials for each current), the publication bias will be assessed by funnel plots. In addition, the impacts of possible selective reporting, reporting deviations from the original protocols, effect of protocol compliance and adherence will be discussed.

DISCUSSION

The study included all existing articles of any type published any time concerning the application of ES currents in

non-specific LBP. Therefore, based on the results of this study, it can be specified how various types of ES currents may improve pain and function in people suffering non-specific CLBP. The results of this study may be cited in the setting of clinical guidelines, legal tariffs for treatment and standard planning of physical therapy sessions. The results will also help physical therapists to decide about low risk and low cost electric stimulus currents with the optimal therapeutic outcome when managing of non-specific CLBP. In the case of reaching enough studies through which various currents were compared, the most effective current for CLBP clients will be identifiable. With regard to the strict but comprehensive selection criteria, the included studies may provide detailed information about parameters and durability of the best-practice ES currents. The studies will be categorized and appraised using various checklist in a peers' consensus to assure high internal validity of article scoring phase. If enough cost-effectiveness study retrieved, the results will also provide field applicable basis for financial preference of selecting electrical stimulus currents in non-specific CLBP.

The search has been already started and the preliminary data shows that in spite of clinical use of some ES currents, they have no scientific research background concerning practical parameter setting, clinical efficacy or durability of the sedative or functional effects; that means that the results will highlight existing research and clinical gaps to design further studies. Development of a meta-analysis will be possible in case of enough homogeneous articles in each field.

ETHICS

The study has been funded and ethically approved by Isfahan University of Medical Sciences (Ethics Code: IR.MUI.REC.1397.090) as a part of a thesis for Master's Degree in Physical Therapy by Sepideh Izadi Laybidi (Registration code: 297051). The sponsor has no role in data collection, analysis of the data and drafting the manuscript. The study meets the ethical standards of the journal of Muscle, Ligament, and Tendon Journal (21).

ACKNOWLEDGMENTS

This study will be developed with the financial support and ethically approval by Isfahan University of Medical Sciences (Ethics Code: IR.MUI.REC.1397.090) as a part of a thesis for Master's Degree in Physical Therapy by Sepideh Izadi Laybidi (Registration code: 297051). The protocol has been registered in International Prospective Register of Systematic Reviews (CRD42019121819). The sponsor will not play a role in data collection, analysis of the data and drafting the manuscript.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

REFERENCES

- Buchmuller A, Navez MF, Millette-Bernardin MF, *et al.* Value of TENS for relief of chronic low back pain with or without radicular pain. *EJP* 2011;16:656-665.
- Comachio J, Oliveira Magalhaes M, Nogueira Burke T, *et al.* Efficacy of acupuncture and electroacupuncture in patients with nonspecific low back pain: study protocol for a randomized controlled trial. *Trials* 2015;16:469.
- Correa JB, Costa LO, Oliveira NT, Lima WP, Sluka KA, Liebano RE. Effects of the carrier frequency of interferential current on pain modulation and central hypersensitivity in people with chronic nonspecific low back pain: a randomized placebo-controlled trial. *EJP* 2016;20:1653-1666.
- Yokoyama M, Sun X, Oku S, *et al.* Comparison of percutaneous electrical nerve stimulation with transcutaneous electrical nerve stimulation for long-term pain relief in patients with chronic low back pain. *Anesth Analg* 2004;98:1552-6.
- Bhadauria EA, Gurudut P. Comparative effectiveness of lumbar stabilization, dynamic strengthening, and Pilates on chronic low back pain: randomized clinical trial. *JER* 2017;13:477-85.
- Rajfur J, Pasternok M, Rajfur K, *et al.* Efficacy of Selected Electrical Therapies on Chronic Low Back Pain: a Comparative Clinical Pilot Study. *Med Sci Monit* 2017;23:85-100.
- Adedoyin RA, Olaogun MOB, Onipede TO, Ikem IC, Egbu MO, Bisiriyu LA. Effects of different swing patterns of interferential currents on patients with low back pain: a single control trial. *Fiz Rehab* 2005-16:61-66.
- Albornoz-Cabello M, Maya-Martin J, Dominguez-Maldonado G, Espejo-Antunez L, Heredia-Rizo AM. Effect of interferential current therapy on pain perception and disability level in subjects with chronic low back pain: a randomized controlled trial. *Clin Rehabil* 2017;31:242-9.
- Elserty N, Kattabei O, Elhafez H. Effect of Fixed Versus Adjusted Transcutaneous Electrical Nerve Stimulation Amplitude on Chronic Mechanical Low Back Pain. *J Altern Complement Med* 2016;22:557-562.
- Itoh K, Itoh S, Katsumi Y, Kitakoji H. A pilot study on using acupuncture and transcutaneous electrical nerve stimulation to treat chronic non-specific low back pain. *Complement Ther Clin Pract* 2009;15:22-5.
- Jarzem PF, Harvey EJ, Arcaro N, Kaczorowski J. Transcutaneous electrical nerve stimulation (TENS) for short term treatment of low back pain: randomized double blind crossover study of sham versus conventional TENS. *JMP* 2005.
- Topuz O, Ozfidan E, Ozgen M, Ardic F. Efficacy of transcutaneous electrical nerve stimulation and percutaneous neuromodulation therapy in chronic low back pain. *J Back Musculoskelet Rehabil* 2004; 17:127-133.
- Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med* 2009;6:2:1006-1012.
- Maher CG, Sherrington C, Herbert RD, Moseley AM, Elkins M. Reliability of the PEDro scale for rating quality of randomized controlled trials. *Phys Ther* 2003;83:713-21.
- Vandenbroucke JP, von EE, Altman DG, *et al.* Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. *Int J Surg* 2014;12:1500-24.
- von EE, Altman DG, Egger M, Pocock SJ, Gotsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Int J Epidemiol* 2007;18:800-4.
- da Costa BR, Cevallos M, Altman DG, Rutjes AW, Egger M. Uses and misuses of the STROBE statement: bibliographic study. *BMJ Open* 2011;1:-000048.
- Moher D, Hopewell S, Schulz KF, *et al.* CONSORT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomised trials. *BMJ* 2010;340:698-702.
- Consort group. <http://www.consort-statement.org/extensions>. 2019.
- Oxford Centre for Triple Value Healthcare Ltd (3V) portfolio. The Critical Appraisals Skills Programme (CASP). Oxford Centre for Triple Value Healthcare Ltd (3V) portfolio 2020.
21. Padulo J, Oliva F, Frizziero A, Maffulli N. Muscles, Ligaments and Tendons Journal. Basic principles and recommendations in clinical and field Science Research: 2018 update. *MLTJ* 2018; 8(3): 305-7.

Asymptomatic Professional Footballers: Prevalence of Ankle Retinacula Injury with Associated Lateral Ligament and Tendon Abnormalities

K. Win¹, M. Gillett², R. Botchu⁵, A.K. Suokas³, A. Seth⁴, S.L. James⁵

¹ Birmingham City Football Club, Cattell Road, Birmingham, United Kingdom

² Nottingham Forest Football Club, The City Ground, Nottingham, United Kingdom

³ Research and Teaching Centre, The Royal Orthopaedic Hospital NHS Foundation Trust, Bristol Road South, Northfield, Birmingham, United Kingdom

⁴ Sports and Exercise Medicine, The Royal Orthopaedic Hospital NHS Foundation Trust, Bristol Road South, Northfield, Birmingham, United Kingdom

⁵ Department of Musculoskeletal Radiology, The Royal Orthopaedic Hospital NHS Foundation Trust, Bristol Road South, Northfield, Birmingham, United Kingdom

CORRESPONDING AUTHOR:

Rajesh Botchu
Department of Musculoskeletal Radiology
The Royal Orthopaedic Hospital
Bristol Road South
Northfield, Birmingham, United Kingdom
E-mail: drbrajesh@yahoo.com

DOI:

10.32098/mltj.03.2020.22

LEVEL OF EVIDENCE: 4

SUMMARY

Background. The retinacula are important dynamic stabilizers around the ankle joint and are susceptible to potential injury in high-level footballers.

Aim. The purpose of this study was to evaluate the prevalence of retinacula injury in asymptomatic professional football players and for the presence of associated ankle tendon or ligament abnormalities.

Methods. Seventeen professional football players from one English premier league club underwent ultrasound examination of both ankles. The retinacula around the ankle were measured for thickness and compared with normal values. Ankle tendons were evaluated for the presence of abnormal vascularity or tendinopathy, tendon sheath effusions, and for peroneal tendon subluxation. Lateral ankle ligaments were also examined.

Results. The results indicated increased thickness of the retinacula at the following sites: superior extensor retinaculum on the medial side (1.5 mm, normal 0.9 mm); superior peroneal retinaculum at the anterior insertion (2.16 mm, normal 1 mm); and flexor retinaculum at the anterior insertion into the tibia (1.78 mm, normal 0.9 mm). Fluid around the tibialis posterior tendon was a frequent finding representing 74%, whilst over 25% revealed fluid within the peroneal tendon sheaths. Over 50% of the scans revealed thickened and abnormal lateral ligament complexes.

Conclusions. The thickening of the retinacula may indicate scarring from previous injury or repetitive micro-trauma, attributable to the ball kicking biomechanics, kinetics and contact injuries during tackles. Furthermore tendon sheath fluid was commonly seen on both the medial and lateral side of the ankle. We conclude that chronic scarring and thickening of these structures should be recognized as a common and often incidental finding and the significance in asymptomatic professional footballers is minimal.

KEY WORDS

Ankle; football; retinaculum; tendon; ligament.

BACKGROUND

Soccer (football) is the world's most popular sport, with an estimated 270 million people (roughly 4% of world's population) actively playing or officiating in the game (1).

At a professional level, high intensity bursts of activity on top of 90 minutes of lower intensity aerobic effort are required with players covering an average distance of 10 km during a 90 min match (2). Union of European Football Associations (UEFA) data indicates that ankle injuries account for 10 to 18% of all injuries in high-level soccer (2) with an incidence of 0.7/ 1000 hour of exposure (3). A professional squad of 25 players is predicted to see an average of 4–5 ankle sprains during a season (3). The most commonly reported mechanism of injury is a tackle on the medial or lateral side of the weight bearing limb (4) with foul play involved in 40% of match-related ankle sprains (3). Despite an anticipated quick return to sport after ankle sprains (mean lay-off 15 days) (3), previous authors have shown that up to 80% of athletes still present with persistent symptoms at 1.5 to 4 year follow up (5-9).

The foot and ankle are a complex anatomical area. Movement in the sagittal plane occurs at the talo-crural joint with dorsi-flexion and plantar-flexion, whilst movement in the coronal plane occurs at the sub-talar joint with inversion and eversion. (10) Active movement is dependent on contraction of the long flexor and extensor muscles of the lower leg, with their tendons undergoing significant angulation and directional changes whilst traversing the ankle joint to insert into the tarsal and meta-tarsal bones. They are held in place by the retinacula of the ankle, which prevent them from a form of subluxation referred to as bowstringing. The retinacula are localized thickenings of the crural fascia and typically heal with fibrosis and scarring after injury. When the muscles contract, the retinacula permit the tendons to glide longitudinally whilst maintaining approximation to the underlying bones, thus augmenting the mechanical strength and stability of the tendon complexes.

Given that ankle ligament injuries are common in professional soccer, accounting for 51 – 81% of all football related ankle injuries (3), our hypothesis was that evidence of a previous retinacula injury should be frequently identified. The aim of this study was to identify the prevalence of abnormal thickening of the retinacula structures of the ankle in asymptomatic professional soccer players during a screening ultrasound examination. The lateral ligament complex as well as the anterior, medial and peroneal tendons was also examined to assess for the prevalence of associated soft tissue abnormalities.

The extensor retinacula is derived from the superficial crural aponeurosis of the leg and serves as a restraint to the extensor tendons at the front of the ankle joint and dorsum

of the foot. It can be divided into the superior extensor and inferior extensor retinacula.

The superior extensor retinaculum is a transverse rectangular aponeurotic band that originates from the anterior border of the fibula and lateral malleolus, crossing over the anterior aspect of the ankle just above the tibio-talar joint to insert into the anterior tibial crest and medial malleolus (11). Deep to it are the extensor tendons (medial to lateral) tibialis anterior, extensor hallucis longus, extensor digitorum longus and peroneus tertius, the dorsalis pedis vessels and the deep peroneal nerve. It is reported that in 25% of cases, the superficial and deep layer fibers form a separate tunnel for the tibialis anterior tendon (12).

The inferior extensor retinaculum is a complex X or Y shaped structure situated at the anterior aspect of ankle and the dorsum of the foot. Previous studies have reported that this structure is difficult to visualize completely on MRI and ultrasound (11), hence it was not included in the ultrasound protocol used in this study.

The peroneal retinacula include the superior and inferior peroneal retinaculum

The superior peroneal retinaculum is a transverse rectangular fibrous band located at the lateral aspect of the ankle. It attaches to the lateral border of the retro-malleolar groove proximally and the lateral wall of the calcaneus distally. It covers the peroneus brevis and peroneus longus tendons when they pass through the retro-malleolar groove to enable functional gliding movements within the groove. It is well documented as a primary restraint for peroneal tendon subluxation and also serves as a secondary restraint to ankle inversion and lateral ankle instability (12). A fibrous triangular cartilage is commonly seen at its distal attachment on the peroneal periosteum.

The inferior peroneal retinaculum is an oblique band, connecting the lateral rim of the sinus tarsi to the lateral calcaneus below the trochlear process. The flexor retinaculum is a triangularly shaped fibrous aponeurosis that extends from the tip of the medial malleolus to the medial calcaneal process. It restrains the flexor tendons (anterior to posterior) tibialis posterior, flexor digitorum longus and flexor hallucis longus.

METHODS

Local ethical committee approval as well approval from Director of performance of the club was obtained for the study (13). The sample comprised of the first team professional players at a single Premier League soccer club. Players were only included in the study if both their ankles were

asymptomatic at the time of examination and they were participating in full unrestricted training. Additionally, if players had undergone previous foot or ankle surgery then they were excluded from the study in order to avoid bias of the retinacula measurements undertaken.

The study consisted of 17 male professional soccer players. The average age was 28 years (range 20-34 years), 11 were right foot and 6 left foot dominant.

Screening medical examinations are common in professional sport and the ultrasounds undertaken for the purposes of this study formed part of an existing and ongoing player screening program which encompasses both clinical and imaging assessment. The ultrasound screening program is approved by the club's governance committee to allow prospective data collection in asymptomatic players.

Ultrasound scanning of both ankles (total 34 scans) was carried out by a fellowship trained specialist musculoskeletal radiologist. The scans were performed with a Siemens Acuson P300 ultrasound system and a linear high frequency probe.

In addition to the retinacula measurements, the ultrasound screening protocol also included examination of the peroneal, flexor and extensor tendons, together with evaluation of the lateral ligament structures reliably visualised with ultrasound namely the anterior inferior tibio-fibular, anterior talo-fibular and calcaneo-fibular ligaments. The tendons were evaluated for the presence of abnormal vascularity/tendinopathy, tendon sheath effusions/tenosynovitis and peroneal tendon subluxation. The lateral ligaments were examined for their integrity and any abnormal thickening indicative of prior injury.

Normal reference values for retinacula structures were compared to the players' measurements. (14) Reference values

were obtained for eight retinacula structures as follows. The mean thickness of the superior extensor retinaculum was 0.9 mm and the range 0.7 -1.3 mm (14) (**figures 1,2**). Measurements for thickness were taken at both the anterior and posterior aspects of the superior peroneal retinaculum, and the mean thickness was 1.0mm and the range 0.7-2.6 mm (14). The mean thickness of the inferior peroneal retinaculum was 0.8mm and the range 0.7-1.0 mm (14). An anterior and posterior measurement of flexor retinaculum thickness was made, and the mean thickness was 0.9mm and the range 0.7-1.0 mm (14) (**figures 2,3,4**).

The mean and range of the thickness of the retinacula were calculated for each of the chosen eight structures. The sample mean and the reference value were compared separately for the dominant and non-dominant foot using the one sample t-test. Shapiro-Wilks tests indicated that the data were not normally distributed, but the one sample t-test was used, as it is fairly robust to departures of normality. P-values < 0.05 were reported as significant. Measurements from each site were plotted and presented with reference values (mean and range) to show the distribution of values. Statistical analysis was carried out using SPSS 22 (IBM Corporation, NY, USA).

RESULTS

The superior extensor retinaculum demonstrated a mean thickness of 1.57mm (range 0.9-2.5 mm) medially and 0.89mm (range 0.5-1.9 mm) laterally. Compared to the reference value, the mean thickness of the medial aspect of the retinaculum was statistically significantly larger for both dominant and non-dominant foot (**table I**). The difference was not statistically significant on the lateral side (**figures 5,6**)

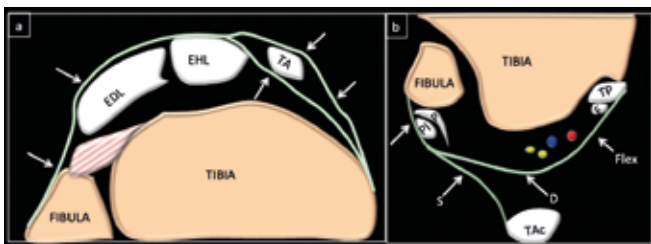


Figure 1. Animations of axial section of 15 mm proximal to ankle joint (a) showing attachment of superior extensor retinaculum (arrow), (b) showing the superior peroneal retinaculum (arrow) continuous with superficial (S) and deep aponeurosis (D) of the posterior ankle and continuous with superior part of the flexor retinaculum. TA (tibialis anterior), EHL (extensor hallucis longus), EDL (extensor digitorum longus), P (peroneus brevis), PI (peroneus longus), TAc (tendoachilles), TP (tibialis posterior), F (flexor digitorum longus).



Figure 2. Transverse image demonstrating a separate tibialis anterior (TA) tunnel. The medial aspect of the superior extensor retinaculum is identified curving around the medial border of the TA tendon (white arrow) and passing deep to the tendon. The retinaculum is seen coursing over the superficial aspect of TA.



Figure 3. Transverse image illustrating a normal flexor retinaculum arising from the tibia medially (white arrow). The site of medial measurement is shown with the tibialis posterior tendon (TP) being identified deep to the retinaculum.



Figure 4. Transverse image demonstrating a thickened flexor retinaculum arising from the tibia (white arrow). There is also an area of bony irregularity associated with the tibial cortex. The tibialis posterior tendon is again illustrated (TP).

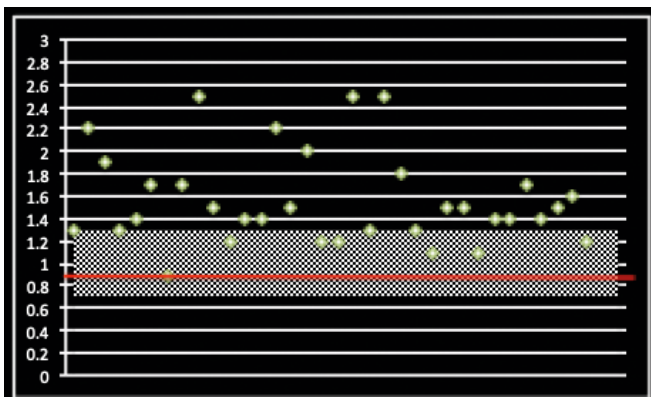


Figure 5. Medial measurement of the superior extensor retinaculum (n = 34 scans). The shaded area represents the normal reference values (mean 0.9mm; range 0.7 – 1.3 mm).

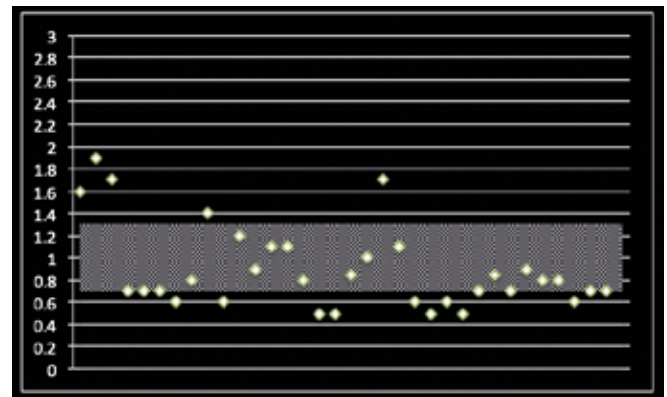


Figure 6. Lateral measurement of the superior extensor retinaculum (n = 34 scans). The shaded area represents the normal reference values (mean 0.9mm; range 0.7 – 1.3 mm).

Table I. One sample t-test comparing the sample mean with the reference value for each retinacula structure by dominant vs. non-dominant foot.

	Dominant foot	Non-dominant foot
Superior extensor retinaculum: medial	t = 6.189, p < 0.001*	t = 7.181, p < 0.001*
Superior extensor retinaculum: lateral	t = -0.143, p = 0.888	t = 0.036, p = 0.972
Superior peroneal retinaculum: anterior	t = 5.227, p < 0.001*	t = 6.403, p < 0.001*
Superior peroneal retinaculum: posterior	t = 1.450, p = 0.167	t = 0.975, p = 0.344
Inferior peroneal retinaculum: anterior	t = -1.628, p = 0.123	t = -1.369, p = 0.190
Inferior peroneal retinaculum: posterior	t = -1.186, p = 0.253	t = -0.466, p = 0.647
Flexor retinaculum: anterior	t = 5.762, p < 0.001*	t = 5.851, p < 0.001*
Flexor retinaculum: posterior	t = 1.255, p = 0.227	t = 2.223, p = 0.041**

* p-values < 0.05 indicate statistically significant difference

** Significant association was caused by an outlier.

11 out of 34 ankles (29%) had a separate tunnel for the tibialis anterior tendon. There was one player, who had a separate tendon tunnel in his dominant leg and 5 players with bilateral separate tibialis anterior tunnels.

The superior peroneal retinaculum demonstrated a mean thickness of 2.16 mm (range 0.7-4.4 mm) anteriorly and 1.18mm (range 0.6-3.6 mm) posteriorly. Compared to the reference value, the mean thickness of the anterior side of the retinaculum was statistically significantly larger for both dominant and non-dominant foot (**table I**). The difference was not statistically significant on the posterior side. (**figures 7,8**) Two ankles, which were completely asymptomatic, demonstrated peroneal tendon dislocation on dynamic ultrasound examination with the foot dorsiflexed and everted implying disruption of the superior peroneal retinaculum.

The inferior peroneal retinaculum demonstrated a mean thickness of 0.71 mm (range 0.5 – 1.3 mm) anteriorly and 0.74mm (Range 0.4 - 1.4 mm) posteriorly. The mean thickness was not statistically significantly different from the reference value for any of the retinacular structures (**table I**) (**figures 9,10**).

The flexor retinaculum demonstrated a mean thickness of 1.78 mm (range 0.8-3.6 mm) anteriorly and 1.06mm (range 0.6-2.5 mm) posteriorly. Compared to the reference value, the mean thickness of the anterior side of the retinaculum was statistically significantly larger for both dominant and non-dominant foot (**table I**). On the posterior side, the difference was only statistically significant for the non-dominant foot. However, this significant result was created by an outlier (2.5 mm) (**figures 11,12**).

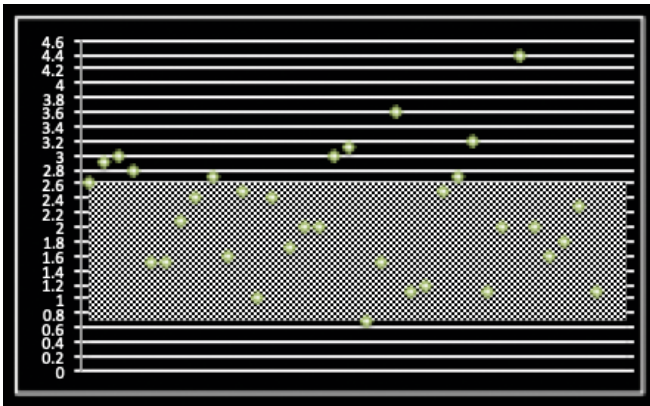


Figure 7. Anterior measurement of the superior peroneal retinaculum (n = 34 scans). The shaded area represents the normal reference values (mean 1.0mm; range 0.7 – 2.6 mm).

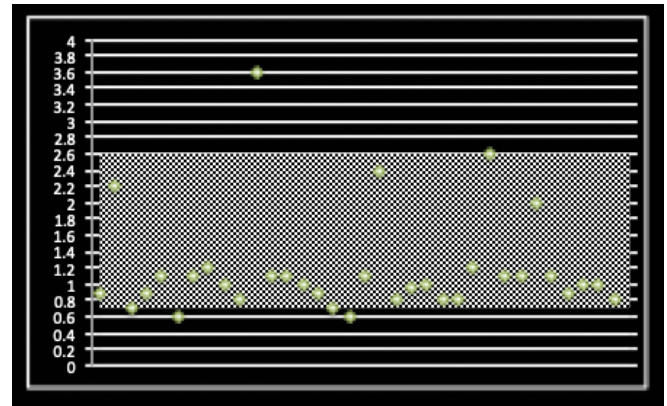


Figure 8. Posterior measurement of the superior peroneal retinaculum (n = 34 scans). The shaded area represents the normal reference values (mean 1.0mm; range 0.7 – 2.6 mm).

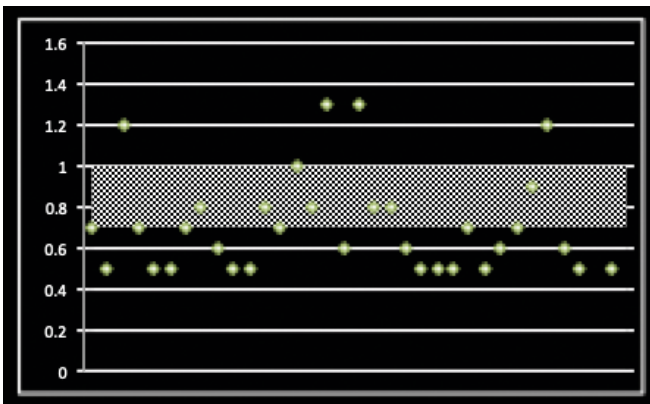


Figure 9. Anterior measurement of the inferior peroneal retinaculum (n = 34 scans). The shaded area represents normal reference values (mean 0.8; range 0.7 – 1.0 mm).

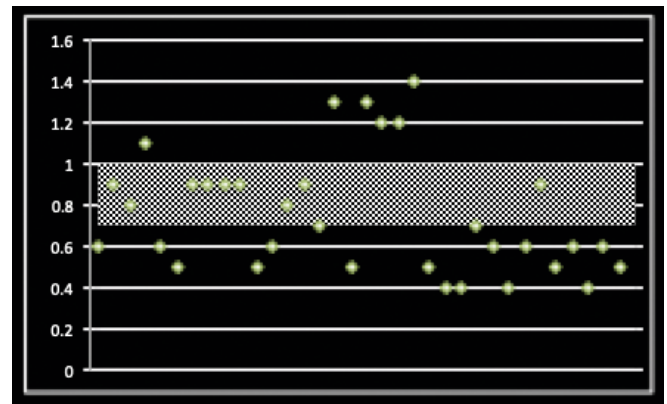


Figure 10. Posterior measurement of the inferior peroneal retinaculum (n = 34 scans). The shaded area represents normal reference values (mean 0.8mm; range 0.7 – 1.0 mm).

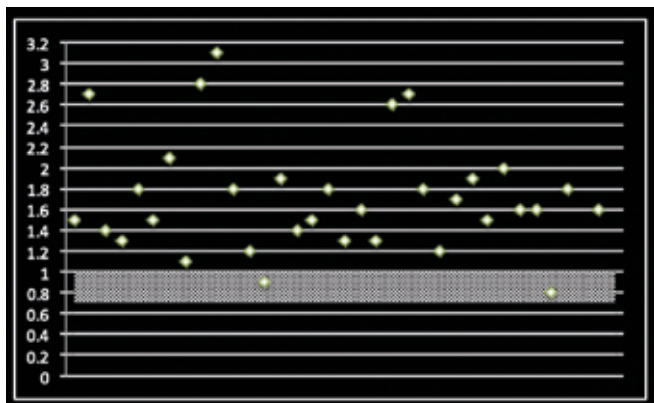


Figure 11. Anterior measurement of the flexor retinaculum (n = 34 scans). The shaded area represents the normal reference values (mean 0.9 mm; range 0.7 – 1 mm).

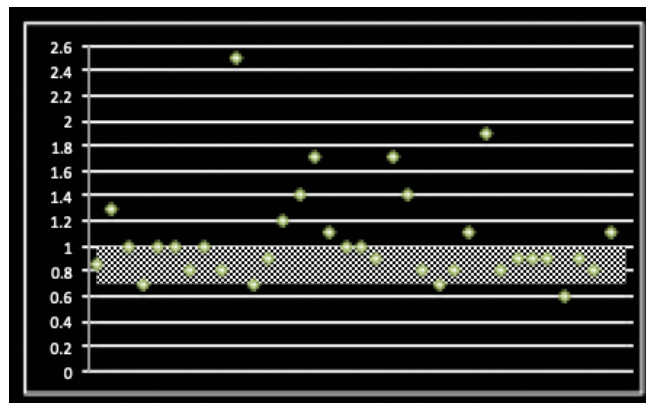


Figure 12. Posterior measurement of the flexor retinaculum (n = 34 scans). The shaded area represents the normal reference values (mean 0.9mm; range 0.7 – 1 mm).

25 out of 34 ankles (74%) elicited fluid within the tibialis posterior tendon sheath. Only one out of these 25 ankles demonstrated increased vascularity around an area of thickened synovium. One player was found to have a low-grade partial tear of the tibialis posterior tendon with mixed intra-substance change indicating associated tendinopathy at the level of the medial malleolus in his dominant foot.

The ultrasound appearance of the tendons of flexor hallucis longus and flexor digitorum longus was normal in most players. Only 3 out of 34 ultrasounds showed fluid around the flexor hallucis longus tendon, whilst a completely different set of 3 players had a tendon sheath effusion in flexor digitorum longus.

4 out of 34 (12%) demonstrated tendon sheath effusions around the tibialis anterior tendons, of which, 2 were associated with separate tibialis anterior tunnels. No significant tendon sheath effusion or tendinopathy was identified in extensor hallucis longus and extensor digitorum longus.

Scanning of the peroneal longus tendon revealed a tendon sheath effusion in 9 out of 34 ankles (26%). This finding was not associated with neovascularisation. A similar result was found in the peroneal brevis tendon, with 10 scans (29%) demonstrating fluid in the tendon sheath.

In this study population, thickening of the lateral ligaments was a common finding. 76% (26/34) revealed a thickened AITFL. However only 2 out of these 26 were associated with neovascularization. 71% (24/34) of scans elicited an abnormal ATFL, with 16 demonstrating a thickened scarred ligament and 8 showing a deficient and attenuated ATFL.

A thickened CFL was seen in 56% of ankle scans (19/34), with one third of players (6/17) demonstrating bilateral ankle involvement.

DISCUSSION

This is the first study to examine the ultrasound appearances of the ankle retinacula in asymptomatic professional soccer players. Our principle findings suggesting increased thickness of the retinacula on the following sites: superior extensor retinaculum on the medial side, superior peroneal retinaculum on the anterior side and flexor retinaculum on the anterior side of the ankle.

Asymptomatic tendon sheath effusions are most commonly identified within the tibialis posterior (74%) and to a lesser extent in the peroneus longus (26%) and brevis tendon (29%) sheaths. Lateral ligament complex injuries are also a common associated finding with the majority of players showing ultrasound evidence of prior injury to the AITFL, ATFL and CFL.

We propose the following two mechanisms as contributing factors to explain the radiological appearances observed in this study:

direct compression of the retinacula can occur during a ball control and kicking manoeuvre, or from contact with another player;

in soccer, significant force is applied through the foot and ankle whilst controlling, passing or blocking the ball using the medial or lateral side of the foot and ankle (15). The repeated sub-maximal stress on normal tissue could induce local tissue inflammation and scar tissue formation in the long-term and may explain the pattern of chronic retinacula scarring observed in this study.

Among different types of ball kicks, the instep kick is normally used for the generation of fast ball speed (16). During ball strike, professional players keep the ankle locked in plantar flexion in order to maximize the force

generated to propel the ball (17). A study conducted by Tol *et al.* (18) that evaluated the relationship of the kicking action in soccer to ankle impingement is highly pertinent to this study. In 39% of kicking actions, the dynamic plantar flexion angle recorded on impact exceeded the maximum static angle. This study also concluded that over 75% of ball strikes involved ball contact with the medial malleolus and the base of the 1st metatarsal. This area is in close anatomical proximity to the medial attachments of the superior extensor retinaculum and the flexor retinaculum, which were found to be significantly thickened in this study. Hence an ankle at the extreme range of plantar flexion contacts the ball at speed on the medial aspect of the foot and ankle, creating an impact force in the region of 1025 Newtons (18). Given the repeated execution of this skill by a professional footballer in both training and matches, the repetitive strain on the antero-medial ankle structures is likely to be significant and a potential cause of retinacula thickening.

Player to player contacts during tackles can result in significant force transmission through the foot and ankle region, which may traumatize both the antero-lateral and medial aspects of the ankle joint (4,19). However, traumatic contacts in professional soccer do not occur with the same frequency as repetitive kicking activity, and it is the authors' hypothesis that this mechanism is likely to be less significant to our findings than repetitive kicking.

High-level soccer places extreme musculo-skeletal demands on the body, especially in the lower limbs. Multidirectional movement patterns including jumping, landing, twisting, turning and cutting are common and induce high forces around the ankle joint (20). The repetitive loading of the foot and ankle during the functional movements of soccer may contribute to the chronic scarring of the retinacula observed around the ankle.

We hypothesized that retinacula injuries may be associated with injuries to the lateral ligament complex.

The most common mechanism of ankle injury in soccer is excessive inversion with plantar flexion, which often leads to lateral ligament complex injuries (20). The lateral ligament injuries are often depicted as isolated injuries to the anterior talofibular ligament (ATFL) and calcaneofibular ligament (CFL). Despite early return to sport, the rate of re-injury and chronic instability are as high as 80% in athletes (5-9,21). Previous literature infers that symptoms such as pain, giving way and reduced ankle range of motion can often persist following acute ankle ligamentous injury (5-9,22-24). In one study (8) 32% of 648 patients attending hospital with an ankle inversion injury reported chronic pain, swelling or recurrent injury 7 years after their initial presentation (5).

In this ultrasound-based study, we have collected evidence that injury of the lateral ligament is a common finding in

soccer players who are clinically asymptomatic. These athletes are still able to function at a high athletic level and hence the significance of this finding in isolation is debatable. A variety of biomechanical changes to the sub-talar and inferior tibio-fibular joints have been shown to follow lateral ligament injury including increased anterior translation and internal rotation of the talus (25,26), excessive pronation and sub-talar laxity (25). Given the extent, variety and frequency of these findings, it is entirely possible that other ankle stabilizing structures are injured at the same time as the lateral ligament and this list may include the ankle retinacula.

At the present time there is inadequate evidence in the medical literature to definitively associate lateral ligament complex injury with retinacula injury. However, in the authors' experience, when ligamentous injuries are imaged in the acute phase oedema is frequently visualized around the retinacula located adjacent to the injured ligaments. It is possible that the thickening of the anterior side of the superior peroneal retinaculum observed in this study is commonly associated with lateral ligament injury and it would be helpful to evaluate this possibility with acute injuries in the future.

The common mechanisms of acute traumatic retinacula injury have been reported in previous studies (11-14,27). An acute injury of the extensor retinaculum is observed with traumatic, forceful dorsiflexion of the foot. Sudden forceful plantar flexion and eversion can cause tearing of the flexor retinaculum, which may be associated with tibialis posterior tendon dislocation (28). Acute traumatic injuries of the extensor and flexor retinacula are infrequent (11) and it is considered unlikely that any of the subjects included in this study sustained a significant acute injury of either of these structures previously.

The peroneal retinaculum serves as a secondary restraint of ankle inversion movement (12,29). An injury of the superior peroneal retinaculum can occur when dorsiflexion of the ankle is coupled with forceful eversion from peroneal muscle contraction (11,12,29). Both peroneal retinaculum injuries and peroneal tendon subluxation are commonly linked to chronic lateral ankle instability (12,12,29). Previous literature suggests that non-operative management of peroneal tendon dislocation can lead to recurrent subluxation of the peroneal tendons, with primary surgical repair recommended for individuals with high athletic demands (29). In our study, 2 players were found to have peroneal retinaculum injuries associated with peroneal tendon dislocations. Interestingly both players were asymptomatic and remained fully functional in professional soccer, challenging the necessity for immediate repair in this patient group.

Tendon sheath effusions were found in 74% of tibialis posterior tendons, 12% of tibialis anterior tendons, 26%

of peroneus longus tendons and 29% of peroneus brevis tendons. Only one of these tendons, a tibialis posterior tendon with a partial tear and intrasubstance change suggestive of tendinopathy, demonstrated imaging evidence of further pathology.

Hence on the basis of this study, tendon sheath effusions can be considered a normal finding in professional footballers and are likely to be reactive and potentially a further consequence of the repeated kicking nature of the sport.

Bianchi and colleagues had described the sonographic features of normal retinacula around the ankle and their pathologies but the study population was not confined to footballers as our study (30).

There are a number of limitations of this study. First and foremost, we are assuming that the reference ranges reported in the cadaveric study reflect an accurate representation of normal retinacula thickness. We do not believe this represents a significant drawback as if there had been previous injuries to the cadaver group this would tend to cause an underestimation of injury prevalence in the screened professional soccer players. The sample size is small as the squad size of football team is limited. We have tried to postulate

potential mechanisms for our findings and this is based on previous literature descriptions of injury mechanisms rather than direct biomechanical analysis. A single experienced radiologist made the ultrasound measurements and there is the potential for inter-observer variability in measurements. Finally, we are also assuming that MRI and ultrasound measurements can be used interchangeably when making comparisons between the cadaver and professional soccer readings.

CONCLUSIONS

Asymptomatic retinacula injuries are common in professional soccer. Despite chronic scarring of the retinacula, the players remain able to function at high level with no reported symptoms. Imaging evidence of lateral ligament complex injury is a common finding as is the presence of fluid within the tendon sheaths of the ankle tendons.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

REFERENCES

1. <http://www.fifa.com/worldfootball/bigcount/index.html>.
2. <http://www.uefa.com/newsfiles/156571>.
3. Walden M, Hagglund M, Ekstrand J. Time-trends and circumstances surrounding ankle injuries in men's professional football: an 11-year follow-up of the UEFA Champions League injury study. *Br J Sports Med* 2013;47(12):748-53 doi: 10.1136/bjsports-2013-092223.
4. Giza E, Fuller C, Junge A, Dvorak J. Mechanisms of foot and ankle injuries in soccer. *Am J Sports Med* 2003;31(4):550-4.
5. Anandacoomarasamy A, Barnsley L. Long term outcomes of inversion ankle injuries. *Br J Sports Med* 2005;39(3):e14; discussion e14 doi: 10.1136/bjism.2004.011676.
6. Gerber JP, Williams GN, Scoville CR, Arciero RA, Taylor DC. Persistent disability associated with ankle sprains: a prospective examination of an athletic population. *Foot Ankle Int* 1998;19(10):653-60.
7. Green T, Refshauge K, Crosbie J, Adams R. A randomized controlled trial of a passive accessory joint mobilization on acute ankle inversion sprains. *Phys Ther* 2001;81(4):984-94.
8. Konradsen L, Bech L, Ehrenbjerg M, Nickelsen T. Seven years follow-up after ankle inversion trauma. *Scand J Med Sci Sports* 2002;12(3):129-35.
9. BL B. Effects of ankle sprain in a general clinical population 6 to 18 months after medical evaluation. *Arch Fam Med* 1999;8:143-8.
10. Brockett CL, Chapman GJ. Biomechanics of the ankle. *Orthop Trauma*. 2016;30(3):232-238. doi:10.1016/j.mporth.2016.04.015.
11. Demondion X, Canella C, Moraux A, Cohen M, Bry R, Cotten A. Retinacular disorders of the ankle and foot. *Semin Musculoskelet Radiol* 2010;14(3):281-91 doi: 10.1055/s-0030-1254518.
12. Geppert MJ, Sobel M, Bohne WH. Lateral ankle instability as a cause of superior peroneal retinacular laxity: an anatomic and biomechanical study of cadaveric feet. *Foot Ankle* 1993;14(6):330-4.
13. Padulo J., Oliva F, Frizziero A., Maffulli N. Muscles, Ligaments and Tendons Journal – Basic principles and recommendations in clinical and field Science Research: 2018 update. *MLTJ* 2018; 8(3): 305 – 307.
14. Numkarunaruote N, Malik A, Aguiar RO, Trudell DJ, Resnick D. Retinacula of the foot and ankle: MRI with anatomic correlation in cadavers. *AJR Am J Roentgenol* 2007;188(4):W348-54 doi: 10.2214/AJR.05.1066.
15. Madden CC. *Netter's sports medicine*. Philadelphia: Saunders/Elsevier, 2010.
16. Levanon J, Dapena J. Comparison of the kinematics of the full-instep and pass kicks in soccer. *Med Sci Sports Exerc* 1998;30(6):917-27.
17. Barfield WR, Kirkendall DT, Yu B. Kinematic instep kicking differences between elite female and male soccer players. *J Sports Sci Med* 2002;1(3):72-9.
18. Tol JL, Slim E, van Soest AJ, van Dijk CN. The relationship of the kicking action in soccer and anterior ankle impingement syndrome. A biomechanical analysis. *Am J Sports Med* 2002;30(1):45-50.

19. Hamilton WG, Geppert MJ, Thompson FM. Pain in the posterior aspect of the ankle in dancers. Differential diagnosis and operative treatment. *J Bone Joint Surg Am* 1996;78(10):1491-500.
20. Kofotolis ND, Kellis E, Vlachopoulos SP. Ankle sprain injuries and risk factors in amateur soccer players during a 2-year period. *Am J Sports Med* 2007;35(3):458-66 doi: 10.1177/0363546506294857.
21. Smith RW, Reischl SF. Treatment of ankle sprains in young athletes. *Am J Sports Med* 1986;14(6):465-71.
22. Gehring D, Faschian K, Lauber B, Lohrer H, Nauck T, Gollhofer A. Mechanical instability destabilises the ankle joint directly in the ankle-sprain mechanism. *Br J Sports Med* 2014;48(5):377-82 doi: 10.1136/bjsports-2013-092626.
23. Gillman SF. The impact of chiropractic manipulative therapy on chronic recurrent lateral ankle sprain syndrome in two young athletes. *J Chiropr Med* 2004;3(4):153-9 doi: 10.1016/S0899-3467(07)60103-7.
24. Loudon JK, Reiman MP, Sylvain J. The efficacy of manual joint mobilisation/manipulation in treatment of lateral ankle sprains: a systematic review. *Br J Sports Med* 2014;48(5):365-70 doi: 10.1136/bjsports-2013-092763.
25. Denegar CR, Miller SJ, 3rd. Can Chronic Ankle Instability Be Prevented? Rethinking Management of Lateral Ankle Sprains. *J Athl Train* 2002;37(4):430-35.
26. Wainright WB, Spritzer CE, Lee JY, et al. The effect of modified Brostrom-Gould repair for lateral ankle instability on in vivo tibiotalar kinematics. *Am J Sports Med* 2012;40(9):2099-104 doi: 10.1177/0363546512454840.
27. Hatch GF, Labib SA, Rolf RH, Hutton WC. Role of the peroneal tendons and superior peroneal retinaculum as static stabilizers of the ankle. *J Surg Orthop Adv* 2007;16(4):187-91.
28. Sharma R, Jomha NM, Otto DD. Recurrent dislocation of the tibialis posterior tendon. *Am J Sports Med* 2006;34(11):1852-4 doi: 10.1177/0363546506288729(published Online First: Epub Date).
29. Maffulli N, Ferran NA, Oliva F, Testa V. Recurrent subluxation of the peroneal tendons. *Am J Sports Med* 2006;34(6):986-92 doi: 10.1177/0363546505283275.
30. Bianchi S, Becciolini M. Ultrasound Features of Ankle Retinacula: Normal Appearance and Pathologic Findings. *J Ultrasound Med.* 2019;38(12):3321-3334.

An Anatomical Perspective of Ulnar Collateral Nerve with Reference to Nerve and Muscle Transfer Surgery

P. J. Jiji., T. Joy, B.V. Murlimanju, M. M. Pai, L. V. Prabhu, R. Rai

Department of Anatomy, Kasturba Medical College, Mangalore, Manipal Academy of Higher Education, Manipal, Karnataka, India

CORRESPONDING AUTHOR:

Teresa Joy
Department of Anatomy
Kasturba Medical College
Mangalore, India
Manipal Academy of Higher Education
575004 Karnataka, India
E-mail: teresa.joy@manipal.edu

DOI:

10.32098/mltj.03.2020.23

LEVEL OF EVIDENCE: 4

SUMMARY

Background. The purpose was to analyze the prevalence, origin, course and termination of the ulnar collateral nerve in anatomical samples from Indian population.

Methods. The present study included 68 adult human cadaveric embalmed upper limbs. The sex of these upper limbs were not known. The initial segment of radial nerve was meticulously dissected in the specimens to observe the ulnar collateral nerve. The digital Vernier caliper was used for the measurements which were performed in this study. The data was given in millimeter and expressed as mean \pm SD.

Results. The ulnar collateral nerve was observed in 57 (83.8%) of our specimens. It originated in the axilla in 35 cases (61.4%), at the brachio-axillary angle in 13 (22.8%) and in the arm in 9 cases (15.8%) respectively. The termination of ulnar collateral nerve by piercing deep into the triceps muscle happened 101.9 ± 4 mm above the medial epicondyle of humerus. The origins of muscular branch to long head of triceps muscle and ulnar collateral nerve were located 44.7 ± 3.7 mm and 66.7 ± 4.4 mm away from the bifurcation of posterior cord respectively.

Conclusions. The present study suggests higher frequency of ulnar collateral nerve in sample Indian population. Ulnar collateral nerve can be used to re-innervate the muscles in case of brachial plexus injuries. Our study provides information about the detailed morphology and topography of the ulnar collateral nerve. The detailed knowledge can help the orthopedic surgeons during the procedures like muscle transfer, nerve transfer and nerve grafting.

KEY WORDS

Elbow surgery; radial nerve; triceps brachii; ulnar collateral nerve.

BACKGROUND

Variant innervations of muscles of upper extremity are comparatively rare, but if present will have clinical, diagnostic and surgical implications (1). Triceps brachii is conventionally believed to be dependent on the radial nerve (RN) for its motor innervation, but few studies (1-6) has confirmed the ulnar innervation for the distal part of its medial head. But there are older descriptions that says there were no branches given by ulnar nerve (UN) in the arm and thus entire nerve supply of triceps is by RN (7). However there are cases reported in which axillary nerve supplied the triceps brachii (8). Naidu *et al.* (9) revealed the independent function of lateral and long heads of triceps brachii

in their electromyographic studies. They restored the flexion of elbow by triceps to biceps tendon transfer procedure. The distal part of medial head of triceps is often affected in subluxation injuries. Posterior approach is commonly performed for the elbow surgery and this requires triceps splitting and triceps reflection (10). In this context, it is important to have the knowledge about anatomical variations in the innervation of triceps brachii. This will prevent inadvertent iatrogenic injury and also provides new modalities for the free functional nerve and muscle transfers for the triceps brachii (11).

Ulnar collateral nerve (UCN) runs along with the UN, but it is a branch of RN (12-14). UCN disappears in the back

of arm by piercing the medial head of triceps brachii¹⁵ and innervates it (16). It was described earlier that the UCN is a sensory nerve, though it pierces medial head of triceps brachii (17). Later, the immunohistochemical studies showed that the UCN is not purely a sensory or motor nerve, it is a mixed nerve (16). UCN also supplies the proximal part of the posterior part of capsular ligament of elbow (18). In brachial plexus injuries, de-innervated muscles can be re-innervated by using the UCN (2). The muscle unit along with its innervation may be transferred to reestablish the flexion of elbow (19). Nerve transfer procedures performed for the brachial plexus reconstruction and peripheral nerve injuries have given better outcomes than the nerve grafting (20). Literature search revealed that there are only a very few studies available about the UCN. This nerve is not highlighted in the anatomical discussions and the morphological data about this nerve are scarce from the Indian population. This was the stimulus to perform this present study. Our goal was to analyze the prevalence, origin, course and termination of the UCN in cadaveric samples.

MATERIALS AND METHODS

The present study included 68 adult human cadaveric embalmed upper limbs with intact axillary region. Among them 43 were right sided and 25 left sided. The source of cadavers is from body donation and unclaimed bodies from the government hospital. The present anatomical investigation has the approval of our institutional ethics committee. We also state that this study is in accordance with the international ethical standards, as per the opinion of Padulo *et al.* (21). The specimens which exhibited surgical scars, external pathological changes and congenital anomalies were excluded from the present study. The upper limbs were positioned with approximately 90° of abduction. The origin, course and termination of UCN was carefully dissected and observed in all the cases. The dissection was performed as per the standard steps given in the manuals of human anatomy. The nerve supply of the medial head of triceps muscle was observed in all the upper extremities. The topographical location of origin of UCN was classified into 3 categories, in the axilla, at the brachio-axillary angle and in the arm as per Hollinshead (12). The distance of origin of UCN was measured from the inferior margin of latissimus dorsi tendon close to its insertion. The topographical location of UCN, where it enters the triceps at its medial head was measured from the tip of the medial epicondyle, with a digital Vernier calliper. The distances of origins of muscular branch to long head and UCN, from the termination of posterior cord, were also measured. The data was given in millimeter and expressed as mean \pm SD.

RESULTS

The present study observed the UCN in 57 (83.8%) cases. Among them 36 (63.2%) were observed in right side upper limbs and 21 (36.8%) over the left side. The UCN was absent in 11 upper limbs (16.2%). It was observed that UCN was arising as the first robust branch to the medial head from the RN and coursing with the UN in the anterior brachium. UCN was innervating the medial head of triceps in each upper extremity, whenever present. The origin of UCN was ranging from 40mm above and 40 mm below the inferior margin of latissimus tendon. It was macroscopically observed that the UCN presented a separate fascial sheath as it coursed close to the UN. We did not observe UN supply (0%) to triceps brachii muscle in this study. Frequency of topographical distribution of the origin of UCN is represented in **figure 1**. The UCN was originating in the axilla (**figures 2,3**) in 35 cases (61.4%), at the brachio-axillary angle (**figure 4**) in 13 (22.8%) and in the arm (**figure 5**) in 9 cases (15.8%) respectively.

Observation of initial segment of RN revealed that its first muscular branch was to the long head of triceps brachii (**figure 2**) and was observed in 65 (95.6%) of upper limbs. In 18 (26.5%) cases, at the entry of spiral groove, RN gave a couple of branches, one each to the lateral and medial head of triceps (**figure 5**). In 22 (32.4%) upper limb specimens, proximal part of medial head of triceps received additional branches either from RN or UCN (**figure 3**).

It was observed that the topography of termination of UCN by piercing deep to the medial head of triceps brachii was extremely variable. This was ranging between 50.5mm to 180.5mm, superiorly to the tip of the medial epicondyle, and the mean distance was 101.9 ± 4 mm. The origins of muscular branch to long head and UCN were located 44.7 ± 3.7 mm and 66.7 ± 4.4 mm from the termination of posterior cord respectively.

The anatomical scheme of the complex variant anatomy of UCN, which was observed in this present study is represented in Fig. 6. The frequency of UCN, which was observed in the present study was compared with the data available in the literature (**table I**).

DISCUSSION

RN is the thickest branch which arises from the posterior cord, which usually provides three branches in the axilla –two muscular and one cutaneous, the cutaneous branch being the posterior cutaneous nerve of arm. The long head of triceps receives the first muscular twig and the second twig goes to the medial head, which is the UCN. RN provides more muscular twigs as it enters the spiral groove -one twig supplies the lateral head and another given to the medial

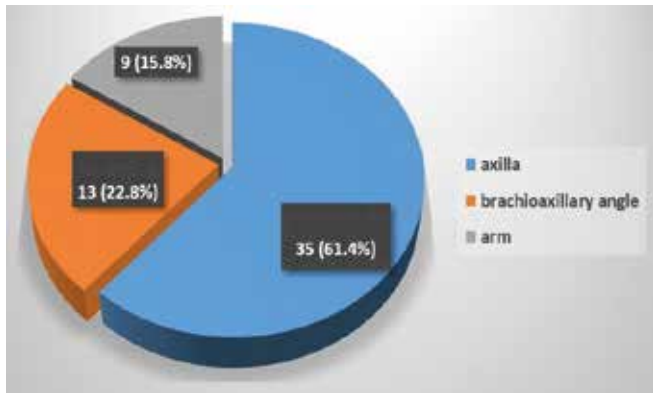


Figure 1. Frequency of topographical distribution of the origin of UCN (n=57).

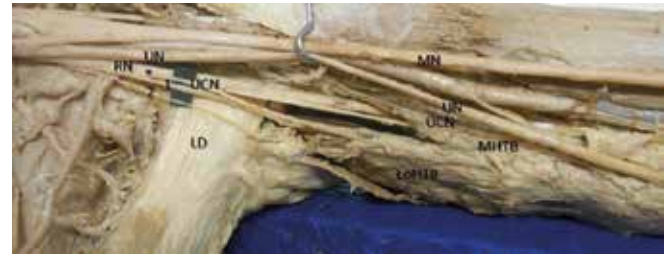


Figure 2. Cadaveric upper limb showing the origin of UCN in the axilla, 61.4% cases.

(*- origin of UCN; RN- radial nerve; UN- ulnar nerve; UCN- ulnar collateral nerve; MN- median nerve; LD- latissimus dorsi; LoHTB- long head of triceps brachii; MHTB- medial head of triceps brachii; 1- branch of radial nerve for LoHTB; 2-posterior cutaneous nerve of the arm).

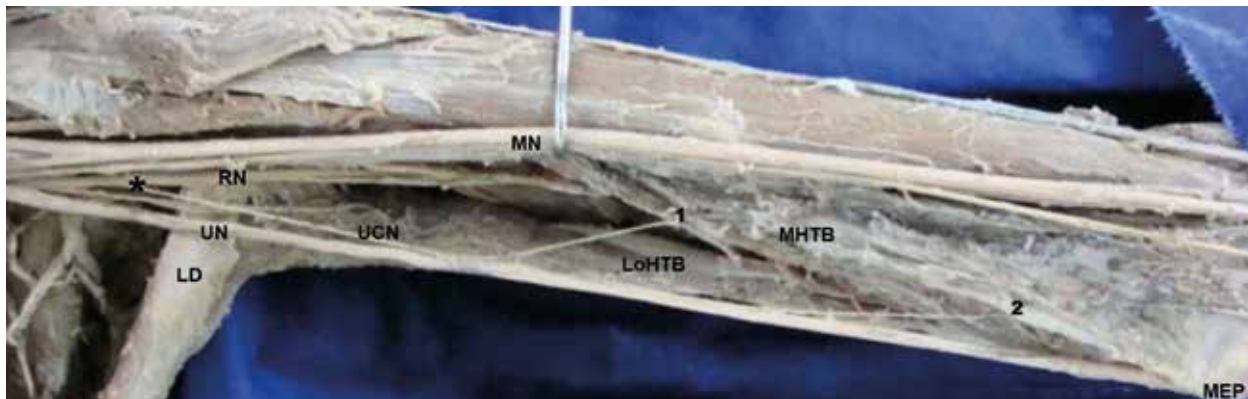


Figure 3. Cadaveric upper limb showing the multiple branches of UCN innervating the medial head of triceps, 32.4% cases. (*- origin of UCN; RN- radial nerve; UN- ulnar nerve; UCN- ulnar collateral nerve; MN- median nerve; LD- latissimus dorsi; LoHTB- long head of triceps brachii; MHTB- medial head of triceps brachii; MEP- medial epicondyle; 1,2- branches from UCN innervating the MHTB).

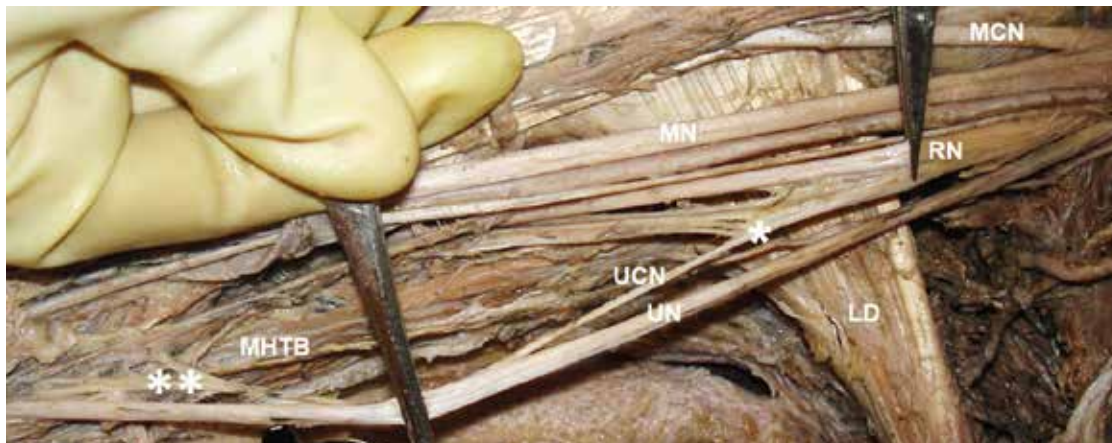


Figure 4. Cadaveric upper limb showing the origin of UCN in the brachio-axillary angle, 22.8% cases. (*- origin of UCN; RN- radial nerve; UN- ulnar nerve; UCN- ulnar collateral nerve; MN- median nerve; MCN- musculocutaneous nerve; LD- latissimus dorsi; MHTB- medial head of triceps brachii; **- UCN piercing MHTB and innervating it).

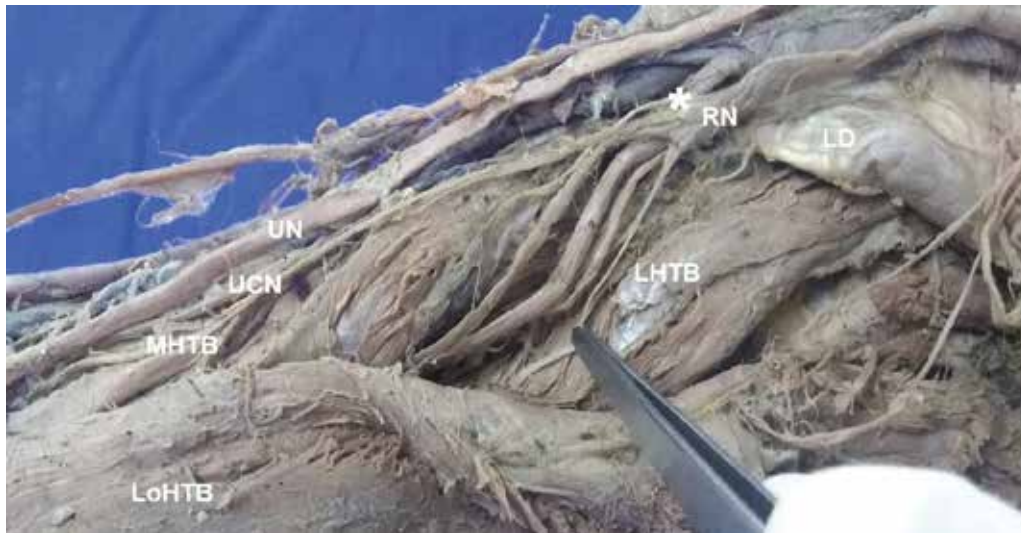


Figure 5. Cadaveric upper limb showing the origin of UCN in the arm, 15.8% cases. (*- origin of UCN; RN- radial nerve; UN- ulnar nerve; UCN- ulnar collateral nerve; LD- latissimus dorsi; MHTB- medial head of triceps brachii; LHTB- lateral head of triceps brachii; LoHTB- long head of triceps brachii).

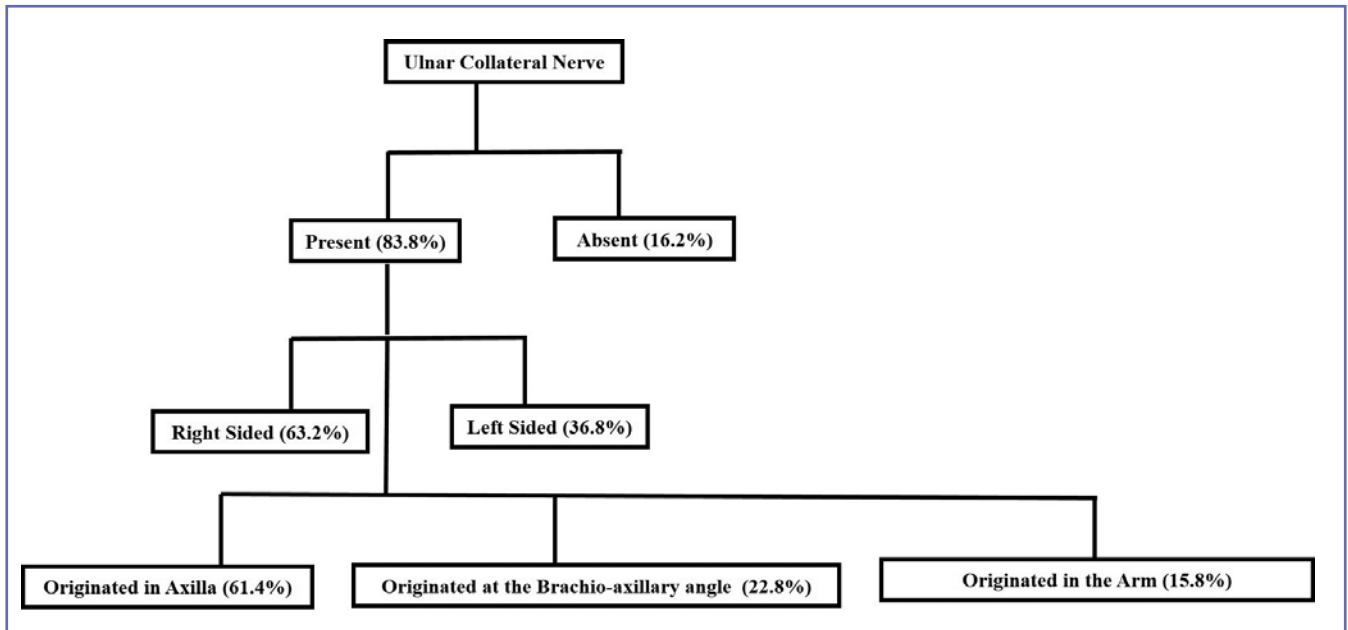


Figure 6. The anatomical scheme of the complex variant anatomy of UCN, which was observed in this study.

head which is considered as major innervation to the medial head (12). According to Linell (17) nerve to long head arises about 7.1 cm below the tip of acromion, the UCN about 9.5 cm, the nerve to lateral head about 10.1 cm and the major nerve to medial head about 11.2 cm below the tip of acromi-

on process. In this present study, the origin and termination of UCN was measured from the terminal part of posterior cord and medial epicondyle respectively. Sunderland (22) agreed with the opinion of Linell (17) about the presence of these four branches of RN but observed five to ten twigs.

In 32.4% of cases of present study, it was observed that RN was giving multiple additional twigs to medial head. According to Johnson and Ellis (13) and Sinnatamby (14), RN gives its major twig to the medial head close to its entry into the radial groove. However many twigs can be given to the medial head at the axilla and proximal part of arm. The first RN branch to medial head is the UCN and this is given usually in the axilla or as it enters the arm (12). Our present study agrees with the description of Hollinshead (12) as it was observed that the first muscular twig to medial head was UCN which was found in 83.8% cases. This frequency of UCN was compared with the global data available in the literature. This rate of 83.8% from Indian population is slightly higher than that of Turkish (4) (60%) and Korean (5) (75%) population. But lesser than Spanish¹⁶ population of 100% prevalence (**table I**).

It was described that the motor supply to the medial head of triceps is complex and controversia (14,11). The UN supply to the distal third of medial head was also advocated by few people in recent years (1-6). It was described that these UN twigs were confined to the distal third of the triceps and were hardly discernible from the fascia to be established macroscopically. The concept of UN supply to the triceps was questioned by Pascual-Font *et al.* (16). from their study on foetal specimens. Their study augmented with immunohistochemistry, reported that triceps was supplied by RN alone. They had further commented that while planning muscle or nerve transpositions by counting on the probable UN supply to medial head, the actual origin should be checked to minimize the failure of the procedure. Pascual-Font *et al.* (16). had further commented that prior confirmation of the real origin of innervation of the medial head, whether it is ulnar or radial is essential for the procedures like muscle and nerve transposition. This helps to prevent the failure of the transposition when performed. In their study, the UCN was present in all of their cases (100%) and it was a branch of RN but coursing along with the UN without intermingling of fibres but sharing a common neural sheath. Our study agrees with this opinion as we did not observe the UN supply of the medial head in any of our specimens. It may be also possible that those could be extremely thin branches and were not discernible from fascial sheath in dissections. It is interesting to note that medial head of triceps inserts separately in a deeper plane at the olecranon process along with the superficial insertion produced jointly by long and lateral heads (23). If there is ulnar innervation of triceps muscle, the clinical implication will be to include the medial head besides the long head of triceps, while performing triceps-to-biceps tendon transfer surgery (6).

The clinical use of RN branch to long head of triceps for nerve reconstruction in brachial plexus injuries was initial-

ly performed by Leechavengvongs *et al.* (24). Develi (4) reported that UCN can also be used for nerve grafting. In the advanced neurotization techniques during motor nerve reconstructions, it was proposed that even UCN can be effectively used as a nerve graft. UCN can be used for the reinnervation in denervated biceps brachii due to brachial plexus palsies and it can also be used for the direct nerve transfer (2).

The distal triceps injuries are rare, however they are seen sometimes in middle aged males particularly in weight-lifters. Because of its relatively rare occurrence, the triceps pathologies may lead to difficulty in the initial diagnosis and management. Snapping of triceps is more common than its tendon rupture in younger population and it is often associated with the ulnar dislocation (25). The distal third of the triceps contains independent motor units with an easy access and it is suggested to be ideal for the motor reconstructions with lesser morbidity (2). UCN can be entrapped due to compression, snapping, rubbing and slipping (4). In interpreting the electrophysiological tests and radiological images, UCN is important (4). Good health and well-being are among the sustainable development goals which are adopted by the BRIC countries and India is among them. In this context, the morphological and topographic knowledge of UCN is important to prevent its iatrogenic injury during the medial and posterior approaches of elbow joint surgery. The present study has certain limitations as the specimens used were from different individuals and hence side-based comparison was not feasible. Since this is a cadaveric study it is prone for alterations in the anatomical structures.

CONCLUSIONS

The present study is suggestive of higher frequency of UCN in Indian ethnic population as it was observed in 83.8% of our anatomical specimens. This study provides the data about the origin, course and termination of UCN, which are useful to the clinicians and operating surgeons to prevent its iatrogenic injury during the shoulder and elbow surgeries. UCN can be used to re-innervate the muscles in case of brachial plexus injuries and reestablishing the flexion of the elbow. Medial head of triceps brachii is the favorite muscle for the plastic surgery, which is being used for the muscle transfer procedures as this is a bulky muscle with multiple innervation. Nerve transfers are preferred over the nerve grafting for the brachial plexus reconstruction and peripheral nerve injuries as it gives better outcomes. In this context, the detailed knowledge about the morphology and topography of UCN can help the orthopedic surgeons during the procedures like muscle transfer, nerve transfer and nerve grafting.

ACKNOWLEDGEMENTS

The authors thank Dr. Soubhagya Ranjan Nayak, Professor of Anatomy, College of Medicine and JNM Hospital, Kalyani, Nadia, West Bengal, India for the help offered during the commencement of this study.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

REFERENCES

- Miguel-Perez MI, Combalia A, Arandes JM. Abnormal innervation of the triceps brachii muscle by the ulnar nerve. *J Hand Surg Eur* 2010;35:430-431.
- Bekler H, Wolfe VM, Rosenwasser MP. A cadaveric study of ulnar nerve innervation of the medial head of the triceps brachii. *Clin Orthop Relat Res* 2009;647:235-238.
- Loukas M, Bellary SS, Yuzbasioglu N, et al. Ulnar nerve innervation of the medial head of the triceps brachii muscle: a cadaveric study. *Clin Anat.* 2013;26:1028-1030.
- Develi S. Co-innervation of triceps brachii muscle with variant branch of ulnar nerve *Turk Neurosurg.* 2018;28:949-953.
- Cho SH, Chung IH, Lee UY. Relationship between the ulnar nerve and the branches of the radial nerve to the medial head of the triceps brachii muscle. *Clin Anat* 2019; 32:137-142.
- Jain DA, Kumar ST, Shetty N. Ulnar nerve innervation to triceps: A cadaveric study and a technical note on partial triceps to biceps transfer. *Indian J Orthop.* 2019;53:353-356.
- Ramström M. Investigations on the innervation of the medial head of the triceps brachii muscle. *Anat Anz* 1918;19:420-431
- Rezzouk J, Durandea A, Vital JM, Fabre T. Long head of the triceps brachii in axillary nerve injury: anatomical and clinical aspects. *Rev Chir Orthop Reparatrice Appar Mot* 2002;88: 561-564.
- Naidu S, Lim A, Poh LK, Kumar VP. Long head of the triceps transfer for elbow flexion. *Plast Reconstr Surg* 2007;119:45e-7e.
- Ozer H, Açar HI, Cömert A, et al. Course of the innervation supply of medial head of triceps muscle and anconeus muscle at the posterior aspect of humerus (anatomical study). *Arch Orthop Trauma Surg* 2006;126:549-553.
- Chaware PN, Santoshi JA, Patel M, et al. Surgical Implications of Innervation Pattern of the Triceps Muscle: A Cadaveric Study. *J Hand Microsurg* 2018;10:139-142.
- Hollinshead HW. *The Back and Limbs.* In: *Anatomy for Surgeons*, Vol. 3, 2nd ed. Philadelphia; Harper & Row 1969;376-378.
- Johnson D, Ellis H. Upper Arm. In: *Standring S, Williams PL, Bannister LH, et al, editors. Gray's Anatomy: The Anatomical Basis of Clinical Practice*, 39th ed. Edinburgh; Churchill Livingstone 2005;857.
- Sinnatamby CS. In: *Last's Anatomy: Regional and Applied*, 11th ed. Edinburgh; Churchill Livingstone 2006;99.
- Taheri MMH, Afshar M. Connection between radial and ulnar nerves at humeral level and its clinical significance: A cadaveric case report. *Int. J. Morphol* 2015;33:1559-1562.
- Pascual-Font A, Vazquez T, Marco F, et al. Ulnar nerve innervation of the triceps muscle: real or apparent? An anatomic study. *Clin Orthop Relat Res* 2013;471:1887-1893.
- Linell EA. The distribution of nerves in the upper limb, with reference to variabilities and their clinical significance. *J Anat* 1921;55:79-112.
- Cavalheiro CS, Filho MR, Rozas J, et al. Anatomical study on the innervation of the elbow capsule. *Rev Bras Ortop* 2015;50:673-679.
- Bertelli JA. Brachialis muscle transfer to the forearm muscles in obstetric brachial plexus palsy. *J Hand Surg Br* 2006;31:261-265.
- Chepla KJ, Bafus BT. Transfer of a radial nerve branch to the brachialis nerve for restoration of elbow flexion. *Tech Hand Up Extrem Surg.* 2018;22:65-67.
- Padulo J, Oliva F, Frizziero A, Maffulli N. *Muscles, Ligaments and Tendons Journal - Basic principles and recommendations in clinical and field Science Research: 2016 Update.* *Muscles Ligaments Tendons J* 2016;6:1-5.
- Sunderland S. Metrical and nonmetrical features of the muscular branches of the radial nerve. *J Comp Neurol* 1946;85:93-111.
- Madsen M, Marx RG, Millett PJ, et al. Surgical anatomy of the triceps brachii tendon: anatomical study and clinical correlation. *Am J Sports Med.* 2006;34:1839-1843.
- Leechavengvongs S, Witoonchart K, Uerpairojkit C, et al. Nerve transfer to deltoid muscle using the nerve to the long head of triceps. Part II: a report of seven cases. *J Hand Surg Am* 2003;28:633-638.
- Spinner RJ, Goldner RD. Snapping of the medial head of the triceps: diagnosis and treatment. *Tech Hand Up Extrem Surg* 2002;6:91-97.

Effects of Flexi bar Training Model to Blood Biochemistry in Overweight Adults

A. Hiruntrakul¹, Y. Phanpheng²

¹ Sports Science program, Faculty of Applied Science and Engineering, Khon Kaen University, Nong Khai Campus, Nong Khai, Thailand

² Exercise and Sport Sciences Program, Graduate School, Khon Kaen University, Khon Kaen, Thailand

CORRESPONDING AUTHOR:

Ashira Hiruntrakul
Sports Science program
Faculty of Applied Science and
Engineering
Khon Kaen University
Nong Khai Campus
43000 Nong Khai, Thailand
E-mail: hashir@kku.ac.th

DOI:

10.32098/mltj.03.2020.24

LEVEL OF EVIDENCE: 1B

SUMMARY

Background. The purpose of this study was to assess the effects of flexi bar training model and moderate running exercise on blood biochemistry in overweight adults.

Methods. The BMI of Asian people were ranged from 23.00 to 24.99 kg/m² was considered overweight. The participants had experienced any orthopedic problems in the spine or in the upper and lower extremities in the previous six months. Forty participants were randomly assigned to an experimental (20 participant performing flexi bar training model (FBT) and control (20 participant performing moderate running exercise (MRE) group. The participant in both groups then underwent program training 50 minute/day, 3 times a week, for 12 weeks.

Results. The main outcome measures were blood biochemistry variable. The result showed significant differences between FBT and MRE group ($p < 0.05$). After 12 weeks FBT showed improve lipid profile variable but not change complete blood count.

Conclusions. Flexi bar training model can improvement body composition and lipid profile in overweight adults.

KEY WORDS

Flexi bar training; blood biochemistry; overweight; body composition; lipid profile.

BACKGROUND

Overweight or being heavier than standard criteria is generally based on Body Mass Index or BMI. It can be calculated by dividing weight (kilograms) by height² (m²). The normal weight who have BMI ranged from 18.50 to 22.99 kilograms per square meter, Meanwhile, those who have BMI ranged from 25.00 to 29.99 kilograms per square meter were considered overweight and more than 30 kilograms per square meter were considered obesity (1). According to BMI information, WHO conducted the research and found that Asian people have smaller physical sizes than American, European, and African people. It is needed to adjust BMI to be appropriate for Asian physical structures. Therefore, the BMI of Asian people was ranged from 18.50 to 22.99 kilograms per square meter. Meanwhile, those who have BMI ranged from 23.00 to 24.99 kilograms per square meter were considered overweight 25.00 to 29.99 were considered obesity level 1 and more than 30 obesity

level 2 (2). In 2017 World Health Organization reported the effects of overweight on chronic disease, stroke, blood pressure, and diabetes type 2 tended to increase for both men and women. To decrease the rate of having health problems in overweight adults, it is focused on promoting appropriate physical behaviors, physical activities, having healthy nutrition, and regular exercises (3).

There are various models of exercises for losing fat such as aerobic dance (4), walking (5,6), running (7,8) and cycling (9). These activities need moving big muscles in different parts together which increase oxygen consumption and basal metabolism rates in overweight adults (10). Vibration exercise an alternative exercising activity to increase lean body mass (11) increasing efficiency of muscle contraction (12,13) increase muscle mass (14,15) and providing positive effects on blood vessels and blood circulatory system (16) since exercising with vibration will stimulate increasing of muscles to correspondingly work as well as increas-

ing of basal metabolism rate (17). Flexi bar exercising is an exercise with vibration equipment and is designed to have low frequency rate at 5 hertz, to vibrate 270 times per minute by using the metronome to control the flexi bar oscillation, with the size of 1.53 meters long, 710 grams, and 9.5 millimeter for circumference (Flexi bar). The experiment was taken place in a laboratory of research and technology, Munich University, Germany and it is certified and tested for the quality of the equipment by National Association of German Back Schools (AGR) that flexi-bar under the brand of Flexisport, Munich is appropriate and safe for exercise (18) because of having been manufactured from high-flexibility fiber glass. While vibrating flexi-bar, the vibration is generated along small amplitude of movement. There is resistance or intensity against vibration along the bar where the weight at both ends were the scale to control the weight of vibration timing. The trainers need to keep the vibration timing rate stable. In this case, the device can maintain stability of force constantly (19). Vibration generated by flexi bar will stimulate core muscle functioning (20,21,22) to generate reaction with the change of cross-sectional area of muscles and increase muscle activate throughout the body (23,24,25). For core muscles in human body, training with flexi bar can help encourage the functioning system of nerve muscles to perform reaction faster (26,27). This condition, body can retrieve energy to be used by burning out energy faster that affects in increasing the rate of more and faster energy expenditure than normal exercises such as brisk walking, running, cycling and *etc.* (28).

There are research reports studying on the function of the group of core muscles in group of healthy people, however, the benefits gained after training have not been found reported indicated about the use of flexi bar with a group of people with overweight people including the variable of blood biochemistry, in addition, there has not been any report found studying on level of intensity exercise models measured by the rate of energy expenditure during the training period. This can link to the change in the variables in different body composition aspects. Consequently, the researcher hopes that this research will be able to provide advantageous information for health promotion and beneficial as another effective exercise for people with overweight.

MATERIALS AND METHODS

Participants

This study was an action research study; it was approved by the Ethics Committee in Human Research, Khon

Kaen University, HE 612319. The research samples were 40 subjects participating in the health promotion project who were staff and students of Loei Rajabhat University, aged 20-45 years old with BMI ranges between 23.00 to 24.99 kg/m², BMI is set as the standard for Asian people is considered overweight. The participants were written informed consent has been obtained from each participant. The 40 samples were identified as overweight, using sample random sampling technique. Inclusion criteria consists of without any operation history or having treated with spinal surgery at least 6 months, being healthy without chronic diseases or health problems that possibly reduce readiness to exercise, evaluated from Physical Activity Readiness Questionnaire (PAR-Q) and pass in physical fitness, being healthy and strong without any affective factors obstructing exercise.

Exclusion criteria were as follows having chronic diseases such as high blood pressure, heart disease, diabetes, and coronary artery diseases, etc., less than 80% participation of the flexi bar training sessions and pregnant or breastfeeding volunteers. Other exercises and food consumption were recorded daily.

Experimental training

Data were collected during three periods: the 1st week, the 8th week, and the 12th week of the experiment. The research instruments included twelfth active vibration with flexi bar training model.

The test periods were performed for 12 weeks and included body composition and blood biochemistry variable included lipid profile and complete blood count; CBC. For the 1st - 12th week of the experiment the participants lived their normal lives.

Flexi bar training model

Twelfth active vibration with flexi bar training model has been developed into a model that is suitable for exercise in people who are overweight. Through consideration of the suitability of sports science experts with qualifications through passing the training course of certified personal trainer from American Council on Exercise (ACE) and National Academy of Sports Medicine (NASM). All participants were asked to perform the 12th week training program by doing Flexi bar training model for 50 minutes per time, 3 times a week on Monday, Wednesday and Friday. This program was conducted from 5 pm to 6 pm. The training program was performed at the Sports complex building, Sport and Exercise Sciences program, Loei Rajabhat University.

MEASUREMENT OF OUTCOMES

Blood biochemistry variable

Data were collected during three periods of the experiment. The test periods were performed for 12 weeks were measured blood biochemistry variable included lipid profile for this research study including Total cholesterol (TC), Low density lipoprotein cholesterol (LDL-C), High density lipoprotein cholesterol (HDL-C), and Triglyceride (TG). Measurement using enzymatic color measurement and chemical analysis by BT1000x chemical analyzer. And complete blood count; CBC, There are 6 parameters to be investigated for this research including Hemoglobin (Hb), using SLS Hemoglobin method, Hematocrit (Hct) using Cumulative pulse height detection method, Red blood cells (RBC), Mean cell volume (MCV), Mean Corpuscular Hemoglobin (MCH) using Hydrodynamic focusing direct current method, and Mean Corpuscular Hemoglobin Concentration (MCHC) measurement using fluorescence flow cytometry method using semiconductor laser with automatic analysis device called Sysmex XN-3000. In addition, the assessment of body composition was carried out through the bioelectrical Impedance Analysis (In body 270) measurement of weight, body mass index (BMI), fat percentage (% Fat), fat mass muscle mass and basal metabolic rate (BMR). The participants' height (without shoes) was measured by a stadiometre. The BMI was calculated by dividing body mass in kilograms by height in square meters (kg/m^2). The waist girth was measured at the level of the umbilicus horizontally

without clothing, Waist hip ratio (WHR) was calculated by dividing the waist girth by the hip girth.

Procedures

Twenty overweight adult subject's active vibration with flexi bar training model (FBT). The exercise sessions were divided into three sessions; at 10 minutes warm up session performing static stretch following dynamic warm up with flexi bar. At 35 minutes workout session performing the other 12 positions, chest, balance abductor, waist, oblique, triceps, biceps, core muscle, deep back extensor, shoulder, hips, lower back and abdominals. The Flexi-Bar (FLEXI-BAR®; Flexi-Sports, Germany) used in this experiment is an exercise tool having weights at the both ends of a glass fiber elastic bar of weight 719 g and length 1.53 m. at the center part, a grip of 17.9 cm, whereas the ends consist of weighty rubber. So the hands and arms holding the middle handle and shaking the flexi bar, by using the metronome to control the flexi bar oscillation rhythm 270 times per minute. In which the speed of 270 vibrations per minute will produce a vibrating response equal to 5 hertz, the subject held the Flexi-Bar with both hands while standing and performed vibration exercise, each position performed its exercise for 30 seconds and rested for 30 seconds, continue to practice with 3 sets of each pose following with a 5 minute cool down and static stretching of major muscle groups. The control group, twenty overweight subjects using moderate running exercise (MRE) were divided into three sessions;

Table I. Flexi bar training model protocol.

Day	Unit of training	Active vibration with flexi bar training model position	Duration of training
Monday Wednesday Friday	Warm up	Static stretching of major muscle groups and dynamic warm up with flexi bar.	10 minutes
	Work out	1. Chest 2. Balance abductor 3. Waist 4. Oblique 5. Triceps 6. Biceps 7. Core muscle 8. Deep back extensor 9. Shoulder 10. Hips 11. Lower back 12. Abdominals	35 minutes - 30 second active vibration - 30 rest in set - 3 sets each position
	Cool down and stretching	Static stretching.	5 minutes

at 10 minutes warm up session performing static stretch following dynamic warm up. At 35 minutes workout session performing moderate running exercise (MRE) with treadmills (T5, Life fitness, America). Manual setting program with control speed and heart rate 60-75% of maximum heart rate. Following with at 5 minutes cool down and static stretching. Both groups used the same duration of training with continue 50 minutes (**table I**).

Intensity control and energy expenditure

Both groups control intensity 65-75% of maximum heart rate throughout the duration of each exercise. Intensity and energy expenditure while training with flexi bar and moderate running exercise. All participants put on heart rate monitor (Polar Team Pro) in which the signal was connected to the receiver (Apple I-pad). Throughout the training period, each subject will show displayed on the screen at the real time for control heart rate. And final results were report training session on the screen while training including Heart Rate (bpm.), Percent of Average Heart Rate (%AVG^{HR}), Percent of Maximum Heart Rate (%AVG^{HRmax}) and Energy Expenditure of Exercise in Kilocalories (Kcal).

STATISTICAL ANALYSES

The data analysis, the statistical package SPSS Version 17 software was used. Data were presented as mean \pm SD, changes within group and within-group variance of baseline data and after-training data. Kolmogorov-Smirnov Test used to normality distribution of the data in both groups before the experiments. The baseline data were the data

before week 1st, week 8th, and week 12th. The paired samples t-test was used to compare pre training and post training variables in each group (FBT or MRE). The independent samples t-test was used to compare basal variables between groups (FBT vs. MRE). Research using two ways ANOVA for analysis with statistical significance level of 0 .05.

RESULTS

The participant group was no baseline differences in demographic and clinical findings between the experimental and control groups. At week 12th, the FBT and MRE group were significantly different ($p < 0.05$) from baseline in body composition variable. For example, for body composition, the weight, percentage of fat decreasing while muscle mass and BMR increase significantly ($p < 0.05$) (**table II**).

Regarding body composition variable there were significant changes ($p < 0.05$) from baseline at week 8th, FBT group showed increase Basal metabolic rate (BMR) found significant difference between group in week 12th, In addition after exercise at 12th week FBT group changes different ($p < 0.05$) in weight, %fat and muscle mass from baseline at week 12th. Also MRE group were showed significantly decreased from baseline in weight, %fat ($p < 0.05$) at 12th week. While blood biochemistry variable there were significant changes ($p < 0.05$) from baseline at week 8th, FBT group showed increase high density lipoprotein (HDL-C) found significant difference between group in week 12th, In addition after 12 week, FBT group showed decreased from baseline different ($p < 0.05$) in triglycerides (TG) and low density lipoprotein (LDL-C). Also MRE group were changes different ($p < 0.05$) in triglycerides (TG), low density lipo-

Table II. Differences of body composition variable.

Body composition Pre test	Moderate Running Exercise (MRE = 20)					
	12 week	Pre test	8 week	12 week		
Body composition variable						
Age (years)	22.70 \pm 1.26	22.70 \pm 1.26	22.70 \pm 1.26	22.40 \pm 1.04	22.40 \pm 1.04	22.40 \pm 1.04
Weight (kg)	67.74 \pm 5.86	67.10 \pm 5.40	66.60 \pm 5.26*	66.64 \pm 6.53	66.40 \pm 6.67	65.72 \pm 6.37*
BMI (kg./m. ²)	24.09 \pm 0.55	23.95 \pm 0.82	23.80 \pm 0.74	24.08 \pm 0.58	23.98 \pm 0.56	23.94 \pm 0.52
Fat % (percent)	20.74 \pm 2.29	20.43 \pm 2.37	19.92 \pm 2.46*	20.07 \pm 1.27	19.89 \pm 1.19	19.69 \pm 1.02*
Fat mass(kg)	15.04 \pm 2.33	14.78 \pm 2.39	14.66 \pm 2.31	15.19 \pm 2.09	14.86 \pm 1.98	14.81 \pm 1.94
Muscle mass (kg)	40.56 \pm 15.08	40.95 \pm 15.39	41.35 \pm 15.41*	40.59 \pm 10.91	39.85 \pm 10.48	39.40 \pm 10.10
WHR (inch)	0.85 \pm 0.53	0.85 \pm 0.52	0.85 \pm 0.52	0.85 \pm 0.23	0.85 \pm 0.52	0.85 \pm 0.52
BMR (Kcal)	1682 \pm 71.32	1690 \pm 88.60*	1693 \pm 86.37* [†]	1687 \pm 68.25	1694 \pm 66.60	1683 \pm 68.22

The data are presented by means \pm SD; *statistically significant difference when compared within group, mean scores at point comparisons from baseline: * $p < 0.05$ and *[†] $p < 0.05$ when comparing the difference between experimental groups

protein (LDL-C) and high density lipoprotein (HDL-C) after exercise at week 12th.

DISCUSSION

This study was aimed to investigate the effect of flexi bar training (FBT) on blood biochemistry variable in overweight adults compared to the moderate running exercise (MRE). There have been studies conducted a study in a passive method using full body vibration exercise equipment, while this study used a flexi-bar to apply active vibration exercise. There were three phases of this study; the pre-training, the 8 week, and 12 week of the experiment. This study showed that flexi bar training significantly improved body composition (**table II**) and blood biochemistry (**table III**). The variables improvements were persisted at 12th week.

According to anatomical and mechanical properties, the exposure of vibration to the skeletal muscular system causes a tonic vibration reflex (TVR) (29). The application of vibration to the tendon or muscle results in a TVR response, as reported previously (30,31). The TVR was initially considered to be a result of frequency stimulation applied directly

to a muscle or tendon for a short period. The application of local vibration to the tendon or muscle also improves muscle function (32,33). From this it can be surmised that the increased effectiveness of the training also influences muscular strength and muscular endurance, additional data include we exercise with flexi bar can activation of the core muscle and increase transvers abdominals thickness (21,22,34). Moreover, active vibration with flexi bar training can improve oxygen building muscle and yield a relevant increase energy expenditure better general exercise in the same amount of time (28).

The active vibration with flexi bar training and moderate running exercise groups provided better body composition outcomes in terms of weight, body mass index (BMI) and fat percentage after training period. The changes in body composition were as follows; the body weight of the participants reduced significantly at the 12th week of training. Because the active vibration with flexi bar training continuously allowed metabolic processes to burn fat cells in the normal weight, overweight and obese people more efficiently. According to principle of aerobic exercise, the active vibration with flexi bar training is a one type of aero-

Table III. Differences of blood biochemistry variable measurements.

Blood biochemistry variable	Flexi Bar Training (FBT = 20)			Moderate Running Exercise (MRE = 20)		
	Pre test	8 week	12 week	Pre test	8 week	12 week
Lipid profile						
Total Cholesterol ; TC (mg/dL)	174.47±18.21	173.65±18.10	173.37±17.72	172.21±16.12	171.61±15.80	171.92±15.52
Triglycerides ; TG (mg/dL)	91.31±7.31	89.62±7.16	88.58±6.32*	91.58±5.70	90.90±5.62	90.14±5.40*
High Density Lipoprotein ; HDL-C (mg/dL)	53.75±10.37	55.10±10.38*	57.65±10.58**	52.85±9.43	53.70±9.25	54.05±9.16*
Low Density Lipoprotein; LDL-C (mg/dL)	123.08±10.92	122.11±10.46	120.73±10.81*	124.63±8.81	122.78±8.75	121.03±8.65*
Complete blood count ; CBC						
Red Blood Cell; RBC (fL)	5.55±0.47	5.53±0.43	5.60±0.49	5.58±0.54	5.50±0.54	5.58±0.57
Hemoglobin; Hb (g/dL)	14.75±1.10	14.75±1.14	14.76±1.18	14.76±1.00	14.76±1.07	14.76±1.08
Hematocrit; Htc (%)	44.77±3.80	44.97±3.63	44.97±3.65	44.73±3.22	44.77±3.08	44.68±3.13
Mean Corpuscular Volume; MCV (fL)	82.76±7.88	82.62±7.32	82.45±7.09	81.85±7.12	81.80±8.19	81.83±8.45
Mean Corpuscular Hemoglobin; MCH (pg)	27.16±1.50	27.12±1.49	27.16±1.47	27.18±1.45	27.22±1.42	27.23±1.44
Mean Corpuscular Hemoglobin Con centration; MCHC (g/dL)	32.74±1.10	32.85±1.16	32.85±1.15	32.72±0.88	32.79±0.94	32.75±0.87

The data are presented by means ± SD; *statistically significant difference when compared within group, mean scores at point comparisons from baseline: *p<0.05 and **p < 0.05 when comparing the difference between experimental groups.

bic exercises. Felix *et al.* (35) suggested that resistance from vibration will help stimulate more blood circulatory transported for muscle and nerve cell nourishment. Moreover, repeating training postures frequently links between nerves and muscles to work together better. To a targeted training program with the active vibration with flexi bar training yields a relevant increase in energy expenditure as a determining factor of weight and fat loss. Moreover, the activation of large muscle groups with moderately high intensity within a given time span as part of a classical flexi bar training module should at least directly correlate with distinct increases in active metabolic rate.

Aerobic exercise helped increase efficiency of heart muscles. Previous studies, aerobic exercise has a positive effect on blood lipids. This is consistent with this study that, after 8 week of training it was found that lipid profile; High density lipoprotein, (HDL-C) and difference statistical significance level 0.05 between groups in 12 weeks. While in both group exercises there were found as follows; Triglycerides (TG), Low Density Lipoprotein (LDL-C) decreased with statistical significance when comparing to before the experiment. However, additionally, not found Total Cholesterol (TC) difference in both groups when comparing to before and after training. Decreasing of lipid profile variable may be resulted from increasing of energy expenditure during exercise with flexi bar in which higher energy is consumed while exercise than moderate running exercise. This activity will stimulate the muscles to use lower energy than constant intensity. According to the assessment of energy expenditure while exercising, comparing between two groups, it can be seen that the group of flexi bar training had higher energy expenditure than running group. Combination of various aerobic energy systems will provide positive effect on energy consumption from body fat and affecting fatty molecules which is lipid transported to parts of body (36). Previous researches showed that different kinds of aerobic exercise activities with continuity and appropriate time will affect the level of lipid profile such as level of Triglyceride, level of low density lipoprotein and increasing of high density lipoprotein (37, 38). Generally, High density lipoprotein (HDL-C) will transport cholesterol along with other kinds of fats totaling 30% in blood, therefore increasing of HDL-C will reduce waste of fat accumulated along blood vessels. This also helps reduce risk factor of Atherosclerosis (39). This is consistent to the finding in this research that the stimulation of muscles to contract by periods of low intensity vibration from flexi bar continuously, approximately 5 Hertz will stimulate muscles to carry out energy for burning fat or fat metabolism. Similarly, Di Loreto A. *et al.* (17) conducted the study to investigate the effects of aerobic exercise with

vibration device. The finding showed that after continuous exercise, it provides positive effects for physical fitness and triglyceride, low density lipoprotein decreased. Moreover, Thorsten *et al.* (4) found that aerobic exercise with use of resistant exercise is beneficial for overweight and fat adults. This exercise helps improve physical fitness and any factors regarding to lipid profile better because muscles need more food substance especially free-fatty acid when doing exercise, blood circulatory along with triglyceride delivered to muscles also increase and turn to be free fatty acid which is a source of energy for muscles. When more triglyceride is oxidized, the level of triglyceride decreases. Moreover, exercise helps stimulate performance of lipoprotein in blood that can also cause in reducing triglyceride as well (40,41). However, change of total cholesterol was not found in this research when comparing with before training. Stein *et al.* (42) suggested about the intensity of aerobic exercise that training the level of 85% of maximal heart rate will affect the decreasing of total cholesterol.

Suitable level of exercise will provide positive effect to blood biochemistry and in this research there is no change regarding Complete Blood Count (CBC) among both groups of overweight adults throughout the period of 12 weeks. Possibly, since the stimulation of flexi bar is at 5 Hertz, as it is low intensity, it doesn't provide effect to change of complete blood count in a short period. This result is consistent with the previous study of Kodama, S *et al.* (43) that there was no statistically significant difference regarding the use of vibration for training in terms of red blood cells, white blood cells, lymphocytes, monocytes, granulocytes, hemoglobin and hematocrit. Additionally, Dorota *et al.* (44) found that after a 12 weeks aerobic exercise, cholesterol in blood of participants reduced while the amount of complete blood counts didn't change. Johannsen *et al.* (45) suggested that after 6 months of aerobic exercise, there was change regarding complete blood counts. Based on the previous research studies, there are supportive results to identify that low flexi bar training with low intensity vibration is a limitation for stimulating blood production process as well as other blood biochemistry components. Limitation of time which is not enough for training cannot provide any change regarding complete blood counts as well as the appropriate level of intensity for exercise in this research study.

Limitation of the study

This study uses samples with body mass index for Asian people only. And training postures, most of which are static training, there should be applies to the training styles for various movements for the effectiveness of the training program.

Suggestions for further research study

The patterns of flexi bar training should be appropriately adjusted for different groups of people such as children, elderly, and patients with chronic diseases.

CONCLUSIONS

To design this Flexi bar training model, the researcher combined exercise and the use of flexi bar which is a pattern of exercise designed by the researcher and proved for appropriateness by the experts based on the principles of sport science and exercise. The model can promote various kinds of body composition and blood biochemistry including lipid profile, complete blood count. This is safe for physical structure and doesn't cause vertical force. Therefore, flexi bar

training is an alternative exercise for those who want to lose weight, strengthen physical fitness and increase effectiveness of blood vessel functioning. Consequently, it should be widely promoted and future applied for enhancing healthiness.

ACKNOWLEDGMENTS

Deeply thanks to all participants in the health promotion project who were staff and students of Loei Rajabhat University and deep gratitude for Graduate School, Khon Kaen University for support in this study.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

REFERENCES

1. American College of Sports Medicine [ACSM]. Guidelines for Exercise Testing and Prescription. 6th ed. Philadelphia: Lippincott Williams and Wilkins 2006.
2. World Health Organization [WHO]. Strategy for oral health in South-East Asia, 2013-2020. India: Indraprastha Estate, Mahatma Gandhi Marg 2016.
3. American College of Sports Medicine [ACSM]. ACSM's Resources for Clinical Exercise Physiology. 2th ed. Philadelphia: Lippincott Williams and Wilkins 2009.
4. Thorsten S, Stefanie S, Billy S. Aerobic dance: health and fitness effects on middle-aged premenopausal women. *J Exerc Physiol* 2008; 11(4): 25-33.
5. Takeshima N, Iyoji T, Fumio K, Takemasa W, Akashi K. Effects of aerobic exercise conditioning at intensities corresponding to lactate threshold in the elderly. *Eur J Appl Physiol* 1993; 67: 138-143.
6. McArdle WD, Katch FI, Katch VL. Exercise Physiology Energy, Nutrition, and Human Performance. 6th ed. Philadelphia: Lippincott Williams & Wilkins 2007.
7. Shaw I, Shaw BS, Krasilshchikov O. Comparison of aerobic and combined aerobic and Resistance training on low-density lipoprotein cholesterol concentrations in men. *Cardiovasc J Afr* 2009; 20(5): 174-189.
8. Nybo L, Sundstrup E, Jakobsen MD, Mohr M, Hornstrup T, Simonsen L. High-Intensity Training Vs. Traditional exercise interventions for promoting health. *Med Sci Sports Exerc* 2010; 42: 225-236.
9. Bergström I, Lombardo C, Brinck J. Physical training decrease waist circumference in the postmenopausal borderline overweight women. *Acta Obstet Gynecol Scand* 2009; 88: 308-313
10. Bell JM, Bassey EJ. A comparison of the relation between oxygen uptake and heart rate during different styles of aerobic dance and a traditional step test in women. Department of physiology and pharmacology, Medical school, Queen's medical centre, Nottingham, England 1993.
11. Olson PT, Donald RD, Arthur SL, Kathryn HS. Moderate resistance training and vascular health in overweight women. *Med Sci Sports Exerc* 2006; 38(9): 1558-1564.
12. Roelants M, Delecluse C, Goris M, Verschueren S. Effects of 24 weeks of whole body vibration training on body composition and muscle strength in untrained females. *Int. J. Sports Med* 2004; 25: 1-5.
13. Cochrane DJ. The potential neural mechanisms of acute indirect vibration. *J Sci Med Sport* 2011; 10: 19-30.
14. Delecluse C, Roelants M, Verschueren S. Strength increase after whole-body vibration compared with resistance training. *Med Sci Sports Exerc* 2003; 35(6): 1033-41.
15. Verschueren SM, Roelants M, Delecluse C, Swinnen S, Vanderschueren D, Boonen S. Effect of 6-month whole body vibration training on hip density, muscle strength, and postural control in postmenopausal women: a randomized controlled pilot study. *J Bone Miner Res* 2004; 19(3): 352-9.
16. Kerschan S, Grampp C, Henk H, Resch E, Preisinger V, Fialka-Moser H. Whole-body vibration exercise leads to alterations in muscle blood volume. *Clinical Physiology* 2001; 21(3): 377-382.
17. Di Loreto A, Ranchelli P, Lucidi G, Murdolo N, Parlanti A, De Cicco O, Tsarpela G, Annino C, Bosco F. Effects of whole-body vibration exercise on the endocrine system of healthy men. *J Endocrinol Invest* 2004; 27(4): 323-327.
18. Markus D, Gunsch F. Deep penetrating 3 D training with the flexi bar: Expert advice concerning the Flexi-Bar performance. Switzerland: Official Training and Therapy Center of the PGA of Germany 2009.
19. Masoud A, Mohammad N, Sajad A, Mohsen A, Mohamad P, Kinda K. A model for flexi-bar to evaluate intervertebral disc and muscle forces in exercises. *Med Eng Phys* 2016; 38: 1076-1082.
20. Jung HK, Ki HS, Yu RB, Byoung HL. A Comparison of Flexi-bar and General Lumbar Stabilizing Exercise Effects on Muscle Activity and Fatigue. *J Phys Ther Sci* 2014; 26: 125-136.

21. Jun SC, Seol P, Ji YK, Ji WP. Effects of flexi-bar and non-flexi-bar exercises on trunk muscles activity in different postures in healthy adults. *J Phys Ther Sci* 2015; 27:2275-2278.
22. Sin HC, Young YY, Hyung JL, Sang HS. Effects of stabilization exercise using flexi-bar on functional disability and transverse abdominis thickness in patients with chronic low back pain *J Phys Ther Sci* 2018; 30:400-404.
23. Seong JL, Yong NK, Dong KL. The effect of flexi-bar exercise with vibration on trunk muscle thickness and balance in university students in their twenties. *J Phys Ther Sci* 2016; 28:1298-1302.
24. Woon SC, Chi BP, Jae HL. The Effect of Trunk Strengthening Exercise using Oscillation on Trunk Muscle Thickness and Balance. *J Korean Med Sci* 2017;12(2): 91-101.
25. Dong KL, Yong NK, Chi BP. The effect of actively induced vibration using shoulder joint on pain and dysfunction in patients with low back pain. *J Phys Ther Sci* 2018; 30:23-26.
26. Torvinen S, Kannus P, Sievanen H, Jarvinen TA, Pasanen M, Kontulainen, S. Effect of a vibration exposure on muscular performance and body balance. *Randomized cross-over study. Clin Physiol Funct Imaging* 2002; 22:145-152.
27. Martin BJ, Park H. Analysis of the tonic vibration reflex: influence of vibration variables on motor unit synchronization and fatigue. *Eur J Appl Physiol* 1997; 75:504-511.
28. Dippert T, Moeller K, Stengel S, Kemmler W. Energy expenditure during a 30-minute exercise unit with the "FLEXI-BAR®" Pilot Study. Nuremberg, Germany: Institute of Medical Physics Friedrich-Alexander-University of Erlangen 2010.
29. Trans T, Aaboe J, Henriksen M, Christensen R, Bliddal H, Lund H. Effect of whole body vibration exercise on muscle strength and proprioception in females with knee osteoarthritis. *Knee* 2009; 16:256-261.
30. Bernard J, Martin SP. Analysis of the tonic vibration reflex: influence of vibration variables on motor unit synchronization and fatigue. *Eur J Appl Physiol Occup Physiol* 1997;75(6):504-511.
31. Couto BP, Silva HR, Filho AG, Da Silveira SR. Acute Effects of Resistance Training with Local Vibration. *Int J Sports Med* 2013;34(9):814-819.
32. Iodice P, Bellomo RG, Gialluca G, Fanò G, Saggini R. Acute and cumulative effects of focused high-frequency vibrations on the endocrinesystem and muscle strength. *Eur J Appl Physiol* 2011; 111:897-904.
33. Mischì M, Cardinale M. The Effects of a 28-Hz Vibration on Arm Muscle Activity during Isometric Exercise. *Med Sci Sports Exerc* 2009;41(3):645-53.
34. Kim YM, Park JH. The effect of whole-body vibration exercise on balance, muscle strength and falls efficacy in the elderly. *J Korean Soc Phys Med* 2017;12(4):61-71.
35. Felix G, Daniel D, Carina G, Ralf B, Thomas V, Tilmann K. Oscillatory whole-body vibration improves exercise capacity and physical performance in pulmonary arterial hypertension: a randomised clinical study. *Heart* 2017; 103: 592-598.
36. Alberga AS, Prud'homme D, Sigal RJ. Effects of aerobic training, resistance training or both on cardiorespiratory and musculoskeletal fitness in adolescents with obesity: the HEARTY trial. *Appl Physiol Nutr Metab* 2016; 2: 514-521.
37. Escalante Y, Saavedra JM, García HA. Improvement of the lipid profile with exercise in obese children: a systematic review. *Prev. Med* 2012; 54: 293-301.
38. Yating W, Danyan X. Effects of aerobic exercise on lipids and lipoproteins. *Lipids in Health and Disease. J Phys Ther Sci* 2017; 3: 116-132.
39. Stefan B, Yevhen P, Cédric E, Celestine NC, Julia K, Stefan A. Solution structure of discoidal high density lipoprotein particles with a shortened apolipoprotein A-I. *Nat. Struct. Mol. Biol* 2017; 24: 187-193.
40. Noble JB. *Physiology of exercise and sport*. St. Louis: Time Mirror/Mosby 1986.
41. Tambalis K, Demosthenes BP, Stavros AK, Labros SS. Responses of Blood Lipids to Aerobic, Resistance, and Combined Aerobic With Resistance Exercise Training: A Systematic Review of Current Evidence. *Angiology* 2009; 60(5): 614-632.
42. Stein RA, Michielli DW, Glantz MD, Sardy H, Cohen A, Goldberg N, Brown CD. Effects of different exercise training intensities on lipoprotein cholesterol fractions in healthy middle-aged men. *Am Heart J* 1990; 119: 277-83.
43. Kodama S, Tanaka S, Saito K, Shu M, Sone Y, Onitake F. Effects of aerobic exercise training on serum level of high-density lipoprotein cholesterol: a meta-analysis. *Clin J Sport Med* 2008; 10: 999-1008.
44. Dorota KN, Robert NZ, Jastrzebski B, Aleksandra Z, Marta B, Izabela D. Effect of 12-week-long aerobic training programme on body composition, aerobic capacity, complete blood count and blood lipid profile among young women. *Biochemia Medica* 2015; 25(1): 103-113.
45. Johannsen NM, Swift DL, Johnson WD, Dixit VD, Earnest CP, Blair SN. Effect of different doses of aerobic exercise on total white blood cell (WBC) and WBC subfraction number in postmenopausal women: results from DREW. *PlosOne* 2012. 7(2): e31319.
46. Padulo J, Oliva F, Frizziero A, Maffulli N. *Muscles, Ligaments and Tendons Journal - Basic principles and recommendations in clinical and field Science Research: 2018 update. MLTJ* 2018; 8(3): 305 - 307.

Study of the Biceps Fatigue after Surgery on the Long Head of Biceps Tendon in Male Heavy Workers. A Prospective Randomized Clinical Trial Comparing Biomechanics and Clinical Outcomes after Tenotomy Versus Tenodesis

J. E. García-Rellán^{1,2}, E. Sánchez-Alepuz³, J. Mudarra-García⁴, A. Silvestre^{4,5}

¹ Department for Orthopedics and Trauma Surgery, Clinic Hospital of Valencia, Valencia, Spain

² CEU-Cardenal Herrera University, Valencia, Spain

³ Hospital of Unión de Mutuas, IMED Hospital, Valencia, Spain

⁴ Department for Orthopaedics and Trauma Surgery, Shoulder Unit, Clinic Hospital of Valencia, Valencia, Spain

⁵ Surgery Department, School of Medicine, University of Valencia, Valencia, Spain

CORRESPONDING AUTHOR:

Jose E Garcia-Rellán
Clinic Hospital of Valencia
Blasco Ibañez Sreet 17
46010, Valencia, Spain
CEU-Cardenal Herrera University
Alfara del Patriarca
46115 Valencia, Spain
E-mail: garcia_josrel@gva.es

DOI:

10.32098/mltj.03.2020.25

LEVEL OF EVIDENCE: 2B

SUMMARY

The aim of this study was to evaluate biceps fatigue and strength after tenotomy vs tenodesis of the LHBT. The hypothesis was that there may be differences when analyzing biceps fatigue between both techniques.

Methods. 70 male heavy workers were initially enrolled to perform a biomechanical study. Preoperatively and 12 months after surgery a maximum elbow flexion force (MVC) and forearm supination test (MVS) were analyzed. Subsequently, a biceps fatigue test was performed by a submaximal contraction to 33% of MVC maintained until claudication. After the claudication, the MVC and MVS were measured again. In addition, the Constant score, SSI functional scale, VAS scale and perceived symptoms were evaluated. 45 patients met de inclusion criteria and were randomized into tenotomy or tenodesis group. 41 of them were followed-up for at least one year.

Results. There were no differences between groups at the end of the follow-up in MVC (246.81 ± 57.4 vs 273.69 ± 58.8 N) nor in MVS (50.73 ± 6.31 vs 177.7 ± 71.6 Nm). The fatigue test was statistically shorter in the tenotomy group (95.18 ± 28.8 vs 122.53 ± 42.1 s). Popeye sign was higher in the tenotomy group. There were no differences in postoperative pain, function scales, groove pain or biceps cramps.

Conclusions. The usual biomechanical study using maximum flexion or supination force has not seen differences, while the study of biceps fatigue has found them. The study of biceps fatigue is necessary to analyze the differences that with the standard study of maximum strength could be hidden.

KEY WORDS

Tenotomy; tenodesis; long head of biceps tendon; biomechanics; supination; fatigue.

INTRODUCTION

Tenotomy and tenodesis are the surgical techniques most commonly used in the treatment of the long head of the biceps tendon (LHBT) pathology. It is frequently associated with rotator cuff lesions due to the immunohistochemical pathogenesis that affects both tendons (1,2). These can be carried out by arthroscopic techniques, when conser-

vative options have failed. Both surgical techniques are widely known in the literature. The LHBT tenotomy (3,4) is a simple and fast technique, with a short rehabilitation time and which produces good results in the functional assessment scales of the shoulder (4,5). But this technique has a high prevalence of producing the Popeye sign (the distal retraction of the muscular belly of the biceps)(6,7),

the presence of muscle spasms and painful fatigue (8-10). The LHBT tenodesis (9,11,12) reattaches the proximal stump of the tendon to prevent the descent of the muscular belly, maintaining tension-length relationship (4,8), but it can produce pain in the place where the tenodesis is performed (13,14).

The presence of muscle weakness after surgery on LHBT has been studied in numerous biomechanical articles using isometric (5,13,15-20) and isokinetic (21-23) measurements. Some authors have reported cases of weakness of the peak flexion force of the elbow (15,21,22,24) or forearm supination (21,22) when the LHBT tenotomy is performed with respect to the healthy arm, although other authors have also noted deficits when performing tenodesis of the tendon (13,17,18).

There are also articles that directly compare tenotomy with tenodesis (13,17-21,24). Generally, the authors of the biomechanical articles base their studies on the measurement of the peak force of elbow flexion or forearm supination, but this may not be the most interesting parameter to know the functional result of the surgery on de LHBT, given that most of the of daily tasks are performed by repeated submaximal contractions (25). This is why other authors have based their biomechanical analysis on the quantification of fatigue of the biceps after the surgery on the LHBT, demonstrating early fatigue in cases operated by tenotomy, with respect to the healthy arm (26).

Therefore, a prospective randomized comparative clinical trial was designed analyzing biomechanical results of 2 different techniques for the treatment of the LHBT: tenotomy vs tenodesis. The aim of this study was to compare the biomechanical results of biceps fatigue (time to claudication) in the patients who underwent LHBT tenotomy in comparison to those who underwent tenodesis. We hypothesized that there would be significant differences in time to claudication among the techniques.

METHODS

The present study was approved by the ethics committees of both hospitals involved, and all the patients provided written informed consent to participate in this study, as required for publication (27). It was a prospective, randomized, controlled clinical trial who underwent arthroscopic repair of the LHBT lesions.

The inclusion criteria were the clinical or radiological diagnosis of LHBT pathology in men between 40 and 65 years of age. Patients older than 65 years as well as women were excluded from the study because they less frequently participated in intense physical work or intense sports activities, which may cause the sequelae to be less symptomatic.

Patients with previous history of contralateral upper limb pathology or with neuropsychiatric pathology were excluded. Associated concomitant injuries, such as those of the rotator cuff, were treated intraoperatively, and the lesion of the LHBT was reconfirmed intraoperatively, excluding from the study patients where the LHBT injury was absent or the lesion was small (less than 25% of the thickness of the tendon), where debridement of the tendon was performed. A total of 70 preoperative assessments of patients candidates for surgical treatment of LHBT were performed. Four patients were eliminated before randomization for not giving their consent for the study, and 21 patients for not meeting the selection criteria.

Randomization by blocks of 10 individuals from a total of 45 patients was carried out, assigning 24 subjects to the tenotomy group and 21 to the tenodesis group. The cases were analyzed by intention to treat. The principal investigator was unaware of the randomization sequence and the technique used until the end of the study. The technique assigned by the randomization process was introduced in a sealed envelope that prevented its reading before the moment of surgery. It was at the time of surgery, once the need to act on the LHBT was verified, when the sealed envelope was opened and the technique specified in the envelope was executed, without the presence of the examiner.

There was a loss of follow-up in 1 patient in the tenotomy group (contact was not achieved) and 3 in the tenodesis group (1 patient did not attend follow-up and 2 did not contact). The patient flow diagram is presented in **figure 1**.

A total of 41 patients met the selection criteria and completed the biomechanical study 12 months after surgery. Patient demographic data and preoperative pain scores, functional scores and level of activity are detailed in **table I**.

In addition with clinical interviews with the surgeon, an external investigator evaluated in the preoperative period all patients where pathology of the LHBT were suspected. If the LHBT injury was confirmed during surgery, the technique specified in the sealed envelope (tenotomy or tenodesis) was performed, and the external investigator examined the patient at 3, 6 and 12 months after surgery.

Population data, and International Physical Activity Questionnaire (IPAQ) responses were recorded in the interviews with the external examiner. Clinical examination was also performed using Shoulder Score Index (SSI) and normalized Constant Score, as well as anthropometric measurements including the presence of Popeye deformity, perceived satisfaction and a biomechanical test.

The biomechanical test (26) consisted of measuring the maximal voluntary flexion of the elbow, maximal voluntary supination of the forearm, a fatigue test, and maximal isometric flexion of the elbow and supination of the forearm

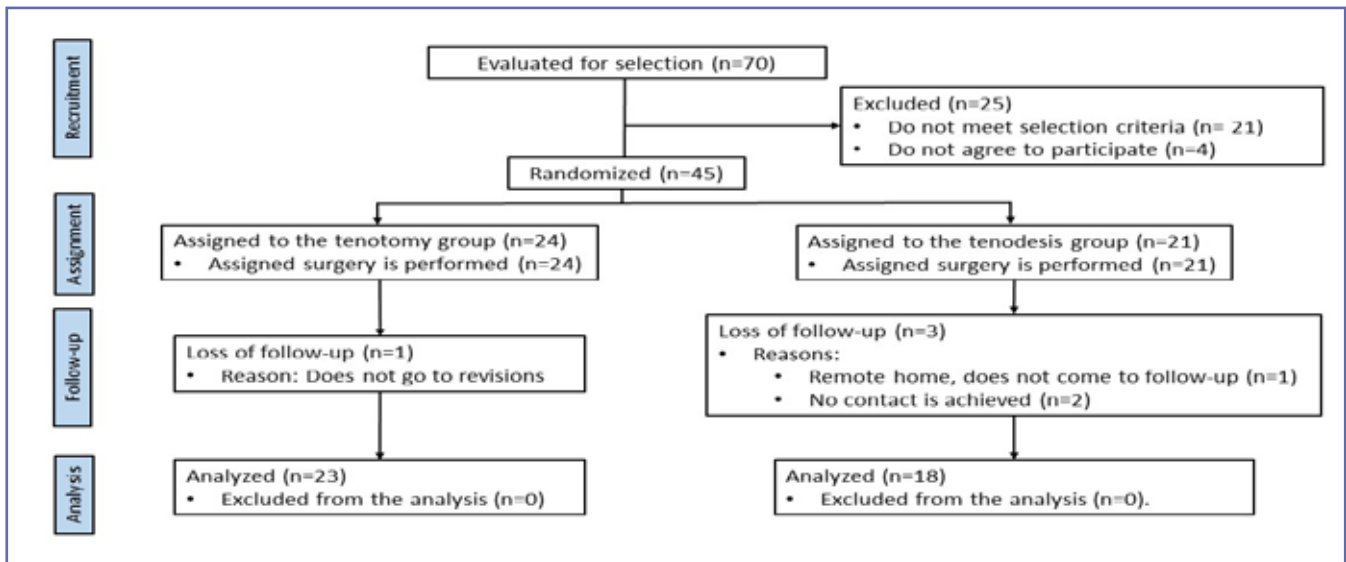


Figure 1. Flowchart of patients.

Table I. Demographic data before surgery in both groups.

	Tenotomy group (n= 23)	Tenodesis group (n=18)	p
Age	54.7 ± 5.78	50.73 ± 6.31	n.s.
Affected shoulder	Right: 14 (60.9%) Left: 9 (39.1%)	Right 11 (61.1%) Left 7 (38.9%)	n.s.
Dominance	Yes: 14 (60.9%) No: 9 (39.1%)	Yes 11 (61.1%) No 6 (38.9%)	n.s.
Size of rotator cuff tear			
· Not affected	n=1 (4.3%)	n=5 (27.7%)	n.s.
· Small tear	n=5 (21.7%)	n=3 (16.6%)	
· Medium tear	n=10 (43.5%)	n=7 (38.8%)	
· Massive tear	n=7 (30.4%)	n=3 (16.6%)	
Level of physical activity (METS)	14608.7 ± 9454.5	16282.6 ± 11223.7	n.s.
Type of work:			
· Construction	4 (17.3%)	3 (16.7%)	n.s.
· Heavy machinery operator	6 (26%)	4 (22.2%)	
· Athletes	3 (13%)	2 (11.1%)	
· Marble, wood or metal workers	4 (17.3%)	3 (16.7%)	
· Security agent	2 (8.7%)	3 (16.7%)	
· Mechanics	2 (8.7%)	2 (11.1%)	
· Farmer	2 (8.7%)	1 (5.5%)	
BMI	29.3 ± 4.8	29.2 ± 3.3	n.s.
Pain (VAS)	7 (3;10)	7 (4;10)	n.s.
SSI	43.33 (12;65)	36.67 (23;72)	n.s.
UCLA score	20.50 (12;25)	20 (9;26)	n.s.
Normalized Constant	70.43 (29;82)	58.51 (34.9;93.75)	n.s.

BMI: Body mass index. VAS: visual analogue scale. SSI Shoulder score index. UCLA: The University of California Los Angeles shoulder score. VAS, Normalized Constant score, UCLA score and SSI scale are expressed in median and ranges.

after-fatigue. Maximal voluntary contraction (MVC) of the elbow was determined in the patients while sitting in a chair, with the elbow flexed at 90°. MVC was determined with the forearm horizontal and in supination, with the transducer positioned on the wrist. The force exerted vertically was quantified using the Commander Muscle Testing dynamometer (JTECH Medical, Salt Lake City, USA) (**figure 2**), and the results were expressed in Newtons. The volunteers performed the maximal contraction test 3 times, trying to reach the highest value possible. The MVC was the largest parameter from among all exercises.

To measure maximal voluntary supination (MVS) force of the forearm a torsion dynamometer has been used (Baseline Hydraulic Wrist Dynamometer, FEI, White Plains, USA) (**figure 3**). The subject was evaluated while seated, with the elbow flexed at 90°, the forearm in pronation, and the hand grasping the lever of the torsion dynamometer. The results of the moment force were expressed in Newtons x m. The subjects performed three measurements on both elbows. The highest parameter of all the exercises was considered the MVS.

After performing the previous exercises, a submaximal isometric fatigue test (26,28-33) was used. This test consisted of performing a contraction in isometric flexion on 33% of the MVC obtained previously, and was maintained as long as possible. The fatigue test concluded when the patient deviated from the target force by - 10% MVC force for more than 5 s. When this occurred, the patient was deemed to have reached exhaustion. The time to claudication was observed in seconds and the patient was not aware of the result until the study was completely finished. Within 10 s after the fatigue test, MVC and MVS were performed again.

During the arthroscopy of the shoulder, a standard posterior and anterior portals were used to explore the joint. LHBT was evaluated for pathological changes at its insertion and also in the beginning of the bicipital groove. If there were others pathologic changes in the glenohumeral joint, they were managed first. In the tenotomy group, surgical technique was performed sectioning the LHBT near of its insertion with an electrocoagulator. The creation of a wide proximal stump was favored to facilitate the stop in the bicipital groove.

In the tenodesis group, all procedures conducted in the glenohumeral joint were similar except for the management of the LHBT. The tenodesis technique was performed by opening the bicipital slider, sectioning the transverse humeral ligament. The LHBT was extracted to appreciate the groove. The execution of the tenodesis was carried out 2 cm inferior to the upper vertex of the greater tuberosity, always inside the slide. The hole was carried out with the help of the Biceptor system (Smith & Nephew, Andover, MA), using a guide wire for the realization of a hole of a diameter similar to the interferential screw to be used, and a length 0.5 cm longer. At that time, the tenotomy of the intraarticular portion of the tendon was performed similar to that defined in the tenotomy group. With the help of the fork of the Biceptor system, the tendon was introduced into the hole, favoring the sliding of the proximal part of the tendon, avoiding the overstretching. Then, the Biosure PK interferential screw (Smith & Nephew, Andover, MA) was introduced and the remnant proximal stump was removed.

Routine postoperative rehabilitation protocol was performed. A sling was used during the first 3 weeks after surgery, allowing passive mobilizations of the shoulder and



Figure 2. Commander muscle testing dynamometer



Figure 3. Baseline Hydraulic Wrist Torsion Dynamometer

Table II. Clinical results 12 months after surgery.

	Tenotomy group	Tenodesis group	p
Pain (VAS)	2 (0.8;3.1)	3 (1.2;4.8)	n.s.
Normalized Constant score	88.9 (84.2;93.5)	89.7 (82.4;97)	n.s.
SSI	84.3 (76.6;92)	76.1 (63.8;88.5)	n.s.
UCLA score	32.8 (28.4;37.1)	31 (28.3;33.8)	n.s.
Popeye sign	56.5%	11.1%	0.01*
Residual groove pain	18.2%	27.7%	n.s.
Biceps cramps	14.3%	22.2%	n.s.
Perceived satisfaction	90.9%	100%	n.s.

VAS: visual analogue scale. SSI Shoulder score index. UCLA: The University of California Los Angeles shoulder score. VAS, normalized Constant score, SSI scale and UCLA score are expressed in median and ranges.

elbow. Gentle active arm movements were allowed from the third week until a full range of mobility was achieved. The present study has been approved by the ethics committees of both hospitals: Hospital Clínico of Valencia (Spain) and Unión de Mutuas of Valencia (Spain).

STATISTICS

Prior to the beginning of the clinical trial, a retrospective pilot study (unpublished data) was conducted to know the variability of the main parameter to be measured in order to know the number of patients needed to be included in the study. The main parameter was established as the time in seconds until claudication when the elbow performs an isometric contraction at 90° of flexion, maintaining the contraction at 33% of the Maximum Voluntary Contraction (in Newtons) previously collected. In this study it was determined that typical deviation of time to claudication was 37 seconds. To detect a measurement difference of 40 seconds (30% of the average fatigue time), using a significance level $\alpha = 0.05$ with a power of 80% and assuming a standard deviation of 37 seconds, it was necessary to observe 13 patients per group, 26 in total.

The SPSS 15.0 program (SPSS Inc, Chicago, IL) has been used for the analysis of the data collected from the sample. An exploratory analysis of the database has been carried out using basic statistical techniques descriptive in both treatment groups, and expressed the quantitative variables in the form of mean \pm standard deviation, and the qualitative variables in absolute number and percentage of the total. The normality of the numerical variables was analyzed using the Kolmogórov-Smirnov test.

For the assessment of the proposed objectives, statistical group comparison techniques have been used. Specifically, for the comparison of continuous variables between the

two groups, we used the t-Student test or its nonparametric alternative, Mann-Whitney U, according to the result of the normality test. For the comparison of proportions, the Chi-square test or its nonparametric alternative, Fisher's exact test, was used, depending on the number of cases. In some cases, and according to the normality of the variables, the dispersion of the data has been expressed, expressing the 95% confidence interval.

RESULTS

Demographic data about groups is expressed in table I. Differences in the base groups have been analyzed for later comparison.

In the comparative study of both techniques 12 months after the surgery, a similar result can be seen in terms of clinical pain parameters, the Constant score and SSI and UCLA scales. Additionally, there were no differences between both techniques in residual groove pain, biceps cramps or perceived satisfaction. Nevertheless, Popeye sign was statistically higher in tenotomy group. These results are displayed in **table II**, showing clinical values between techniques 12 months after surgery.

Biomechanical data are expressed in **tables III and IV**. A significant increase has been proved in MVC in both groups with respect to the preoperative values, both in the pre-fatigue study and in the after-fatigue study. This increase with respect to the preoperative values has also been seen in the measurement of the MVS, except in the tenotomy group in the pre-fatigue measurement, where no differences have been found.

A significant loss of fatigue time (time to claudication) was found in the group where LHBT tenotomy was performed (decrease from 139.1 ± 71.6 s to 95.18 ± 28.8 s; $p < 0.01$). This deficit has not been evidenced in the group where tenodesis was performed (137.6 ± 99.2 s in the preoperative

Table III. Biomechanical results 12 months after surgery.

	Tenotomy group	Tenodesis group	p
MVC at rest (N)	246.81 ± 57.4	273.69 ± 58.8	n.s.
MVS at rest (Nm)	147.5 ± 56.98	177.7 ± 71.6	n.s.
Fatigue time (s)	95.18 ± 28.8	122.53 ± 42.1	0.03*
MVC after-fatigue (N)	207.3 ± 51.8	219.3 ± 65.77	n.s.
MVS after-fatigue (Nm)	137.1 ± 49.9	194.7 ± 72.9	0.01*

MVC: Maximal voluntary contraction (in elbow flexion). MVS: Maximal voluntary supination (of the forearm).

Table IV. Differences in biomechanics before and after surgery.

		Before surgery	12 m after surgery	p
MVC at rest (N)	Tt	193.18 ± 57.9	246.81 ± 57.4	<0.01*
	Td	193.36 ± 88.6	273.69 ± 58.8	<0.01*
MVS at rest (N)	Tt	149.95 ± 73.2	147.5 ± 56.98	n.s.
	Td	129.18 ± 77	177.7 ± 71.6	0.01*
Fatigue time (s)	Tt	139.1 ± 71.6	95.18 ± 28.8	<0.01*
	Td	137.6 ± 99.2	122.53 ± 42.1	n.s.
MVC after-fatigue (N)	Tt	169.5 ± 6	207.3 ± 51.8	<0.01*
	Td	158.8 ± 76.8	219.3 ± 65.77	0.01
MVS after-fatigue (N)	Tt	146.9 ± 77.5	137.1 ± 49.9	n.s.
	Td	118 ± 68.6	194.7 ± 72.9	<0.01*

MVC: Maximal voluntary contraction (in elbow flexion). MVS: Maximal voluntary supination (of the forearm). Tt: tenotomy group. Td: tenodesis group.

period and 122.53 ± 42.1 s at the end of the follow-up; p = n.s.). Statistical differences were found between groups at the end of the follow-up in terms of fatigue time (95.18 ± 28.8 s vs. 122.53 ± 42.1; p = 0.03).

DISCUSSION

The most important finding in this study has been the difference in fatigue time between groups found at the end of the follow-up (95.18 ± 28.8 s in the tenotomy group vs 122.53 ± 42.1 s in the tenodesis group; p = 0.03). To date, no clinical trial has focused on the study of biceps fatigue as the main study parameter. The different biomechanical studies carried out to date analyze the results of the surgery based on the analysis of basic biomechanical parameters (MVC, MVS). Maximum peak force in elbow flexion or supination of the forearm are important biomechanical parameters, but they may not be the most relevant parameters in clinical practice, because most of the daily activities are performed by repeated submaximal contractions (25).

There is controversy in whether there is a decrease in MVC or MVS after the surgery on the LHBT. There are different studies that show a decrease in flexion strength between

4.4% and 14% (15,21,22,24) in patients where tenotomy where performed with respect to the healthy arm. But this decrease is very similar to the data provided by studies that analyze patients undergoing tenodesis with respect to the healthy arm (decrease between 9% and 15%)(21,22). Shank *et al.* (21) and Sentürk *et al.* (23) studied the biomechanical differences between patients undergoing tenotomy or tenodesis of LHBT. Both authors, using the Cybex isokinetic dynamometer, found no differences in flexion strength or supination. In the same way, other authors have also not found differences when manual dynamometers were used. Lee *et al.* (19) and Oh *et al.* (20) did not find differences between groups in flexion, but in supination, in favor of performing tenodesis.

In the present study, no differences in pre-fatigue flexion strength were observed between the groups (246.81 ± 57.4 N vs 273.69 ± 58.8 N), nor in the pre-fatigue supination force of the forearm (147.5 ± 56.98 N vs 177.7 ± 71.6 N). Generally, force measurements in biomechanical studies are analyzed with the arm in baseline (not fatigued). Friedman *et al.* (13) trying to find differences between tenotomy and tenodesis, analyzed the MVC and MVS in the fatigued arm. For this, they performed a fatigue test by lifting dumbbells and subsequently quantified the MVC and the MVS

in the fatigued arm, without finding differences between the groups. In the present study, no differences were found between groups in the after-fatigue MVC (207.3 ± 51.8 N vs. 219.3 ± 65.77 N), but statistical differences have been observed in the after-fatigue MVS (137.1 ± 49.9 N vs. 194.7 ± 72.9 N; $p=0.01$). These differences were not appreciated in the pre-fatigue study. This suggests that the biomechanical study in the fatigued arm is useful for demonstrating differences that were not appreciated with the arm at baseline. This particularity in the biomechanical measurement after-fatigue has its relevance in the clinic, given that manual workers do not carry out their job in a baseline situation, but are progressively more fatigued when performing their workday.

It is interesting to note that in the present study differences between groups in after-fatigue supination force were observed (**table III**), while no differences were observed in terms of muscle cramps or residual pain in the slider (**table II**). This suggests that the strength deficit is not related to pain. This supination deficit was only seen in the after-fatigue study. This means that, in basal conditions (non-fatigued arm), the generation of supination force does not differ between the groups, while differences in time to claudication and supination force after fatigue have been found. An electromyographic study of the operated patients could determine if the deficit is due to a failure in neuromuscular transmission (inhibition of the motor neuron, central fatigue) or if it is due by a cellular cause (peripheral fatigue).

To date, no author has focused the study on fatigue time until biceps claudication when analyzing the differences between tenotomy and LHBT tenodesis.

Some authors have tried to make an approximation to biceps fatigue by lifting dumbbells, measuring the number of repetitions until claudication of the arm. Kelly *et al.* (9) appreciated differences in the number of elevations two years after performing biceps tenotomy (32.3 repetitions) compared to the healthy arm (34.5 repetitions). In a similar way, Drakos *et al.* (34) did not appreciate differences in the number of dumbbell elevations in the arm where the tenodesis was performed (33.2 repetitions) with respect to the healthy arm (34.3 repetitions).

Using dumbbells to perform the fatigue test is an easy, accessible and reproducible way to perform the biomechanical test, but it can lead to bias. This is because it does not report the test time or the frequency of repetitions. It does not take into account the biomechanical characteristics of each subject (all subjects lift 10lbs without taking into account biometrics) nor do the authors report the position of the forearm during the dumbbell lift. Other studies induce biceps fatigue by isometric contraction of the elbow,

using a manual dynamometer (28,31,32,35). To do this, the MVC is determined first and, depending on it, perform a submaximal percentage isometric contraction maintained over time. This fatigue induction model has been used in basic studies and also in studies that analyze the results after the surgery on LHBT²⁶. This biceps fatigue induction model has been used in the present study. It estimates the fatigue test based on the biomechanical characteristics of the individual, in a dynamic way over time, according to their MVC. It pays special attention to the duration of the test until the claudication and allows the performance of the MVC and the MVS after-fatigue. The study of the time until claudication after performing the surgical techniques on LHBT has clinical relevance because it provides information on the possible fatigue of the biceps in patients who require a physical activity maintained during the workday. This must be taken into account for the choice of surgical technique on the LHBT by the surgeon.

Avoiding the Popeye sign is one of the reasons why LHBT tenodesis is performed. In a recent systematic review, Slenker *et al.* (6) quantified this deformity in 42% of the patients where tenotomy had been performed, compared to 8% of the patients operated by tenodesis. In the present study, this difference between groups has been significant (56.5% vs. 11.1%; $p = 0.01$). The data obtained have been similar to other studies in the literature (5,9,13,20,36), but slightly higher than those provided by the systematic review. This can be justified by the mean age (50.73 ± 6.31 years in the tenodesis group), lower than in the systematic review (over 60 years). It can also be justified by the male sex of all subjects, more prone to deformity after surgery on the LHBT (9,15,20,37). There were no differences between groups in terms of pain (VAS), functional scales (normalized Constant score, SSI, UCLA), residual pain in the slide, biceps cramps or perceived satisfaction.

It has been analyzed whether in the presence of aesthetic deformity there are differences in function, pain, side effects or biomechanical parameters with respect to subjects without deformity. In the function scales, differences were found in favor of the group with deformity in the SSI scale (75.68 ± 18.54 in the group without deformity vs 89.44 ± 18.15 in the group with deformity; $p = 0.03$), but not in normalized Constant score nor UCLA. No differences were observed in terms of pain, muscle cramps, pain in the slide or biomechanical parameters.

The evolution of postoperative pain after LHBT surgery is a parameter to be taken into account, because a higher incidence of initial surgical pain has been observed in patients where tenodesis have been performed⁷. In the present study, there were no differences in the VAS scale in the postoperative period at 3 months (3.06 ± 2.28 in tenotomies vs 4.33

± 2.28 in tenodesis), 6 months (2.67 ± 2.49 vs 2.83 ± 2.44), nor at 12 months. (2 ± 2.65 vs 3 ± 2.97).

In this study some methodological weaknesses have been assumed. The first is the small sample size. Although the population studied is small, it reaches the minimum required by the power study that was conducted prior to the clinical trial. The second weakness is the short period of follow-up of patients (1 year). Longer temporal evolution can lead to adaptive changes that modify the clinical and biomechanical results. There is also a weakness induced by the age range of the sample studied and sex. This can lead to bias in extrapolation of data to the general population.

As mentioned in this discussion section, the results of this clinical trial are important in daily clinical practice, because they offer biomechanical and clinical data to be taken into account in the choice of surgical technique to be used in the pathology of LHBT.

REFERENCES

1. Oliva F, Piccirilli E, Bossa M, et al. I.S.Mu.L.T - Rotator Cuff Tears Guidelines. *Muscles, ligaments and tendons journal* 2015;5:227-63.
2. Cipollaro L, Sahemey R, Oliva F, Maffulli N. Immunohistochemical features of rotator cuff tendinopathy. *British medical bulletin* 2019;130:105-23.
3. Maynou C, Mehdi N, Cassagnaud X, Audebert S, Mestdagh H. [Clinical results of arthroscopic tenotomy of the long head of the biceps brachii in full thickness tears of the rotator cuff without repair: 40 cases]. *Rev Chir Orthop Reparatrice Appar Mot* 2005;91:300-6.
4. Walch G, Edwards TB, Boulahia A, Nove-Josserand L, Neyton L, Szabo I. Arthroscopic tenotomy of the long head of the biceps in the treatment of rotator cuff tears: clinical and radiographic results of 307 cases. *J Shoulder Elbow Surg* 2005;14:238-46.
5. Duff SJ, Campbell PT. Patient acceptance of long head of biceps brachii tenotomy. *J Shoulder Elbow Surg* 2012;21:61-5.
6. Slenker NR, Lawson K, Ciccotti MG, Dodson CC, Cohen SB. Biceps tenotomy versus tenodesis: clinical outcomes. *Arthroscopy* 2012;28:576-82.
7. Hsu AR, Ghodadra NS, Provencher MT, Lewis PB, Bach BR. Biceps tenotomy versus tenodesis: a review of clinical outcomes and biomechanical results. *J Shoulder Elbow Surg* 2011;20:326-32.
8. Boileau P, Bague F, Valerio L, Ahrens P, Chuinard C, Trojani C. Isolated arthroscopic biceps tenotomy or tenodesis improves symptoms in patients with massive irreparable rotator cuff tears. *J Bone Joint Surg Am* 2007;89:747-57.
9. Kelly AM, Drakos MC, Fealy S, Taylor SA, O'Brien SJ. Arthroscopic release of the long head of the biceps tendon: functional outcome and clinical results. *Am J Sports Med* 2005;33:208-13.
10. Carroll RE. *HLRotbbacmotJBSA*.

CONCLUSIONS

The most important finding of this study was that differences in fatigue time were found between the tenotomy and tenodesis groups at 12 months after the intervention.

Differences were seen in the MVS after-fatigue in favor of performing tenodesis. No differences were found in the study of MVC or MVS before fatigue.

The study of fatigue should be considered in subsequent biomechanical studies.

Clinical relevance. Despite of the absence of differences in the maximum strength of flexion or supination of the elbow, the presence of early fatigue in the tenotomy group should be taken into account when choosing the surgical technique on LHBT.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

11. Berlemann U, Bayley I. Tenodesis of the long head of biceps brachii in the painful shoulder: improving results in the long term. *J Shoulder Elbow Surg* 1995;4:429-35.
12. Boileau P, Krishnan SG, Coste JS, Walch G. Arthroscopic biceps tenodesis: a new technique using bioabsorbable interference screw fixation. *Arthroscopy* 2002;18:1002-12.
13. Friedman JL, FitzPatrick JL, Rylander LS, Bennett C, Vidal AF, McCarty EC. Biceps Tenotomy Versus Tenodesis in Active Patients Younger Than 55 Years: Is There a Difference in Strength and Outcomes? *Orthop J Sports Med* 2015;3:2325967115570848.
14. Franceschi F, Longo UG, Ruzzini L, Papalia R, Rizzello G, Denaro V. To detach the long head of the biceps tendon after tenodesis or not: outcome analysis at the 4-year follow-up of two different techniques. *Int Orthop* 2007;31:537-45.
15. Lim TK, Moon ES, Koh KH, Yoo JC. Patient-related factors and complications after arthroscopic tenotomy of the long head of the biceps tendon. *Am J Sports Med* 2011;39:783-9.
16. De Carli A, Zanzotto E, Vadala AP, Luzon D, Di Salvo M, Ferretti A. Surgical repair of the distal biceps brachii tendon: clinical and isokinetic long-term follow-up. *Knee surgery, sports traumatology, arthroscopy : official journal of the ESSKA* 2009;17:850-6.
17. Koh KH, Ahn JH, Kim SM, Yoo JC. Treatment of biceps tendon lesions in the setting of rotator cuff tears: prospective cohort study of tenotomy versus tenodesis. *Am J Sports Med* 2010;38:1584-90.
18. Zhang Q, Zhou J, Ge H, Cheng B. Tenotomy or tenodesis for long head biceps lesions in shoulders with reparable rotator cuff tears: a prospective randomised trial. *Knee surgery, sports traumatology, arthroscopy : official journal of the ESSKA* 2015;23:464-9.
19. Lee HJ, Jeong JY, Kim CK, Kim YS. Surgical treatment of lesions of the long head of the biceps brachii tendon with rota-

- tor cuff tear: a prospective randomized clinical trial comparing the clinical results of tenotomy and tenodesis. *J Shoulder Elbow Surg* 2016;25:1107-14.
20. Oh JH, Lee YH, Kim SH, et al. Comparison of Treatments for Superior Labrum-Biceps Complex Lesions With Concomitant Rotator Cuff Repair: A Prospective, Randomized, Comparative Analysis of Debridement, Biceps Tenotomy, and Biceps Tenodesis. *Arthroscopy* 2016;32:958-67.
 21. Shank JR, Singleton SB, Braun S, et al. A comparison of forearm supination and elbow flexion strength in patients with long head of the biceps tenotomy or tenodesis. *Arthroscopy* 2011;27:9-16.
 22. The B, Brutty M, Wang A, Campbell PT, Halliday MJ, Ackland TR. Long-term functional results and isokinetic strength evaluation after arthroscopic tenotomy of the long head of biceps tendon. *Int J Shoulder Surg* 2014;8:76-80.
 23. Senturk I, Ozalay M, Akpınar S, Leblebici B, Cınar BM, Tuncay C. Clinical and isokinetic comparison between tenotomy and tenodesis in biceps pathologies. *Acta Orthop Traumatol Turc* 2011;45:41-6.
 24. De Carli A, Vadala A, Zanzotto E, et al. Reparable rotator cuff tears with concomitant long-head biceps lesions: tenotomy or tenotomy/tenodesis? *Knee surgery, sports traumatology, arthroscopy : official journal of the ESSKA* 2012;20:2553-8.
 25. Grabiner MD, Enoka RM. Changes in movement capabilities with aging. *Exerc Sport Sci Rev* 1995;23:65-104.
 26. Garcia-Rellan JE, Sanchez-Alepuz E, Mudarra-Garcia J. Increased fatigue of the biceps after tenotomy of the long head of biceps tendon. *Knee surgery, sports traumatology, arthroscopy : official journal of the ESSKA* 2018;26:3826-31.
 27. Padulo J, Oliva F, Frizziero A, Maffulli N. Muscles, Ligaments and Tendons Journal - Basic principles and recommendations in clinical and field Science Research: 2016 Update. *Muscles, ligaments and tendons journal* 2016;6:1-5.
 28. Seghers J, Spaepen A, Delecluse C, Colman V. Habitual level of physical activity and muscle fatigue of the elbow flexor muscles in older men. *Eur J Appl Physiol* 2003;89:427-34.
 29. Bilodeau M, Henderson TK, Nolte BE, Pursley PJ, Sandfort GL. Effect of aging on fatigue characteristics of elbow flexor muscles during sustained submaximal contraction. *J Appl Physiol (1985)* 2001;91:2654-64.
 30. Hunter SK, Enoka RM. Sex differences in the fatigability of arm muscles depends on absolute force during isometric contractions. *J Appl Physiol (1985)* 2001;91:2686-94.
 31. Hunter SK, Critchlow A, Shin IS, Enoka RM. Fatigability of the elbow flexor muscles for a sustained submaximal contraction is similar in men and women matched for strength. *J Appl Physiol (1985)* 2004;96:195-202.
 32. Hunter SK, Critchlow A, Enoka RM. Muscle endurance is greater for old men compared with strength-matched young men. *J Appl Physiol (1985)* 2005;99:890-7.
 33. Bilodeau M, Erb MD, Nichols JM, Joiner KL, Weeks JB. Fatigue of elbow flexor muscles in younger and older adults. *Muscle Nerve* 2001;24:98-106.
 34. Drakos MC, Verma NN, Gulotta LV, et al. Arthroscopic transfer of the long head of the biceps tendon: functional outcome and clinical results. *Arthroscopy* 2008;24:217-23.
 35. Fuglevand AJ, Zackowski KM, Huey KA, Enoka RM. Impairment of neuromuscular propagation during human fatiguing contractions at submaximal forces. *J Physiol* 1993;460:549-72.
 36. Franceschi F, Longo UG, Ruzzini L, Rizzello G, Maffulli N, Denaro V. No advantages in repairing a type II superior labrum anterior and posterior (SLAP) lesion when associated with rotator cuff repair in patients over age 50: a randomized controlled trial. *Am J Sports Med* 2008;36:247-53.
 37. Biz C, Vinanti GB, Rossato A, Arnaldi E, Aldegheri R. Prospective study of three surgical procedures for long head biceps tendinopathy associated with rotator cuff tears. *Muscles, ligaments and tendons journal* 2012;2:133-

Assessment of Post-Stroke Biceps Brachialis Muscle Stiffness by Shear-Wave Elastography: a Pilot Study

P. D. Analan¹, H. Ozdemir²

¹ Department of Physical Medicine and Rehabilitation, School of Medicine, Baskent University, Adana, Turkey

² Department of Radiology, Baskent University Faculty of Medicine, Adana, Turkey

CORRESPONDING AUTHOR:

Pinar Doruk Analan
Department of Physical Medicine and
Rehabilitation
School of Medicine
Baskent University
Adana Dr. Turgut Noyan Teaching
Medical Research Center
Dadaloglu Mh
01250, Adana, Turkey
E-mail: doruk.pinar@gmail.com

DOI:

10.32098/mltj.03.2020.26

LEVEL OF EVIDENCE: 3B

SUMMARY

Objectives. Spasticity following stroke tend to increase muscle stiffness. Recent clinical measures of post-stroke biceps brachialis muscle spasticity are subjective. We aimed to measure post-stroke biceps brachialis muscle stiffness quantitatively by using shear-wave elastography (SWE).

Methods. This prospective study included 24 post-stroke patients with unilateral biceps brachialis muscle spasticity. The Modified Ashworth Scale of the biceps muscle, Brunnstrom stages of the upper extremity of the patients were evaluated as the clinical outcomes. Biceps brachialis muscle stiffness was compared among affected and non-affected upper extremities by SWE. The correlations between SWE findings and clinical outcomes were analyzed.

Results. Shear Wave Velocity (SWV) on the spastic side did not show any significant differences from those on the non-spastic side ($p>0.05$). SWV findings were not correlated with clinical outcomes ($r \leq 0.3$, $p>0.05$).

Conclusions. Biceps brachialis muscle stiffness was found similar among the affected and non-affected extremities. In addition, SWV was not correlated with clinical outcomes. SWE may not be helpful in detecting the changes in biceps muscle stiffness following stroke.

KEY WORDS

Elastography; spasticity; biceps brachialis muscle; modified Ashworth Scale.

INTRODUCTION

Spasticity is determined as a velocity-dependent increase in muscle tone and increased resistance to passive movement. It can affect daily living activities, such as mobility, transferring, toileting, and dressing. Also, it may cause pain, contracture, deformity, and limitations on the range of motion (ROM), function and muscle strength (1-4). Muscle hardness may increase with post-stroke spasticity (5,6).

The Modified Ashworth Scale (MAS) is the most commonly accepted clinical spasticity assessment method (1). Several studies in the literature have assessed its validity and reliability (7-11). However, these studies have emphasized that MAS can not detect small changes in muscle spasticity (9,12). Although widely used, the MAS can not evaluate muscle stiffness which has the potential to be related to muscle function (13). Therefore, a non-invasive radiological technique for the quantifying stiffness of the muscles may be useful.

Ultrasound (US) elastography has been shown to assess muscle architectural and mechanical alterations (14,15).

Elastography is a recently developed US-based method to evaluate tissue stiffness. Strain (compression) elastography and shear-wave elastography (SWE) are the two common elastography techniques in clinical practice. Strain elastography produces an image based on the displacement of the tissue from an external or patient source. Point-SWE using acoustic radiation force impulse (ARFI), imaging is a new technique enabling quantitative analysis of tissue elasticity without compression. In ARFI-Imaging, the acoustic pulses generate localized tissue displacements and the displacements cause lateral shear wave propagation which is tracked using laterally positioned US tracking beams. The shear wave velocity (SWV) of the tissue can be reconstructed by estimating the maximum displacement at each lateral location. The shear wave propagation velocity is proportional

to the square root of tissue elasticity. By SWE, shear wave velocity is expressed in meters per second (16-18).

SWE has been used in studies searching patients with neurological disorders including Parkinson's disease and cerebral palsy (CP), as well as in studies on the effects of dynamic exercise on muscles (15,19,20). Previously reported studies showed that elastography is feasible in the quantitative assessment of spastic biceps brachii muscle (21,22).

Herein, we aimed to evaluate the biceps brachialis muscle by SWE and to analyze the correlation of stroke-related outcomes with elastography measurements.

MATERIALS AND METHODS

This study was approved by the Baskent University Institutional Review Board and Ethics Committee (number of the project: KA16/149) and supported by Institutional Research Fund. Written informed consent was obtained from all subjects before the study. Power analysis measured before the ethics committee application and biostatistics preliminary evaluation revealed that 24 patients should be included in the study.

24 post-stroke patients with biceps brachialis muscle spasticity above 18 years old were evaluated for the present study. Exclusion criteria were; bilateral and multiple stroke attacks healed with sequelae; anti-spastic injections in the last 6 months; established contracture of the elbow; previous treatment with oral or intramuscular anti-spastic treatment medications, intrathecal baclofen, or surgery for spasticity; and other neurological or muscular diseases. Also, subjects who underwent arm and shoulder surgeries were excluded from the present study.

Immediately before performing SWE, clinical outcomes were evaluated by a single physiatrist with 16 years of experience in neurologic rehabilitation.

CLINICAL OUTCOME MEASURES

Modified Ashworth Scale (MAS)

The MAS is most commonly used tool of spasticity and includes these categories: **0 (MAS 0)**, normal muscle tone; **1 (MAS 1)**, minimal increased in muscle tone; **2 (MAS 1+)**, minimal increased in muscle tone that seen throughout less than half of the ROM; **3 (MAS 2)**, more marked increase in muscle tone through most of the ROM; **4 (MAS 3)**, considerable resistance, difficulty of passive movement; **5 (MAS 4)**, rigid elbow flexion.²³

Brunnstrom motor recovery stages

Brunnstrom motor recovery stages (BS) of the upper extremity was used in this study (24). BS comprises six stages: **stage I**, flaccidity, no active movement on the affected side; **stage II**, appearance of spasticity, muscles begin involuntary, abnormal, small movement with synergy; **stage III**, spasticity increases and synergy arising maximum level, muscles begin voluntary and synergic contractions; **stage IV**, decreased spasticity, movement is seen voluntary, more complex and non-synergic features; **stage V**, spasticity decreased and minimal, the patient has isolated active movement; and **stage VI**, no spasticity, muscle tone is normal, all of the isolated active movement is seen. A higher stage of BS represents better recovery (25).

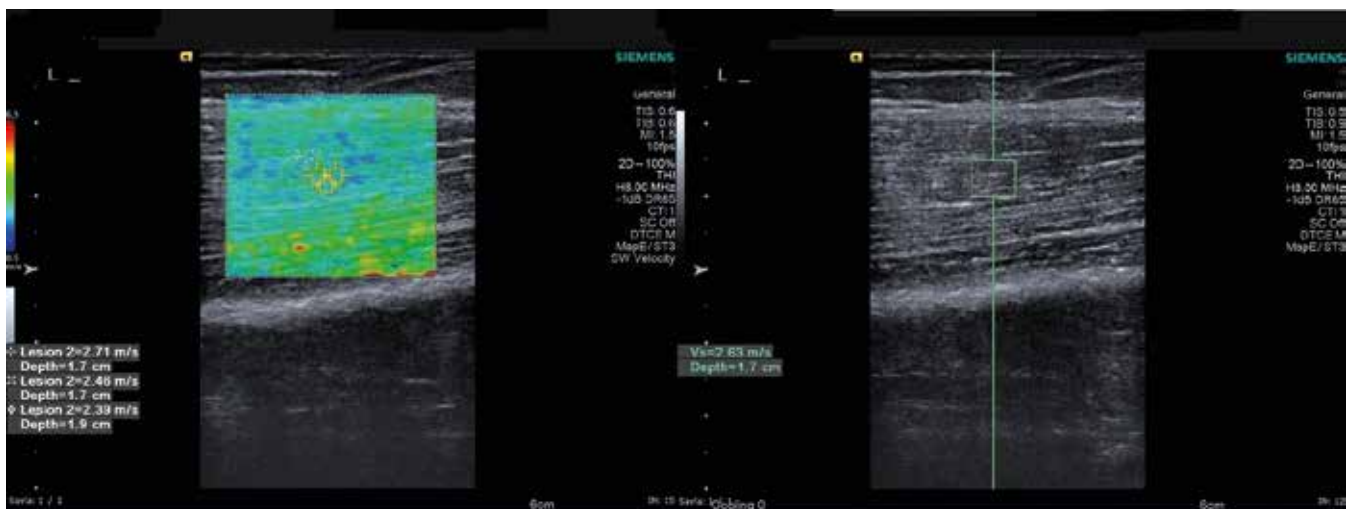


Figure 1. 45 years old patient with right sided upper limb spasticity following stroke. SWV of the spastic biceps muscle measured by VTIQ method (right) and VTQ method (left) are shown.

Shear Wave Elastography (SWE)

US and SWE examinations were performed using a US system (Acuson S 2000; Siemens, Erlangen, Germany). SWE was performed using a probe with an L9-4 linear array. SWE was performed with the patient in a sitting position in front of an examination bed. The patient was asked to keep their elbow in extension and their hand in a pronated position to keep the biceps muscles relaxed. The affected arm was positioned with the wrist in a rest position. All patients were requested to remain as relaxed as possible throughout the testing (approximately 1-2 minutes). Evaluations were repeated for the unaffected side in the same manner.

A standard US examination was initially performed to view the biceps muscle in the axial plane. Measurements were performed from the belly point of the muscle while the muscle was seen on the longitudinal plane (**figure 1**). All patients were evaluated by both VTQ and VTIQ methods. Scanning was repeated if any movement or muscle contraction occurred during SWE. Image quality was assessed using quality maps produced by the US system. The image having the highest quality map was used to measure SWV. A

rectangular electronic box-shaped region of interest (ROI) was used for measurements. At least three boxes were set at the mid-depth of the muscle by VTIQ. Maximum SWVs were recorded. As measured by SWE, faster velocity refers to greater stiffness (hardness) and lower velocity indicates less stiffness (softness).

STATISTICAL ANALYSIS

The statistical package SPSS software (Version 17.0, SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Normal continuous variables were described as the mean \pm standard deviation ($p > 0.05$ in Kolmogorov-Smirnov test or Shapira-Wilk [$n < 30$]) and variables that were not normal were described as the median. The Student T-test was used to compare groups for normally distributed data and the Mann Whitney U test for not normally distributed data. The Pearson's correlation test was used for correlation analyses. The correlation coefficients were interpreted either excellent $r \geq 0.91$; good $0.90 \geq r \geq 0.71$; fair $0.70 \geq r \geq 0.51$; weak $0.50 \geq r \geq 0.31$; or little or none $r \leq 0.3$. Values of $p < 0.05$ were considered statistically significant.

Table I. Clinical characteristics of the study population.

Characteristics	Value
Age (years) *	57.46 \pm 10.27
Gender (Females/ Males), (n)	14/10
Time since stroke, months*	23.08 \pm 26.06
Body mass index, kg/m ² *	27.04 \pm 7.35
Paretic side, Right/left, (n)	9/15
Stroke type, (n)	
Ischemic	18
Hemorrhagic	4
Hemorrhagic transformation following ischemic stroke	2

*: mean \pm standard deviation

Table II. Clinical outcomes of study population, (n:24).

Characteristics	n
Brunnstrom's Stages of Motor Recovery of the upper extremity	
Stage 2	7
Stage 3	9
Stage 4	5
Stage 5	3
Spasticity degree of biceps brachialis measured using the Modified Ashworth Scale	
Stage 1+	8
Stage 2	13
Stage 3	3

Table III. Comparisons of Shear-Wave Elastography measurements of biceps brachialis muscle, (m/s), (mean \pm standard deviation).

Characteristics	Value	p
Shear Wave Velocity (VTIQ*)		
Spastic side	3.22 \pm 1.2	0.73
Non-spastic side	3.15 \pm 0.91	
Shear Wave Velocity (VTQ**)		
Spastic side	2.63 \pm 1.06	0.74
Non-spastic side	2.95 \pm 0.185	

*: Virtual Touch Imaging Quantification

**: Virtual Touch Quantification

RESULTS

The study population consisted of 10 male and 14 female patients with a mean age of 57.46 \pm 10.27 years. The major etiology of stroke was hypertension in 12 patients, followed by diabetes mellitus in 2 and a history of mitral valve surgery in 1. Multifactorial etiologies were responsible for 5 patients and the etiology was unknown in 4. Clinical features are noted in **table I**. BS and MAS scores are summarized in **table II**.

16 of the patients (66.6%) had stage 2 and 3 disease whereas 8 patients (33.4%) had stage 4 and 5 disease. 13 of the patients had (54.2 %) score 2 disease by MAS.

The SWV findings were statistically similar among the groups. Comparisons of SWV measurements are categorized in **table III**.

There were not any correlations between the both SWV (measured both VTIQ and VTQ methods) of the biceps brachialis muscle with the duration of a stroke, MAS, or BS scores ($r \leq 0.3$, $p > 0.05$). Pearson correlation coefficients were as follows for VTIQ measurements: duration of stroke ($r = -0.18$), MAS score ($r = 0.0073$) and BS score ($r = 0.056$). Pearson correlation coefficients were as follows for VTQ measurements: duration of stroke ($r = -0.196$), MAS score ($r = -0.048$) and BS score ($r = 0.13$).

DISCUSSION

This study showed that post-stroke biceps brachialis muscle stiffness was not different from the non-spastic side. Also, SWV findings were not correlated with clinical spasticity and motor recovery stage evaluations. We believe that the inactivity and disuse of the muscles may lead to muscle atrophy. In our study, the standard deviation of the duration of stroke was high as a result; the difference in muscle pathological changes including muscle degeneration could widely vary among our study population. Thus, muscle atrophy may neutralize the effects of muscle stiffness.

In the literature, there are some studies analyzing the effects of some neurological disorders on muscles using elastogra-

phy. Parkinson's disease was reported to cause increased biceps muscle stiffness when compared with healthy subjects (15).

Moreover, Kesikburun *et al.* (2) found a significant increase of post-stroke gastrocnemius muscle stiffness compared with the non-spastic side by strain elastography. In addition, a recent study reported that medial gastrocnemius muscle stiffness of patients with CP was higher than that of healthy children using SWE (13).

Another study including children with CP proposed that elastography can detect the stiffer areas in the spastic muscles and guide the description of optimal injection sites for botulinum toxin therapy (19).

Not only the neurological disorders but also posture, exercise, gender or anatomical properties were reported to affect the muscle stiffness. Nordez *et al.* (26) reported that stretching of the ankle plantar flexors increases the gastrocnemius muscle elasticity. Also, biceps brachii muscle stiffness measured by SWE can also be affected by the flexion or extension posture of the elbow and gender of the patients (27). The posture of the extremities, stretching degree of the tendons and muscle contractions can, therefore, be considered to potentially affect elastography measurements. It may be difficult to standardize all factors among the studies. Another study showed a reduction in the strain ratio after exercise (20). Due to the difficulty in controlling all parameters during examinations, differences in measurements may depend on the complexity of muscle function and anatomy. The limited number of patients may be considered the first limitation of our study. Also, we could not categorize the study population according to MAS or BS scores.

The level of evidence of the study is 3B, so randomized controlled trials are needed (28).

CONCLUSIONS

In conclusion, SWE seems not an applicable tool for the evaluation of changes in muscle stiffness in the spastic biceps brachialis muscle following stroke.

AUTHOR CONTRIBUTIONS

PDA conceived the study. PDA and HO collected the data, conducted statistical analysis and wrote the first draft of the manuscript. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

ETHICS

This study was approved by Baskent University Institutional Review Board and Ethics Committee (number of the project: KA16/149) and supported by Institutional Research Fund.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

REFERENCES

- Kaku M, Simpson DM. Spotlight on botulinum toxin and its potential in the treatment of stroke-related spasticity. *Drug Des Devel Ther.*2016;10:1085-1099.
- Kesikburun S, Yaşar E, Adıgüzel E, Güzelküçük Ü, Alaca R, Tan AK. Assessment of Spasticity With Sonoelastography Following Stroke: A Feasibility Study. *PM R.*2015;7(12):1254-60.
- Brainin M, Norrving B, Sunnerhagen KS, Goldstein LB, Cramer SC, Donnan GA, et al. International PSS Disability Study Group: Poststroke chronic disease management: Towards improved identification and interventions for poststroke spasticity-related complications. *Int J Stroke.* 2011;6(1): 42-46.
- Sommerfeld DK, Eek EU, Svensson AK, Holmqvist LW, von Arbin MH.. Spasticity after stroke: Its occurrence and association with motor impairments and activity limitations. *Stroke.* 2004;35(1): 134-139.
- Rydahl SJ, Brouwer BJ. Ankle stiffness and tissue compliance in stroke survivors: a validation of myotonometer measurements. *Arch Phys Med Rehabil.* 2004;85(10):1631-1637.
- Leonard CT, Stephens JU, Stroppel SL. Assessing the spastic condition of individuals with upper motorneuron involvement: validity of the myotonometer. *Arch Phys Med Rehabil.* 2001;82(10): 1419-1420.
- Brashear A, Zafonte R, Corcoran M, et al. Inter- and intrarater reliability of the ashworth scale and the disability assessment scale in patients with upper-limb poststroke spasticity. *Acad Am J Phys Med Rehabil.* 2002;83(10):1349-1354.
- Pandyan AD, Johnson GR, Price CI, Curless RH, Barnes MP, Rodgers H. A review of the properties and limitations of the ashworth and modified ashworth scales as measures of spasticity. *Clin Rehabil.* 1999; 13(5): 373-383.
- Mehrholz J, Wagner K, Meissner D, Grundmann K, Zange C, Koch R, Pohl M. Reliability of the modified tardieu scale and the modified ashworth scale in adult patients with severe brain injury: a comparison study. *Clin Rehabil.* 2005;19(7):751-759.
- Biering-Sorensen F, Nielsen JB, Klinge K. Spasticity-assessment: a review. *Spinal Cord.* 2006;44(12):708-722.
- Abolhasani H, Ansari NN, Naghdi S, Mansouri K, Ghotbi N, Hasson S. Comparing the validity of the Modified Modified Ashworth Scale (MMAS) and the Modified Tardieu Scale (MTS) in the assessment of wrist flexor spasticity in patients with stroke: protocol for a neurophysiological study. *BMJ Open.* 2012;2(6).
- Seth N, Johnson D, Taylor GW, Allen OB, Abdullah HA. Robotic pilot study for analysing spasticity: clinical data versus healthy controls. *J Neuroeng Rehabil.* 2015;12:109.
- Kwon DR, ParkGY, Lee SU, Chung I. Spastic cerebral palsy in children: dynamic sonoelastographic findings of medial gastrocnemius. *Radiology.* 2012;263(3): 794-801.
- Lee SS, Spear S, Rymer WZ. Quantifying changes in material properties of stroke-impaired muscle. *Clin Biomech (Bristol, Avon).* 2015;30(3): 269-275.
- Du LJ, He W, Cheng LG, Li S, Pan YS, Gao J. Ultrasound shear wave elastography in assessment of muscle stiffness in patients with Parkinson's disease: A primary observation. *Clin Imaging.* 2016;40(6):1075-1080.
- Nightingale K, Soo MS, Nightingale R, Trahey G. Acoustic radiation force impulse imaging: in vivo demonstration of clinical feasibility. *Ultrasound Med. Biol.* 2002;28(2): 227-235.
- Dahl JJ, Palmeri ML, Nightingale KR, et al. 4K-5 Shear Wave Velocity Estimation Using Acoustic Radiation Force Impulsive Excitation in Liver In Vivo. 2006 IEEE Ultrason. Symp, IEEE, -2006: pp. 1156-1160.
- Sarvazyan AP, Rudenko OV, Swanson SD, Fowlkes JB, Emelianov SY. Shear wave elasticity imaging: a new ultrasonic technology of medical diagnostics. *Ultrasound Med. Biol.* 1998;24(9):1419-1435.
- Vasilescu D, Vasilescu D, Ducea S, Botar-Jid C, Sfrângeu S, Cosma D. Sonoelastography contribution in cerebral palsy spasticity treatment assessment, preliminary report: a systematic review of the literature apropos of seven patients. *Med Ultrason.* 2010;12(4):306-310.
- Yanagisawa O, Niitsu M, Kurihara T, Fukubayashi T. Evaluation of human muscle hardness after dynamic exercise with ultrasound real-time tissue elastography: a feasibility study. *Clin Radiol.* 2011;66(9):815-819.
- Gao J, He W, Du LJ, Chen J, Park D, Wells M, Fowlkes B, O'Dell M. Quantitative Ultrasound Imaging to Assess the Biceps Brachii Muscle in Chronic Post-Stroke Spasticity: Preliminary Observation. *Ultrasound Med Biol.* 2018;44(9):1931-1940.
- Wu CH, Ho YC, Hsiao MY, Chen WS, Wang TG. Evaluation of Post-Stroke Spastic Muscle Stiffness Using Shear Wave Ultrasound Elastography. *Ultrasound Med Biol.* 2017;43(6):1105-1111.
- Bohannon RW, Smith MB. Interrater reliability of a modified Ashworth scale of muscle spasticity. *Phys Ther.* 1987;67(2):206-207.
- Brandstater ME. Stroke rehabilitation. In: DeLisa JA, ed: Rehabilitation Medicine principles and practice, Philadelphia, Lippincott Raven Publishers, 1998; 1165-1189.
- Kuptniratsaikul V, Kovindha A, Suethanapornkul S, et al. Motor recovery of stroke patients after rehabilitation: one-year follow-up study. *Int J Neurosci.* 2017;127(1):37-43.
- Nordez A, Gennisson JL, Casari P, Catheline S, Cornu C. Characterization of muscle belly elastic properties during passive stretching using transient elastography. *J Biomech.* 2008;41(10): 2305-2311.
- Chen J, O'Dell M, He W, Du LJ, Li PC, Gao J. Ultrasound shear wave elastography in the assessment of passive biceps brachii muscle stiffness: influences of sex and elbow position. *Clin Imaging.* 2017;45:26-29.
- Padulo J, Oliva F, Frizziero A, Maffulli N. Muscles, Ligaments and Tendons Journal - Basic principles and recommendations in clinical and field Science Research: 2018 update. *MLTJ* 2018; 8(3): 305 - 307.

Ancient Textual Sources on Ligamentum Teres: Context and Transmission

S.V. Arkhipov¹, I.V. Prolygina²

¹ Laboratory of Clinical Physiology and Biomechanics, Federal State Budgetary Institution N.N. Priorov National Medical Research Center of Traumatology and Orthopaedics of the Ministry of Health of the Russian Federation, Moscow, Russia

² A.I. Yevdokimov Moscow State University of Medicine and Dentistry, Moscow, Russia

CORRESPONDING AUTHOR:

Sergey V. Arkhipov
Laboratory of Clinical Physiology
and Biomechanics
Federal State Budgetary Institution
N.N. Priorov National
Medical Research Center
of Traumatology and Orthopaedics
of the Ministry of Health of the Russian
Federation
10 Priorova Street, Moscow, Russia, 127299
E-mail: archipovsv@gmail.com

DOI:

10.32098/mltj.03.2020.27

LEVEL OF EVIDENCE: 1A

SUMMARY

Background. One of the least researched anatomical structures of the human body is the ligament of head of femur, most often referred to as *ligamentum teres*. The history of the nomination of this term, medical contexts of its use, the etymology and the first synonyms are not sufficiently understood.

Purpose. The purpose of the article is to present the most complete collection of evidence from ancient medical authors about the term *ligamentum teres*, trace the history of its nomination and analyze the gradual changes in the level of knowledge about the anatomy, mechanical and geometric properties of this structure, its pathology and treatment methods.

Methods. The study is based on an interdisciplinary approach, comprising a combination of linguistic and medical analysis of the texts in ancient Greek and Latin, which contain references to *ligamentum teres*.

Results. Text analysis showed that *ligamentum teres* was known in Palestine at the time of the compilation of the Book of Genesis. In the medical sources written by the Greek physicians, references to it and the description of its properties and role date back to the V-IV cent. BC. The study of textual sources and their medical contexts showed the evolution of the development of this term from the general concept of “sinew” to a narrowly defined “ligament” with detailed qualitative characteristics of the structure and function of this anatomical structure. It has been noted that since ancient times *ligamentum teres* has been recognized as an important element of biomechanics of hip joint and the action of walking.

Conclusions. This work will serve as a basis for further studies and treatment of the interesting and mysterious structure that is *ligamentum teres* – a true *ligamentum incognitum*.

KEY WORDS

Ancient medicine; ancient traumatology; Galen; Hippocrates; hip joint; ligamentum capitis femoris; ligament of head of femur; ligamentum teres.

INTRODUCTION

Recently, the attention of researchers and clinicians has been increasingly attracted by the under-investigated anatomical structure *ligamentum teres* (LT), which connects the femoral head and the acetabulum (1,2). Despite the fact that LT has been known for over three millennia, its anatomy and function have not been fully studied and continue to be refined (3-6). For instance, its role in the musculoskeletal system is still not clear, and opinions on this issue are often very

divided (7-10). Some authors concluded that LT is a rudimentary structure of hip joint (HJ) (11,12). The contemporary studies, however, regard it as a fully functional ligament with the strength comparable to that of the anterior cruciate ligament of the knee joint (8). The recent data show the importance of LT as an HJ-stabilizing structure, which is involved in maintaining vertical positions (13-17). LT is currently recognized as a potential source of pain as well as mechanical symptoms in HJ, including gait impairment

(3,18-24). The evasive knowledge about LT is reflected in dozens of its synonyms, with its original names unknown not only to laymen, but even to experts (7,23,25). In some publications, including those claiming to be comprehensive, the earliest extant evidence regarding LT is, in our opinion, insufficiently covered (26,27). Therefore, this work attempts to provide the most complete review of LT-related sources from ancient authors. We believe that this will make it possible to clarify the earliest period of studying LT and knowledge about it and will help experts to better understand the basis of their research.

MATERIALS AND METHODS

This study is based on an interdisciplinary approach involving a combination of linguistic and medical knowledge. The original fragments of ancient physicians on LT, written in ancient Greek and Latin, as preserved in the electronic platform *Thesaurus Linguae Graecae* (University of California, Irvine, CA, USA), were investigated in relation to the modern views on normal and pathological anatomy of this anatomical element, its role in the musculoskeletal system, its mechanical and geometric properties. The pathology treatment methods are also considered.

This work submits to the ethical standards of the Muscles, Ligaments and Tendons Journal (28).

RESULTS

Biblical tradition: Genesis 32:32

Perhaps the earliest known mention of LT is found in the *Book of Genesis*. The period of the compilation of the *Pentateuch*, which includes the *Book of Genesis*, can be approximated only with a very relative accuracy. Given the period of codification and the long oral tradition of its sources, it can be dated to the period of the XIV-VI centuries BC (29-31). In this ancient written source, LT is mentioned in the context of the narrative of the Patriarch Jacob wrestling with God and acquiring the name of Israel. *Genesis* 32:32 says:

«That is why to this day the Israelites do not eat the thigh sinew which is at the hip socket: because he had struck Jacob at the hip socket on the thigh sinew».

NJB.

In the Masoretic text of the *Biblia Hebraica*, the expression *הַרְגֵּהוּ כִּי לֹעַ הַשֵּׁנָה הַשְּׂמֵנָה דִּישָׁנָה* is used. It can literally be translated as “the thigh sinew, which [is located] in the socket of the hip joint”. The etymology of the term *הַשֵּׁנָה*

(*hannasbeh*) is unclear, and the term *גִּיד* (*gid*) is translated as “sinew” (32).

The authors of the *Septuagint* (LXX), the Greek translation of the *Old Testament* (III-I cent. BC in Alexandria) from a pre-masoretic version of the Hebrew text, rendered this text as: «τὸ νεῦρον ὃ ἐνάρκησεν ὃ ἐστὶν ἐπὶ τοῦ πλάτους τοῦ μηροῦ», that is, “the sinew which shrank, which is upon the hollow of the thigh” (KJV). The translators, apparently, linked the word *hannasbeh* (LXX: ἐνάρκησεν) with the root *nashab*, interpreting it as “to dislocate”, “become weak”. From the point of view of modern medicine, it can be assumed that in this case we are talking about damage to acetabulum.

The Latin translation of the *Bible*, the so-called *Vulgate*, was completed in the IV century AD by St. Jerome, who translated the *Bible* directly from Hebrew, but was also very familiar with the Greek text of the *Septuagint*. This fragment described above is conveyed there as follows: *nervum qui emarcuit in femore Iacob*, “sinew, which weakened in Jacob’s thigh”. The Hebrew, the Greek and the Latin texts formed the basis for all the subsequent translations into other ancient and modern languages, as well as for patristic, Jewish and modern biblical comments.

Most biblical commentators agree that *Genesis* 32:32 mentions the sciatic nerve (*nervus ischiadicus*), the large nerve trunk of the femoral region, which the Greeks called the Achilles tendon (33-35). Other commentators argue that this word refers to the tendon which holds the head of the femur in the acetabulum, along with the surrounding muscles (36). However, neither in the *Pentateuch* nor in the other *Old Testament* books there is any further mention of the anatomical structure of HJ, so it is difficult to establish the exact meaning. In our opinion, consistent with the point of view of some other authors (37), this “sinew” is LT, since no muscle tendons are attached in acetabulum and no large nerve trunks are present. Moreover, the transverse section of the proximal end of LT reveals an approximately cylindrical shape, resembling a tendon or a nerve fragment.

The quote from *Genesis* 32:32 has an important historical and medical implication for anatomy, traumatology, and biomechanics of HJ. This fragment is one of the earliest mentions of HJ and, possibly, the first written reference in the history of mankind to a special anatomical element in it, that is, LT. It can be assumed that in Palestine of the biblical times it was known that LT could be damaged by an indirect trauma mechanism, for example, by a forced hip rotation, its subluxation and dislocation. This is also the first known description of one of the earliest visual symptoms of LT injury, that is, lameness. In addition, *Genesis* provides perhaps the first “medical history” of HJ and LT injury. As befits the modern medical record, the patient’s

name, marital status, personal characteristics, approximate age and working conditions are described, as well as the circumstances, time and location of the injury, localization of the damage, its mechanism, consequences and pathomorphology. From further narration (*Genesis* 50:2), we learn that the Patriarch Jacob was posthumously embalmed by Egyptian physicians, first mentioned in *Genesis* as representatives of a specific and separate profession (25). This allows us to conclude that the diagnosis of LT injury could be morphologically verified by means of some prototype of pathological study. From the biblical text it follows that the resulting biomechanical ambulation disorder turned out to be persistent, and the cause of it was damage to HJ, or, more precisely, LT.

Paradoxical as it may seem, an analysis of this ancient, not at all medical source, allows us to make a significant assumption for modern science: LT is an important functional relation of HJ, and only with it intact, normal-walking can be achieved, making LT a part in the organization of this type of human locomotion.

The terms νεῦρον/nervus: classical tradition

Ancient authors of the Classical period did not initially distinguish between such anatomical structures as tendon, ligament and nerve neither in terminology, nor, apparently, in practice, as indicated by the absence of special names for each one of them. There was a single term τὸ νεῦρον, to which the multivalent term “sinew” corresponds most closely. It can mean “tendon”, “ligament”, “nerve”, as well as “vein” and “artery”. The Latin term *nervus* has the same root as the Greek τὸ νεῦρον and generally has the same initial meanings as “string”, “thread”, “fiber”, from which, by metaphorical transfer, they acquired the medical meaning of “white fibrous threads that support the muscles, connect the joints and transmit nerve or motor impulses” (38). The term τὸ νεῦρον, in the meaning of “sinew”, which can mean “tendons”, “ligaments”, and “muscles”, and possibly “large nerves” and “blood vessels”), is found in Homer’s *Iliad*:

«[...] Phylides, taking note / That bold Amphiclus bent at him, prevented him, and smote / His thigh’s extreme part, where of man his fattest muscle lies, / The nerves torn with his lance’s pile, and darkness clos’d his eyes».

Iliad XVI: 313-316.

Hippocrates (born 460 BC), expounding in the treatise *Places in Man* on certain types of sinews, mentions those that are “connected with joints,” meaning tendons, and “hollow veins”, that is, blood vessels, lymphatic vessels, various ducts and bronchi (*De loc. in hom.*, 5) (39).

Apparently, it is about the articular ligaments connecting two, and in some cases more, bone organs, that Aristotle (IV cent. BC) writes in the *History of Animals*, mentioning that:

«They aid in the support of the body <...> The sinews around the joints have not received any name, for all the bones where they are contiguous are bound together by the sinew (νεῦροις). And there are many sinews round all the bones».

Hist. anim. III, V, 50 (515b 11).

The distinction between nerves, muscles, tendons and ligaments first appears only in the anatomy of the first Alexandrian school (III cent. BC). Herophilus (IV-III cent. BC) did not yet have a clear distinction between nerves and muscles, since he recognized both muscles and nerves as organs of voluntary movement (40). The idea of the physiology of muscle contraction first appears in Erasistratus (IV-III cent. BC). He believed that muscle contracted under the influence of the pneuma that is contained within, and the nerves were deprived of any function in voluntary movement (*Gal. De loc. aff.*) (41,42). In terminology, Erasistratus distinguished between sensory (αἰσθητικά) and motor nerves (πρακτικά, κινητικά).

Apparently, it was Pliny the Elder (1st cent. AD) that used the Latin term *nervus* as applied to tendons in *Natural History*. When speaking about the Achilles tendon, he calls it a “sinew” (*nervus*), which is defined as “flat” (*platys*) (*Hist. nat.* 26, 90). He uses the same term with reference to ligaments:

«In all animals they (*nervi*) are fastened to the lubricous surface of the bones, and so serve to fasten those knots in the body which are known as articulations or joints, sometimes lying between them, sometimes surrounding them, and sometimes running from one to the another; in one place they are long, and in another broad, according as the necessity of each case may demand».

Hist. nat. 11, 217.

Here we have one of the first classifications of ligaments and the first use of the concept of “round” (lat. *teres, rotundum*) used to describe ligaments in general. As for the nerves, since they do resemble thin tendons, the ancient anatomists thought that they served for flexion and extension of various parts of the body.

Aulus Cornelius Celsus (25 BC – 50 AD) writes about a pathology of HJ ligaments, called “sinews” (*nervis*), which are damaged and stretched when hip is dislocated:

«When the bone is replaced nothing further need be done, but the patient must be kept in bed for a rather long time or

the thigh may become displaced again on moving while the sinews (nervis) are still relaxed».

De med. VIII, 20, 8.

Galen of Pergamon (II-III cent. AD) provides a more clear systematization of various kinds of sinews. He often accompanies the term νεῦρον with clarifying definitions or gives one-word synonyms. In the treatise *On Bones for Beginners* Galen describes three types of sinews: those originating in the brain and spinal cord - προαιρειακὰ νεῦρα, “sinews responsible for voluntary movements”; originating in bones - συνδετικὰ νεῦρα, “connecting sinews” or συνδέσμους, “ligaments”; and originating in the muscles - τένοντας, “tendons” (*De oss. ad tir.*, 24) (41). Speaking in the treatise *On Movement of Muscles* on the origin of this term, he associates it with the verb νέω, “bow one’s head, nod” (*De motu musc.* 1, 1) (41).

13 centuries later, the etymology and medical ambiguity of the term *nervus* would be discussed by Andreas Vesalius (1514–1564) in his anatomical atlas *On the Fabric of the Human Body* (1543), in which he’d accept and repeat the definitions of Galen (*De humani fabr. corp.* IV, 1). The modern etymological dictionaries consider this interpretation to be a paronymy and correlate the term νεῦρον with the verb νέω, “spin” (43), implying the pulling and twisting of yarn from a tuft of wool.

In a later treatise, *On the Usefulness of Parts of the Body*, Galen already clearly differentiates both the terms and the functions of ligaments and nerves:

«Where a member needs only a connection, there is only a ligament (σύνδεσμος), and where it needs only a sensation, there is only a nerve (νεῦρον). On the contrary, in organs that could benefit from having voluntary movement, you can see them together: a nerve that transmits an order received from the center of thought and determines the principle of movement, and a ligament that provides the nerve with its power to maintain the joints brought into a state of movement».

De usu part. XII, 3, 7 (41)

Ancient medical authors (prior to Galen) about *ligamentum teres*

In the history of ancient medicine, LT and its topographic anatomy were first mentioned in the treatise *Instruments of Redactions* by Hippocrates:

«The femur itself bends outward and forward; its head is a round epiphysis which gives origin to ligament (νεῦρον) inserted in the acetabulum of the hip-joint. This

bone is articulated somewhat obliquely, but less so than the humerus».

Vectiar. 1 (39).

Indeed, LT attaches to the bottom of *acetabular fossa* at an acute angle, and its counterpart in the shoulder joint is at a right angle. This so-called “*ligamentum teres* in the shoulder joint” is clearly visible in certain animal species and is otherwise called *ligamentum gleno-humerale* (syn. *lig. interarticulare humeri*) (44,45). Its counterpart in humans is not very prominent and is called *ligamentum glenohumerale superius* (46,47). This fragment indirectly indicates that Hippocrates studied the anatomy primarily by dissecting animal bodies.

It should be noted that Hippocrates describes LT as an already well-known element, noting its location and attachment to the bone of HJ. How could Hippocrates, who did not dissect humans, observe LT? It is likely that he could have seen this anatomical element when examining a patient with an open hip dislocation. This pathology is a rare type of trauma that currently occurs with high energy trauma as a result of a car accident and fall (48,49). Similar high-energy traumas could be observed in the time of Hippocrates, for example, when one fell from height, was hit by a large animal or by a heavy part of rigging of a ship.

In Galen’s IV commentary on Hippocrates’ book *On Joints*, a fragment of the treatise *On External Treatment* by the Greek physician Heraclides of Tarentum (III-II cent. BC) was preserved (50). Speaking about the reduction of a dislocated hip, he gives the earliest description of the pathology of LT resulting from this injury:

«Those who believe that the hip does not remain set because the ligament (νεῦρον) connecting the femur to the acetabulum is torn, do not know things of general knowledge, when expressing their negation. For neither Hippocrates nor Diocles would have described the reductions, and neither would Phylotimus, Evenor, Nileus, Molpis, Nymphodorus and some others. And we have achieved this goal [reduction] in two children, although in adults the joint is dislocated again more often. This case should be judged not from hearsay, but since the thigh sometimes remains [set], it must be assumed that this ligament does not always rupture, but that it stretches and contracts again [...]» In Hipp. de artic. IV, 40 (41).

From this fragment we learn that in the days of Heraclides of Tarentum physicians already knew not only about LT, its attachment to the hip and the acetabulum, as well as its connecting function, but also about its injury incurred by a traumatic hip dislocation. It is safe to assume that the author personally performed anatomical studies of both normal and pathologically altered HJ. He also opined that LT could

stretch and contract, thus remaining intact after hip dislocation. A few modern studies partly confirm the Heraclides of Tarentum's guess and indicate the possibility of LT recovery after hip dislocation (18,51,52).

There is an extant fragment of the text by another Greek surgeon, Hegetor, a native of Alexandria (II century BC). The Apollonius of Citium's (90-15 BC) commentary on the Hippocrates' book *On Joints* provides an excerpt from it:

«In the book "On Causes", Hegetor mentioned hip dislocation in the following passage: "Why don't those who rely only on experience seek to find any other [way] of reduction the femoral head for those who have it dislocated, in such a way that every time it dislocates, it can be reset? After all, we can observe that the lower jaw, and the humeral head, the elbow, and the knee, and each finger, and the majority of joints that may dislocate, can be reset in a similar way. For, not being able to understand why this joint only, after dislocation and repeated reduction, won't remain in its place, and seeing what often happens with other joints, they will probably come to the conclusion that there might be a better way of reduction, after which the joint will stay [set]. If they only thought about the reason from the point of view of anatomy, - because the femoral head is a foundation for the ligament (νεύρον) [of femoral head], which grows into the middle of acetabulum; and when it remains [intact], the femur cannot dislocate, but when it ruptures, the hip cannot provide a firm connection; and when there is a lack of connection, the joint cannot remain in place. Since the reason has been clarified, one can refrain altogether from a reduction of a dislocated femur and not make attempts doomed to failure».

In Hipp. de art. (53).

Hegetor repeats the previously known information about LT about the areas of its attachment and its anchoring role, but, unlike Heraclides of Tarentum, he is somewhat pessimistic about the possibility of reduction of hip dislocation. Perhaps the author in his practice encountered cases of complicated dislocation accompanied by a fracture of the wall of acetabulum or femoral head, or perhaps he was not able to differentiate the dislocation from the femoral neck fracture. For these reasons, his attempts to "set it" were not successful, which served as the basis for therapeutic pessimism.

Further, Apollonius of Citium expresses its own opinion on this issue:

«If the dislocated and displaced hip could not remain in place, then the physician [Hippocrates] would have clearly indicated the incurability of this case, so that we would not be led astray. Don't those who hold the opposite opinion know about the nature of joints, ligaments and the teachings about

these things in general? After all, the physician considered the cause of the mild or, on the contrary, complex displacement and reduction of the joints to lie in the natural structure, condition, and strength or stretching of the ligaments (νεύρον), associated with fluid, so that, with respect to femur dislocation, if it does not remain [in place], this happens not because the ligament (νεύρον) [of the femoral head] is torn, but because of the natural weakening or stretching of the ligaments (νεύρον), just as he says about the bulls that their joints are mobile by nature». In Hipp. de art. (53).

In this fragment, the author agrees with the views of Heraclides and Hippocrates, noting the possibility of lengthening, weakening of strength and elasticity of LT. Apollonius of Citium talks (already with confidence) about the possibility of pathological changes in LT and transformation of its geometric and mechanical properties. These changes, registered over 2000 years ago, are still the cause of differences in the description of normal LT. It should also be noted that Apollonius of Citium says, like Hippocrates, that ligaments, and hence LT, are present in animals (cf. *De artic.* 8, 14-15; 52; *Vectiar.* 5, 5-6) (39).

Another mention of LT is found in the Roman anatomist and physician Rufus of Ephesus (I-II cent. AD). Speaking about the names of different body parts in the treatise *De appellationibus partium corporis humani*, he writes:

«The name of pelvis (ισχίον) [is given to] the ligament (νεύρον) that attaches to acetabulum and secures the entire joint» (54).

The statement by Rufus suggests that the author was aware of the existence of LT, its attachment to acetabulum and its connective function.

Galen and the Byzantine authors

Galen, who, along with Hippocrates, had remained the main authority in the field of anatomy until the time of Andreas Vesalius, mentions LT in a number of his treatises. In one of his early essays, *On Bones for Beginners*, written for those who are just beginning to study anatomy, Galen, explaining the structure of HJ, says:

«There is a socket of large size in each of the ischia, attached by a very stout ligament (σύνδεσμος) to the head of the femur». *De oss. ad tir.* XX, 2 (41).

This quote is interesting in that Galen notes, like the previous authors, that LT is attached directly to the bones that form HJ, and not to its capsule or transverse acetabular liga-

ment. He also specifically mentions the considerable strength of LT, which was not pointed out by his predecessors. This can be explained by the fact that this anatomical element was personally studied by Galen in somatically healthy young people who died in battle, i.e. physically well-developed warriors and gladiators (which is currently impossible for ethical reasons). The latest information on the low mechanical strength of LT, refers, as a rule, to senior persons (55-58). In his main work on anatomy, *On Anatomical Procedures*, Galen gives a more detailed description of the structure of HJ and LT yet:

«As with the arm you examined the ligaments of the bones, so now examine those of all the exposed joints and first of the hip. This has one ligament (σύνδεσμον) embracing it [capsular ligament], as with all joints. A second, hidden in the depths of the joint [ligamentum teres], ties the head of the femur to the hollow in the hip-bone [acetabulum]. It is so tough (σκληρός) that it could be called a “cartilaginous sinew” (νεῦρον χονδρῶδες)».

De anat. adm. II, 10 (41,59).

By the “ligament embracing the hip” Galen clearly means the HJ capsule (*capsula articularis*) with the external ligaments woven into it. The word “tough” describes the tension and high elastic modulus of LT (57). In the described specimen, Galen encountered LT of the “cartilage consistence”. Modern histological studies have established that “near their attachments the structure of ligaments undergoes a transition into fibrocartilage [...] and the fibroblasts become encapsulated and resembled chondrocytes” (60).

Galen provides a detailed description of LT in his work on physiology *On the Usefulness of the Parts of the Body*:

«In the femoral joint Nature created a ligament (σύνδεσμος), which is round, very strong, extending from the femoral head and connecting in the middle with the acetabulum, but did not create this in the shoulder joint, arranging it so that it could perform various movements».

De usu part. XII, 5, 17 (41).

This quotation from Galen echoes the statement made by Hippocrates in the treatise *Instruments of Redactions* (§ 1), where he also compares the anatomical features of HJ and the shoulder joint. Galen was undoubtedly familiar with this treatise by Hippocrates, although he left no comment on this work. Calling the ligament “round” (στρογγύλος) (lat. *teres, rotundum*), Galen must have meant not only the cross-sectional shape of LT, but also the interweaving of thin fibers, the fibrils of its stroma, since the Greek verb

στρογγύλλω also means “to twist, rotate”. These thin fibers or, more precisely, bundles of fibers, were observed by Galen in ligaments and nerves.

Two more references to LT are found in Galenic *Commentary on Hippocrates' On Joints*, considered as one of the earlier works compiled between 177 and 180 AD. In the third chapter of the first Galen's comment we read:

«The femur has a small head and an elongated neck. It is located in the acetabulum, which is [quite] deep and surrounded by protruding edges; and at the top of its head [there is] a strongest ligament (σύνδεσμον ισχυρότατον) connecting with the acetabulum in the deepest place. That is why the femur is rarely dislocated as opposed to the shoulder that has no ligament (σύνδεσμον) and does not enter a deep cavity».

In Hipp. de art. I, 3 (41).

This passage, as the ones before it, refers to the writings of Hippocrates, namely, to the treatise *On Joints* (*De artic.*, 79) and *Instruments of Redactions* (*Vectiar.*, 42), in which the articular cavity of the shoulder joint is compared to the one of the HJ (39). The above quote shows that Galen had a good idea of the structure of HJ and distinguished *acetabular labrum* as well as its “deepest place” – *acetabular fossa*. He also notes the strength of LT and one of its functions, the retention of femoral head in acetabulum.

In the fourth Galenic *Commentary on Hippocrates' On Joints*, § 40, entitled “How to correct hip dislocation when it is dislocated inward,” the author writes this about HJ:

«In this joint, the ligament [of the femoral head] is extremely strong, rounded and hidden in it, connecting the top of the femoral head with the deepest inner part of the acetabulum. Therefore, without even seeing the ligament itself, but based only on this reasoning, we can understand that it is short: since the hip always rotates - as Hippocrates said: “it rotates in the pelvic bone” - and never leaves the acetabulum, the ligament ought to be very short. And so, it is the ligament that prevents the hip from being dislocated, at least while it is in its natural state. And not only can the ligament rupture, but also, due to the abundance of fluid that has unnaturally accumulated in the cavity [joint], become so stretched that it sometimes allows the femur to dislocate from its natural place. And if, due to a rupture of the ligament, the hip is dislocated, then even with immediate reduction it will not be able to remain in its place. <...> This is how the nature of things teaches us that with a torn ligament, the reduction of femur cannot remain in its place. It should also be added that on the outside of the knee joint there are several tendinous ligaments and in the hip joint there is only this ligament, since neither anything of this kind, nor any muscles support

the joint from the outside. <...> And Hippocrates himself said at the beginning of this book that even in bulls it is the hip that get dislocated when they grow old and lose weight, because when the ligament of the hip is torn, the thigh joint cannot remain in its place even after reduction, the thing that especially applies to the emaciated [animals].

Next, let's look at what can happen if the ligament, weakened due to the abundance of fluid [in the joint], allows the hip to dislocate, but then is reset. It seems to me that it is quite obvious that in the presence of fluid the joint will dislocate again, and when it dries up, it will regain its natural strength. And the fact that dislocation of the hip can happen due to the fluid can be learned from Hippocrates himself, who writes in the "Aphorisms" thus: "Whoever, due to the chronic sciatica, has the femoral head dislocated and reset again, will have an accumulation of mucus formed there". And when mucus builds up in the joint, then the ligament softens and weakens as a result. And it does not matter whether you call it a ligament (σύνδεσμον) or a connecting sinew (νεῦρον συνδετικόν). For those ligaments that have a rounded shape like tendons are usually called connecting sinews by anatomists. And we have already cured this kind of hip dislocation twice, and it did not dislocate again. Drying medications should be applied over the joint for a long time until the connective sinew is dry enough to stop stretching together with the femur extending beyond the edge of the acetabulum and keep it in its natural position».

In Hipp. de art. IV, 40 (41).

Such an extensive quotation is provided in view of its exceptional significance. Here Galen describes in detail the geometric and mechanical properties of LT, its topographic anatomy and function. Regarding the attachment areas, the author notes that LT connects with the bones, that is, with the femoral head and the inside of the acetabulum (*In Hipp. de art. IV, 40; cf. ibid. I, 3*) (41), undoubtedly meaning acetabular fossa (lat. *fossa acetabuli*). Perhaps it is from this treatise that the term "round ligament" (lat. *ligamentum teres, ligamentum rotundum*) first entered the academic vocabulary and is still very much in use.

The author also dwelled on the pathology, that is, a rupture resulting from a traumatic hip dislocation and its "weakening" due to accumulation of pathological fluid, possibly implying synovitis. Galen also informs us of his own successful experience in treating a (presumably) recurring hip dislocation in children. Here we find, for the first time in history of medicine, a description of the conservative treatment of LT pathology. Galen in this case used some "drying medications", possibly in the form of compresses or ointment dressings, which, according to his clinical plan, "dried" the LT, eliminating its hyperelasticity, and was conducive to its contracting.

Galen's texts were well known to the Byzantine medical authors, who composed various kinds of encyclopedic codices and compendia containing extracts from the writings of ancient physicians, mainly Hippocrates and Galen. So, for example, Oribasius (IV-V cent.), when describing the structure of the femur in his essay "Medical Collections" (*Coll. Med. XXV, 19*) gives an exact quote from the aforementioned Galen's treatise *On Bones for Beginners* (41).

Important refinements in the description of the structure of LT are also found in the Pseudo-Galen's treatise *Introduction, or the Physician*:

«The hip has one bone. And its head, somewhat curved, enters the deep acetabulum of the pelvis; and it is connected by a sinew (νεῦρον), growing from the middle of this cavity and growing into the middle of the femoral head».

Introd. s. Med. XII (41,61,62).

It should be noted that in this case, Pseudo-Galen, in reference to LT, uses not the word σύνδεσμος, which is more characteristic of Galen, but the old term νεῦρον, which was employed by his predecessors Hippocrates, Heraclides of Tarentum, Hegetor, Apollonius of Citium and Rufus of Ephesus. Apparently, Pseudo-Galen was well versed in the normal anatomy of HJ, as he correctly describes the significant depth of acetabulum and the attachment of LT to its middle, that is, to the bottom of acetabular fossa. The latter circumstance is extremely important for biomechanics of HJ, since only with this type of connection can LT fully perform its important functions: limiting movements, supporting the pelvis, the hip abductor muscle group and the upper part of the femoral head (15,17,23).

Another description of LT, similar to Galen's, is found in the works of Byzantine physician Theophilus Protospatharius (circa VII cent.), who, in book V of the treatise *On the Construction of the Human Being*, wrote:

«For the sake of this, the kindness and creation of God grew from the bottom of the acetabulum a round sinew (νεῦρον), a cartilaginous ligament (σύνδεσμον χονδρώδη), growing into the head of the femur and holding it there to avoid dislocation».

De corp. hum. fabr. XIII, 204.

In the above passage, Theophilus Protospatharius does not provide any new information, but, undoubtedly under the influence of Galen, uses the epithet "round" and "cartilaginous", describing the beginning of LT from the bottom of acetabulum, and also agreeing with its role of holding the femoral head and preventing dislocation.

CONCLUSIONS

Ancient physicians wrote predominantly in Greek, with medical literature in Latin being presented but scarcely, and terminology developed poorly (63). The term *ligamentum* in the anatomical meaning of “ligament” did not yet exist in Latin literature. The word *nervus*, “sinew”, was used to denote ligaments in Latin. By the end of the V century the four basic terms for LT that Galen mentions had already been known: νεῦρον (sinew), σύνδεσμος (ligament), νεῦρον συνδετικόν (connecting sinew) and νεῦρον χονδροῶδες (cartilaginous sinew).

The physicians of the Classical period clearly established that LT was present in both humans and animals, charted its topographic anatomy, as well as established its main mechanical and geometric properties. In particular, they noted that it “is located in the acetabulum”, and is “deeply hidden in the joint”, for some authors it “grows from the femoral head”, for others it “starts from the middle of the acetabulum”. These physicians also drew attention to its mechanical properties, that is, very high strength, resilience, consistence (cartilage density) and, at the same time, flexibility, allowing movements of the femoral head. The geometric features were also indicated, in particular, that LT has a short length, and in shape it is “round”. The latter characteristic described by Galen subsequently led to the emergence of the well-known Latin term for LT, i.e. “round ligament”, which is still applied. Regarding the role of LT, it was noted that it connects the acetabulum with the femoral head and prevents its dislocation, that is, limits rotational and forward movements in HJ.

In Palestine of the biblical times, it was believed that LT provided a normal gait, participating in the organization of this type of human locomotion, and an injury to it caused lameness. Ancient medical authors also knew about its traumatic rupture, and identified other types of LT pathology, in particular, “soaking”, weakening (possibly a dystrophic change), stretching (that is, lengthening), suggested the possibility of its constriction (in other words, shortening) and drying (dehydration with increasing elasticity). The factors leading to pathological changes in LT were identified as trauma, namely hip dislocation, exhaustion, advanced age, and an excessive accumulation of “fluid” in HJ. For the treatment of LT pathology, the application of drying compresses was used. An injury to LT was seen as the cause of recurring hip dislocation. It was regarding this issue that the first correspondence discussion on LT took place, lasting five centuries and involving Heraclides of Tarentum, Hegetor, Apollonius of Citium and Galen of Pergamon.

STRENGTHS & WEAKNESSES

Strengths

Our work based on an interdisciplinary approach, allowing us to propose a combination of linguistic and medical analysis of various Greek and Latin texts containing references to *ligamentum teres*. A linguistic analysis has enabled us to trace the transformation of the term from 5 cent. BC to 3 cent. AD, as well as the history of the term, medical contexts of its usage, its etymology and its early synonyms. Due to descriptive study of different sources we have created a new field of researching and new questions to acquire a better understanding of how ancient authors considered normal and pathological anatomy of the *ligamentum teres*, its physical and geometric features to make possible a description of the conservative treatment of its pathology.

Weaknesses

Works of some ancient authors (Heraclides of Tarentum, Hegetor), which we have considered as genuine, preserved only in short fragments and quotations by the later writers, so that the genuineness of these works and our conclusions are still in question, and the information we have extracted from them is often suggestive rather than definitive. Not all of the passages we quoted (such as Galenus *In Hipp. De art. IV, 40*, ed. Kuhn XVIII A: 731-736) have been published in modern critical editions; an English translation of these passages are our own. Some authors reiterate or rethink the concepts of their predecessors, but we considered it necessary to present a complete collection of the quotations, providing them with comments.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

LIST OF ABBREVIATIONS

- Apollonius:
In Hipp. de art. – In Hippocratis de articulis commentarius
- Aristoteles:
Hist. anim. – Historia animalium
- Celsus:
De med. – De medicina
- Galenus
De anat. adm. – De anatomicis administrationibus
De loc. aff. – De locis affectis
De motu musc. – De motu musculorum

De usu part. – De usu partium

In Hipp. de art. – In Hippocratis librum de articulis et Galeni in eum commentarii IV

• Hippocrates:

De artic. – De articulis

De loc. in hom. – De locis in homine

Vectiar. – Vectiarius

• Oribasius:

Coll. med. – Collectiones medicae

• Plinius Major:

Hist. nat. – Historia naturalis

• Ps.-Galenus:

Introd. s. Med. – Introductio seu Medicus

• Theophilus Protospatharius:

De corp. hum. fabr. – De corporis humani fabrica

REFERENCES

1. Netter F. Atlas of human anatomy. 6th ed. Philadelphia; Saunders Elsevier 2011;474. InternetArchive
2. Waugh A, Grant A. Ross & Wilson Anatomy and physiology in health and illness. Edinburg [etc.]; Elsevier Health Sciences 2014;418. Google Books
3. Cerezal L, Arnaiz J, Canga A, et al. Emerging topics on the hip: ligamentum teres and hip microinstability. *Eur J Radiol.* 2012;81(12):3745–3754. PubMed
4. Martin HD, Hatem MA, Kivlan BR, Martin RL. Function of the ligamentum teres in limiting hip rotation: a cadaveric study. *Arthroscopy.* 2014;30(9):1085–1091. PubMed
5. Perumal V, Woodley SJ, Nicholson HD. Ligament of the head of femur: A Comprehensive Review of its Anatomy, Embryology, and Potential Function. *Clin Anat.* 2015;19(2):247–255. PubMed
6. Brady AW, Mikula JD, Chahla J, et al. Anatomic analysis of the native ligamentum teres. *J Hip Preserv Surg.* 2016;3(1):hnw030.012. GoogleScholar
7. Arhipov SV. Funkciya svyazki golovki bedrennoj kosti: obzor lit. *Genij ortopedii.* 2006;4:105–107. [in Russ.] eLIBRARY.RU
8. Wenger DR, Miyanji F, Mahar A, Oka R. The mechanical properties of the ligamentum teres: a pilot study to assess its potential for improving stability in children's hip surgery. *J Pediatr Orthop.* 2007;27(4):408–410. PubMed
9. Wenger DR, Mubarak SJ, Henderson PC, Miyanji F. Ligamentum teres maintenance and transfer as a stabilizer in open reduction for pediatric hip dislocation: surgical technique and early clinical results. *J Child Orthop.* 2008;2(3):177–185. PubMed
10. Lampert C. Läsionen des lig. capitis femoris: pathologie und therapie. *Arthroscopie.* 2009;22(4):293–298. SpringerLink
11. Sutton JB. The nature of certain ligaments. *J Anat Physio.* 1884;18:225–238. PubMed
12. Kapandji IA. The physiology of the ligamentum teres. In: Kapandji IA, Ed. *The physiology of the joints.* Vol. 2. 2-nd ed. New York; Churchill Livingstone 1978. GoogleScholar
13. Chen HH, Li KC, Li AFY, et al. Effects of ligamentum teres on the hip stability. *Biomed Eng Appl Basis & Comm.* 1994;6:276–279. GoogleScholar
14. Rao J, Zhou YX, Villar RN. Injury to the ligamentum teres. Mechanism, findings, and results of treatment. *Clin Sports Med.* 2001;20(4):791–799. PubMed
15. Arkhipov SV. On the role of the ligamentum capitis femoris in the maintenance of different types of erect posture. *Hum Physiol.* 2008;34(1):79–85. SpringerLink
16. Dodds MK, Lee J, McCormack D. Transarticular stabilization of the immature femoral head: assessment of a novel surgical approach to the dislocating pediatric hip in a porcine model. *J Pediatr Orthop.* 2008;28(1):36–42. PubMed
17. Arkhipov SV, Zagorodny NV, Skvortsov DV. Ligamentum capitis femoris a pilot an experimental study. *Am J Biomed Sci & Res.* 2019;5(2):92–94. AmJBiomedSciRes
18. Bardakos NV, Villar RN. The ligamentum teres of the adult hip. *J Bone Joint Surg. Br.* 2009;91(1):8–15. PubMed
19. Cerezal L, Kassarian A, Canga A, et al. Anatomy, biomechanics, imaging, and management of ligamentum teres injuries. *Radiographics.* 2010;30(6):1637–1651. PubMed
20. Botser IB, Martin DE, Stout CE, Domb BG. Tears of the ligamentum teres: prevalence in hip arthroscopy using 2 classification systems. *Am J Sports Med.* 2011;39(1) (Suppl):117S–125S. PubMed
21. Byrd JW, Jones KS. Traumatic rupture of the ligamentum teres as a source of hip pain. *Arthroscopy.* 2004;20(4):385–391.

PubMed

22. Haviv B, O'Donnell J. Arthroscopic debridement of the isolated Ligamentum Teres rupture. *Knee Surg Sports Traumatol Arthrosc: Off J ESSKA*. 2011;19(9):1510–1513.

PubMed

23. Arkhipov SV. Svyazka golovki bedrennoi kosti. Funktsiya i rol' v patogeneze koksartroza. Saarbrücken; Lambert Academic Publishing 2013. [in Russ.]

MoreBooks

24. O'Donnell J, Klaber I, Takla A. Ligamentum teres reconstruction: indications, technique and minimum 1-year results in nine patients. *J Hip Preserv Surg*. 2020;0(0):1–7.

GoogleScholar

25. Arkhipov SV, Skvortsov DV. Ligamentum capitis femoris: first written mentions. *MLTJ*. 2019;9(2):156–164.

GoogleScholar

26. O'Donnell JM, Pritchard M, Salas AP, Singh PJ. The ligamentum teres - its increasing importance. *J Hip Preserv Surg*. 2014;1(1):3–11.

GoogleScholar

27. Rosinsky PJ, Shapira J, Lall AC, Domb BG. All About the Ligamentum Teres: From Biomechanical Role to Surgical Reconstruction. *J Am Acad Orthop Surg*. 2020;28(8):e328–e339.

PubMed

28. Padulo J, Oliva F, Frizziero A, Maffulli N. Muscles, Ligaments and Tendons Journal – Basic principles and recommendations in clinical and field Science Research: 2018 update. *MLTJ* 2018;8(3):305–307.

GoogleScholar

29. Shchedrovickij D. Vvedenie v Vethij Zavet. I. Kniga Bytiya. Moskva; Terevinf 1994. [in Russ.]

GoogleScholar

30. Haudebert P (Ed.). *Le Pentateuque: Débats et recherches*. XIVe congrès de l'ACFEB, Angers (1991) (coll. *Lectio divina*, 151). Paris; Le Cerf 1992.

Persée

31. Bräumer H. *Das erste Buch Mose*. Bd. I. Wuppertaler Studienbibel. Reihe Altes Testament. Berlin; Evangelische Versandbuchhandlung O. Ekelmann Nachf 1986.

Booklooker

32. Brown F. *The new Brown-Driver-Broggs-Gesenius Hebrew and English Lexicon*. Peabody; Hendrickson Publishers 1979:161;674–675.

GoogleScholar

33. Jamieson R, Fausset AR, Brown D. *Commentary Critical and Explanatory on the Whole Bible*. Vol. I. Hartford; 1871.

GoogleScholar

34. *The Pulpit Commentary*. / Ed. by H.D.M. Spence and J.S. Exell. Vol. I. New York, Toronto; Funk & Wagnalls Company 1880.

InternetArchive

35. *A Concise Hebrew and Aramaic Lexicon of the Old Testament*. / By W.L. Holladay. Leiden; Brill 1988.

InternetArchive

36. *Exposition of the Old and New Testament*. / Ed. by J. Gill. Vol. 1. London; 1748–1763.

WebArchive

37. Preuss J. *Biblical and Talmudic Medicine*. Transl. and ed. by Dr. F. Rosner. New York [etc.]; A Jason Aronson Book, Rowman & Littlefield Publishers 2004.

GoogleBooks

38. André J. *Le vocabulaire latin de l'anatomie*. Paris; Belles lettres 1991:208–209.

GoogleScholar

39. *Oeuvres complètes d'Hippocrate* / Par É. Littré. Vol. 1–10. Paris; 1839–1861 (repr. Amsterdam, 1961–1973).

HathiTrust

40. von Staden H. *Herophilus. The Art of Medicine in Early Alexandria*. Cambridge [etc.]; Cambridge University Press 1989:256.

GoogleBooks

41. Kühn CG. *Claudii Galeni opera omnia*. Leipzig; 1821–1833 (repr. Hildesheim, 1965).

HathiTrust

42. Garofalo I. *Erasistrati fragmenta*. Pisa; Gardini 1988.

GoogleBooks

43. Marcovecchio E. *Dizionario etimologico storico dei termini medici*. Firenze; Festina lente 1993:578.

GoogleScholar

44. Sutton JB. The nature of ligaments. Part IV. *J Anat Physiol*. 1885;20(Pt 1):38.1–75.

PubMed

45. Welcker H. Nachweis eines ligamentum interarticulare ("teres") humeri, sowie eines Lig. sessile Femoris. *Z Anat Entwicklungsgesch*. 1877;2:98–107.

GoogleScholar

46. Kolts I, Busch LC, Tomusk H, et al. Macroscopical anatomy of the so-called "rotator interval". A cadaver study on 19 shoulder joints. *Ann Anat*. 2002;184(1):9–14.

GoogleScholar

47. Reuther F. Anatomie, Biomechanik und Klassifikation der Schultergelenkverletzung. *Trauma Berufskrankh*. 2006;8(3):S241–S246.

GoogleScholar

48. Sabat D, Singh D, Kumar V, Gupta A. Open perineal dislocation of hip in a child. *Eur J Orthop Surg Traumatol*. 2009;19:277–279.

GoogleScholar

49. Muzaffar N, Hafeez A, Bashir N, Singh S. Open anterior hip dislocation in a young adult with exposed femo-

- ral head and no neurovascular damage. *Malays Orthop J*. 2012;6(1):40–42.
PMC
50. Arkhipov SV, Prolygina IV. Galen o vyvikhe bedra i svyazke golovki bedrennoi kosti. *Opera medica historica. Trudy po istorii meditsiny. Al'manakh ROIM. Vypusk 4. Moskva*; 2019:89–96. [in Russ.]
ResearchGate
51. Schaumkel JV, Villar RN. Healing of the ruptured ligamentum teres after hip dislocation-an arthroscopic finding. *HIP Int*. 2009;19(1):64–66.
GoogleScholar
52. Davarinos N, Bonvin A, Christofilopoulos P. Ligamentum teres reattachment post-surgical dislocation of the hip: a case report. Regenerative capacity reaffirming its greater role in hip stability and function? *J Hip Preserv Surg*. 2017;4(4):337–340.
GoogleScholar
53. Kollesch J, Kudlien F. Apollonii Citiensis In Hippocratis De articulis commentaries; ediderunt J.Kollesch et F.Kudlien, in linguam Germanicam transtulerunt J.Kollesch et D.Nickel. Berolini; in aedibus Academiae Scientiarum 1965.
CMG
54. Daremberg Ch, Ruelle Ch. *Oeuvres de Rufus d'Ephèse*. Paris; 1879.
GoogleBooks
55. Podrushnyak EP. *Vozrastnye izmeneniya sustavov cheloveka*. Kiev; Zdorov'ya 1972. [In Russ.]
GoogleScholar
56. Chen HH, Li AF, Li KC, Wu JJ, Chen TS, Lee MC. Adaptations of ligamentum teres in ischemic necrosis of human femoral head. *Clin Orthop Relat Res*. 1996;328:268–275.
PubMed
57. Philippon MJ, Rasmussen MT, Turnbull TL, et al. Structural Properties of the Native Ligamentum Teres. *Orthop J Sports Med*. 2014;2(12):2325967114561962.
PubMed
58. Perumal V, Scholze M, Hammer N, Woodley S, Nicholson H. Load-deformation properties of the ligament of the head of femur in situ. *Clin Anat*. 2019:1–9.
GoogleScholar
59. Singer C. *Galen: On Anatomical Procedures (I-IX 6)*. London [etc.]; Oxford University Press 1956.
InternetArchiv
60. Ham AW. *Histology*. Philadelphia, Toronto; J.B. Lippincott Company 1974.
InternetArchive
61. Everett N. *The Alphabet of Galen: Pharmacy from Antiquity to the Middle Ages: a Critical Edition of the Latin text with English translation and commentary*. Toronto [etc.]; University of Toronto Press 2012.
GoogleBooks
62. Petit C. What does pseudo-Galen tell us that Galen does not? Ancient medical schools in the Roman Empire. In *Philosophical themes in Galen*. Eds. P. Adamson, R. Hansbeger, J. Wilberding. London; The Institute of Classical Studies University of London 2014:269–290.
GoogleScholar
63. Langslow DR. *Medical Latin in the Roman Empire*. Oxford; University Press 2000.
GoogleBooks